Flour Strength as Influenced by the Addition of Diastatic Ferments

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE
SCHOOL OF THE UNIVERSITY OF MINNESOTA

By

FERDINAND A. COLLATZ, B. S., M. S.

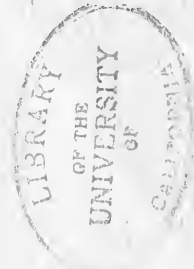IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
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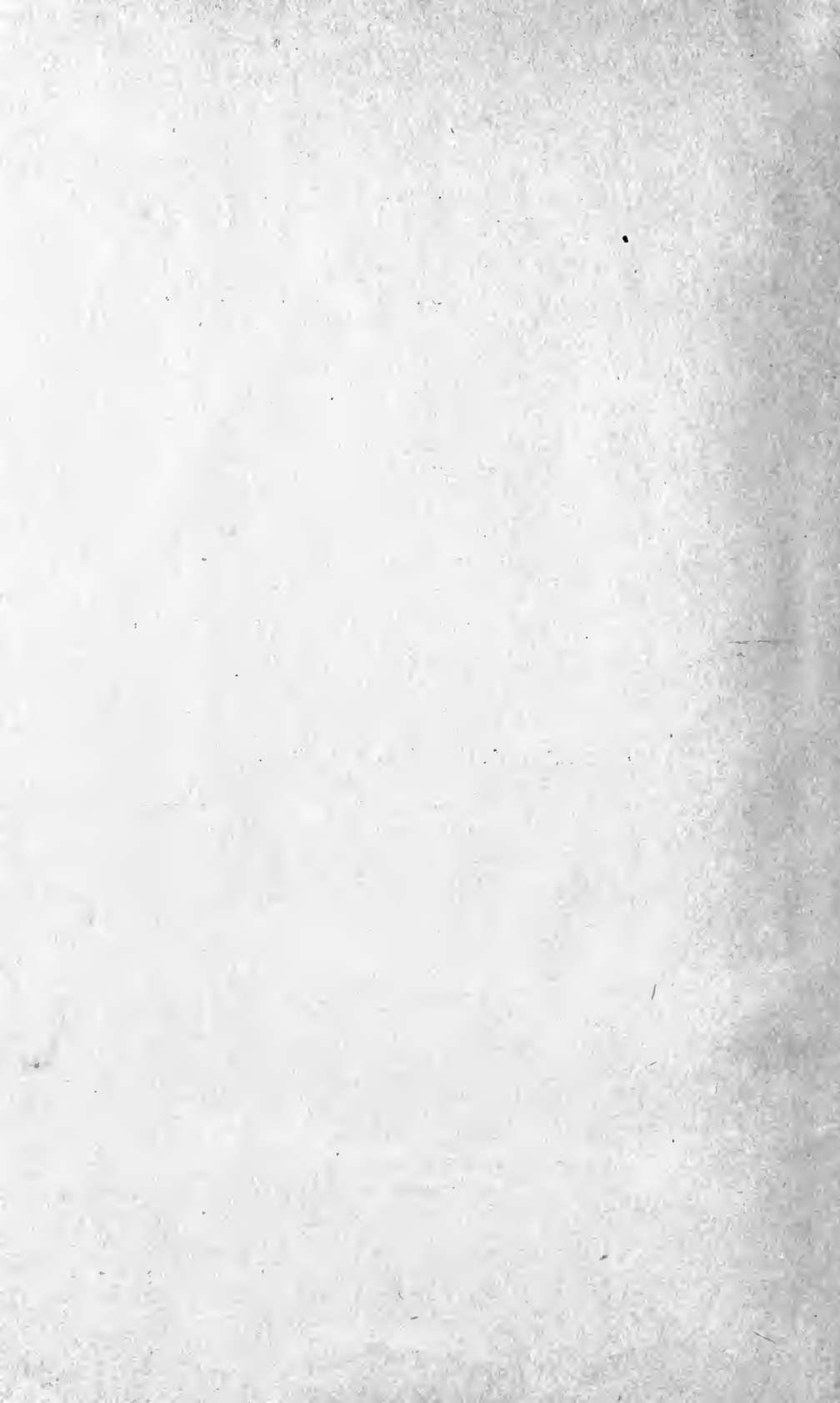# Flour Strength as Influenced by the Addition of Diastatic Ferments

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By Ferdinand A. Collatz

I. INTRODUCTION.

The baking strength of flour has received a great deal of attention by scientific workers in the last twenty-five years, due primarily to the economic importance of bread. A number of factors have been thoroughly investigated, in their relation to baking strength, in order to draw some conclusions as to why some flours give a large, light, palatable loaf of bread and others an inferior loaf. Certain factors which have been investigated in their relation to baking strength are total nitrogen, ratio of water-soluble nitrogen to total nitrogen, chemical composition of the individual proteins, total gluten, total gliadin, ratio of gliadin to glutenin, ratio of gliadin to total nitrogen, ratio of wet to dry gluten, effect of electrolytes, hydrogen-ion concentration, total amount of gas evolved during fermentation, and the effects of diastatic and proteolytic enzymes of the flour.

Flours which bake out well have been given the arbitrary term of strong flours while the others are termed weak. Naturally a great number of definitions of strength have found their way into the literature, but the definition that has been most generally accepted is that of Humphries and Biffin (1907), who state that "a strong wheat is one which yields flour capable of making large, well-piled loaves." Flours which do not measure up to this empirical standard are classed as weak. This definition indicates that strength in flour is more desirable than weakness for the baking of bread. Wood (1907), has called attention to two factors in strength, namely, size and shape of the loaf. This has stimulated a great deal of research by Ford and Guthrie (1908), Baker and Hulton (1908), on the diastatic and proteolytic enzymes in wheat flour, and also by Upson and Calvin (1915) (1916), Gortner and Doherty (1918), and Sharp and Gortner (1922), on the colloidal properties of wheat gluten as affecting flour strength. Today we must recognize three groups of factors dealing with strength or weakness in flour, According to Sharp and Gortner (1922). we have "at least three classes of weak flour, i. e., (1) weakness due to an adequate quantity of gluten but of inferior quality, (2) weakness due to an inadequate quantity of a good quality gluten and (3) weakness due to factors influencing yeast activity."

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HISTORICAL.

Not until Osborn and Voorhees (1893) (1894), established the composition and properties of the wheat proteins was any great advance made in regard to flour strength, and naturally attention was then directed to the two main proteins, gliadin and glutenin. Fluerent (1896) asserted that flour strength depended upon the proportions of gliadin to glutenin, the ratio of 75 percent to 25 percent or 3 to 1 being most nearly ideal. Snyder (1899) came to similar conclusions but stated that the ideal ratio was 65 percent gliadin and 35 percent glutenin. In a later publication Snyder (1901) claims quality rather than quantity of protein is of the greater importance. Shutt (1904) (1907) points out that after several years of research "it appears extremely doubtful if the gliadin number (percentage of albuminoids in the form of gliadin) constitutes a factor that can be correlated with bread making values as obtained by baking trials." These conclusions are again verified in a later report. Thatcher's (1907) results show that no single factor or group of chemical tests which he tried give results from which the comparative baking qualities of flour can be determined. Blish (1915) states that the gliadin-glutenin ratio is much more constant in flours of different baking strength than has heretofore been supposed. Blish found, after careful investigation, that the individual proteins of weak and strong flours are chemically identical.

The soluble proteins of flour have also had their share of investigations as to their relation to baking strength. Snyder (1897), Bremer (1907), Wood (1907) and others have found no relation of baking strength to the amounts of water soluble proteins.

Quite recently Martin (1920) has attempted to correlate certain properties of flours with baking strength. He finds "for flours having a satisfactory gas producing capacity, bakers' marks, gas retaining capacity, and amended gliadin content are closely related. and it is considered that the estimation of the gas producing capacity will indicate the strength of the flour."

Martin's "amended gliadin" content is the difference in protein (Nx5.7) between the amounts extracted by 50 percent alcohol and that extracted by water acting for three hours at 24-25°. Sharp and Gortner (1922) find that "amended gliadin" values were not correlated with the strength of the flours with which they worked.

The Proteins of Wheat Flour and Their Physical Relation to Flour Strength.

As far as we know, all proteins belong to that class of colloids known as emulsoids and more recently termed "hydrophylic colloids." As the latter term suggests, this class of colloids has a great affinity for

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water, which, however, can be modified to a great extent by the addition of acids, bases and salts to the dispersion medium.

Hofmeister was one of the first to investigate the swelling of proteins. He found that in solutions of sulphates, tartrates, acetates, alcohol and cane sugar, gelatin-plates take up less water than they do when immersed in distilled water, while in solutions of potassium, sodium or ammonium chlorides, sodium chlorate, sodium nitrate and sodium bromide, they take up more water than they do when immersed in distilled water. Hofmeister's work has been enlarged upon by others, notably Pauli (1899) (1902) (1903) (1905) (1906) and Fischer (1915) (1918).

Wood (1907) and Wood and Hardy (1908) have demonstrated wheat gluten to be an emulsoid colloid and as has already been noted the water-holding capacity of hydrophylic colloids can be altered to a marked degree by the addition of electrolytes. Acids and bases cause imbibition up to a certain point, while neutral salts tend to inhibit the imbibition of water.

It appears that Wood (1907) was the first to call attention to the physical properties of the proteins in wheat flours rather than the chemical differences in relation to flour strength. He investigated the possible chemical differences between the glutenin and the gliadin of these two classes of flours. From this he concludes that strength (particularly the shape of the loaf) is closely related to the physical state of the gluten, which in turn is affected by the presence of electrolytes.

Wood, and Wood and Hardy determined the effects of acids, with and without salts, by suspending bits of gluten from glass rods in the liquid to be investigated. They found that dispersion of the gluten starts immediately when immersed even in the lowest concentration of acid, and dispersion increased with increase in concentration within certain limits. This holds good for sulphuric, phosphoric and oxalic acids, but not for hydrochloric. When the concentration of the latter exceeded N/30 the dispersion began to decrease until at a concentration of N/12 the gluten was more elastic and coherent than in its original condition. The addition of salts decreases dispersion in all cases and such amounts of salts can be added which will prevent the dispersion of the gluten. From this Wood suggests "that the variation in coherence, elasticity and water content, observed in gluten extracted from different flours, is due to varying cancentrations of acids and salts in the natural surroundings of the gluten, rather than to any intrinsic difference in the composition of the glutens themselves." Wood thinks that the direct addition of acids or salts to the flour, in order to strengthen it, is impractical as they would have to be in con-

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tact for forty-eight hours before baking. He found that this was the time required for the gluten to come into equilibrium with its surroundings. He, therefore, advocates the blending of such flours which will supply each flour's requirements in this respect.

In a later paper Wood and Hardy (1908) take up the theoretical discussion of the effects of acids, alkalis, and salts upon gluten.

Upson and Calvin were the next to study the colloidal swelling of gluten. They atacked the problem in a slightly different manner, washing out the gluten with distilled water and pressing it out into thin layers. They next cut out discs of uniform size and weighed them immediately. The discs are then immersed in acid and acid-salt solutions of varying concentrations for a definite period of time when they are taken out, drained and weighed. They find that "flours containing acids and salts in such combinations as to favor water absorption will behave as weak flours, whereas those containing acids and salts in such combinations as inhibit water absorption will behave as strong flours." Their conclusions are very similar to those of Wood.

Gortner and Doherty were the next to investigate the colloidal properties of wheat flour gluten. Their method of attack was like that of Upson and Calvin, in that they recorded the increase in imbibition by weighing discs of gluten after immersion in acid and acid-salt solutions of various concentrations. They worked with five different flours, namely a high grade patent flour milled from hard spring wheat, a clear flour and three typically soft flours milled from Oregon wheat. Their results show that the gluten from a weak flour has a much lower rate of imbibition and a much lower hydration capacity than the gluten from a strong flour; also that inorganic salts when added to an acid solution lower the relative imbibition of gluten placed in such solutions. Glutens from the different flours react differently to the addition of inorganic salts. This leads them to believe "that a weak gluten does not owe its 'weakness' nor its imbibition curve its 'flatness,' to either the acid or the salt content of the flour from which it is derived, but rather to the fact that a weak gluten has inherently inferior colloidal properties."

In 1918 and 1919, a series of articles dealing with the physical properties of wheat flours were published by Henderson and his co-workers. Inasmuch as they fail to describe their flours, no conclusions can be drawn as to their effect on flour strength. It is of interest to note, however, that at a hydrogen ion concentration of about pH 5, (the optimum hydrogen ion concentration in the making of bread as found by Jessen-Hanson) the viscosity of dough, made from four different flours is at a minimum. They further note that salts such as sodium lactate, Na_2SO_4 $MgSO_4$, $KBrO^3$ tend to decrease the viscosity of the

dough, while NaCl, $MgCl_2$ NH_4Cl tend to decrease the viscosity when used in small amounts, but on further addition the viscosity increases.

Ostwald (1919) and Lüers (1919) and Lüers and Ostwald (1919) (1920) in a series of papers show the remarkable parallelism existing between viscosity measurements of flour-water mixtures and grade of flour. They found that flours divide themselves into three distinct groups when measured in this way. The low extraction flours (40%-60%) constitutes one group, the high extraction flours (60%-94%) a second, and the tailing flours (remains of 60 % - 94 %) constitute the third group. They also find that acids and bases tend to increase the viscosity of the flour-water mixtures while salts tend to depress the viscosity. Their results, although obtained by a different method, confirm the work of Wood, Wood and Hardy, and Upson and Calvin. It must be emphasized, however, that their results were obtained on flours of different milling extraction and the differences they obtained refer only to the grade or degree of extraction of the flours, and do not necessarily apply directly to the problem of flour strength.

Sharp and Gortner have continued the work of Gortner and Doherty, and confirm the findings of the latter in regard to the action of acids upon gluten. They find a marked difference in the rate of imbibition when using the various alkali hydroxides. The action of alkalis on gluten is markedly different from that of acids, as dispersion takes place at much lower concentrations. They remark in this reinspect, "Indeed dispersion and imbibition are here almost coincident." During the course of the work Sharp and Gortner washed out the gluten from their strong and weak flours and dried it at low temperature in vacuo. After pulverizing, they found that the dried glutens of various flours were much more alike than in the wet state. This observation is in accord with their theory "that the strong gluten is strong because of its colloidal properties; inasmuch as it is well known that the alternate wetting and drying of a colloidal gel, breaks down the gel structure." They also find that the optimum hydrogen ion concentration for imbibition is the same for the different acids used.

In their next paper Sharp and Gortner (1921) extend their work on the hydration capacity of glutens. They find that the viscosimeter gives an accurate measure of imbibition in that the curve obtained followed the previous curves by weighing out the discs of gluten. As might be expected, they found that the strong flours give much greater viscosity measurements than do the weak flours.

The Carbohydrates of Wheat Flour and Their Relation to Flour Strength.

The carbohydrates in the flour have also been investigated with ref-

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erence to their role in flour strength. Wood was perhaps the first to make any definite statement in this respect, although Girard and Fleurent (1903) called attention to the great variations in the amounts of sugars in the flours. They found on analysis that glucose and cane sugar were present, the former varying from 0.09% to 0.81%, the latter 0.63% to 1.89%. Bruying in 1906 considered that the sugar present in flour is not glucose and sucrose, but almost entirely maltose. Liebig (1909) supports the views of Girard and Fleurent in that he found wheat flour to contain from 1-1.5% sucrose and 0.1-0.4% dextrose. Wood thinks that strength hinges to a great extent, as far as volume and size of the loaf is concerned, upon the sugar content and the diastatic enzymes that the flour contains. He sums up that factor in strength dealing with volume of the loaf as follows: "The factor which primarily determines the size of the loaf which a flour can make is quite distinct. The size of the loaf is shown to depend in the first instance on the amount of sugar contained in the flour together with that formed in the dough by diastatic action. Particular attention should be paid to the rate of gas evolution in the later stages of fermentation, as this is shown to be more directly connected with the size of the loaf." Wood's method of measuring the gas-producing capacity of a flour consists of mixing 20 grams of flour and 0.5 grams of yeast with 20 cc of water in stoppered flasks and measuring the liberated carbon dioxide under brine.

Shutt (1907) shortly after Wood's article, determined the sugar extracted by 70 per cent alcohol and by water and could find no evidence "that with increase of sugars there is increase of volume in loaf, but rather the reverse." Shutt's data is shown in Table I.

TABLE I.

Sugars in flour extracted by 70 per cent alcohol and water. (Data taken from Shutt [1907] page 20).

| | In aqueous extract | | | In alcoholic extract | | | |
Designation of Sample	Directly reducing as Maltose %	After Inversion as Sucrose %	Total Sugars %	Directly reducing as Maltose %	After Inversion as Sucrose %	Total Sugars %	Vol. Bakers Mark
No. 1 Hard	1.96	2.14	4.10	.13	.91	1.04	492
No. 1 Northern	2.73	1.95	4.68	.20	.94	1.14	443
No. 2 Northern	1.87	1.79	3.66	.18	.87	1.05	438
No. 3 Northern	3.42	2.10	5.22	.26	1.21	1.47	383
No. 4 Com'cial Gr.	3.62	2.43	6.05	.05	1.34	1.39	397
No. 5 Com'cial Gr.	3.63	2.43	6.06	.06	1.42	1.48	366
No. 6 Com'cial Gr.	4.07	2.43	6.50	.06	1.36	1.42	363

Shortly after the appearance of Wood's paper, Baker and Hulton (1908) reported a paper in which they investigated the action of en-

zymes contained in flour with regard to their effect on flour strength. Unable to demonstrate experimentally the existence of proteolytic enzymes in flour, they concluded that any which are present have no effect upon the gluten during the time of fermentation. They did show, however, that the proteolytic enzymes contained in yeast play an important part, since one dough containing yeast showed 2.7% soluble nitrogen as protein, while a similar dough without yeast had 1.9% soluble nitrogen as protein. Although the fact was known that wheat flour contained amylolytic enzymes, Baker and Hulton demonstrated their presence in dough by an increase in maltose, extracting the latter with water and preparing the maltosazone. They found that contrary to expectations, the diastatic activity of flours increased with age. *

Baker and Hulton (1908) show that the total volume of gas produced (same method as outlined by Wood) increases roughly with increase in baking strength (Baker's Mark) of the flour. They also point out that a weak flour may have a diastatic power as high or even higher than a strong flour. This is explained by the fact that in reality the weak flour is deficient in liquifying enzymes and that by the addition of liquifying enzymes a much greater volume of gas is given off, while a strong flour shows no increase in gas production when a diastatic enzyme is added. The liquifying enzymes were added in the form of a malt extract and Table II shows that even such smaller amounts as 0.25 and 1.0 percent caused the gas production to increase enormously in a weak flour. They did not state whether the addition of the malt extract did actually increase the baking strength of the flour. From the data submitted, Baker and Hulton concluded that weak flours in some instances give as great a gas production as do strong flours, and that gas production is not a function of the quantity of diastases but, as they show, (Table III) it is intimately connected with "the additional matter rendered soluble during the process of doughing," i. e. maltose.

