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A STUDY OF THE STARCH-DIGESTING AND SUGAR-FORMING ENZYMES OF WHEAT

BY

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I. THE AMYLOCLASTIC AND SACCHAROGENIC ENZYMES OF WHEAT

A. Historical

Early experiments have shown that starch is not a single substance, but is composed of at least two constituents, amylopectin and amylose (29); this fact alone might be considered to lend some support to the two enzyme theory.

No enzyme has received closer study than amylase. As early as 1815, Kirchhoff (22) stated that starch is not wholly degraded to sugar, but that an uncrystallizable, degraded form of starch remains in the conversion liquor. This was the first important description of amylase activity.

Guérin-Varry (18) found that this gummy material is not saccharified even with an excess of the enzyme, but that after its isolation or separation it can be converted almost completely into sugar.

Musculus (30) in 1860 observed that sugar and dextrins are produced by a process of hydration.

Petit (36) found that the action of amylase on starch at 50° yields the fermentable, reducing sugar maltose and also another sugar which is fermentable but non-reducing.

Grützner (17) observed that heating salivary diastase for a short period at 80° inhibited the saccharifying activity without affecting its power of liquefying starch.

Bourquelot (4) found that normal diastase and diastase which had been heated to 68° show equal activity during the

first stages of starch hydrolysis, but the production of reducing substances by the heated diastase was very much slower than by the normal diastase. These experiments seem to show that either the quantity of diastase is not diminished by heat (but its properties are changed) or else that diastase consists of several soluble ferments which are destroyed one after another as the temperature rises.

Naereker (28) early stated that at 60° four molecules of starch, yield three of maltose and one of dextrin; hydrolysis at 65° gave a reduced yield of maltose and at higher temperatures he found the proportion of maltose to dextrin to be as two to one. He therefore considered the process to involve two enzymes, of which the one chiefly responsible for sugar production was more sensitive to heat than the other.

Chittenden (6) and his co-workers found that the enzymic degradation of starch does not lead smoothly to the formation of the theoretical quantity of maltose, but that the reaction comes to a standstill considerably before this point is reached.

Wijsman (52) was the first to express the two enzyme theory in a definite form. He concluded that diastase is a mixture of two enzymes, one hydrolyzing starch to maltose and erythro-granulose, and another which converts this erythro-granulose to louco-dextrin and starch to malto-dextrin, from evidence based on some diffusion experiments.

Brown and Morris (5) also considered that two enzymes

were concerned and these were named by them 'translocation diastase' and 'secretion diastase' respectively. The 'translocation diastase' acted only on soluble starch but did not hydrolyze starch paste. Other workers have opposed this view considering that variations in the property of the enzyme would be explained by physical changes induced in the latter by the treatment to which it had been subjected.

Gruss (16) confirmed the presence of three distinct enzyme actions in germinating barley, namely, a starch dissolving, a cell wall dissolving, and an inverting action.

Seyffert (42) carried out determinations at various temperatures, using four starches and dextrines as substrates. Results indicate that in barley malt there are at least three enzymes which differ in their activity toward starch, soluble starch, erythro dextrin and amylo dextrin; glucasic enzymes do not play an important part.

Pottevin (37) separated amylo dextrin, by precipitation with alcohol into "α amylodextrin" and "β amylo dextrin". He gave experimental evidence that diastase is composed of two enzymes, one capable of converting starch to dextrin and the other, dextrin to maltose. Microscopic examination showed that even after gelatinization, the starch granules are not uniform, causing an uneven rate of saccharification and leaving a stable, residual dextrin.

Teichek (49) determined the ratio of amyloclastic to saccharogenic activity during various stages of malting. He

gave elementary analysis of active preparations obtained. His results favor the two enzyme theory.

Prior (40) presented evidence favoring the existence of two amylolytic enzymes in malt.

Sherman and Schlesinger (43) and Kendall and Sherman have demonstrated that with pancreatic amylase the liquefying action is predominant and that with malt amylase, on the contrary, the saccharifying action is predominant.

Frankel and Hamburg (14) claimed that they had separated malt diastase into two components by dialysis, the sugar producing enzyme diffusing out and the liquefying enzyme remaining behind in the dialyser.

Reinitzer (41) working with acacia gum diastase made the observation that the saccharifying enzyme was held back by Pukall filters, while the liquefying enzyme passed through.

Bertrand and Compton (2) hydrolyzed amygdalin by the action of emulsin at varying temperatures. After fifteen hours it was found that curves showing the rate of hydrolysis are superimposable. If, however, the proportion of ferment is increased and the hydrolysis measured after two hours the curves are no longer superimposable. This is further evidence of the existence of two distinct diastatic ferments in emulsin.