TABLE II.
Effects of added malt extract upon a weak flour with reference to an increase in gas production. (From data of Baker and Hulton [1908] page 372).

Time Hours	Flour Alone c.c. CO_2	0.25 Percent of Malt c.c. CO_2	1.0 Per cent of Malt c.c. CO_2
0.5	28	30	32
1.0	47	66	69
1.5	55	101	115
14.0	113	245	362

*(It is a well-known fact that flours on aging show greater baking strength and this increase in diastatic activity may therefore be the primary cause for increase in baking strength as the flour ages. At any rate it seems to be in accord with Wood's theory).

TABLE III.

The relation between gas volume and the additional matter rendered soluble during the process of doughing.   (From Baker and Hulton (1908) page 372).

Flour	Percent of matter soluble in water at 15.5°C.	Percent of Matter soluble in dough when kept at 40°C. for 3 hours	Differences Maltose formed during doughing	Volume of gas obtained from dough in 3 hours	Bakers Mark
1	2.12	3.60	1.48	78	45
X	2.03	4.41	1.38	84	40
W	2.83	5.38	2.53	145	76
3	2.49	5.53	3.04	155	80
Y	2.69	6.57	3.88	164	95
2	3.19	6.66	3.45	175	78
4	4.19	10.95	6.75	193	90
V	2.83	8.26	5.42	217	90
T	2.84	7.66	4.82	220	80
U	2.65	7.68	5.02	230	91
Z	3.54	11.65	8.11	270	90

It would seem from the above table that low strength flours are deficient in liquifying enzymes and the authors conclude that the liquifying enzymes are the limiting factors in the production of maltose in the dough stage.

Simultaneously with the appearance of Baker and Hulton's article, the work of Ford and Guthrie (1908) was published on the relation of enzymes contained in flour to its baking strength.   They conducted experiments of extraction and found that amylases could be greatly stimulated by the use of KCl and also by active and by boiled papain. In testing amylase values from twelve flours, they found differences (using KCl and papain extracts) varying from 22.1 to 46.8 expressed in grams maltose per gram of dry flour.   They could not correlate diastase activity with flour strength and state "It however indicates that in developing a method of evaluation, the total amylase is one important factor, also that the presence of a proteolytic ferment is another and possibly more valuable consideration."

Ford and Guthrie (1908) were probably the first to demonstrate the action of proteolytic enzymes in flour.   They were unable to secure results with nitrogen determinations or with the viscosimeter, so they tried 1 percent gelatin.   The liquification of the gelatin gave them positive proof of proteolysis.   They also conducted baking tests with a large amount of protease added, and naturally the loaf did not rise during the fermentation period, the resultant bread being a soggy mass.   They concluded that proteases decrease gas holding properties of the gluten and point out that this is the chief reason for failure in the use of malt extracts in baking practise.

Bailey and Weigley (1922) found that flour strength depended

upon factors which control the rate of carbon dioxide production and the amount of carbon dioxide lost during fermentation. They found that "the loss of carbon dioxide per unit increase in volume under controlled conditions affords a useful measure of gas-holding capacity of dough."

In some unpublished data Thatcher and Kennedy show that when flour was digested with water, the amounts of reducing sugars in the extract increased regularly with increase in temperature. A centrifuged aliquot of a flour water extract likewise increases in soluble nitrogen with increase in temperature of extraction. They also found that no increase in reducing sugars takes place when a filtered extract (O°-5°C) of flour is allowed to act on soluble starch when incubated at 40°, 50° and 60°C. Under these conditions they assume that absorption of the enzyme or activator has taken place upon the filter paper or upon the gluten colloids.

Historical Review of the Study of Diastatic Enzymes.

In taking up the history of the diastases, one is confronted with a voluminous and at times conflicting literature, which extends back over a period of one hundred years or more. Naturally, a great deal has to be discarded, as it would be impossible to review any but the most important papers submitted on this question. Nevertheless it is my intention to cite a number of papers which are of interest from a purely historical viewpoint.

Vauquelin in 1811 was the first to record the fact that when starch was heated in water, it gave an opaque solution and had the characteristics of gum arabic. In the same year Kirchoff found that when starch was boiled with dilute H_2SO_4, a crystallized sugar was formed. Two years later he noted that the protein of the embryo of the seed, particularly if the seed had been germinated, acted on starch in much the same manner as did the acid. He really was the first to record diastatic activity but did not realize the importance of his observations. Vogel in 1812 found that when starch was boiled with acid, it gave two products, a sugar and a gummy substance, the latter now known as dextrin. Stromeyer in 1813 found that iodine was a specific reagent for starch and visa versa, while the action of alkaline copper sulphate was found by Trommer in 1841 to be a means of distinguishing sugar from starches and gums.

The gummy substance found by Vogel was investigated by Biot and Persoz in 1853 and was found to turn the plane of polarized light to the right. For this reason it was given the name of dextrin. It is of interest to note that the work of Biot and Persoz formed the basis for the development of our present day polaris-

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cope. In the same year Payen and Persoz conclusively established the fact that an extract of malted grain had a powerful action in liquifying and saccharifying starch. They ascribed this function to some inner substance and named it diastase. It had been the impression of chemists up until this time that glucose was the sugar formed when starch was acted upon by diastase and it was not until 1872-1876 that O'Sullivan showed it to be maltose. O'Sullivan found the optical rotation to be too high and the reducing power too small to correspond with glucose. It might be of interest to call attention to the discovery of maltose at this time. Although DeSassure had accurately described maltose in 1819 the fact had evidently been forgotten until Dubrunfaut called attention to it in 1847 and named it maltose. This rediscovery was again forgotten until it was again described by O'Sullivan in 1872.

Marker in 1877 states that at a temperature of 60°C four molecules of starch yield three of maltose and one of dextrin, under the influence of diastatic ferments. At 65° the yield of maltose is lowered and at still higher temperatures two molecules of starch yield one of dextrin and one of maltose. Marker concludes that there are two diastatic ferments, one producing dextrin and the other maltose. This is our present day conception of the diastases, one being termed the liquifying and the other the saccharifying enzyme. Musculus and Gruber in 1878 regarded starch as a polysaccharide, containing five or six times the group $C_{12}H_{20}O_{10}$. Under the action of diastase or acids, the carbohydrate undergoes a series of changes of hydration and successive decomposition, resulting in maltose and dextrin of less molecular weight. Brown and Heron in 1879 found that the heating of a diastatic solution diminishes its activity and that an increase in temperature increases its activity up to 66°, beyond which not much activity is shown. They also found that alkalies markedly reduce the activity of a malt extract.

It was in 1879 that Kjeldahl stated his law of proportionality in regard to the action of diastases. He determined the reducing power of malt extract and saliva on an excess of starch at 57°-59°. He conisdered that the reduced copper was directly proportional to the amount of amylase present and was a true measure of diastatic power so long as digestion was not carried above 40 per cent of the starch present. Griefsmayer a year later, confirmed Kjeldahl's work and the law of proportionality.

Up to about this time the polariscope and the cupric reducing method had been used to estimate the diastatic power of malt extract by the amount of maltose formed. The iodine reaction was made use of by Roberts in 1881, however, and he defined the dias-

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tatic power of pancreatic extracts, saliva, and malt extracts, as the number of cubic centimeters of a standard starch paste which could be converted by one cubic centimeter of the active solution during five minutes at 40° into products giving no color reaction with iodine.

Jungk two years later published a method of determining the diastatic activity of a malt extract by the iodine method which was similar to the method of Roberts. He determined the time required for 10 cc of extract to convert 10 grams of starch which he considered should not exceed 10 minutes, for a good malt. His temperature of digestion was 40°.

From the literature already cited, two methods of determining the diastatic activity of an amylase preparation had come into use, namely the iodine or the so-called liquifaction method, which measures the power of the amylase to completely convert the starch into products which no longer give the characteristic color with iodine, and the reduction or saccharification method in which the amounts of reducing sugars are estimated by means of alkaline copper sulphate. The development of the iodine method from the time of Jungk will be the first considered.

Iodine Method for the Estimation of Diastatic Activity.

Francis in 1898 made the next improvement in the iodine method by laying down very exact rules for the determination of the end point in the iodine starch reaction. He also extended the time of digestion from 10 minutes to half an hour. Takamine in the same year developed a different procedure. He first standardized a sample of taka-diastase, which was found to keep its diastatic power for a considerable length of time. He then determined the relative amounts of standard and sample to be tested, which are required to accomplish the same amount of conversion in the same length of time. In these determinations he used iodine to test for the end point.

The next important work was carried out by Wohlgemuth in 1908. He established a new standard which was based on the number of cubic centimeters of 1 percent starch solution which 1 cc of diastatic ferment could convert in 30 minutes at 40°C. The method consists of measuring out 5 cc of a 1 percent starch solution into each of a series of test tubes and then adding different amounts of a diastatic solution to each. At the end of a half hour at 40° the test tubes are transferred to an ice bath. After cooling each tube was shaken up with a definite amount of iodine solution and the one which showed no trace of color was taken for the end point.

Johnson in 1908 improved the technique of Francis, and Jungk, by

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preparing a starch paste (potato) of constant value. He added his diastatic preparations to a fixed amount of starch and withdrew portions and tested with iodine, at the end of ten minutes. When close to the end point, he tested more frequently, repeating with smaller increments of sample, until the amount was found which would in ten minutes just convert the starch.

Sherman and Schlesinger (1913) and Sherman and Thomas (1915), used the method of Wohlgemuth in determining amyloclastic activity. They stipulate a definite color for the end point of the iodine reaction, using the Milton Bradley color chart as given by Mullikin in his "Identification of Pure Organic Compounds."

If the iodine method is to be used it appears probable that the most accurate results can be obtained by following the technic described by Sherman and his various co-workers.

Copper Reduction Method for the Determination of Reducing Sugars Formed by the Action of Diastase.

Although Kjeldahl showed that the reducing sugars formed by the action of diastatic enzymes upon an excess of starch was a measure of their activity, no great advance was made in the exact valuation of diastase preparations until 1886, when Lintner modified Kjeldahl's method. He first prepared a soluble starch possessing rather definite characteristics. He was then enabled to base his calculations of diastatic power upon the production of a constant quantity of maltose formed by the action of the malt extract upon a definite amount of soluble starch. His method consisted of measuring 10 cc. of a 2 per cent soluble starch solution into a series of ten test tubes, to each tube he added a slightly different amount of malt extract. After digesting exactly one hour at 21°C., 5 cc. of Fehling solution is added to each tube and the whole series of tubes are placed in boiling water for ten minutes, and then examined to determine the first tube in which all the copper was reduced. Lintner prepared a diastase, of which .12 mg. was able to produce the required amount of maltose, under the above conditions, to completely reduce 5 cc. of Fehling's solution. To this preparation Lintner gave the value of 100 and the power of the samples were calculated as inversely proportional to the amount of sample required to produce this fixed amount of reducing sugar.

Ford (1904) prepared a soluble starch from various sources and after careful purification, similar to the Lintner method, concluded that no difference was attributable to the source of the starch. He specified that soluble starch should be neutral to rosolic acid in order to give concordant results by the Lintner method.

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Ford and Guthrie (1908) expressed amylolytic activity as grams of maltose produced by a filtered extract of one gram of mashed barley in an excess of soluble starch for one hour at 40°C.

Several other copper reduction methods have found their way into use since Lintner established his method for determining diastatic activity by measuring the reducing sugars formed. The most notable is that of Sherman, Kendall and Clark (1910). This method consists of placing different amounts of enzyme e. g., 0.2, 0.5, 0.8, and 1.0 mgms. into four erlenmeyer flasks, to this is added 100 cc. of a 2 per cent soluble starch and the whole digested 30 minutes at 40°C. At the end of this time, 50 cc. of Fehling solution are added and the flasks are immersed in a boiling water bath for 15 minutes. The reduced Cu_2O is then quickly filtered and determined gravimetrically. They supply a table which gives diastatic activity of the preparation called the "new scale."

Influence of Temperature on Diastatic Activity.

In all of the earlier work high temperatures were employed, that is between 40°-60°C. Märker found that at 60° four molecules of starch formed three of maltose and one of dextrin, while at 65° the yield was lowered and only one molecule of maltose and one of dextrin were formed from two of starch. It is quite obvious from our present day knowledge that the optimum temperature for diastatic activity lies between 63°-65°C., which would explain why Märker got a decreased production of maltose at 65°C.

Brown and Heron (1879) found that previous heating of a malt extract decreased its activity to a great extent. They found that it showed very litle activity when heated above 66°C.

Vernon (1901-1902) gives the optimum temperature for the activity of diastase as 35°C. with continued activity up to 65°C.

Kendall and Sherman (1910) find the optimum temperature of purified amylases to be 40° in the presence of salts and a trace of alkali. They find that between 20°-40° the activity is about doubled for each 10° increase in temperature with a considerable falling off in rate of increase of activity betwen 40°-50°, where the maximum activity was found.

Influence of Acids, Bases and Salts on Diastatic Activity.

The effects of acids and bases upon diastatic activity has received a great deal of attention, some investigators claiming that an acid medium was the most favorable, while others advocate a neutral medium, for the optimum activity of the enzyme.

Baswitz (1878-1879) and Mohr (1903) found that when carbon dioxide was passed through the reacting medium, a great increase in

diastatic activity took place. Detmer (1882) reached the same conclusion and noted that small amounts of citric, phosphoric and hydrochloric acids had an activating effect. Reychler (1889) found that KH_2PO_4 had a stimulating effect, while Effront noted that HCl, HF, H_2SO_4 and phosphoric acid as well as KH_2PO_4 had a very favorable action. Petit (1904) found an acid medium to be the best. Kjeldahl (1880) reported that H_2SO_4 when in a concentration of .005 N. increased diastatic activity, but was decidedly detrimental when .01 N. was used. The same concentrations hold for citric and acetic acids as reported by Schneidewind, Meyer and Münter (1906).

Heyl finds that KH_2PO_4 has an activating as well as a conserving action, while Hawkins (1913) reports that small additions of phosphoric, acetic and tartaric acids increase the saccharogenic power of the malt extract but did not effect the amyloclastic activity.

Chittenden and Cummins (1884), Duggan (1885), Lintner (1885), Ford (1904), Maquenne and Roux (1906) and Fernbach and Wolff (1907) are all agreed that a neutral medium is the most favorable for diastatic activity.

Sherman and Thomas (1915) and Sherman, Thomas and Baldwin (1919) find that the optimum diastatic activity for various diastatic preparations, depending upon their source, is at a pH of 4.2-4.6. They report an optimum hydrogen ion concentration of about pH 4.4 for a diastase prepared from malt extract, a pH of about 4.7 for the amylase of Aspergillus oryzae and a pH of 6.8-7.0 for pancreatic amylase. After reaching the optimum pH the activity falls off very sharply on the alkaline side.

Many substances have likewise been reported which activate diastatic preparations such as asparagine, aspartic acid, different amino acids and proteins. Chief among the workers who have reported such results are Effront (1904), Ford (1904), Rockwood (1917), Sherman and Walker (1921) and Sherman and Caldwell (1921).

Very little work is reported on the action of alkalis upon diastatic activity. Brown and Heron report a decided falling off in diastatic activity when $Ba(OH)_2$, KOH or NaOH is added to the medium. In fact, it has been the custom to use Alkalies to stop diastatic activity in solutions being analyzed.

Effects of Proteolytic Enzymes.

The action of proteolytic enzymes upon protein material is well understood today and needs very little mention. In regard to the action of proteolytic enzymes in flour, Ford and Guthrie were not able to show by any chemical means that they existed in wheat flour. However, when 1 per cent gelatin was added to the flour and then

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allowed to solidify, proteolysis could be followed by the gradual liquification of the gelatin.

Baker and Hulton could find no method to establish the presence of proteolytic enzymes in flour and therefore claim that any which may be present would have very little effect upon a dough. They did demonstrate, however, that yeast contains a very powerful protein splitting enzyme, as shown by the soluble nitrogen of two identical doughs, one with and the other without yeast. In the dough to which yeast had been added, they report 2.7 per cent soluble nitrogen as protein, while in the other dough they found only 1.9 per cent soluble nitrogen as protein.

Hydrogen Ion Concentration.

From the foregoing literature, it has been shown that acids, bases and salts are of the utmost importance in relation to the activity of diastatic ferments. Jessen Hanson has also shown that the optimum conditions for the baking of bread occur when the dough is at a hydrogen ion concentration of about pH 5.0.

Bailey and Peterson (1921) find that when acid or alkali is added to buffered water extracts of flour, a characteristic curve is obtained which indicates very accurately the grade and baking qualities of a flour. Bailey and Collatz (1921) have shown that a remarkable parallelism exists between grade of flour and the electrical conductivity of a water extract of flour when digested one hour at 25°C.

Viscosity.

Although Ford and Guthrie attempted to demonstrate the proteolytic action of enzymes in wheat flour, by digesting the flour in water at a set temperature by means of viscosity measurements, they report no success in this method.

Ostwald and Lüers in a series of papers show that different mill grades of flours can be distinguished by means of a viscosimeter. From their data, the flours group themselves according to the degree of extraction in milling.

Quite recently Sharp and Gortner have demonstrated the efficiency of the viscosimeter in measuring differences in the imbibitional capacity of strong and weak flours when treated with various acids, bases and salts. They find that strong flours show a greater viscosity under the conditions of their experiments than do the weak flours.

From the literature cited one may judge the amount of work expended upon the question of flour strength. Although the work of the last few years shows progress on this question, we do not have a single test which gives us an absolute criterion of flour strength

and it is still necessary to fall back upon the baking tests to have a final answer to the question.

II. EXPERIMENTAL.

(a) The Problem.

It has been shown in the historical review that flour strength has been studied in a variety of ways. Two points of attack are outstanding, however, the work of Wood and Hardy, Upson and Calvin, Gortner and Doherty, and Sharp and Gortner, who have concerned themselves with the physico-chemical properties of the gluten; and of Baker and Hulton, and Ford and Guthrie who have attacked the problem from the enzymic standpoint. In this Thesis we are concerned with enzyme relationships. From the data presented by Ford and Guthrie it would appear that the diastatic enzymes were of more importance than the proteolytic enzymes with regard to flour strength.

Baker and Hulton indicate in their excellent work that the amyloclastic enzymes were perhaps the limiting factor in the production of maltose. I have taken up the problem at this point and am concerned with the effect upon the baking strength of wheaten flours when diastatic ferments are added to the dough. As the diastatic preparations available for the baker are in the form of malt flours and malt extracts which contain proteolytic enzymes, the problem is at once broadened to include the later as well as the starch splitting ferments.

(b) Material.

The present investigation was conducted with a commercial malt flour, a representative malt extract, a commercial sample of wheat starch, and a series of seven wheat flours of different grades milled from wheats grown in different regions of North America. All of the flour samples and malt preparations were submitted to careful chemical analysis and in most cases were rechecked by other investigators using the same materials. The buffer values of the flour extracts were also determined by the method of Bailey and Peterson in order to have additional data as to the grade of the flour. This data is given in Table VII.