Chrzaszcz (7) by fractional precipitation with ammonium sulfate arrived at the conclusions (1) that diastase consists fundamentally of two different substances one of which liquefies, and the other saccharifies, starch; (2) that, as the

fractions of the saccharifying portion of diastase differ so widely in their properties, the saccharifying enzyme is a complex body.

Bartholomew (1) presents evidence to show that diastase is not composed of a single enzyme but a series of amylases and dextrinases.

Chrzaszcz and Jascht (8) regard malt amylase as being compounded of the action of two distinct ferments, one producing the liquefaction of starch and the other its degradation to sugars.

The more recent investigations by McGuigan (27) by Euler and Svanberg (13) and by Lellrs and Wasmund (25) have shown the saccharification limit or "limited degredation" lies at the position of about three quarters of the theoretical quantity of maltose.

Olsson (29) proposed a method which depends on the time required for a glass sphere to fall to the bottom of an evacuated tube containing the starch enzyme mixture. The great difference between the time required for solution and for saccharification is taken as evidence favoring the two enzyme theory of malt amylase.

Ohlsson (33) ascribes the action of malt amylase to two individual enzymes, a "dextrinogenase" and a "saccharogenase". A method of separation is based on the fact that the dextrinogenase is rapidly destroyed in a solution at pH 4 and the saccharogenase is almost completely destroyed by heating at pH 6

for twenty minutes at 70°C.

The considerable differences in the liquefaction and saccharification capacities of the different types of amylase seemed to justify the theory of two enzymes occurring in varying proportions.

Effront (12) and Chrzaszcz (9) observed that the liquefying activity is almost entirely lacking in resting seeds of cereals, while the saccharifying action is found to be normal; therefore a strong increase in liquefying capacity takes place upon germination.

Sjöberg (47) compares the saccharogenic and amyloclastic activities of the amylase of bean by determining the influence of acidity, temperature coefficient, relation of heat resistance to acidity, time course of inactivation, relation of inactivating coefficient to temperature, temperature coefficient of inactivating constant, and influence of sodium chloride. Results indicate that two enzymes are engaged in the conversion of starch to sugar.

Windisch and co-workers (53) treated amylopectin from wheat starch with malt extract at 60° in a buffer solution at pH 4.8, the liquid filtered, the filtrate hydrolyzed with hydrochloric acid and the resulting dextrose determined iodimetrically. The amount of starch liquefied by one gram of a malt is taken as a measure of its liquefying power. A number of determinations show that liquefaction follows Kjeldahl's law of proportionality, that optimum activity occurs at pH 5.03 and that the amyloclastic activity of malt does not bear a constant

relation to their saccharogenic activity. He accepts the possibility of a second enzyme, dextrinase.

In 1923, Norris and Viswanath (32) compared the action of barley diastase with the enzyme derived from cholam (sorghum vulgare) and showed that their relative activity varied according to whether the saccharifying or liquefying power was under consideration, cholam enzyme bringing about much more rapid liquefaction of starch than barley enzyme, though the formation of sugar was much slower. This observation supports the idea that two enzymes are concerned and that the proportion in which the two components are present varies in different grains.

Pringsheim and Fuchs (38) have pointed out that this saccharification depends upon the action of a complement contained in the yeast, which the amylase requires in order to complete the hydrolysis of certain starch degradation products.

Ling and Nanji (23) have shown that while the enzyme of unmalted barley can hydrolyze amylose it is without action on amylopectin, suggesting that the enzyme capable of hydrolyzing amylopectin is only developed on germination of the grain. This corresponds to the secretion diastase of Brown and Morris.

Chrzaszcz (9) found that amylases were completely destroyed by heating the aqueous solution one hour at 75°. The temperatures of commencement of the destructive process were determined for the various amylases; they varied from 61-66°.

Sjöberg and co-workers (48) studied the hydrolyses of soluble starch, amylose and amylopectin by the amylases of ger-

minated and ungerminated barley. In no case was more then eighty per cent maltose obtained. He determined the optimum pH for hydrolysis of amylose and amylopectin. From a determination of the inhibition of amyloclastic and saccharogenic activities by maltose and glucose he concluded that there is more than one enzyme in malt amylase.

Tomioka (50) found that the optimum pH for the activity of pancreatic amylase is between 6.3 and 6.9. The destructive action of hydrochloric, acetic and propionic acids was found to be a function of hydrogen ion concentration. Only amylase has three stability zones namely at a pH 1.5, 4.4-4.9 and over 6.0. From these data Tomioka concludes that there are three or more enzymes instead of one.

Chrzaszcz (10) recognized three distinct stages in starch hydrolysis, namely, liquefaction, dextrin formation, and sugar production. He could effect a selective alternation of any of these processes by alcohol precipitation, application of high temperature, or aging of the enzyme. He claims to have effected a separation of the enzymes by fractional precipitation with ammonium sulphate. He also observed that the relation between saccharification, liquefaction and iodine coloration varied with the origin of the malt.

Effront (12) found the ratio

$$100 \times \frac{\text{saccharification power (SP)}}{\text{liquefaction power (LP)}}$$ to vary from 0.1 to 2,400,

depending on the source of the enzyme.

Windisch, Dietrich and Beyer (53) also suggest that the

ratio $\frac{SP}{LP}$ is not constant for all malts.

Holmberg (20) has shown with liver amylase that the presence of iodides inhibits sugar formation but activates the liquefaction process.

The experiments of Hizume (19) indicated on the contrary that the ions Cl^-, Br^-, NO_3^-, SO_4^{--} affect liquefaction and saccharification by diastase in the same manner.

Fricke and Kaja (15) found that during electro-dialysis of barley malt diastase, with increasing purification the liquefying power decreased with an increase in the saccharifying power.

Pringsheim (39) used the terms amylase and amylobiase referring to a special saccharifying enzyme, besides the starch-liquefying or depolymerizing.

Investigations of Sherman and Schlesinger (43) with pancreatic amylase, and of Lilors and Sollner (24) with malt amylase, showed that in their preparations, the ratio of liquefying to saccharifying enzyme is usually found constant, even though marked differences are found between the malt and pancreas enzymes themselves.

Windisch (54) produced maltose and achroodextrin by the action of amylase on starch. He noted that there is a definite relation existing between the concentration of enzyme and the rate of reaction and found that the enzyme is inhibited by the presence of quantities of maltose. At a temperature of 56-63° destruction occurs, and if the acidity is below pH 3.2, the

activity is greatly decreased and at pH 2.1 and 8.2 entirely destroyed. Thus he concludes that amylase may consist of two enzymes a liquefying and a saccharifying.

Ohlsson in 1930 (34) stated that malt amylase is not an individual enzyme but a mixture of two different enzymes, a dextrinogenic and a saccharogenic amylase. Among the products formed by hydrolysis of starch with dextrinogenic amylase the dextrins predominate, while with saccharogenic amylase the main product is maltose from the very start. When malt solution is dialyzed, the dextrinogenic enzyme is almost completely destroyed by the time the saccharogenic enzyme has lost half of its original activity. The stability of the two enzymes is not dependent in the same manner upon hydrogen ion concentration and temperature. With lower temperature and higher hydrogen ion concentration the saccharogenic amylase is essentially the more stable, while with higher temperature and alkaline reaction the relative stability is reversed. If a malt solution at zero degrees is acidified to pH 3.3 with hydrochloric acid and after fifteen minutes brought to pH of 6.0 by addition of di sodium hydrogen phosphate the dextrinogenic amylase is almost completely destroyed while the saccharogenic still retains seventy to eighty per cent of its activity. If, on the other hand, a malt solution at pH 6-7 is heated fifteen minutes at 70° the saccharogenic enzyme is almost completely destroyed while the dextrinogenic enzyme remains seventy-five per cent active. Saccharogenic amylase has an activity curve with a

broad optimal zone between pH 4 and 5.75, while the optimum for dextrinogenic amylase is pH 5.5-6.0. Studies on the changes in osmotic pressure during hydrolysis indicate that in dextrinogenic hydrolysis the starch molecule splits into two or more dextrin molecules which then break up into smaller molecules until maltose results. Saccharogenic hydrolysis on the other hand, liberates maltose at the start; the starch molecule breaks down into one or more molecules of maltose, and a residue of dextrin which then undergoes further hydrolysis into maltose and dextrin. Dextrinogenic amylase is therefore an α-amylase; saccharogenic amylase a β-amylase.

Borchardt and Pringsheim (3) prepared the amylase of potato from the press juice of the ground potato. This was deproteinized with colloidal ferric hydroxide. The potato amylase occupies an intermediate position between the malt amylase particularly rich in β-amylase and the taka-amylase which is pure α-amylase, as it contains less β- than α-amylase.