Description of Materials Used in These Studies.

The flours used in this investigation were flours milled from authentic samples of wheat, grown in different regions of North America under different climatic conditions. The A. O. A. C. methods were followed in analyzing the flours and malt preparations, with the results shown in Tables IV, V, and VI. A description of the wheat flours is as follows:

Flour 1001 was milled from a sample of hard Kansas wheat from the crop of 1921. The baking tests showed it to be of good strength and the low ash content. Hydrogen ion concentration, in terms of pH, show it to be a patent of low extraction. The protein content, which is a trifle low, reflects directly upon its baking strength.

TABLE IV.
Analysis of Wheat Flours on Air Dry Basis.

Flour Samples Laboratory No.	Moisture Percent	Ash Percent	Protein (Nx5.7) Percent	Milling Grade	pH of Water Extract
1001	12.15	.40	11.34	Patent	5.816
1002	12.14	.61	13.00	Clear	6.052
1003	13.06	.46	8.83	Patent	6.002
1007	11.06	.64	14.12	Clear	6.103
1008	11.70	.46	15.32	Patent	6.133
1009	11.61	.42	13.81	Patent	5.981
1011	11.44	.56	10.77	Patent	6.050

TABLE V.
Analysis of Malt Flour on Air Dry Basis.

Malt Flour Laboratory No.	Moisture Percent	Ash Percent	Protein Percent	Reducing Sugars as Dextrose Percent	Total Sugars as Dextrose Percent	Diastatic Value Degrees Lintner
24	8.8	1.26	11.25	4.75	10.62	177.05

TABLE VI.
Analysis of Malt Extract.

Extract No.	Moisture Percent	Ash Percent	Reducing Sugars Calculated Maltose Percent	Reducing Sugars Calculated Dextrose Percent	Total Sugars Calculated as Maltose Percent	Total Sugars Calculated as Dextrose Percent	Protein Percent	Diastatic Value Degrees Lintner	Specific Gravity
D	25.63	1.35	73.74	42.52	73.80	42.62	6.06	37.1	1.384

Flour 1002 was milled from a sample of hard Kansas wheat. The high ash content and the pH of the water extract indicate a clear flour. The baking tests show it to have a fair degree of strength.

Flour 1003 is a patent milled from soft, white winter, Washington wheat. The ash content and the pH of the water extract indicate a patent flour, while the protein content and the baking tests show it to be an exceptionally weak flour.

Flour 1007 is a clear flour milled from a sample of selected hard spring wheat grown near Calgary, Canada. The ash content and the pH, of the water extract, show it to be a clear flour. Although the protein content is high the baking tests show it to be of poor baking strength.

Flour 1008 is a patent milled from selected hard spring Canadian

wheat, and shows up exceptionally strong in the baking tests. This flour, it would seem, is too strong for any ordinary baking purposes and would have to be blended with a weaker flour.

Flour 1009 is a composite, commercial patent flour, milled from hard spring wheat for a select trade. The ash content and pH values show it to be a low extraction patent, while the baking tests show it to be a very strong flour. Flour 1009 does not give the volume of loaf that flour 1008 does, but it produces bread with a better grain and texture. This flour is the only one of the series in which the origin of the wheat is not known.

Flour 1011 is a patent flour milled from Ohio winter wheat. The baking tests show it to be of rather poor baking strength.

TABLE VII.

Hydrogen Ion Concentration after addition of Acid and Alkali to Flour Extracts as an Index of Buffer Value.

Flour No.	1001	1002	1003	1007	1008	1009	1011	Malt Flour
cc. N/50 HCl Added	pH	pH	pH	pH	pH	pH	pH	pH
12.5	2.519	2.958	2.654	2.894	2.514	2.510	2.789	3.904
10.0	2.654	3.006	2.874	3.359	2.876	2.705	2.977	4.225
7.5	2.925	3.320	3.144	3.799	3.192	2.992	3.210	4.428
5.0	3.388	3.685	3.761	4.471	3.545	3.496	3.630	4.727
2.5	4.150	4.666	4.623	5.158	4.502	4.272	4.426	5.166
0.0	5.816	6.052	6.002	6.103	6.133	5.981	6.050	5.491
(cc.N/50 NaOH)								
2.5	7.371	6.931	7.726	6.938	7.499	7.792	7.048	6.071
5.0	9.045	8.021	9.653	7.852	8.906	8.892	8.883	6.390
7.5	9.755	9.146	10.557	8.926	9.609	9.915	9.540	6.652
10.0	10.253	9.772	10.877	9.535	9.919	10.448	10.177	6.888
12.5	10.617	10.202	11.022	10.062	10.249	10.769	10.464	7.136

(c) The Methods.

The Munson and Walker method for the determination of reducing sugars was used throughout the investigation for the estimation of sugar resulting from diastatic activity, and all the results are calculated as dextrose. In the cases where proteolytic activity is determined, the amino nitrogen method of Van Slyke, and the viscosity method of Sharp and Gortner were used.

The Method of Determining Diastatic Activity.

It was evident from the very first that the method of Lintner, for the determination of diastatic activity was out of the question, as were also the other methods which have since been developed. It was also evident that **the raw starch of the flours was the natural**

substrate of the enzymes and consequently should be used to duplicate, as far as possible, the changes taking place in the fermentation process. Many difficulties were involved and clarification of the solution was necessary to obtain uniform results. The method finally adopted was one which was developed and perfected in this laboratory. It consisted of adding 3 cc. of 15% Na_2WO_4 to the digestion mixture, transferring to a 200 cc. volumetric flask and then adding 20 drops of concentrated H_2SO_4 and filling up to the mark with water. After careful and thorough shaking the contents were transferred to centrifuge tubes and whirled 5 minutes. The resulting clear supernatant liquid contained all the soluble sugars and was practically free of soluble protein as demonstrated by repeated Kjeldahl determinations. For further data, see report on diastatic enzymes of wheat flour and their relation to flour strength. (Rumsey, 1922).

In determining the diastatic activity of a malt preparation and the amount of soluble sugars produced from the flours by its action, the following method was used: Ten grams of flour were weighed out and transferred to a 400 cc. erlenmeyer flask, the specified amount of flour or malt extract was then added. One hundred cc. of water, previously brought to temperature, was then added and the whole was thoroughly mixed and placed in a water bath, for 1 hour at 27°C. The flasks were agitated every five minutes and at the end of the digestion period the contents of the digestion flasks were transferred to a 200 cc. volumetric flask (any starch particles adhering to the sides of the flask can be removed with a rubber policeman and a stream of water from the wash bottle) and clarified as described above. After clarification 50 cc. aliquots were transferred to 400 cc. beakers and the reducing sugars determined by the Munson and Walker method.

In determining the reducing sugars formed in the dough at various stages of fermentation essentially the same methods were followed. At the time specified, ten grams of dough are pinched off from the fermenting mass and rubbed up in a mortar with a little water until a homogeneous suspension is secured, this is then transferred to a 250 cc. volumetric flask and the same procedure followed as outlined above.

The Method of Determination of Proteolytic Activity.

In the determination of proteolytic activity eighteen grams of flour (calculated on the dry basis) were weighed and transferred to a 500 cc. erlenmeyer flask, malt flour or malt extract was added and 100 cc. of water, previously brought to temperature of digestion, was then added and the whole digested 4 hours at 40°C. The mixture was

then cooled to 25°C, at the close of digestion, and poured into the cup of the MacMichael viscosimeter and the average of three readings taken. Then 5 cc. of N/1 lactic acid was added, the contents thoroughly mixed, and the average of three successive readings taken. From the calibrated scale reading of the MacMichael Viscosimeter, which is denoted as M, any values such as centipoise or absolute viscosity can be determined by calculation.

Method of Determining Buffer Value of Flours.

In the determination of the buffer values of the flours, 80 grams of flour are weighed into a 2 liter flask and 400 cc of water added. The whole is well shaken up to get rid of any lumps and digested 1 hour at 25°C. The digestion mixture is then centrifuged to throw down the suspended matter. Aliquot portions of 25 cc. volume were treated with 2.5, 5.0, 7.5, 10.0 and 12.5 cc. respectively of N/50 HCl or NaOH, then brought to a volume of 50 cc. and the hydrogen ion concentration determined by the use of the Bailey electrode and a Leeds and Northrup type K potentiometer in conjunction with an N/10 KCl calomel electrode and a flowing junction of saturated KCl.

Method for Determination of Gas Producing Capacity of Flour.

In determining the gas producing capacity of a flour, twenty grams of flour are weighed out and transferred to a wide mouthed bottle. To this is added .5 grams of fresh yeast suspended in 20 cc. of distilled water and the whole is thoroughly stirred and the bottle stoppered with a cork containing a delivery tube. The bottles are then put in a water bath kept at 37°C. and the liberated gas is measured in an inverted cylinder under concentrated brine. Readings are taken every half hour. When malt extract is added it is first incorporated with the yeast and water and added to the flour in this way.

Baking Tests.

The baking tests were carried out according to the standard formula adopted by the American Institue of Baking with one exception and that consisted of leaving out sucrose in one set of baking experiments. The formula of the dough was as follows:

Flour ....325 grams
Water ....173 grams(varied depending upon absorption of the flour)
Yeast .... 10 grams
Sugar .... 10 grams
Salt .... 5 grams
Lard .... 6.5 grams

This formula was corrected for the sugar content of the malt flour and malt extract used, the total sugar content being always equivalent to that stated in the formula. The doughs were mixed with a small

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bench mixer, fermented and baked under as accurately controlled conditions as possible. After the baked loaves were withdrawn from the oven they were placed in a cabinet to cool and were weighed 1 hour after baking. The volumes of the bread were taken the next day and each loaf was then cut and judged for grain, texture, color, flavor and odor. In the case where the development of sugar formation was followed during the course of fermentation, a double portion of dough was mixed and then divided. The samples for anlysis were taken from one portion of the dough while the other was baked, and then judged as were those described above. In these experiments dough was fermented, with and without yeast, to estimate the total production of sugar and that used by the yeast in normal fermentation. The weight and temperature of the dough were taken at stated periods of fermentation.

Certain changes in the hydrophyllic properties of the gluten colloids of this series of doughs, as measured by the viscosity of dough suspensions in water, were followed by Mr. P. F. Sharp, and will be reported by him in a separate paper.

(d) Influence of Varying Conditions on Diastatic Activity. Determination of the Optimum pH for the Amylase of Malt Flour.

This analysis was made to determine what relation existed between the optimum pH of dough and the optimum pH for the maximum production of maltose by the malt flour used. Sherman and his collaborators determined the optimum pH for a purified malt amylase and it was thought of interest to know how a commercial preparation behaved in this regard. The process of manipulation varied slightly from that described above for the determination of buffer values, so will be described at this time. Ten grams of malt flour containing both the enzyme and the raw-starch substrate were weighed out into a flask. Enough water was then added so that when the mixture was brought to the desired pH by acid or alkali, the total volume of liquid added would be 50 cc. The mixture was then digested for one hour at 25°C in an accurately regulated water bath. After digestion the whole was centrifuged and 25 cc. (half of sample) was pipetted into a 200 cc. volumetric flask, 2 cc. of 15% Na_2WO_4 were added and thorughly shaken, and 20 drops of concentrated H_2SO_4 is added and made up to the mark. The preparation was centrifuged again and 50 cc. taken for reducing sugars as described above. The other portion of the unclarified extract was used to determine the pH values.

The experimental data showing the influence of diastatic activity by change in pH are given in Table VIII and illustrated graphically in Figure 1. The optimum activity occurred at pH=4.26.

TABLE VIII.

Relation between hydrogen ion concentration (as pH) and the
diastatic activity of malt flour as expressed in grams of
dextrose per 1.5 grams of malt flour.

Normality of HC1	cc. used	pH	Wt. of Cu_2O Grams	Wt. of Dextrose Grams	Dextrose Per Cent
N/10	28.0	1.988	.1075	.0527	3.52
N/10	26.0	2.099	.1115	.0548	3.65
N/10	24.0	2.139	.1147	.0563	3.75
N/10	22.0	2.437	.1111	.0545	3.64
N/25	50.0	2.572	.1070	.0525	3.50
N/25	45.0	2.745	.1123	.0552	3.68
N/25	40.0	2.970	.1239	.0611	4.07
N/25	35.0	3.156	.1438	.0714	4.76
N/10	13.0	3.224	.1526	.0759	5.03
N/10	12.0	3.420	.1734	.0868	5.78
N/25	30.0	3.499	.1828	.0917	6.12
N/10	11.0	3.613	.1937	.0976	6.51
N/25	25.0	3.704	.1935	.0974	6.50
N/25	22.5	3.870	.1984	.1000	6.67
N/25	20.0	4.159	.2238	.1137	7.58
N/25	15.0	4.260	.2305	.1173	7.82
N/25	12.5	4.542	.2224	.1129	7.52
N/25	10.0	4.621	.2190	.1111	7.41
N/25	5.0	5.069	.2081	.1051	7.01
NaOH	0.0	5.548	.1827	.0917	6.11
N/25	5.0	6.069	.1670	.0834	5.56
N/25	10.0	6.489	.1548	.0769	5.12
N/25	15.0	6.830	.1438	.0713	4.75
N/25	20.0	7.359	.1336	.0661	4.41
N/25	25.0	7.871	.1230	.0605	4.03
N/25	30.0	8.419	.1196	.0589	3.93
N/25	35.0	8.920	.1180	.0581	3.87
N/25	40.0	9.306	.1163	.0572	3.81
N/25	45.0	9.649	.1090	.0535	3.57
N/25	50.0	9.991	.1028	.0503	3.37

Influence of Time of Digestion on the Diastatic Activity of Malt Flour.

The influence of time on the activity of malt flour was investigated
to ascertain at what point, or length of time, the activity would
decrease. Heyl has noted that at first the reaction is logarithmic, but
deviates as the products of hydrolysis accumulate. This particular
experiment was planned in order to find the optimum length of time
for future periods of digestion. It developed that at the end of eight
hours the reaction was proceeding at about the same rate of speed
as that of one hour so it was decided to make one hour the standard
period of all digestions.

The effects of time of digestion up to eight hours is given in Table
IX and presented graphically in Figure 2.
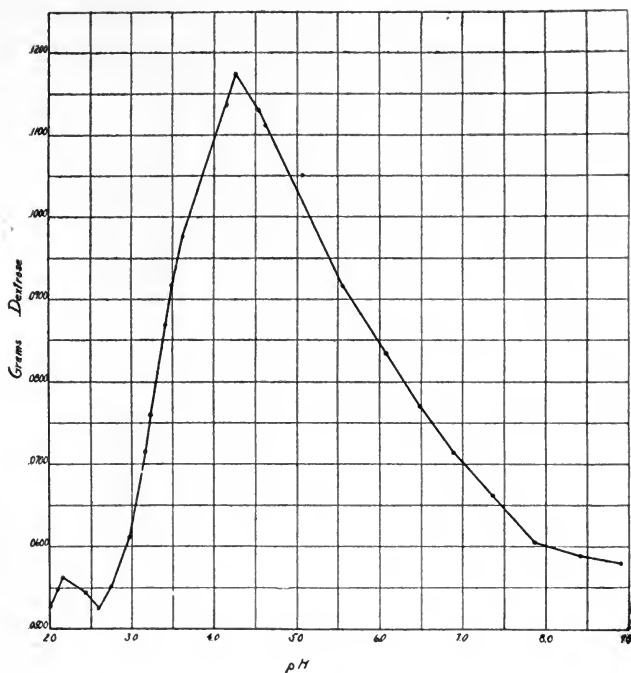
Figure 1.

Effect of pH on the activity of diastase in malt flour expressed as
grams dextrose per 1.5 grams malt flour.

TABLE IX.

Effects of time of digestion on diastatic activity as expressed in grams
of dextrose per 10 grams of malt flour.

Time of Digestion Hours	Weight of Cu_2O Grams	Wt. of Dextrose per 10 grams of flour Grams	Dextrose Per Cent
0.00	.1112	.3844	3.86
.25	.1462	.5128	5.13
.50	.1622	.5752	5.55
1.00	.1962	.6992	7.00
1.50	.2230	.7992	8.00
2.00	.2457	.8848	8.85
3.00	.2931	1.0696	10.70
4.00	.3331	1.2296	12.30
5.00	.3650	1.3608	13.61
6.00	.3991	1.5024	15.03
7.00	.4264	1.6200	16.20
8.00	.4560	1.7480	17.48
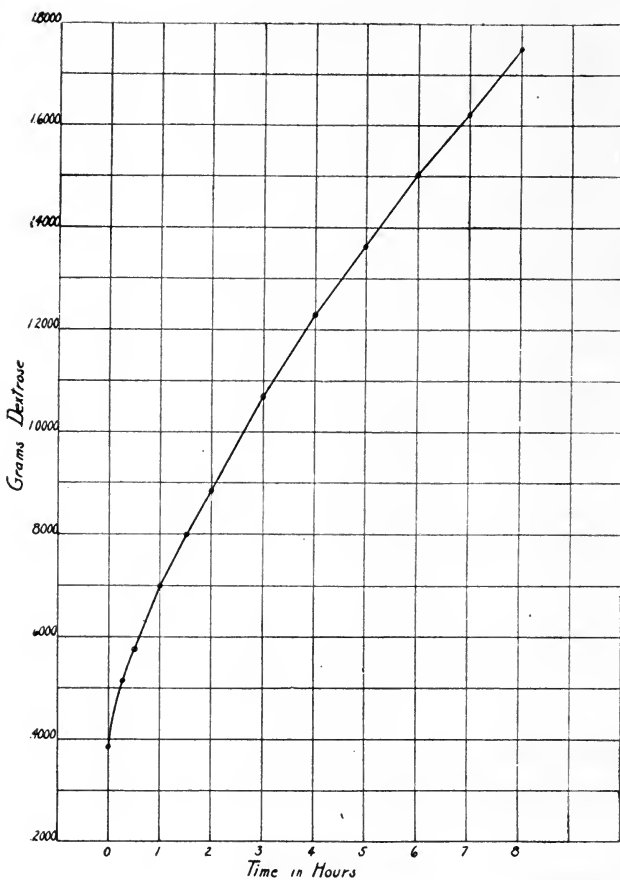
Figure 2.

Diastatic activity as influenced by time of digestion, expressed in
grams of dextrose per 10.000 grams of malt flour.

Effect of Temperature Upon the Activity of Diastase.

Practically all of the work in this investigation was carried out at
27°C, which is the temperature of fermentation used in the bake shop;
however, it was necessary to find the optimum temperature of the
diastase in the malt flour, as Sherman notes that 40° is the optimum
temperature with a maximum at 55° for pancreatic amylase. Most
of the investigators quoted above found 65°C to be the optimum for
malt amylase, while Swanson and Calvin found 62.5°C to be the
optimum for wheat diastase. It was thought to be of interest to
determine at what temperature the diastase in malt flour exerted its
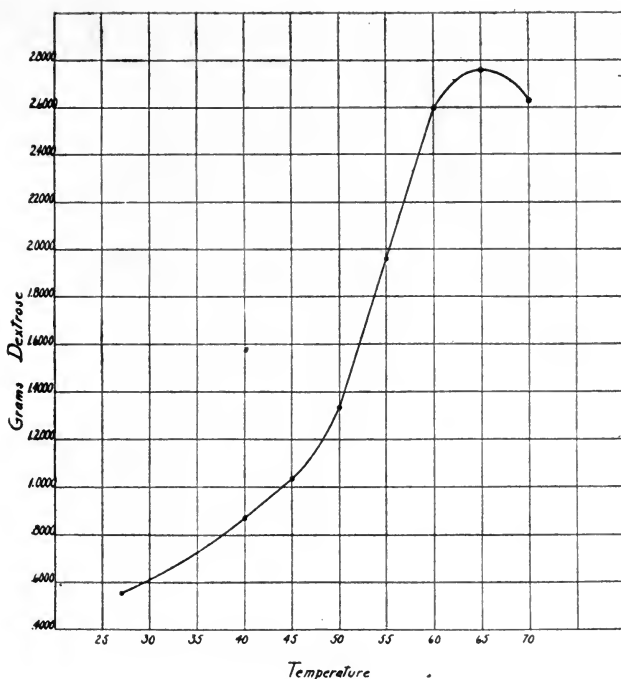
Figure 3.

Effects of temperature upon the activity of diastase in malt flour when 10.00 grams are digested one hour at various temperatures.

TABLE X.

Effect of temperature upon the activity of diastase in malt flour when 10 grams are digested for 1 hour at various temperatures.

		———–Series 1————				————Series 2————		
Temp. of Diges-tion Degrees C	Wt. of Cu₂O Grams	Wt. of Dex-trose Grams	Wt. of Dex-trose per 10 Grams Malt Flour Grams	Dex-trose Per Cent	Wt. of Cu₂O Grams	Wt. of Dex-trose Grams	Wt. of Dex-trose per 10 Grams Malt Flour Grams	Dex-trose Per Cent
27	.1279	.0559	.5590	5.59	.1280	.0559	.5590	5.59
40	.1951	.0867	.8670	8.67	.1940	.0862	.8620	8.62
45	.2305	.1034	1.0340	10.34	.2320	.1041	1.0410	10.41
50	.2922	.1333	1.3330	13.33	.2914	.1329	1.3290	13.29
55	.4144	.1960	1.9600	19.60	.4136	.1956	1.9560	19.56
60x	.5704	.2598	2.5980	25.98	.5692	.2592	2.5920	25.92
65x	.6030	.2759	2.7590	27.59	.6018	.2754	2.7540	27.54
70x	.5760	.2626	2.6260	26.26	.5762	.2627	2.6270	26.27

x Aliquots of one-half the usual quantity were used, and the resulting values multiplied by two.

maximum effect. The procedure was as follows: 10 grams of malt flour were weighed out and digested at temperatures of 27°, 40°, 45°, 50°, 55°, 60°, 65 , and 70 for one hour with 100 cc. of water (previously brought to temperature). After clarifying and bringing to a volume of 250 cc. a 25 cc. aliquot was taken for reducing sugars and determined by the Munson and Walker method. These data are given in Table X and illustrated graphically in Figure 3.

Effect of Concentration of Diastase on Hydrolysis of Starch in Wheat Flour.

As the concentration of diastatic ferments added to the dough is of great importance, the effects of different concentrations of malt flour up to 50% were tried by mixing definite proportions of wheat and malt flour and digesting it at 27°C. for one hour. It was necessary to first determine the amounts of dextrose formed when different amounts of malt flour were digested separately in order to apply corrections for the autolysis of malt flour in the succeeding experiments with the wheat flour. This data is given in Tables XI and XII, and presented graphically in Figure 4.

TABLE XI.

Autolysis of malt flour at 27°C. for one hour.

Amount of Malt Flour used, grams	0.25	0.50	0.75	1.0	1.25
Wt. Cu_2O, grams	.0337	.0744	.1080	.1519	.1980
Wt. Dextrose, grams	.0142	.0320	.0469	.0668	.0852
Dextrose, per cent	5.68	6.40	6.25	6.68	6.90

TABLE XII.

Relation of concentration of malt flour to the production of reducing Sugars from wheat flour when digested 1 hour at 27°C.

Proportion of wheat flour to malt flour	Wt. of Cu_2O per 2.5 gms. flour Grams	weight of Dextrose formed per 2.5 gms. flour Grams	Dextrose corrected for dextrose of malt flour Grams	Malt flour Percent	Dextrose formed Percent
10:0	.0944	.0408	.0408	0.0	1.63
9:1	.2167	.0968	.0826	10.0	3.67
8:2	.2583	.1168	.0848	20.0	4.24
7:3	.2918	.1331	.0862	33.3	4.92
6:4	.3030	.1387	.0719	40.0	4.80
5:5	.3279	.1512	.0760	50.0	6.06

30
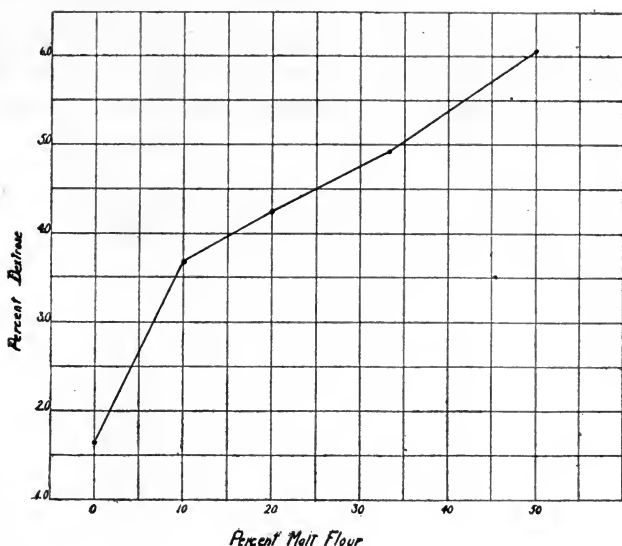
Figure 4.

Relation of increasing concentration of malt flour to the production of reducing sugars, when wheat flour is digested one hour at 27°C.

Effects of Increasing Amounts of Malt Flour When Digested With a Constant Quantity of Wheat Flour.

Table XII shows the effects of large quantities of malt flour upon wheat starch, but the amounts are out of all proportion to that used in practice. In the experiments following, the sugar producing capacity of the malt flour was measured upon a series of seven flours and a commercial wheat starch with concentrations varying from 0.2 percent to 5.0 percent.

The experimental data showing percent dextrose produced, when 10 grams of flour are digested with 0.02 - 0.50 grams of malt flour, is given in Table XIII and illustrated graphically in Figure 5.

TABLE XIII.

Percent dextrose produced from 10 grams of flours 1001, 1002, 1003, 1007, 1008, 1009, 1011, and a commercial wheat starch when digested with 0.02 - 0.50 grams of malt flour for 1 hour at 27°C.

| | | Flour Sample Number | | | | | | | Commercial Wheat Starch |
Grams	Malt Flour Used %	1001 %	1002 %	1003 %	1007 %	1008 %	1009 %	1011 %	%
.0000	0.00	1.85	1.34	.40	.94	2.28	1.36		.18
.0200	.20					2.35	1.72		
.0250	.25	2.15	1.50	.74	1.15				.32
.0400	.40						1.80		
.0500	.50	2.32	1.64	.88	1.71	2.38		.85	.42
.0600	.60						1.95		
.0750	.75	2.47	1.74	1.03	2.04	2.48			.50
.0800	.80						1.98		
.1000	.99	2.59	1.85	1.09	2.10	2.54	2.12	1.00	.59
.1200	1.19						2.19		
.1250	1.24	2.69	1.94	1.14	2.32			1.06	.62
.1500	1.48	2.77	2.04	1.20	2.50	2.56	2.28	1.14	.69
.1750	1.72					2.58	2.46	1.16	
.2000	1.96	2.90	2.11	1.36	2.80	2.62	2.62	1.47	.84
.2250	2.20						2.64		
.2500	2.44	3.02	2.21	1.40	2.95	2.63	2.67	1.51	.91
.3000	2.91	3.15	2.31	1.49	3.21	2.72	2.84	1.56	1.07
.4000	3.84	3.34	2.48	1.64	3.35	2.83	3.06	1.72	
.5000	4.76	3.47	2.61	1.73	3.73	3.03	3.24	1.93	1.53

The Production of Reducing Sugars in the Dough During Fermentation.

The production of reducing sugars in an actively fermenting dough and its subsequent use by the yeast was followed at various stages of fermentation This necessitated running two parallel doughs, one having the required amount of yeast, while the other had no yeast added but identical in every other respect. After mixing the dough, a ten gram sample was pinched off, shaken to a homogeneous suspension, clarified and the reducing sugars determined. At stated periods 10 gram samples were taken and submitted to analysis, as after the mix. Twice the usual quantity of each dough (with and without yeast) was mixed, divided into two equal parts and the samples taken from one portion only in order to have one dough to bake in the usual way.

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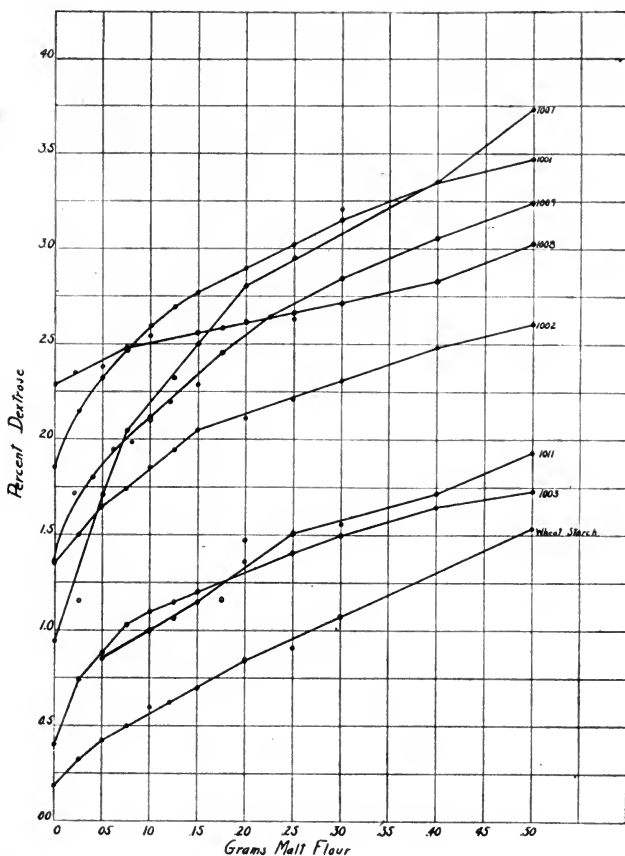
Figure 5.

Percent of dextrose formed from a series of flours when digested one
hour at 27°C. with various amounts of malt flour.

Tables XIV-XXII give this data on three flours representing a
strong type Canadian patent flour (1008), a decidedly weak Pacific
Coast patent flour (1003), and a clear flour from Kansas (1002). Each
flour was mixed with varying amounts of diastatic preparations as fol-
lows. 1.5 percent malt flour, 4.0 percent malt flour and 3.0 percent
malt extract. Controls with no diastase were included in each series.
No sugars were added to any of the doughs except that amount con-
tained in the malt flour and malt extract used, but this amount was
deemed negligible in its effect upon frementation.

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TABLE XIV.

Production of reducing sugars in the fermentation of flour 1008 with and without yeast. No diastase or sucrose added.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough Dextrose from 10 gm. Dough Grams	Dextrose on dry basis Perct.	Total sugars Calc. as Dextrose Grams	Total sugars on dry basis Perct.	Moisture Perct.	No Yeast Dough Dextrose from 10 gm. Dough Grams	Dextrose on dry basis Perct.	Total sugars Calc. as Dextrose Grams	Total sugars on dry basis Perct.	Moisture Perct.
Mix	0	00	.0900	2.12			57.48	.0670	1.59			57.95
1 hr. later	1	00	.1405	3.31	.1625	3.82	57.48	.1085	2.58	.1875	4.46	57.95
1st punch	2	30	.1500	3.53	.1685	3.96	57.48	.1390	3.31	.2060	4.90	57.95
2d punch	3	40	.1380	3.24			57.48	.1540	3.66			57.95
To proof	4	35	.1095	2.52	.1140	2.63	56.57	.1760	4.21	.2425	5.80	58.18
After proof (to oven)	5	08	.1015	2.34	.1065	2.45	56.59	.1745	4.18	.2245	5.37	58.20

TABLE XV.

Production of reducing sugars in the fermentation of flour 1008 with and without yeast, with 1.5 percent added malt flour. No sugar added.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Perct.	Moisture Perct.	No Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Perct.	Moisture Perct.
Mix	0	00	.1290	3.04	57.62	.0845	2.07	59.23
1 hr. later	1	00	.1610	3.80	57.62	.1190	2.92	59.23
1st punch	2	30	.1555	3.66	57.50	.1405	3.50	59.87
2d punch	3	40	.1380	3.25	57.50	.1590	3.96	59.87
To proof	4	25	.1230	2.87	57.13	.1775	4.39	59.61
To oven	5	05	.1135	2.65	57.13	.2000	4.95	59.61

TABLE XVI.

Production of reducing sugars in the fermentation of flour 1008 with and without yeast, with 4 percent added malt flour. No sugar added.

Stage of Fermentation	Time of Fermentation Hours	Time of Fermentation Mins.	Yeast Dough Dextrose from 10 gms. dough Grams	Yeast Dough Dextrose on dry basis Perct.	Yeast Dough Moisture Perct.	No Yeast Dough Dextrose from 10 gms. dough Grams	No Yeast Dough Dextrose on dry basis Perct.	No Yeast Dough Moisture Perct.
Mix	0	00	.1285	3.06	57.99	.0835	2.03	58.86
1 hr. later	1	00	.1535	3.65	57.99	.1240	3.01	58.86
1st punch	2	00	.1680	3.91	57.01	.1500	3.67	59.10
2d punch	3	25	.1500	3.49	57.01	.1790	4.38	59.10
To proof	4	04	.1350	3.14	57.01	.1940	4.74	59.10
To oven	4	44	.1150	2.67	57.01	.2155	5.27	59.10

TABLE XVII.

Production of reducing sugars in the fermentation of flour 1008 with and without yeast, with 3 percent added malt extract.

Stage of Fermentation	Time of Fermentation Hours	Time of Fermentation Mins.	Yeast Dough Dextrose from 10 gms. dough Grams	Yeast Dough Dextrose on dry basis Percent	Yeast Dough Moisture Percent	No Yeast Dough Dextrose from 10 gms. dough Grams	No Yeast Dough Percent Dextrose on dry basis	No Yeast Dough Moisture Percent
Mix	0	00	.1670	3.96	57.82	.1455	3.57	59.25
1 hr. later	1	00	.1895	4.49	57.82	.1800	4.42	59.25
1st punch	2	30	.1985	4.70	57.72	.2285	5.50	58.49
2d punch	3	40	.1780	4.21	57.72	.2350	5.66	58.49
To proof	4	20	.1620	3.81	57.44	.2445	5.93	58.78
To oven	5	00	.1405	3.30	57.44	.2490	6.04	58.78

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TABLE XVIII.

Production of reducing sugars in the fermentation of flour 1003 (weak Pacific Coast flour) with and without yeast with no added diastase or sugar.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough					No Yeast Dough				
			Dextrose from 10 gm. Dough Grams	Dextrose on dry basis Percent	Total sugars Calc. as Dextrose Grams	Total sugars on dry basis Percent	Moisture Percent	Dextrose from 10 gm. Dough Grams	Dextrose on dry basis Percent	Total sugars as Dextrose Grams	Total sugars on dry basis Percent	Moisture Percent
Mix	0	00	.0420	.97	.0910	2.10	56.58	.0275	.64	.1010	2.35	57.11
1 hr. later	1	00	.0390	.90	.0870	2.00	56.58	.0350	.82			57.11
1st punch	2	00	.0330	.76	.0515	1.19	56.57	.0425	.99			57.15
2d punch	3	00	.0175	.40	.0265	.61	56.57	.0525	1.23			57.15
To proof	3	35	.0105	.24	.0210	.48	56.52	.0525	1.23	.1250	2.92	57.15
To oven	4	15	.0075	.17	....	..	56.52	.0530	1.24	.1070	2.50	57.15

TABLE XIX.

Production of reducing sugars in fermentation of flour 1003, with and without yeast, with 4 percent malt flour. No sucrose added.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough			No Yeast Dough		
			Dextrose from 10 gms. dough Grams	Dextrose on dry basis Perct.	Moisture Perct.	Dextrose from 10 gms. dough Grams	Dextrose on dry basis Perct.	Moisture Perct.
Mix	0	00	.0930	2.20	57.79	.0675	1.61	58.12
1 hr. later	1	00	.1170	2.77	57.79	.0800	1.91	58.12
1st punch	2	00	.1190	2.78	57.18	.1195	2.83	57.74
To proof	3	20	.0935	2.18	57.17	.1310	3.12	57.96
To oven	4	05	.0805	1.88	57.17	.1460	3.47	57.96

TABLE XX.

Production of reducing sugars in the fermentation of flour 1003, with and without yeast, with 3 percent malt extract added. No sucrose added.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Percent	Moisture Percent	No Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Percent	Moisture Percent
Mix	0	00	.1390	3.28	57.60	.1350	3.39	60.10
1 hr. later	1	00	.1425	3.36	57.60	.1440	3.61	60.10
To proof	2	25	.1330	3.12	58.32	.1572	3.85	59.10
To oven	3	30	.0970	2.27	58.32	.1725	4.22	59.10

TABLE XXI.

Production of reducing sugars in the fermentation of flour 1002 (a clear flour) with and without yeast, with no added diastase or sucrose.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough Dextrose from 10 gm. Dough Grams	Dextrose on dry basis Percent	Total sugars Calc. as Dextrose Grams	Total sugars on dry basis Percent	Moisture Percent	No Yeast Dough Dextrose from 10 gm. Dough Grams	Dextrose on dry basis Percent	Total sugars as Dextrose Grams	Total sugars on dry basis Percent	Moisture Percent
Mix	0	00	.0795	1.87	.1225	2.87	57.38	.0605	1.43	.1230	2.91	57.75
1 hr. later	1	00	.1090	2.56	.1460	3.42	57.38	.0905	2.14	.1800	4.24	57.75
1st punch	2	15	.1065	2.46	.1245	2.88	56.71	.1160	2.73			57.58
2d punch	3	25	.0875	2.02	.1010	2.33	56.71	.1305	3.08			57.58
To proof	4	05	.0780	1.80	.0990	2.88	56.56	.1350	3.22	.1875	4.48	58.10
To oven	4	45	.0590	1.36	.0690	1.59	56.56	.1370	3.27	.1910	4.56	58.10

TABLE XXII.