Van Klinkenberg (51) prepared α-amylase by precipitating an aqueous extract of malt at a concentration of sixty per cent ethyl alcohol, filtering, drying, redissolving in water, heating at seventy degrees for fifteen minutes to destroy any β-amylase, cooling quickly, filtering, precipitating again at sixty per cent ethyl alcohol, filtering and drying. β-amylase was prepared by extracting husked, ground barley with fifty per cent ethyl alcohol, filtering to remove a trace of α-amylase, increasing the ethyl alcohol to eighty per cent, filter-

ing, drying, repeating the process. The substrate for the β-amylase was ordinary two per cent potato starch paste. The substrate for the α-amylase was prepared by digesting potato starch with β-amylase to completion, at which point thirty-six per cent of the starch had not been saccharified. This residue was precipitated by ethyl alcohol and called "erythro-granulose". The optimum pH for the α-amylase was 4.55-5.15; that of the β- was 5.65-5.85. β-maltose reduced the action of β-amylase more than it did the action of α-amylase. When β-amylase acts upon soluble starch, it liberates a maximum of sixty-four per cent of the theoretical amount of maltose, irrespective of the amount of enzyme used. The residue gives a strong iodine reaction. When α-amylase acts upon soluble starch, it quickly liberates about thirty-six per cent of the theoretical amount of maltose, and then slowly liberates up to about fifty per cent. Glycogen is a very poor substrate for the β-amylase, but a good one for the α-amylase. This coincides with the facts that pancreatic and fungus amylases are of the α-form, and both animal and fungi contain glycogen. On the other hand, malt amylase is a mixture of the α- and β- forms.

B. Preparation of the Two Amylases of Wheat

1. Statement of the Problem.

The unique work of Erik Ohlsson (34) on the preparation of the two malt amylases has opened the two-enzyme theory with renewed enthusiasm. In many properties, the amylases of malt and of wheat have been found to be similar (31) . Thus the work of Ohlsson has suggested the possibility of two amylases in other grains, such as wheat. The above named investigator has shown that the sugar-forming (saccharogenic) property of a malt extract can be almost entirely destroyed by heating for fifteen minutes at a temperature of 70°, and that this treatment affects but slightly the starch-digesting (amyloclastic) property. This being the case, then it should be possible to treat a wheat extract in exactly the same manner and obtain an extract with little saccharogenic activity and nearly normal amyloclastic activity. From such an extract, fractional precipitation with alcohol should yield a solid enzyme preparation with power to digest starch to dextrins but with very little sugar-forming power.

Ohlsson's work further indicated a treatment of the malt extract which destroyed much of the amyloclastic activity without injuring the saccharogenic activity of the enzyme. Working at 0°C., he brought the malt extract to a pH of 3.3 by the addition of hydrochloric acid, and then adjusted the solution to pH 6 by addition of disodium hydrogen phosphate. Likewise

it should be possible to treat wheat extract exactly as above mentioned and obtain an extract with little amyloclastic activity and with nearly normal saccharogenic activity. From such a solution, fractional precipitation with alcohol should yield a solid enzyme preparation with power to digest starch directly to sugars, but with little amyloclastic activity. Thus the object of this part of the investigation was to prepare two solid enzymes, one showing strong starch-digesting (amyloclastic) properties, and the other showing strong sugar-forming (saccharogenic) properties.

2. Preparation of Materials.

Germination of the Grain. The wheat was soaked in distilled water for ten to fifteen hours. It was then spread out in enamel pans on paper towels which had been moistened with distilled water. The seeds germinated more rapidly if they were kept wet without floating them in water. Germination proceeded more rapidly and uniformly at a room temperature of 25-30°C. The grain was germinated for a period of four to five days. It was washed several times in distilled water and spread out on a clean glass plate. The plate was placed directly in front of an electric fan and thus dried rapidly to stop the germination. It was ground to a fine powder and was then ready for experimental use.

Preparation of Standard Enzyme. In the methods part of the thesis, experiments are described for the preparation of

solid amyloclastic and saccharogenic enzyme according to Ohlsson's procedure. In order to have a standard wheat enzyme for purposes of comparison, it was necessary to make preparations from untreated wheat extract, according to the method used by House (31) . The grain used in these preparations was germinated over a longer period of time than that used in previous work. This procedure seemed to facilitate the purification of the enzyme. Experiments indicated that the longer period of germination gave a clearer enzyme extract, from which active enzyme could be precipitated with alcohol without previous fractionation with ammonium sulfate.

A weighed quantity of finely ground germinated wheat, usually 500 grams, was extracted with two and one-half times its weight of cold distilled water. This mixture was stirred and then placed in the ice box over night. A clear extract was obtained by filtering through Buchner funnels. This extract contained the active enzyme. Extracts prepared in this way were used for standard enzyme preparations, and for the treated enzyme solutions described later. All solutions were kept ice cold throughout every procedure. The standard enzyme preparation was made by fractional precipitation with absolute alcohol. The precipitate was centrifuged out and dried over sulfuric acid in a vacuum desiccator.

A standard enzyme precipitate was made at the same time and under the same conditions as each treated precipitate. This furnished a comparison for each method of treatment.

In making a solution of the solid enzyme, 0.15 gram of solid enzyme was used per 100 cc. of solution. The enzyme is mixed with a few drops of ice cold water, and then thoroughly dispersed in the entire volume.