Production of reducing sugars in the fermentation of flour 1002 with and without yeast, with 1.5 percent added malt flour. No added sucrose.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Percent	Moisture Percent	No Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Percent	Moisture Percent
Mix	0	00	.1035	2.42	57.21	.0830	1.95	57.43
1 hr. later	1	00	.1370	3.20	57.21	.1210	2.84	57.43
1st punch	2	15	.1405	3.26	56.82	.1390	3.29	57.75
2d punch	3	25	.1200	2.78	56.82	.1565	3.70	57.75
To proof	4	05	.1100	2.54	56.75	.1605	3.80	57.77
To oven	4	45	.0895	2.07	56.75	.1700	4.03	57.77

TABLE XXIII.

Production of reducing sugars in the fermentation of flour 1002, with and without yeast, with 4 percent added malt flour. No sucrose added.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Percent	Moisture Percent	No Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Percent	Moisture Percent
Mix	0	00	.1075	2.55	57.82	.0900	2.11	57.44
1 hr. later	1	00	.1665	3.95	57.82	.1600	3.76	57.44
1st punch	2	15	.1720	4.07	57.72	.1695	3.95	57.11
To proof	3	40	.1395	3.30	57.72	.1895	4.42	57.11
To oven	4	20	.1230	2.91	57.72	.2260	5.27	57.11

TABLE XXIV.

Production of reducing sugars in the fermentation of flour 1002, with and without yeast, with 3 percent added malt extract. No added sucrose.

Stage of Fermentation	Time of Fermentation Hours	Mins.	Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on Dry basis Percent	Moisture Percent	No Yeast Dough Dextrose from 10 gms. dough Grams	Dextrose on dry basis Percent	Moisture Percent
Mix	0	00	.1735	4.08	57.48	.1525	3.74	59.20
1 hr. later	1	00	.1900	4.47	57.48	.1925	4.72	59.20
1st punch	2	30	.1795	4.21	57.36	.2055	4.93	58.34
To proof	3	45	.1595	3.74	57.36	.2190	5.26	58.34
To oven	4	40	.1360	3.19	57.36	.2300	5.52	58.34

Figure 6.

Productions of reducing sugars in the panary fermentation of flour 1008 with different concentrations of malt flour and malt extract. (Full lines indicate doughs to which yeast was added, dotted lines no yeast dough).

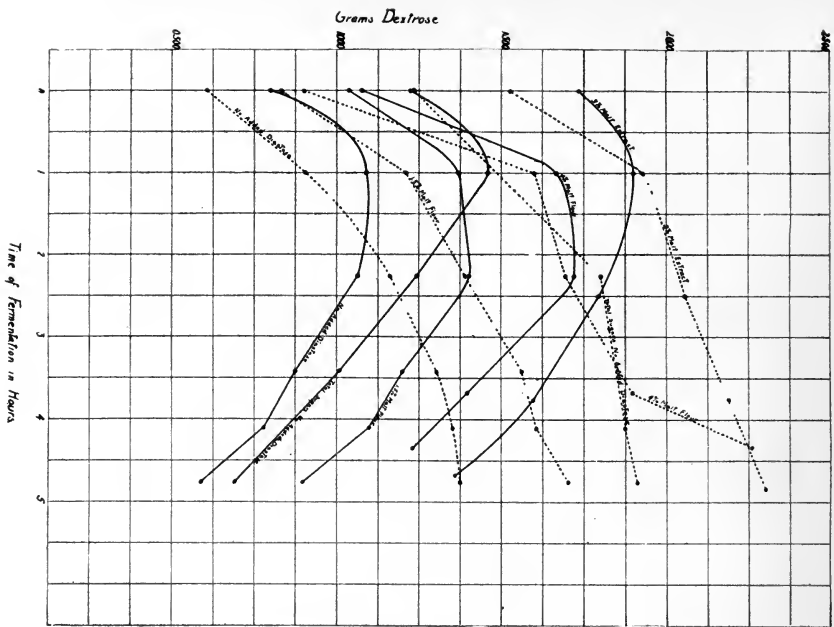
Figure 7.

Production of reducing sugars in the panary fermentation of flour 1003 with different concentrations of malt flour and malt extract. (Full lines indicate doughs to which yeast was added, dotted lines no yeast dough).

Figure 8.

Productions of reducing sugars in the panary fermentation of flour 1002, with different concentrations of malt flour and malt extract. (Full lines indicate doughs to which yeast was added, dotted lines no yeast dough).
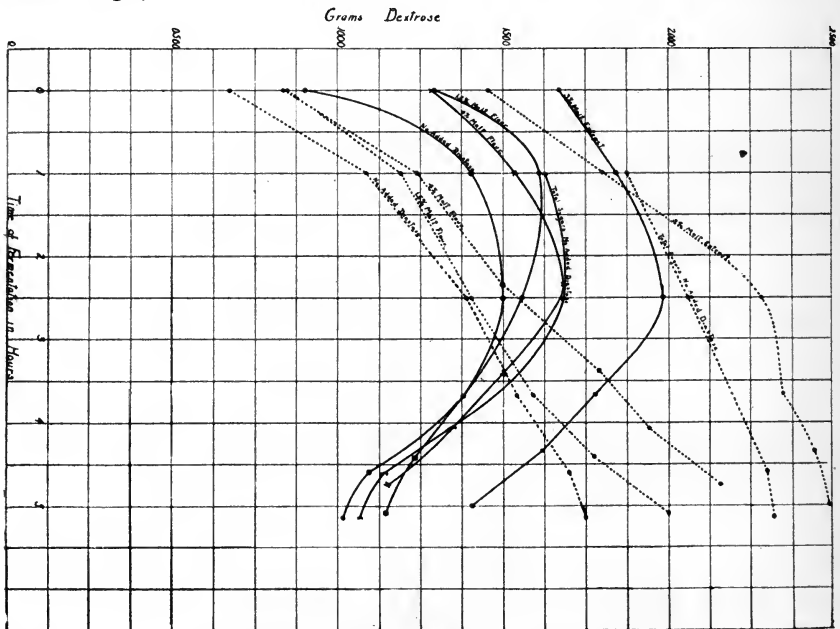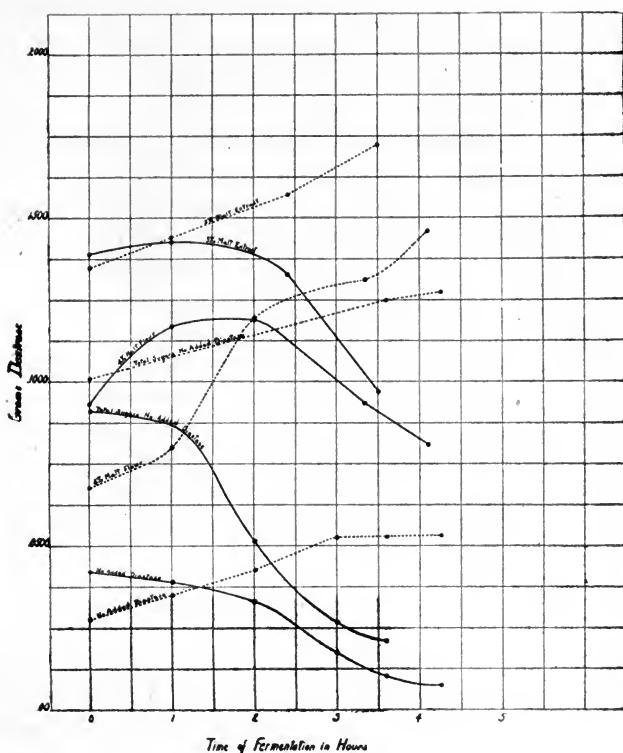
Determination of Proteolytic Activity as Measured by the Fall in Viscosity of Flour-water Suspensions, when Digested with Diastatic Preparations.

The action of proteolytic ferments upon gluten is accompanied by a breaking down of the protein material into simpler compounds such as protesoses, peptones and amino acids. As the latter can be easily measured by the amount of free amino nitrogen, it was thought that an increase in amino nitrogen measured by VanSlyke's method, would be a measure of the amount of proteolysis taking place. This, however, was not the case as no appreciable difference in amino nitrogen could be detected when the flour was digested with and without malt preparations.

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It is well known that dough becomes slack or less viscous during the fermentation period. This has been attributed to the action of the proteolytic enzymes of the yeast and partially to those in malt preparations. Ford and Guthrie were unable to demonstrate proteolytic enzymes in flour by means of the viscosimeter and concluded that any which were present would have little or no effect upon the gluten during fermentation. Sharp and Gortner have shown that the hydration capacity and the quality of the gluten can be accurately determined by the use of the viscosimeter. They have shown that the viscosity of a flour-water suspension is tremendously increased and increases to a well defined maximum by the addition of lactic acid in small amounts, while the further addition of lactic acid causes no appreciable change in viscosity value. The suspensions of various flours in water differed slightly in initial viscosity and it was only after the addition of the lactic acid that these differences were increased to such an extent as to make the results of significance. Under the conditions of Sharp and Gortner's experiments the addition of 5 cc N/1 lactic acid was

TABLE XXV.

The measurements of proteolytic activity in a flour-water suspension showing the effects of different periods of digestion (1-5 hours) at 30°C. with 100 cc of water and concentrations of malt flour (2 and 4 percent) on viscosity (degrees MacMichael) with the addition of various amounts of lactic acid.

Viscosity in degrees MacMichael on addition of N/1 lactic acid.

N1 Lactic Acid added cc.		0.0	0.5	1.0	1.5	2.0	3.0	5.0
Digestion used Hours	Malt Flour Percent	Degr's M	Degr's M	Degr's M	Degr's M	Degr's M	Degr's M	Degr's M
1	0.0	33	83	119	134	144	152	160
2	0.0	41	107	139	151	156	161	167
3	0.0	36	86	120	133	139	148	156
4	0.0	38	86	117	129	136	144	160
5	0.0	34	83	115	127	133	141	153
1	2.0	35	86	126	135	141	147	151
2	2.0	29	70	101	115	122	130	135
3	2.0	29	67	96	108	116	122	127
4	2.0	30	69	97	106	112	118	122
5	2.0	31	70	95	104	109	115	120
1	4.0	32	71	104	119	127	134	138
2	4.0	25	57	88	101	109	117	121
3	4.0	22	51	77	90	97	105	111
4	4.0	24	48	72	83	88	96	100
5	4.0	22	45	68	77	83	90	96

sufficient to bring any flour to the point of highest viscosity; accordingly their method was followed to see whether any differences could be detected when flour was digested alone and when digested with malt preparations and if any differences which existed in the flour-water extracts could be increased by the addition of lactic acid.

A few preliminary experiments in which flour was digested with and without malt preparations for different periods of time showed that the flour-water suspensions of the different flours varied very little in their initial viscosities, thus showing why Ford and Guthrie were unsuccessful in measuring proteolysis by means of the viscosimeter. With the addition of lactic acid, however, great differences were noticeable between the different flours and the results justified the following experiments.
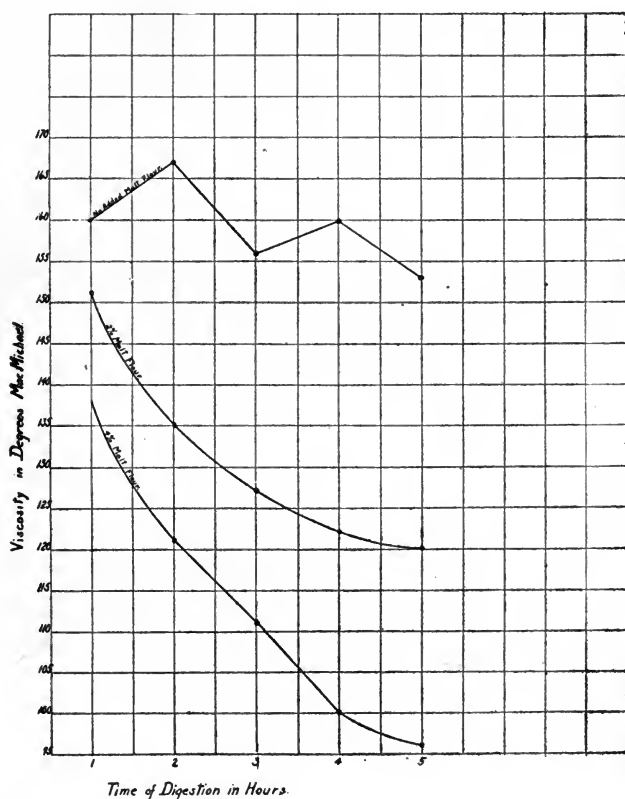

Figure 9.

Effect of the proteolytic enzymes, contained in malt flour, upon wheat flour as measured by the fall in viscosity when digested 1 to 5 hours at 30°C. With lactic acid added.

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The effect of time of digestion was first tried to determine the optimum time of digestion. The experiment consisted of digesting flour 1001 (a strong Kansas patent) for 1 to 5 hours with 100 cc of water, and repeating with the same flour after adding 2 and 4 percent of malt flour. After digesting for the stated period of time the flour-water suspensions were transferred to the cup of the MacMichael viscosimeter and the average of three readings taken, N/1 lactic acid was then added in small amounts up to 5 cc. The mixture was thoroughly stirred after each addition of acid and the average of three viscosity readings taken.

The experimental data of this series when digested at 30°C. is given in Table XXV and the data in the last column of this Table is shown graphically in Figure 9.

It appears from this table that four hour digestion is ample time to secure evidence of proteolytic action, and this time of digestion was used in all subsequent work. Also the temperature of digestion was increased from 30° to 40° in order to procure greater activity of the enzymes.

The data given in Table XXVI and illustrated graphically in Figure 10 was obtained by digesting a series of flours (1001-1002-1003-1007 and 1008) with varying amounts of malt flour (1.0-1.5-2.0-2.5-3.0 and 4 percent) with 100 cc of water at 40°C. and determining the viscosity after adding 5 cc of N/1 lactic acid. Table XXVII and Figure 10A show similar data when malt extract was used.

It has been shown in the literature cited that even minute traces of salts have a marked affect upon the imbibition capacities of gluten. It was, therefore, thought advisable to see how the viscosity of the flour reacted when treated as above but with the salts of the flour and malt flour washed out after the digestion period. The precedure was as follows: The equivalent of 18 grams of water-free flour 1002, was weighed into each of six flasks and digested with 0.0, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 percent malt flour in 100 cc of water at 40°C. After four hours 900 cc of distilled water were added, the whole well shaken and centrifuged. The supernatant liquid was decanted and the flour residue shaken up with water and after complete disintegration made up to 100 cc, placed in viscosimeter cup, and the readings taken after adding varying amounts of lactic acid.

The data showing the viscosity in the presence of lactic acid of flour 1002 with the salts washed out is given in Table XXVIII.

In order to demonstrate still further that the salt content of the added malt flour did not vitiate the results obtained in Table XXVI when flour (for example) 1002 was digested 4 hours with 4 percent

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TABLE XXVI.

Effect of varying amounts of malt flour upon the viscosity of 18 grams
(calculated on the dry basis) of wheat flour when digested for
four hours at 40°C. with 100 cc of water (5 cc N/1 lactic
added in each instance). Viscosity readings in
degrees MacMichael.

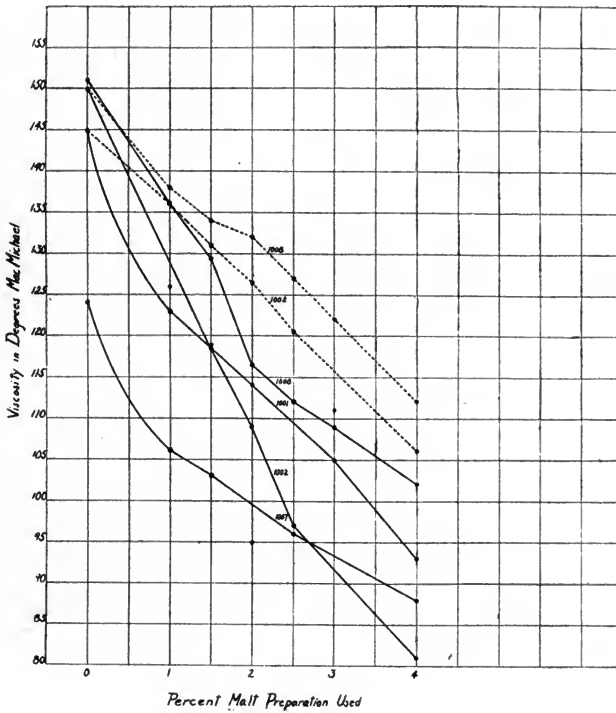| % Malt Flour Used.....	0.0 Degr's M	1.0 Degr's M	1.5 Degr's M	2.0 Degr's M	2.5 Degr's M	3.0 Degr's M	4.0 Degr's M
Flour No.							
1001	145	123	119	114	112	105	93
1002	150	126	118	109	97	99	81
1003	50	44	42.5	40	36.5	37	28
1007	124	106	103	95	97	103	88
1008	151	136	129.5	117.5	112	109	102

Figure 10.

Changes in viscosity of flour water suspensions when digested 4
hours at 40°C. with increasing amounts of malt flour and malt extract,
as measured in degrees MacMichael with lactic acid added. (Full
line curves flours digested with malt flour, dotted line curves those
digested with malt extract).

TABLE XXVII.

Effect of varying amounts of malt extract upon the viscosity of 18 grams (calculated to the dry basis) of wheat flour when digested 4 hours at 40 C. with 100 cc of water. (5 cc of N/1 lactic acid added in each instance). Viscosity readings in degrees MacMichael.

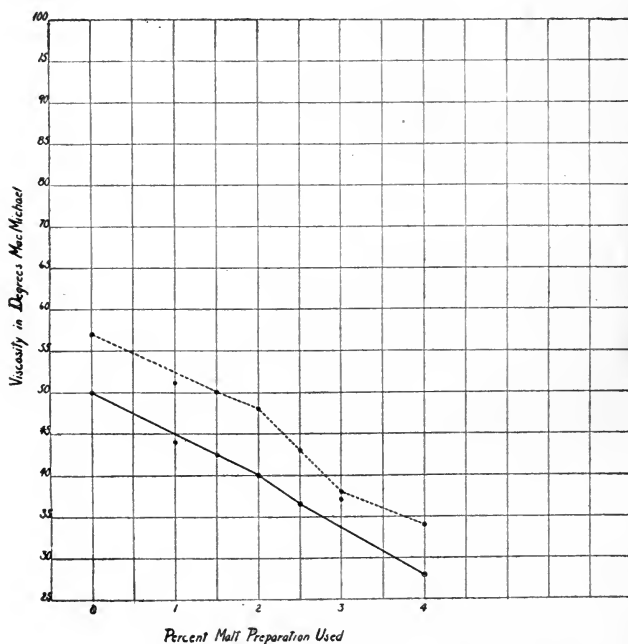| % Malt Ext. Used......	0.0 Degr's M	1.0 Degr's M	1.5 Degr's M	2.0 Degr's M	2.5 Degr's M	3.0 Degr's M	4.0 Degr's M
Flour No.							
1002	145	136	131	127.5	120.5	111	106
1003	57	51	50	48	43	38	34
1008	150	138	134	132	127	122	112

Figure 10-A.

Changes in viscosity of water suspensions of flour 1003 when digested 4 hours at 40° with increasing amounts of malt flour and malt extract, as measured in degrees MacMichael. With lactic acid added. (Full line curve digestions with malt flour, dotted line curve digestion with malt extract).

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malt flour three samples of 1002 were weighed out, two were used as checks and to the third 4 percent malt flour was added. At the end of three and one-half hours 4 percent malt flour was added to one of the checks and the mixture well shaken. At the end of 4 hours digestion, the viscosities of all three preparations were determined as usual.

The data showing the viscosities of this experiment are given in Table XXIX, and leave no doubt but that proteolysis has taken place.

TABLE XXVIII.

The effect on the viscosity of flour 1002 with the salts washed out after digesting with varying amounts of malt flour for 4 hours at 40°C. with 100 cc of water (lactic acid added in each instance.)

Lactic Acid cc. Percent Malt Flour	0.5 cc. Viscosity Degrees M	1.0 cc. Viscosity Degrees M	1.5 cc. Viscosity Degrees M
0	388	411	405
1	299	328	340
2	295	312	315
3	273	295	305
4	245	263	271

TABLE XXIX.

Effect of three and one-half hour digestion without, and half an hour digestion with malt flour as compared to a 4 hour digestion with and without 4 percent malt flour upon the viscosity of suspensions in water.

Cubic Centimeters N/1 Lactic Acid Added Flour No.	0.0 Degrees M	5.0 Degrees M
1002 digested without malt flour..................	38	145
1002 digested with 4% malt flour................	19	81
1002 digested 3.5 hrs. without and 3 minutes with 4% malt flour............................	30	127

Gas Production Capacity of Wheat Flour in Relation to Strength.

Although Wood pointed out that the gas production capacity of a flour was an index to one of the factors in flour strength and Baker and Hulton pointed out that weak flours were low in liquifying enzymes, they did not submit sufficient data to show that this was actually the case. In the following work the gas producing capacities of a series of flours was determined, according to the method of Wood, and with and without the addition of malt extract. The flours selected consisted of two typically strong patent flours 1008 and 1009, two clear flours of fair baking strength 1002 and 1007, and one typically weak

patent flour 1003, milled from Washington wheat. The method followed was the same as that described in the methods under gas production.

The data giving the cubic centimeters of gas produced from flours 1002, 1003, 1007, 1008 and 1009 when fermented with and without the addition of 1 percent malt extract, is given in Table XXX, and illustrated graphically in Figures 11 and 12.

TABLE XXX.

Effect of added malt extract upon the gas producing capacity of flours 1002, 1003, 1007, 1008 and 1009 when fermented with 2.5 percent yeast for four hours at 35°.

Flour No........	No Added Malt Extract					One Percent Malt Extract				
	1002	1003	1007	1008	1009	1002	1003	1007	1008	1009
Time of Fermentation Hours	Gas cc.	Gas cc.	Gas cc.	Gas cc.	Gas cc.	Gas cc.	Gas cc.	Gas cc.	Gas cc.	Gas cc.
0.5	13	14	10	10	7	14	15	12	14	8
1.0	34	33	31	27	28	35	34	33	36	31
1.5	62	57	58	53	60	64	59	60	66	61
2.0	98	80	92		90	99	88	94	105	101
2.5	140	95	132	148	116	141	115	134	153	125
3.0	177	104	174	202	148	180	140	178	203	153
3.5	214	117	212	250	176	218	184	223	252	181
4.0	245	123	243		192	254	196	270		196
4.5	.	128				..	208			

TABLE XXXI.

Changes in pH during the fermentation of the dough with values for flour extract and the extract of bread crumb.

Baking Data.

Flour No.	Ash %	pH Values						
		Flour	Mix	1st Pch.	2nd Pch.	3rd Pch.	After Pf.	Bread
1001	.40	5.81	5.33	5.18	5.09	5.02	4.79	4.96
1002	.61	6.05	5.65	5.25	5.25	5.24	5.05	5.29
1003	.46	6.00	5.24	5.16	5.19	5.37	5.05	5.16
1004	.83	6.17	5.91	5.92	5.87	5.85	5.80	5.52
1005	.43	5.84	5.75	5.40	5.22	5.23	5.17	5.29
1006	.38	5.78	5.70	5.28	5.22	5.17	5.03	5.30
1007	.64	6.10	5.76	5.63	5.58	5.59	5.53	5.58
1008	.42	5.98	5.33	5.23	5.17	5.19		5.25
1011	.56	6.15	5.47	5.33	5.30	5.20	4.92	5.28

Change in Hydrogen Ion Concentration of Fermenting Dough.

As considerable data has been accumulated upon this series of flours it was thought that the changes in hydrogen ion concentration during the fermentation period would be of considerable value, inasmuch as the speed of diastatic and proteolytic activity is influenced to such a great degree by changes in hydrogen ion concentration and many ir-
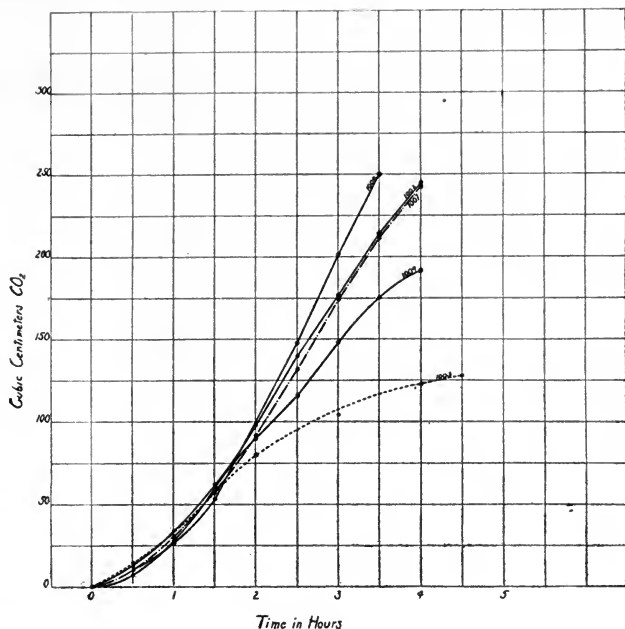
Figure 11.
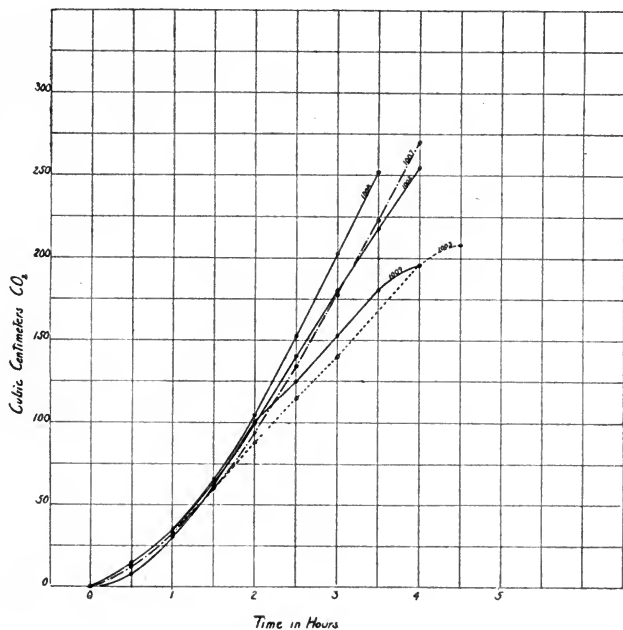Gas producing capacity of flours 1002, 1003, 1007, 1008 and 1009, fermented with 2.5% yeast.

Figure 12.
Effect of 1.% added malt extract upon the gas producing capacity of flours 1002, 1003, 1007, 1008 and 1009, when fermented with 25.% yeast.

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regularities in the data might be accounted for in this manner. The activity of yeast is also very much influenced by changes in the pH of its medium. The doughs were made from the same flours and in the same manner as that reported in the section on reducing sugars formed during fermentation. The procedure consisted of taking 10 grams of dough and shaking it up with 50 cc of water until a homogeneous suspension was secured. The whole was then centrifuged and the pH of the supernatant liquid was then determined in the manner described above. Samples were taken at the mix, first punch, second punch, third punch and after proof. The pH value of the flour extract is also given as is that of a water extract of the finished bread. The data is shown in Table XXXI.

In the past all chemical and physical data accumulated on the strength of flour has been accompanied by baking tests which in the final analysis have been the criterion of flour strength. Inasmuch as it was imperative to have accurate knowledge of the flours and the effects of diastatic ferments, a series of baking tests was conducted in addition to the baking tests made in studying formation of reducing sugars and the change in hydrogen ion concentration during the fermentation process. All baking tests were conducted as nearly alike as possible to secure comparable data.

In the baking experiments conducted to test the effects of added diastatic ferments upon wheat flours, the two diastatic preparations used throughout the entire work were employed, namely a malt flour and a malt extract. These were added in amounts of .5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4 per cent to each of a series of flours and the results are recorded as total time of fermentation, valume of the loaf, color, grain and texture of the crumb, flavor and odor. The doughs were made in the manner described under the methods of baking tests and where fermentation is spoken of, total fermentation is meant including both the actively fermenting and proofing periods.

The data showing the effects of varying amounts of malt flour and malt extracts upon flours 1001, 1002, 1003, 1007, and 1008 is given in tables XXXII to XLI.

TABLE XXXII.
The effects of varying amounts of malt flour upon the baking qualities of flour 1001.

Malt Flour %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	5:00	4:56	4:52	4:48	4:44	4:40	4:36	4:32
Sucrose added gms.	10.00	9.790	9.580	9.370	9.155	8.940	8.730	8.310
Wt. of dough gms.	513	515	516	517	517	521	522	525
Wt. of loaf gms.	454	452	443	446	450	446	459	457
Vol of loaf cc.	1870	1890	2020	1955	2030	2060	1910	2010

General Remarks: Loaves grade down with respect to grain and texture with each added increment of malt flour. Color of the crumb is markedly influenced by each increase of malt flour. The crumb was full of large gas holes which was probably due to localized yeast activity. Mean average temperature of fermentation was 81°F., and the temperature of baking 440°F. Time of baking 26 minutes .

TABLE XXXIII.

The effects of varying amounts of malt extract upon the baking qualities of flour 1001.

Malt Extract %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	5:00	4:52	4:48	4:44	4:38	4:34	4:30	4:26
Sucrose added gms.	10.00	8.94	7.88	6.820	5.76	4.70	3.64	1.52
Wt. of dough gms.	513	514	514	515	516	514	515	517
Wt. of loaf gms.	454	457	450	448	447	450	453	457
Vol. of loaf cc.	1870	1810	1880	1895	1925	1920	1970	1880

General Remarks: The texture and grain was excellent throughout, but the loaf made with 1 percent malt extract seemed to have better grain than any other. Color was very good up to 3 percent of malt extract where increase in the malt extract darkens the color, or shade. The volume of the loaf also increases up to 3 percent malt extract and then drops. A very decided sweet honeyed flavor was imparted to the bake which grew more pronounced as the percentage of malt extract increased. Mean temperature of fermentation 81°-82°. Time of baking 25 minutes. Temperature of baking 440°-430°C.

TABLE XXXIV.

The effects of varying amounts of malt flour upon the baking qaulities of flour 1002.

Malt Flour %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	5:18	5:13	5:08	5:03	4:58	4:53	4:48	4:44
Sucrose added gms.	10.00	9.79	9.58	9.37	9.155	8.94	9.73	8.31
Wt. of dough gms.	519	523	525	528	529	530	532	535
Wt. of loaf gms.	454	451	454	451	460	458	470	471
Vol. of loaf cc.	1740	1890	2040	2010	2050	2010	2050	2030

General Remarks: Color of crumb grades down very quickly with each addition of malt flour. Grain very much alike throughout while texture was even. Color of the crust improves with increase in malt flour; very good smell and good taste while malt flavor is not in evidence. Mean Temperature of fermentation 82°-83° and baking 470°F. Time of baking 24 minutes.

TABLE XXXV.

The effect of varying amounts of malt extract upon the baking qualities of flour 1002.

Malt Extract %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	5:19	5:12	5:07	5:02	4:57	4:52	4:47	4:42
Sucrose added gms.	10.00	8.94	7.88	6.82	5.76	4.70	3.64	1.52
Wt. of dough gms.	519	514	513	513	513	514	515	521
Wt. of loaf gms.	454	441	448	445	437	436	440	447
Vol. of loaf cc.	1740	1900	2050	2010	2010	1980	1960	1940

General Remarks: Color of crumb grades off a trifle as percentage of malt extract increases. Texture increases in fineness with increase in malt extract. Grain is decidedly improved with an addition of malt extract up to 2 percent and then falls off. Odor and flavor of malt extract increases as the percentage of malt extract increases. No noticeable difference in the color of the crust between the various bakes. Mean temperature of fermentation 82°-83° and baking 479°F. Time of baking 25 minutes.

<p align="center">## TABLE XXXVI.

The effect of varying amounts of malt flour upon the baking qualities of flour 1003.

Malt Flour %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	4:42	4:38	4:34	4:28	4:24	4:20	4:16	4:12
Sucrose added gms.	10.00	9.79	9.58	9.37	9.155	8.94	8.73	8.31
Wt. of dough gms.	511	512	515	515	513	514	521	523
Wt. of loaf gms.	468	467	468	464	457	463	469	476
Vol. of loaf cc.	1660	1760	1675	1860	1710	1690	1650	1550

General Remarks: Standard loaf had by far the best color, which grades down very quickly with increase in malt flour. Loaf made with 0.5 percent malt flour possessed the best texture, flavor and odor. That with 1 percent malt flour had the best grain and those loaves with increased quantities grade off to a very coarse grain. Loaves were soggy and heavy. Mean temperature of fermentation was 81° and that of baking 470°F. Time of baking 21 minutes.

<p align="center">## TABLE XXXVII.

The effects of varying amounts of malt extract upon the baking qualities of flour 1003.

Malt Extract %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	4:42	4:44	4:40	4:36	4:32	4:28	4:25	4:21
Sucrose added gms.	10.00	8.94	7.88	6.82	5.76	4.70	3.64	1.52
Wt. of dough gms.	511	504	507	504	506	508	508	492
Wt. of loaf gms.	468	460	461	454	448	453	460	448
Vol. of loaf cc.	1660	1730	1775	1810	1770	1700	1750	1680

General Remarks: Color was decidedly the best in the loaf made with 0.5 percent malt extract while the texture and flavor were best in that with 2.5 percent. Best grain was secured when 1.5 percent malt extract was used and seemed to run off as percent malt extract increased but nearly as bad as that of the malt flour. The malt flavor was not as pronounced as in the previous bakes when using malt extract. Color of crust good throughout. Mean temperature of fermentation 81° and that of baking 525°F. Time of baking 20 minutes

TABLE XXXVIII.
The effect of varying amounts malt flour upon the baking qualities of flour 1007.

Malt Flour %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	5:22	5:15	5:10	5:06	5:02	4:58	4:52	4:47
Sucrose added gms.	10.00	9.79	9.50	9.37	9.155	8.94	8.73	8.21
Wt. of dough gms.								
Wt. of loaf gms.								
Vol. of loaf cc.	1640	1750	1750	1780	1680	1665	1610	1690

General Remarks: 1.5 percent malt flour gave the largest volume, finest texture, color and grain. This appeared to be the high point in quality, since all factors decreased as percentage of malt flour increased. Mean temperature of fermentation was 84° while that of baking was 470°F. Time of baking 22 minutes.

TABLE XXXIX.
The effect of varying amounts of malt extract upon the baking qualities of flour 1007.

Malt Extract %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	5:22	5:17	5:12	5:07	5:02	4:57	4:52	4:47
Sucrose added gms.	10.00	8.94	7.88	6.82	5.76	4.70	3.64	1.52
Wt. of dough gms.								
Wt. of loaf gms.								
Vol. of loaf cc.	1640	1690	1900	1620	1660	1850	1890	1760

General Remarks: The use of 3 percent malt extract gives the best loaf for color, texture and grain and the best general appearing loaf. Malt extract increased the bloom, color of crumb and volume, throughout the bake. Mean temperature of fermentation was 84° while that of baking was 470°F. The time of baking was 25 minutes.

TABLE XL.
The effect of varying amounts of malt flour upon the baking qualities of flour 1008.

Malt Flour %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	4:53	4:57	4:52	4:47	4:42	4:37	4:32	4:27
Sucrose added gms.	10.00	8.94	7.88	6.82	5.76	4.70	3.64	1.52
Wt. of dough gms.	524	524	525	526	630	530	531	532
Wt. of loaf gms.	450	460	455	460	467	466	467	464
Vol. of loaf cc.	2160	2070	2000	2120	2100	1950	1860	1885

General Remarks: The color of the crumb was affected by the addition of malt flour as those preceeding. Best texture and grain was secured by the use of 1.5 percent malt flour. There was a very marked difference between those loaves made with 1.5 and 2.0 percent in texture and grain. A distinct wheaty smell was noticed in the loaves having malt flour. The color and bloom were about alike. Temperature of fermentation was 83° and the temperature of baking 480°F. Time of baking was 24 minutes.

TABLE XLI.
The effect of varying amounts of malt extract upon the baking qualities of flour 1008.

Malt Extract %	Standard	.5	1.0	1.5	2.0	2.5	3.0	5.0
Fermentation Hrs.	4:53	4:57	4:52	4:47	4:42	4:37	4:32	4:27
Sucrose added gms.	10.00	8.94	7.88	6.82	5.76	4.70	3.64	1.52
Wt. of dough gms.	524	513	513	514	518	521	519	525
Wt. of loaf gms.	450	457	457	454	450	450	453	460
Vol. of loaf cc.	2160	2020	2040	2070	1995	2130	2180	2130

General Remarks: Color was good throughout the bake, the bread made with 3 percent had the best grain and texture. Added malt extract gave the bread a slight sweet odor and taste. Bloom was even throughout the whole bake. Temperature of fermentation 83° and was baked out in 22 minutes at a temperature of 500°F.

III. DISCUSSION.
Changes in pH, Temperature, Time and Concentration and Their Effects Upon the Activity of the Diastases Contained in a Commercial Malt Flour.

As already noted in the historical review of the diastase literature, Sherman and his co-workers found that the pH for the optimum activity of diastase of different origins were not the same and it could hardly be expected that the diastases derived from different sources of barley would have the same activity at the same hydrogen ion concentration. A study of Table VIII, and Figure 1 show that the greatest activity of the diastases, in the malt flour used in this investigation, was at a pH of 4.26, while that found by Sherman for a highly purified malt amylase was very close to a pH of 4.4 which shows relatively close agreement. It will be noted in Table XXXI, where the changes in hydrogen ion concentration of fermenting dough was followed, that in the later stages of fermentation the dough was, with two exceptions. at a pH of about 5.0. Although this is not at the optimum for diastatic activity, it will be noted from Figure 1 that the rate of reaction was very high at this point. This is of significance when we consider that the sugars formed in the later stages of fermentation are important factors in determining the size of the resulting loaf.