Preparation of Starch Dispersions. Lintner's soluble starch, prepared from potato starch by the method of treatment with dilute hydrochloric acid, was used. The moisture of the air dried starch was determined, and enough of the starch was used in each starch dispersion to make one gram of anhydrous starch in every 100 cc. portion used in the actual hydrolysis. In making the dispersion the required amount of starch was weighed out and transferred to a beaker, rubbed to a smooth thin paste with distilled water and poured into a comparatively large volume of hot distilled water in an Erlenmeyer flask, brought quickly to the boiling point and boiled for three minutes. The flask containing the starch paste was tightly closed to prevent contact with the air and was cooled to room temperature. After cooling to room temperature and adding the required amounts of sodium hydroxide, mono- and disodium hydrogen phosphates, the dispersion was diluted to volume with distilled water. The amounts of sodium hydroxide and of phosphate used were those required to bring the starch to the optimum hydrogen ion concentration for amyloclastic and saccharogenic enzyme activity.

3. Methods and Data.

Determination of Amyloclastic Power. For determination of amyloclastic power of the amylase a procedure based upon the method of Wohlgemuth was used. This procedure is as follows: Forty test tubes, very carefully cleaned and dried, are placed in a special wire frame and the whole set in a bath of ice water. Varying quantities of enzyme are carefully measured into each test tube by means of a calibrated Bureau of Standards pipette. Then 5 cc. of one per cent starch solutions prepared as described are carefully measured into each of the tubes by means of a burette which has a very long delivery tip reaching to the bottom of the test tubes, thus avoiding the lodging of any of the solution on the sides of the tubes. The tubes are then agitated to insure a perfect mixture. No reaction takes place because the solutions are kept cold by the ice box in which the basket of tubes rests.

The basket of tubes is transferred from the ice bath to a constant temperature bath at 40°C., and at the expiration of 30 minutes it is taken out and placed in the ice bath to stop the enzyme action. The tubes are subjected to a temperature of 40°C. for 30 minutes. One drop of 0.01 N iodine in potassium iodide solution is added to each tube. Distilled water is poured into each tube, filling it to within about 2 cc. of the top, and the contents thoroughly mixed. The tube of lowest enzyme concentration which shows none of the familiar blue color due to starch is taken as the end point. To obtain the

value of the amyloclastic power of the enzyme, the weight of one per cent starch paste, 5000 milligrams, is divided by the weight in milligrams of enzyme in the tube showing the end point.

Determination of Saccharogenic Power. Throughout the investigation two per cent starch dispersions were used in the determination of saccharogenic activity. The starch dispersions were always freshly prepared. One hundred cubic centimeter portions of those starch dispersions were hydrolyzed for 30 minutes at 40°C. The temperature was kept constant by means of a water bath controlled by a thermostat. The starch solutions were brought to 40°C. in the water bath before they were added to the enzyme solution. The enzyme solutions were prepared just before using. The enzyme solutions were measured directly into 250 cc. Erlenmeyer flasks by means of a Bureau of Standard one cubic centimeter graduated pipette. The concentration of enzyme preparations was kept constant throughout the investigation, i.e., 0.15 grams of enzyme was made up to a volume of 100 cc. and varying amounts of this solution were used in the experiments. The reducing sugars formed by the enzymic hydrolysis of starch were determined by use of mixed Fehling's solution (11) . The Shaffer and Hartman method was tried but this method gave very inconsistent results and was therefore not used. The flasks were transferred to a boiling water bath which was kept boiling vigorously for 15 minutes. The cuprous oxide precipitated by the reducing sugars

formed in the hydrolysis of the starch was collected on the asbestos pads of Gooch crucibles, and washed with hot water, alcohol and ether. The crucibles were dried in an electric oven at 100°C. and weighed to constant weight. Crucibles were cleaned with nitric acid and reweighed each time before they were used.

The saccharogenic power was calculated on all solid enzyme precipitates. It is defined as the ratio of the milligrams of maltose per milligrams of enzyme obtained in 30 minute digestions at 40° under optimum conditions. The amount of maltose formed in a digestion is determined by converting the cuprous oxide to cupric oxide and this in turn to maltose by the use of Defren's tables