Table IX and Figure 2 show that the diastatic activity of the malt flour was practically constant over a period of eight hours digestion. A slight decrease in activity was noticeable as time of digestion proceeds, but for all practical purposes, the rate of reaction showed a straight line when the quantity of dextrose formed was plotted against time.

Table X and Figure 3 show that increase in temperature up to 65°C. increased the activity of the diastase. From 27° - 45°C. the rate of reaction increased quite regularly with each increment of rise in temperature. Between 45° and 50° the rate was greatly increased, while between 50° and 60° the increase was very rapid, following quite closely the Vant Hoff and LaBelle law. After 60° the increase in activity was not so marked and the diastatic activity was apparently at a maximum at 65°C., as a decline in activity was noted with further increase in temperature. Table XII shows that when the percentage of malt flour was increased from 0-50 per cent, the percentage of dextrose formed from malt flour increased from 1.63 to 6.06 percent. This was calculated to show the quantity of raw starch converted to dextrose. The greatest effect of added diastase was in the first 10 percent of added malt flour, which gave an increase in dextrose from 1.03 to 3.67 percent.

The Effect of Diastatic Enzymes Upon Starch of Different Flours.

The addition of diastatic ferments to wheat flours increased the reducing sugars, when digested at 27°C. for 1 hour. As the amount of diastatic ferment was increased a corresponding increase in reducing sugars was noted. All of the flours used in the experiment did not react in the same way to the addition of malt flour, as great differences were shown not only in the initial amounts of reducing sugars (1 hour digestion without diastase) contained, but with an increase in malt flour more starch was converted in some flours than in others. In general, but for one exception, the weaker flours produce less reducing sugars than do the stronger, when digested with the same amounts of malt flour. From the data presented in Table XIII and Figure 5, it will be noted that the commercial wheat starch shows the least amount of initial reducing sugars and responds less to the action of malt flour than any of the wheat flours. Flour 1003, a decidedly weak Pacific Coast flour, is next in the series; it shows a slightly greater amount of initial reducing sugars (digestion 1 hour without added diastases) and responds a trifle more readily to conversion by the malt flour, than does the wheat starch. The next flour, 1011, is a patent milled from a soft winter wheat and runs just a trifle higher in initial sugar content and appears to be more easily converted than flour 1003. These two flours, and the wheat starch constitute a

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special group as far as sugar content is concerned. While the wheat starch has no baking value, the other two, namely 1003 and 1011, showed very poor baking qualities.

The patent flours of good baking strength are highest in the list of initial sugar content and with one exception produce under the action of diastatic enzymes, more dextrose than do the weaker flours. The exception noted was flour 1008, which showed the largest volume in the baking test, had a greater initial sugar content than any of the other flours, but did not produce as much dextrose as flours 1001 and 1009 when digested with malt flour. Flour 1007, a clear flour of very poor baking qualities, milled from Canadian wheat stood fourth in the series in regard to initial sugar content. Under the action of the diastase in malt flour, however, the reducing sugars increased out of all proportion to its baking strength and on the addition of .5000 grams of malt flour, it contained more reducing sugars than any of the other flours with a like concentration of malt flour.

In general the initial sugar content (digestion 1 hour without added diastases) indicated the baking qualities of the flour quite accurately. This in turn depends to a large extent upon the diastatic enzymes contained in the flour itself. From the data presented, it would seem that the starch of the strong flours was generally more easily converted than that of weak flours. This was not invariable, as flour 1008, a particularly strong patent flour showed only a very slight increase in soluble sugars when digested with approximately 5 percent malt flour, whereas flour 1007, a clear flour of notably poor baking qualities, showed a phenomenal increase under the same experimental conditions.

The Production of Reducing Sugars in the Panary Fermentation of Bread and the Effects of Diastases Added in the Dough.

In this phase of the investigation, flours 1008 a strong patent, 1003 a weak flour, and 1002 a clear flour of good baking strength, were used. The data in Tables XIV to XXI and in Figures 6, 7 and 8 show how these three typical flours behave with regard to producing reducing sugars, when fermented normally and with different amounts of added malt preparations. In order to have a check on the reducing sugars actually produced during the fermentation period, a check series was run at the same time, identical in all respects but having no yeast. In every instance where a diastatic enzyme was added, there was an increase in reducing sugars, over that of the normal dough, throughout the fermentation period.

Flour 1008 had by far the greater amounts of reducing sugars, when the same additions of malt flour or malt extract were made, than did

the other two flours. Flour 1002 was next and 1003 was at the bottom of the list. The curves in Figure 6, representing the production of sugars in Flour 1008, are all of the same general type, that is, the yeast dough curves are alike and the "no yeast" curves are alike. In the yeast doughs the peak of sugar formation or the point where the diastases were producing as much available sugar as the yeast was using up, was at the first punch after two and one-half hours of fermentation. From this time on the yeast seems to be stimulated and uses up the sugar faster than it is produced, steadily diminishing the surplus that the diastases have piled up in the first half of the fermentation period. In the doughs which have no yeast, conversion seems to be slightly faster in the later stages of fermentation than in the beginning. If this is the case in the yeast dough, the yeast undoubtedly increases in activity as fermentation proceeds. When 3 percent malt extract is used, the sugar content is decidedly higher than in any of the other doughs. This is of decided advantage as the yeast has a large surplus of sugars to draw from.

The curves for flour 1002 (Figure 8), are very similar to those for flour 1008 (Figure 6), with the exception that sugar production appeared to have reached a maximum at the end of 1 hour of fermentation in the dough to which no diastatic enzymes were added and the one to which 3 percent malt extract was added. The doughs, to which 1.5 and 4.0 percent malt flour were added, reached their maximum of sugar production at the first punch after a fermentation period of 2 hours and fifteen minutes. The amounts of sugars produced by the diastatic enzymes are remarkably constant during the time of fermentation, with the greatest conversion produced by the addition of 4 percent malt flour. As in the preceding series, the dough to which the malt extract was added showed a larger amount of reducing sugars throughout the entire fermentation period.

A difference in the shape of the curves is at once noticed (Figure 7) where the sugars produced, in the fermentation of flour 1003, are followed. Instead of an initial increase in reducing sugars, as was the case with the preceding strong flours, the yeast utiltizes all available sugars immediately, with a continued decrease in sugar content as fermentation proceeded. A slight increase in reducing sugars was secured by the use of 4 percent malt flour, which reached the highest point after 2 hours fermentation. The addition of 3 percent malt extract seemed to be able to convert enough starch to hold the available reducing sugars constant for one hour, but after this time the yeast increased in activity and the available sugars dropped off rapidly.

From an inspection of Tables XIV-XVI and XXI-XXIV, and Figures 6 and 8, it appeared that an addition of diastatic enzymes to a dough resulted in a surplus of reducing sugars during the earlier stages of fermentation. This surplus was used up in the later stages of fermentation along with the sugars simultaneously produced by the diastatic enzymes. It also appeared that yeast activity was increased to a considerable extent when the carbon dioxide was punched out of the dough; at least it seems to have been coincident with the punching of the dough in the case of the stronger flours. It was also evident from the amounts of reducing sugars available, that the flours which showed good baking qualities had a greater diastatic activity than did the flour of poor baking strength. The malt preparations when added to weak flours produced less sugars in proportion than when added to strong flours. Whether or not the starch of the former was harder to hydrolyze than that of the latter is problematical but the data presented in section 2, and also in this section, might be taken as indicating such a possibility.

Effects of the Proteolytic Enzymes, Contained in Malt Preparations, Upon the Viscosity of Strong and Weak Flours Following the Addition of Various Amounts of N/1 Lactic Acid.

A very decided difference in the viscosity of a flour-water suspension was noted when a flour was digested, with and without malt flour, for different periods of time, as shown in Table XXV, and illustrated graphically in Figure 9. The higher the concentration of malt flour added, the lower was the resulting viscosity reading, and when digestion was carried out for varied lengths of time, a steady decrease in viscosity occurred as time of digestion progressed. This was very noticeable as the percentage of malt flour was increased.

When flours of different baking strengths were digested with increasing amounts of malt flour and malt extract, the viscosity of their suspensions in water (plus lactic acid) decreased quite decidedly as shown in Tables XXVI and XXVII, and graphically in Figures 10 and 10A. The strongest flours grouped themselves, and their suspensions in acidified water have a much higher viscosity than those of the medium or weak flours, and when treated with 4 percent of malt flour or malt extract the strength of the flours was indicated by its position on the curve. The malt extract used did not decrease the viscosity as much as a like concentration of malt flour, and the conclusion was that it did not contain as large an amount of proteolytic enzymes as did the malt flour. It might be expected that the stronger flours would not show as great a decrease in viscosity as the weak flours. When digested with 4 per cent malt flour or malt extract over the range given in Tables XXVI and XXVII, but the opposite seems to be

actually the case. Flour 1008, the strongest flour in the series, showed a decrease in viscosity of 49° MacMichael, when digested with 4 percent extract, while the decrease found for flour 1003 under the same conditions is 22° and 24° M. respectively. Clear flour 1007 is intermediate in this particular and showed a decrease of 36° MacMichael, when digested with 4 percent malt flour.

Although it has been demonstrated that salts have a profound influence upon the viscosity of flour-water suspensions, the results in Table XXVIII show that while the viscosity readings were very much higher in a flour-water suspension, from which the salts have been washed out, the same relative values hold, and the results recorded above were not vitiated by the electrolyte content of the flours. This has been demonstrated in another way where a flour was digested alone for four hours, with 4 percent malt flour for four hours, and another sample digested alone for three and one-half hours and at the end of this time 4 percent malt flour was added and digested thirty minutes longer. It was thought that the salts of the added malt flour would be extracted in thirty minutes and would exert their maximum effect in depressing the viscosity. Also, that in this length of time only a small amount of proteolytic activity would take place, thus showing a difference in viscosity between the flour which was digested four hours with 4 percent malt flour and the other which was digested three and one-half hours alone, and thirty minutes with 4 percent malt flour. These expectations were justified, as demonstrated in Table XXIX, where the flour digested alone gaves a reading of 145°M., and that digested with 4 percent malt flour for four hours gave a reading of 81°M., while that digested alone for three and one-half hours and then thirty minutes more with malt flour gave a reading of 127°M. These data show that the increase in viscosity was not due entirely to the electrolytes but to the partial disintegration of the protein.

From the data presented in Tables XXVI and XXVII it has been shown that suspensions of strong flours in water have a higher viscosity than weak flours when digested with and without added malt preparations. It has also been shown that suspensions of strong flours in water show a greater decrease in viscosity than do similar suspensions of weak flours when digested with malt preparations and that the decreases in viscosity recorded above were not due entirely to the electrolyte content of the flours but to the cleavage of the gluten, thus decreasing its imbibitional capacity and consequently its viscosity.

The Gas Producing Capacities of Strong and Weak Flours and the Effect of Added Malt Extract Upon Them.

Wood has shown that the gas produced by a flour, especially in the later stages of fermentation, was a factor in strength, while Baker and

Hulton have shown that in some cases a weak flour produces as much, and in some cases even more, gas than does a strong flour. They believed that weak flours were deficient in liquifying enzymes and that an addition of liquifying enzymes would increase the gas production of a weak flour to a considerable extent, while they would have little or no effect upon a strong flour. The data in Table XXX supports their theory and shows that flours 1008 and 1009, which showed very good baking qualities, did not increase to any extent in gas producing capacity when malt extract was added, while flour 1002, a strong clear flour increased only 9 cc. under the same conditions, and 1007, a clear flour of poor baking quality increased 37 cc. under the same treatment. The test seems to be conclusive by the increase shown by flour 1003, a notably weak flour, which increased 80 cc. when 1 percent malt extract was added.

The Changes in Hydrogen Ion Concentration Taking Place During the Fermentation of the Dough.

The changes in hydrogen ion concentration taking place during the fermentation of the dough, are recorded in Table XXXI and show that steady increase in hydorgen ion concentration takes place as fermentation proceeds. With two exceptions the doughs when ready for the oven had a hydrogen ion concentration of approximately pH 5. The two flours which had a higher pH were clear flours of very poor baking strength.

The Effects of Malt Flour and Malt Extract Upon the Baking Value of Flour.

In flour 1001, a strong patent flour, the volume was considerably increased by the use of 2.5 percent malt flour. This advantage was materially offset by the decrease in color. With the use of the malt extract, the volume increased with additions up to 3 percent with not much decrease in color, while a sweet honey-like flavor is evident which adds to the value of the loaf. The data in Table XXXIII shows that the baking qualities of the flour were improved when 3 percent malt extract was used.

Flour 1002, a fairly strong clear flour, increased in volume with the addition of malt flour. The greatest volume was secured by the use of 2 percent malt flour for the weight of the dough baked out. While the grain and texture were uniform throughout, the decrease in color value offset the advantages secured by the increase in volume. In using malt extract the greatest volume was secured by the use of 1.5 and 2.0 percent. and decidedly the best loaves were thus produced, since texture and grain increased in fineness as the amount of malt extract increased. The slight decrease in color value was not a serious

objection and the addition of 1.5 to 2.0 percent malt extract had a decided beneficial effect upon the baking qualities of flour 1002.

With the use of 1.5 percent malt flour the largest volume was secured in baking flour 1003. As the grain was coarse and the color off, however, the advantages gained by the increase in volume were offset. A decided increase in volume and grain was secured by the use of 1.5 percent malt extract in this weak flour in my opinion, the baking quality of this flour was thus greatly increased.

In clear flour 1007, the use of 1.5 percent malt flour increased the volume as well as the texture and grain, and in this flour the addition of malt flour was beneficial. The use of 3 percent malt extract gave a decided improvement in the baking qualities of flour 1007 as far as volume, grain, texture and color is concerned.

In flour 1008, the strongest flour of the series, the use of 1.5 percent malt flour improved the texture and grain but darkened the color considerably. The use of malt flour did not increase the baking qualities of this flour, while on the other hand the use of 3 percent malt extract increased the volume slightly, improved the texture and grain thus improving the baking qualities to a marked extent.

IV. SUMMARY

This paper deals with the effects of diastatic ferments upon the strength of wheat flours. Tables and graphs have been presented, showing:

1. Optimum temperature for the diastatic activity of the malt flour used was at temperature of 65°C.

2. Optimum hydrogen ion concentration for the diastatic enzymes in malt flour was at a pH of 4.26.

3. Constant diastatic activity was shown by the malt flour over a period of eight hours when digested at 27°C.

4. Concentrations of added diastase exert their greatest effect in the first 10 percent of added malt flour, giving an increase in dextrose from 1.63 to 3.67 percent.

5. Diastatic ferments when added to wheat flours increase the reducing sugars, when digested at 27°C for 1 hour. The strong flours showed a higher sugar content and greater diastatic activity than did the weaker flours. The starch of the strong flours appeared to be more easily hydrolyzed by diastatic ferments than that of the weaker flours.

6. Addition of diastatic ferments to a dough convert the starch to reducing sugars and in the earlier stages of fermentation, produce a surplus of fermentable sugars in the doughs made from strong flours. This surplus soon disappears as the activity of the yeast increases, and at the end of the fermentation period the dough is nearly depleted of available sugars.

7. Suspensions of strong flours in water had a higher viscosity (on the addition of lactic acid) than similar suspensions of weak flours when incubated alone or with added diastatic ferments in the form of malt flour and malt extract. The course of proteolytic activity could be accurately followed by the change in viscosity when wheat flour was digested with added malt flour. The presence of naturally occurring salts of the wheat flours did not vitiate the viscosity readings.

8. Gas producing capacity of weak flours was greatly increased when fermented with added malt extract. This was not the case when strong flours were fermented with added malt extract.

9. Hydrogen ion concentration of the dough steadily increased as fermentation proceeded. With two exceptions, the doughs were at approximately a pH of 5.0 when ready for the oven.

10. Addition of malt flour and malt extract to doughs increased the volume of the resulting bread. In all cases the use of malt extract gave a superior loaf of bread in volume, grain and texture, thus increasing the baking strength of the flours.

V. LITERATURE CITED

Bailey, C. H.
  1920. A simple hydrogen electrode. Jour. Amer. Chem. Soc., 42, 45-48.
Bailey, C. H., and Collatz, F. A.
  1921. Studies of wheat flour grades 1. .Electrical conductivity of water extracts. Jour. Indus. Engin. Chem., 13, 319-321.
Bailey, C. H., and Peterson, A. C.
  Studies of wheat flour grades II. Buffer action of water Extracts. Jour. Indus. Engin. Chem., 13, 916-918.
Bailey, C. H., and Weigley, Mildred.
  1922. Loss of carbon dioxide from dough as an index of flour strength. Jour. Indus. Engin. Chem., 14, 147-150.
Baker, J. L., and Hulton, H. E. F.
  1908. Conditions affecting the strength of wheaten flour. Jour. Soc. Chem. Indus., 27, 368-376.
Baker, J. L., and Hulton, H. E. F.
  1908. Action of malt enzymes on the enzymes of ungerminated cereals. Analyst 33, 392.
Baker, J. L., and Hulton, H. E. F.
  1914. Action of diastase on starch granules. Jour. Chem. Soc. (London), 105, 1529-1536.
Baswitz, M.
  1878. Zur Kenntniss der Diastase. Ber., 11, 1443-1447.
Baswitz, M.
  1879. Zur Kenntniss der Diastase. Ber., 12, 1827-1831.
Biot, and Persoz.
  1883. Mémoire sur les modifications que la fécule et la gomme subissent sous l'influence des acids. Ann. chim et phys., 52, 72-90.
Blish, M. J.
  1915. On· the chemical constitution of the proteins of wheat flour and its relation to baking strength. Jour. Indus. Engin. Chem., 7, 138-144.
Bremer, H.
  1907. Hat der Gehalt des Weizenmehles an Wasserlöslichen Stickstoff einen Einfluss auf seinen Backwert. Z. Unter. Nahr. Genussm., 13, 69-74.
Brown, H. T., and Heron, J.
  1879. Contributions to the history of starch and its transformations. Jour. Chem. Soc. (London), 35, 596-654.

Brown, H. T., and Heron, J.
1879. Beitrage zur Geschichte der Stärke und der Verwendung derselben. Annalen, 199, 165-253.
Bruying, F. F.
1905. La valeur boulangere du froment. Archives Teyler, 2, p. 2. (Cited from T. B. Wood, page 270).
Chamberlain, J. S.
1904. Determination of gliadin and glutenin in flour by the Fleurent-Manget method. U. S. Dept. of Agr., Bur. Chem. Bul. 81, 118-125.
Chamberlain, J. S.
1906. Investigations on the properties of wheat proteins. Jour. Amer. Chem. Soc., 28, 1657-1667.
Cohn, E. J., and Henderson, L. J.
1918. The physical chemistry of bread making. Science, 48, 501-505.
Cohn, E. J., Wohlback, S. B., Henderson, L. J., and Cathcart, P. H.
1918. On the control of rope in bread. Jour. Gen. Physiol., 1, 221-230.
Cohn, E. J., Cathcart, P. H., and Henderson, L. J.
1918. The measurement of the acidity of bread. Jour. Biol. Chem., 36, 581-586.
de Sassure, Theodore.
1819. Sur l'action de l'amidon a la temperature atmospherique par l'action de l'air et de l'eau. Ann. chim. et phys., 11, 379-408.
Detmer, W.
1882. Ueber den Einfluss der Reaction Amylum sowie Diastase enthaltender Flussigkeiten auf den Verlauf der fermentativen Prozesse. Z. Physiol. Chem., 7, 1-6.
Dubrunfaut.
1847. Note sur le glucose. Ann. chim. et phys., 21 (III), 178-180.
Duggan, J. R.
1885-86 On the determination of diastatic activity. Amer. Chem. Jour., 7, 306-312.
Effront, J.
1892. Sur les conditions chimique de l'action des diastase. Compt. rend., 115, 1324-1326.
Effront, J.
1893. Sur les conditions chimiques de l'action de la diastase. Bull. soc. chim., 9 (III), 151-156.
Effront, J.
1895. Sur l'amylase. Comp. rend., 120, 1281-1283.

Effront, J.
   1904. Action des acides amids l'amylase. Bull. soc. chim., 31,
      (III), 1230-1233.
Fernbach, A., and Wolff, J.
   1906. Sur la transformation presque integrale en maltose en dex-
      trine provenant de la saccharification de l'amidon. Compt.
      rend., 142, 1216-1218.
Fernbach, A., Wolff, J.
   1907. Etude sur la liquefaction diastasique des empois de fécule.
      Compt. rend., 145, 261-263.
Fischer, M.
   1915. OEdema and Nephritis. Ed. 2. New York, 1915.
Fischer, M.
   1918. On the swelling of gelatin in polybasic acids and their salts.
      Jour. Amer. Chem. Soc., 40, 272-292.
Fischer, M.
   1918. On the swelling of fibrin in polybasic acids and their salts.
      Jour. Amer. Chem. Soc., 40, 292-303.
Fischer, M.
   1918. On the liquifaction or "solution" of gelatin in polybasic
      acids and their salts. Jour. Amer. Chem. Soc., 40, 303-312.
Fleurent.
   1896. Sur une method chimique d'appreciation de la valuer bou-
      langers des farines de blé. Compt. rend., 123, 755-758.
Ford, J. S.
   1904. Lintner's soluble starch and the estimation of diastatic
      power. Jour. Soc. Chem. Indus., 23, 414-422.
Ford, J. S., and Guthrie, J. M.
   1908. Contributions to the biochemistry of barley 1. Amylases of
      resting barley. Jour Inst. Brew., 14, 61-85. Abst. in
      Jour. Soc. Chem. Indus. 27, 239-240.
Ford, J. S., and Guthrie, J. M.
   1908. The amylolytic and proteolytic ferments of wheaten flours,
      and their relation to baking value. Jour. Soc. Chem. In-
      dus., 27, 389-393.
Francis, J. M.
   1898. Testing of diastatic digestants. Bull. Pharm., 12, 52-54.
Girard and Fleurent.
   1903. Le froment et sa mouture. Gauthier-Villars, Paris, 1903.
Gortner, R. A., and Doherty, E. H.
   1918. Hydration capacity of gluten from "strong" and "weak"
      flours. Jour. Agr. Res., 13, 389-419.

Gortner, R. A., and Sharp, P. F.

    1922.   Physico-chemical properties of strong and weak flours III, Viscosity as a measure of hydration capacity and the relation of the hydrogen ion concentration to imbibition in the different acids. Reported before Amer. Chem. Soc.. New York, Sept., 1921. Abstract in Science N. S. 54, p. 558, 1921.

Griefsmeyer, V.

    1880.   Rundschau auf dem Gebiete der Bierbrauerei. Dingler's Polytechn. Jour., 235, 379-387; 452-460.

Hawkins, Lon A.

    1913.   The effect of certain chlorides singly and combined in pairs on the activity of malt diastase. Bot. Gaz., 55, 265-285.

Henderson, L. J., Fenn, W. O., and Cohn, E. J.

    1919.   Influence of electrolytes upon the viscosity of dough. Jour. Gen. Physiol., 1, 387-397.

Henderson, L .J., Cohn, E. J., Cathcart, P. H., Wachman, J. D., and Fenn, W. O.

    1919.   A study of the action of acid and alkali on gluten. Jour. Gen. Physiol., 1, 459-472.

Hofmeister, Franz.

    1890.   Zur Lehre von der Wirkung der Salze V. Untersuchungen über den Quellungsvorgang. Arch. fur Expt. Path. u. Pharmakol., 27, 395-413. (Cited from Gortner and Doherty).

Humphries, A. E., and Biffen, R. H.

    1907.   The improvement of English Wheat. Jour. Agr. Sci., 1, 1-16.

Humphries, A. E., and Biffen, R. H.

    1910.   Quality in wheaten flours. Jour. Board Agr., 17, No. 3, Supplement No. 4, 39-45.

Jessen-Hansen, H.

    1911.   Etudes sur la farine de froment. 1. Influence de la concentration en ions hydrogen sur la boulangere de la farine. Compt. rend. trav. lab. Carlsberg, 10, 170-206.

Johnson, W. A.

    1908.   A proposed method for the routine valuation of diastase preparations. Jour. Amer. Chem. Soc., 30, 798-805.

Jungk, J. F. C.

    1884.   Analysis of malt-extract. Jour. Chem. Soc. (London), 46, 529-531.

Kendall, E. C., and Sherman, H. C.

    1910.   Studies on amylases. 11. A study of the action of pancreatic amylase. Jour. Amer. Chem. Soc., 32, 1087-1105.

Kirchhoff, G. S.
1811. Ueber die neue von Kirchhoff entdeckte Zuckergewinnung. Schweigger's Jour. f. Chem. u. Phys., 4, 108-110.

Kirchhoff, G. S.
1815. Ueber die Zuckerbildung beim Malzen des Getreides und beim bebrühen seines Mehls mit kochenden Wasser. Schweigger's Jour. f. Chem. u. Phys., 14, 389-398.

Kjeldahl, J.
1879. Recherches sur le ferment producteur du sucre. Compt. rend. trav. lab. Carlsberg., (not read; cited by Griefsmayer).

Kjeldahl, J.
1880. Mitteilung aus dem Carlsberger Laboratorium. Dingler's Polytechn. Jour., 235, 379-387; 452-460.

Koch, Geo.
1914. The diastase and invertase content of wheat flour and their relation to baking strength. A thesis submitted to the graduate school of the University of Minn. for the degree of Master of Science.

Lintner, C. J.
1885. Zur bestimmung der Diastasewirkung. Z. ges. Brauwesen, n. s. 8, 281-285.

Lintner, C. J.
1886. Studien über Diastase. Jour. Prakt. Chem., 34, (2) 378-394.

Lintner, C. J.
1887. Studien über Diastase II. Jour. Prakt. Chem., 36, (2) 481-498.

Liebig, H. J. von.
1909. Uber den Zuckergehalt der feinen Weizenmehle der Weizenmehle teige und der vergorener Mehlteige sowie über die diastatische Kraft der Weizenmehle. Land. Jahr., 38, 251-271. (Abst. from C. A. 3, 2327).

Lüers, H., and Ostwald, Wo.
1919. Beiträge zur Kolloidchemie des Brotes II; Zur Viskosimetrie der Mehle. Kolloid Z., 25, 82-136.

Lüers, H.
1919. Beiträge zur Kolloidchemie des Brotes III; Kolloidchemische Studien am Riggen und Weizen gliadin mit besonderer Berucksichtigung des Kleber und Bachfähigkeits problems. Kolloid Z., 25, 177-240.

Lüers, H., and Ostwald, Wo.
1920. Beiträge zur Kolloidchemie des Brotes, IV; Zur kenntnis von Mehlen von schlechter Backfähigkeit. Kolloid Z., 26, 66-67.

Lüers, H., and Ostwald, Wo.
    1920. Beitrage zur Kolloidchemie des Brotes V. Die Kolloid Quel
        lung des Weizenklebers. Kolloid Z., 27, 34-37.

Manquenne, L., and Roux, E.
    1906. Influence de la reaction du milieu sur l'activite de l'amylase
        et la composition des empois saccharifies. Compt. rend.,
        142, 124-129.

Maquenne, L., and Roux, E.
    1906. Nouvelles recherches sur la saccharification diastatique.
        Compt. rend., 142, 1059-1055.

Märker.
    1877. Standpunct unsere Kenntnisse der diastatischen Vorgänge.
        Landw. Versuchs Sta., 23, 69-70; Ber. der Naturfor., Mün-
        chen 1877.

Martin, F. J.
    1920. Properties effecting strength in wheaten flours. Jour. Soc.
        Chem. Indus. 39, 246T-251T.

Mohr, O.
    1903. Einfluss der Kohlensaure auf die Diastasewirkung. Ber., 35,
        1024-1029.

Musculus, M. F.
    1860. Remarques sur la transformation de la matiere amylases en
        glucose et dextrine. Ann. chim. et phys., 60, 203-207.

Musculus, M. F.
    1862. Nouvelle note sur la transformation de l'amidon en dextrin
        et glucose. Compt. rend., 54, 194-197.

Musculus, M. F., and Gruber, D.
    1878. Sur l'amidon. Bull. Soc. Chim. Soc., 30, 54-70.

Norton, F. A.
    1906. Crude gluten. Jour. Am. Chem. Soc., 28, 8-25.

Osborn, T. B., and Voorhees, C. G.
    1893. The proteins of wheat kernels. Amer. Chem. Jour., 15, 392-
        471.

Osborn, T. B., and Voorhees, C. G.
    1894. The proteins of wheat kernels. Amer. Chem. Jour., 16, 524-
        535.

Osborn, T. B.
    1907. The proteins of the wheat kernel. Carnegie Inst., Washing-
        ton Bul. 84.

Ostwald, Wo.
    1919. Beitrage zur Kolloidchemie des Brotes 1. Kolloid Z., 25,
        26-45.

O'Sullivan, C.
  1872. On the transformation products of starch. Jour. Chem. Soc.
    (London), 25, 579-588.
O'Sullivan, C.
  1876. On the action of malt-extract on starch. Jour. Chem. Soc.
    (London), 30, 125-144.
Payene and Persoz.
  1833. Memoire sur la diastase, les principaux produits de ses reac-
    tions, et leurs applications aux arts industriels. Ann.
    chim. et phys. 53, 73-92.
Pauli, Wo.
  1899. Die physikalischen Zustandsänderungen der Eiweisskörper.
    Arch. ges. Physiol., 78, 315-345.
Pauli, Wo.
  1902. Untersuchungen über physikalische Zustandsänderungen der
    Kolloide. Verhalten der Eiweisskörper gegen elektrolyte.
    Beitr. z. Chem. Physiol. u. Path., 3, 225-246.
Pauli, Wo.
  1903. Untersuchungen über physikalische Zustandesänderungen
    der Kolloide. Irreversible Eiweissfällung durch elektro-
    lyte. Beitr. z. Chem. Physiol. u. Path., 5, 27-55.
Pauli, Wo.
  1905. Untersuchungen über physikalische Zustandsänderungen
    der Kolloide. Eiweissfällung durch Schwermettalle.
    Beitr. z. Chem. Physiol. u. Path., 6, 233-259.
Pauli, Wo.
  1906. Untersuchungen über physikalische Zustandsänderungen
    der Kolloide. Die Eletrische Sadung von Eiweiss. Beitr. z.
    Chem. Physiol. u. Path., 7, 531-547.
Petit, P.
  1904. Action de la chaleur et de l'acidite sur l'amylase. Compt.
    rend., 138, 1231-1233.
Reychler, M. A.
  1889. La saccharification diastasique. Bull. Soc. Chim., 1, (III),
    286-295.
Roberts, W.
  1881. On the estimation of amylolytic and proteolytic activity of
    pancreatic extracts. Proc. Roy. Soc., 32, 145-161.
Rockwood, E. W.
  1917. Some nitrogeneous auxoamylases. Jour. Amer. Chem. Soc.,
    39, 2745-2752.
Rumsey, L. A.
  1922. The diastatic enzymes of wheat flour and their relation to
    flour strength. Am. Inst. Baking. Bul. 8.

Schmidt, C. L. A., and Hoagland, D. R.
  1919. Table of pH, H⁺ and OH⁻ values corresponding to electromotive forces determined in hydrogen electrode measurements, with bibliography. Pub. Univ. of Cal., 5, No. 4, 23-69.

Schutt, F. T.
  1904. A chemical study of the grain and flour of the grades of wheat. Centr. Expt. Farm (Ottawa, Can.), Bul. 50, pt. 2.

Schutt F. T.
  1907. The relationship of composition to bread-making value. Centr. Expt. Farm (Ottawa, Can.), Bul. 57, pt. 2.

Schutt F. T.
  1908. A chemical study of the grain and flour of the grades of wheat—1907. Centr. Expt. Farm (Ottawa, Can.), Bul. 60, pt. 2.

Sharp, P. F., and Gortner, R. A.
  1922. Physico-chemical studies of strong and weak flours II. The imbibitional properties of the glutens from strong and weak flours. J. Phys. Chem., 26, 101-136.

Sherman, H. C., Kendall, E. C., and Clark, E. D.
  1910. Study on amylases 1. An examination of methods for the determniation of diastatic power. Jour. Amer. Chem. Soc., 32, 1073-1087.

Sherman, E. C., and Schlesinger, M. D.
  1913. Study on amylases V. Experiments upon the purification of the amylases of malt. Jour. Amer. Chem. Soc., 35, 1617-1624.

Sherman, H. C., and Schlesinger, M. D.
  1913. Study on amylases VI. A comparison of amyloclastic and saccharogenic powers. Jour. Amer. Chem. Soc., 35, 1784-1790.

Sherman, H. C., and Thomas, A. W.
  1915. Study on amylases VIII. The influence of certain acids and salts upon the activity of malt amylase. Jour. Amer. Chem. Soc., 37, 623-642.

Sherman, H. C., Thomas, A. W., and Baldwin, M. E.
  1919. Influence of hydrogen-ion concentration upon enzymic activity of three typical amylases. Jour. Amer. Chem. Soc., 41, 231-235.

Sherman, H. C., and Neun, Dora E.
  1919. The proteolytic activity of pancreatic amylase preparations. Jour. Amer. Chem. Soc., 41, 1855-1862.

Sherman, H. C., and Neun, Dora E.
1921. The influence of certain amino acids upon the enzymic hy drolysis of starch. Jour. Amer. Chem. Soc., 43, 2461-2469.
Sherman, E. C., and Caldwell, M. L.
1921. A study of the influence of arginine, histidine, tryptophane and cystine upon the hydrolysis of starch by purified pancreatic amylase. Jour. Amer. Chem. Soc., 43, 2469-2476.
Snyder, Harry.
1899. Proteids of wheat flour. Minn. Exp. Sta., Bull. 63, 519-533.
Snyder, Harry.
1901. Studies of bread and bread making. U. S. Dept. Agr., Bull. 101.
Snyder, Harry.
1903. Wheat flour and bread. U. S. Dept. Agr., Year Book, 347-362.
Snyder, Harry.
1904. The determination of gliadin in wheat flour by means of the polariscope. Jour. Amer. Chem. Soc., 26, 263-266.
Snyder, Harry.
1905. Milling tests of wheat. Minn. Agr. Exp. Sta., Bull. 90.
Schneidewind, W., Meyer, D., and Münter, F.
1906. Ueber Enzyme. Landw. Jahr., 35, 911-922.
Stromeyer.
1813. Ueber eine in Ungarn gefundere Gediegen-Eisenmasse uber Jodine und Platina-verarbeitung. Schweigger's Jour. Chemie u. Physik, 12, 349.
Swanson, C. O., and Calvin, J. W.
1913. A preliminary study on the conditions which affect the activity of the amylolytic enzymes in wheat flour. Jour. Amer. Chem. Soc., 35, 1635-1643.
Thatcher, R. W.
1907. A comparison of various methods of estimating the baking qualities of flour. Jour. Amer. Chem. Soc., 29, 910-921.
Thatcher, R. W., and Koch, Geo.
1914. The quantitative extraction of diastases from plant tissues. Jour. Amer. Chem. Soc , 36, 759-770.
Trommer, H.
1841. Unterscheidung von Gummi, Dextrin, Traubenzucker und Rohrzucker. Annalen, 39, 360-362.
Upson, F. W., and Calvin, J. W.
1915. Colloidal swelling of wheat gluten. Jour. Amer. Chem. Soc. 37, 1295-1304.

Upson, F. W., and Calvin, J. W.
  1916. The colloidal swelling of wheat gluten in relation to milling
       and baking. Nebr. Agr. Exp. Sta., Res. Bull., No. 3.
Vauquellin, M.
  1811. Experiences comparatives sur le sucre, la gomme et le sucre
    ⸀   de lait. Bull. Pharm, 3, 54-56. 28, 156-174.
Vernon, H. M.
  1901. The conditions of action of pancreatic rennin and diastase.
       Jour. Physiol. 27, 174-199.
Vernon, H. M.
  1902. The difference of action of various diastases. Jour. Physiol..
       28, 156-174.
Vogel, H. A.
  1812. Untersuchungen über den flussigen Zucker aus Stärkmehl
       und über Umwandlung susser Materien in gährungsfa-
       higen Zucker. Schweigger's Jour. Chemie u. Physik, 5.
       80-93.
Wohlgemuth, J.
  1908. Uber eine methode zur quantitativen Bestimmung der diasta-
       tischen Fermente. Biochem. Z., 9, 1-9.
Wood, T. B.
  1907 The chemistry of strength of wheat flour. Jour. Agr. Sci., 2,
       139-160; 267-277.
Wood, T. B., and Hardy, W. B.
  1908. Electrolytes and colloids. The physical state of gluten.
       Proc. Roy. Soc. (London), B., 81, 38-43.

BIOGRAPHICAL.

Ferdinand Albert Collatz was born in Duluth, Minnesota. He graduated from the Duluth Central High School in June, 1914, and entered the University of Minnesota the same fall, where he received the degree of Bachelor of Science in June, 1918. Shortly after this he entered the Army and was assigned to the Physiological Laboratory at the Lakeside Hospital, Cleveland, Ohio, under the direction of Major Roy G. Pierce. During 1919-1920, he held the position of Assistant in Agricultural Biochemistry, University of Minnesota, and in June, 1920, received the degree of Master of Science from this department. During 1920-21 he held the American Institute of Baking Research Fellowship, where the experimental work in this Thesis was done, at the same time continuing his graduate work in the department of Agricultural Biochemistry, University of Minnesota. Here he studied for the degree of Doctor of Philosophy.

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