Preparation of Amyloclastic Enzyme. The amyloclastic enzyme was prepared in the following manner. A large Erlenmeyer flask was placed in a water thermostat at 70°C. In order to heat the flask to the temperature of the bath before introducing the extract. The cold extract was poured into the flask and heated for a period of fifteen minutes. During this time the solution was gently shaken. In the process of heating a white precipitate came down. The activity of this precipitate was tested at a later time and was found to be inactive. The solution was quickly removed from the bath and poured into a cold vessel surrounded by ice. After cooling, the solution was filtered. Two hundred cubic centimeter portions of the heated and unheated extracts were placed in 500 cc. collodion

sacs and dialyzed in running tap water for thirty-six hours. By making the dialyzates up to 65 per cent and 85 per cent alcohol by volume, white flocculent precipitates were obtained. The precipitates came down immediately upon the addition of the alcohol. These solutions were allowed to stand for ten to fifteen hours in the ice box in order to allow the precipitate to settle. The supernatant liquid was siphoned off in each case. The precipitates were centrifuged off and dried over sulfuric acid in a vacuum desiccator. In some of the experiments, the enzyme solution was added to the amount of alcohol necessary to make a 65 per cent solution. In this way, the active enzyme precipitate came down at once. Any further precipitate was less active than the first one obtained.

Throughout these experiments, similarities between the amylases of wheat and malt have been observed. The extract of wheat is affected to about the same extent as that of malt by using the method for preparing this enzyme as proposed by Ohlsson. The amyloclastic and saccharogenic activities of the heated and standard extracts were always tested before precipitating the solid enzyme. Ohlsson carried his work only as far as the preparation of amyloclastic and saccharogenic extracts. The solid amyloclastic enzyme of wheat was prepared repeatedly with very consistent results. The amyloclastic activity was affected slightly in proportion to the saccharogenic destruction on the liquid extract. This difference was even more pronounced with the solid precipitate. These pre-

parations were always compared to the activity of a standard enzyme made at the same time and under the same laboratory conditions. Tables I to IV show the experimental results obtained in testing the amyloclastic and saccharogenic activity of the amyloclastic enzyme. The points just mentioned are indicated in these tables, and are summarized in Table V.

Table I

Amyloclastic Activity of Amyloclastic Liquid Extract

Number of Experiment	Type of Solution	Volume of Enzyme in Cubic Centimeters									Per Cent Loss in Activity
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	
	Heated	Blue	Blue	Blue	Blue	Violet-blue*	Violet	Violet-red	Violet-red	Red	
0	Standard	Blue	Blue	Violet-blue*	Violet	Violet-red	Red	Red	Orange-red	Red-orange	40.00
	Heated	Blue	Blue	Blue	Blue	Violet-blue*	Violet	Violet-red	Violet-red	Red	
1	Standard	Blue	Blue	Violet-blue*	Violet	Violet-red	Red	Red	Orange-red	Red-orange	40.00
	Heated	Blue	Blue	Violet*	Red-violet	Violet-red	Violet-red	Red	Orange-red	Orange-red	
2	Standard	Blue	Violet*	Violet-red	Violet-red	Violet-red	Red	Orange-red	Orange-red	Orange-red	33.33
	Heated	Blue	Blue	Blue	Blue	Violet-blue*	Violet	Violet-red	Violet-red	Red	
3	Standard	Blue	Blue	Blue	Violet-blue*	Violet	Violet-red	Red	Red	Orange-red	20.00
	Heated	Blue	Blue	Blue	Blue	Blue	Violet-blue*	Violet	Violet-red	Red	
4	Standard	Blue	Blue	Blue	Blue	Violet-blue*	Violet	Violet-red	Red	Orange-red	16.66

*Indicates the end point tube.

Table II

Saccharogenic Activity of Amyloclastic Liquid

Number of Preparation	Volume of Enzyme in cc. 0.2 Heated $MgCu_2O$	0.2 Standard $MgCu_2O$	Per Cent Loss in Activity	Volume of Enzyme in cc. 0.4 Heated $MgCu_2O$	0.4 Standard $MgCu_2O$	Per Cent Loss in Activity	Volume of Enzyme in cc. 0.6 Heated $MgCu_2O$	0.6 Standard $MgCu_2O$	Per Cent Loss in Activity
0	20.3	147.4	86.22	35.5	291.1	87.80	52.4	443.6	88.18
1	20.7	136.6	84.84	35.5	264.0	86.55	51.9	409.5	87.32
2	24.9	254.8	90.22	45.3	Over Reduced	---	67.1	Over Reduced	---
3	16.0	141.2	88.66	22.4	267.1	91.61	33.0	399.1	91.73
4	19.3	124.0	84.43	27.6	240.5	88.52	40.2	358.2	88.77

TABLE

AMYLOCLASTIC ACTIVITY OF

Number of Preparation	Type of Solution	0.15	0.30	0.45	0.60	0.75	0.90
1	65% Heated	Blue	Blue	Blue	Blue	Violet-* blue	Violet
	60% Standard	Blue	Blue	Blue	Violet-* blue	Violet	Red-violet
	85% Heated	Blue	Blue	Blue	Blue	Blue	Blue
	85% Standard	Blue	Blue	Blue	Blue	Violet-* blue	Violet
2	75% Heated	Blue	Blue	Blue	Blue	Blue	Blue
	75% Standard	Blue	Blue	Blue	Blue	Blue	Blue
	85% Heated	Blue	Blue	Blue	Blue	Blue	Blue
	85% Standard	Blue	Blue	Blue	Blue	Blue	Violet
3	65% Heated	Blue	Blue	Blue	Blue	Violet-* blue	Violet-red
	65% Standard	Destroyed by Fire					
	85% Heated						
	85% Standard	Destroyed by Fire					
4	65% Heated	Blue	Blue	Blue	Blue	Blue	Violet-* blue
	65% Standard	Blue	Blue	Blue	Violet-* blue	Violet	Red-violet
	85% Heated						
	85% Standard	Blue	Blue	Blue	Blue	Blue	Blue

(* indicates the end point tube)

TABLE III

TIVITY OF SOLID AMYLOCLASTIC ENZYME.

Weights of enzyme in milligrams								
0.90	1.05	1.20	1.35	1.50	3.00	4.50	6.00	7.50
Violet	Violet-red	Violet-red	Red					
Red-violet	Red-violet	Violet-red	Red	Orange	Orange	Orange	Orange	Orange
Blue	Blue	Blue	Blue	Blue	Blue	Violet*	Red	Red
Violet	Violet-red	Violet-red	Red					
Blue	Violet-blue	Violet-red	Red					
Blue	Violet-blue	Violet-red	Red					
Blue	Blue	Blue	Blue	Blue	Violet-blue	Red	Orange	Orange
Violet	Violet-red	Red	Orange	Orange				
Violet-red	Violet-red		Red	Red				
					Blue	Blue	Blue	Blue	Blue
Violet-blue	Violet-Red	Violet-red	Red	Red				
Red-violet	Red-violet	Violet-red	Red	Red				
				Blue	Blue	Blue	Blue	Blue
Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue

a m s						Amyloclastic Power	Per cent Loss in Activity
6.00	7.50	9.00	10.50	12.00	13.50		
						66,666	
Orange	Orange	Orange	Orange	Orange	Orange	83.333	20.00
Red	Red	Orange	Orange	Orange	Orange	11.111	
						66,666	83.33
						47.618	
						47.618	00.00
Orange	Orange	Orange	Orange			16.666	
						55.555	70.00
						66.660	
Blue	Blue	Blue	Blue	Violet-blue		416	
						55.555	
						83.333	33.33
Blue	Blue	Blue	Blue	Blue			
Blue	Blue	Blue	Blue	Blue	Blue		

TABLE IV

SACCHAROGENIC ACTIVITY OF SOLID AMYLOCLA

Number of Preparation	Volume of Enzyme in cc.		Saccharogenic Power		Per cent	1
	1.0 Heated (65%)	0.2 Standard (65%)	Heated	Standard	Loss in activity	5.
	$Mg \cdot Cu_2O$	$Mg \cdot Cu_2O$				
1	64.4	34.2	35.85	91.66	60.88	
2	46.6	27.7	25.13	74.66	66.34	
3*	63.7	Destroyed by fire	35.73	-- --	-- --	
4*	57.7	73.3	31.00	198.66	84.39	

* (Enzyme precipitated by adding alcohol to the enzyme extract)

D AMYLOCLASTIC ENZYME.

cent	Volume of Enzyme in cc.		Saccharogenic Power		Per cent	Volume of
s in ivity	5.0 Heated (65%)	0.4 Standard (65%)	Heated	Standard	Loss in activity	1.0 Heated (85%)
	$Mg \cdot Cu_2O$	$Mg \cdot Cu_2O$				$Mg \cdot Cu_2O$
.88	284.9	57.8	31.32	92.6	66.17	27.5
.34	203.4	46.1	22.13	65.33	66.12	23.3
—	305.6	Destroyed by fire	33.57	— —	— —	12.1
.39	258.0	134.0	28.28	181.16	84.33	11.7

Volume of Enzyme in cc.		Saccharogenic Power		Per cent	Volume of Enzyme in	
1.0 Heated (85%)	0.2 Standard (85%)	Heated	Standard	Loss in activity	5.0 Heated (85%)	0.4 Stan (85%)
Mg. Cu_2O	Mg. Cu_2O				Mg. Cu_2O	Mg. Cu_2
27.5	82.7	14.93	223.0	93.30	103.5	158.8
23.3	50.1	12.48	135.0	90.75	85.5	90.3
12.1	Destroyed by fire	6.48	--- -	-- --	35.4	Destroy by fira
11.7	10.1	6.26	27.06	76.86	22.0	5.0 Stan (85%) 71.1

Volume of Enzyme in cc.		Saccharogenic Power		Per cent
5.0 Heated (85%)	0.4 Standard (85%)	Heated	Standard	Loss In Activity
$Mg \cdot Cu_2O$	$Mg \cdot Cu_2O$			
103.5	158.8	11.17	211.00	94.70
85.5	90.3	9.21	121.30	92.40
35.4	Destroyed by fire	3.77	--- --	-- --
22.0	5.0 Standard (85%) 71.1	2.12	7.65	72.28

Table V

Summary of Work on Amyloclastic Enzyme

Number of Preparation	Per Cent Loss in Amyloclastic Activity of Amyloclastic Liquid Extract.	Per Cent Loss in Saccharogenic Activity of Amyloclastic Liquid Extract.	Per Cent Loss in Amyloclastic Activity of Solid Amyloclastic Enzyme.	Per Cent Loss in Saccharogenic Activity of Solid Amyloclastic Enzyme.
1	40.00	84.84	20.00	94.70
2	33.33	90.22	00.00	92.40
3	20.00	88.66	---	---
4	16.66	84.43	33.33	84.39

Preparation of Saccharogenic Enzyme. The saccharogenic amylase was prepared by adding to a wheat extract at 6 to 8°C. sufficient quantity of hydrochloric acid to give a pH of about 3.3. The acid was added slowly in such a way that the last acid was added within fifteen minutes from the time of starting. Immediately the hydrogen ion concentration was adjusted to pH of 6 by the addition of a saturated disodium hydrogen phosphate. Great care was taken throughout this work to keep all solutions ice cold.

Difficulty was experienced in obtaining preparations with consistont saccharogenic powers. For this reason a series of preparations have been made varying different factors such as rate of addition of acid, adding the reagents in an atmosphere of carbon dioxide etc., as shown by the descriptions which follow.

Preparation 1. Eight cubic centimeters of hydrochloric acid was added drop by drop with slow stirring to 100 cc. portions of wheat extract. The disodium hydrogen phosphate was added to the above treated solution. The flasks were kept in an ice bath throughout the whole experiment.

Preparation 2 was carried out in the same manner except the hydrochloric acid was added drop by drop to a large volume of extract. The solution was shaken vigorously after the addition of each drop of acid.

Preparation 3. The extract in this case was placed in a large open beaker and stirred vigorously with electric motor and hydrochloric acid was added at rate of eighty drops per minute. A precipitate came down after the addition of a small amount of acid.

Preparation 4. The acid in this preparation was added to 200 cc. portions of extract at the rate of 132 drops per minute. The flask was shaken by hand at a slow and constant rate. The phosphate was added very rapidly.

Preparation 5. The acid was added to a large volume of extract at rate of 65 drops per minute. The phosphate solution was added rapidly. A precipitate did not come down in this preparation.

Preparation 6. In this preparation the acid was added at the rate of 10 drops per minute to a large volume of extract. The phosphate solution was added very rapidly. Much foam was produced in stirring.

Preparation 7. This preparation is the exact duplicate of preparation 6 except the reagents were added to the extract in an atmosphere of carbon-dioxide.

Preparation 8. In this case the acid was added to 100 cc. portions of extract at the rate of 20 drops per minute in the presence of nitrogen.

Preparations 9 and 10. These preparations were carried out in exactly the same way as preparation 8 except the reagents were added in an atmosphere of carbon dioxide.

Preparation 11. This preparation was carried out in exactly the same manner as preparations 9 and 10.

Preparations 12 and 14 were prepared exactly as 11 except the reagents were added in an atmosphere of air.

Preparations 13 and 15. In these preparations sodium hydroxide was used in place of hydrochloric acid, otherwise the procedure was the same.

Preparation 16 was prepared in the same way as preparations 9 and 10.

Preparation 17 was made in same way as preparation 16 except reagents were added in an atmosphere of air.

Preparation 18. Compressed air was bubbled through the extract while the reagents were added. Otherwise the procedure was the same.

The preparation of the saccharogenic amylase from this point is exactly the same as that of the amyloclastic enzyme.

The purification of the saccharogenic enzyme by fractional precipitation with alcohol has been a difficult problem as

viewed by many workers. As soon as purification is attempted, the enzyme is removed from its normal environment and loses activity. Success has been attained only through observance of many precautions, such as keeping the solutions very cold and working quickly. However, even though the same technique be used, it is difficult to obtain the same standard of activity in every preparation made.

The saccharogenic enzyme is considered to be more complex than the amyloclastic (7) . It may be this complexity which accounts for the difficulties encountered in the precipitation of the saccharogenic enzyme. An unusual result was that the saccharogenic precipitate had a higher power than the precipitate from the untreated extract made at the same time. It may be interpreted that the treatment makes it possible to precipitate saccharogenic enzyme with less inactive material, and hence more pure than under other conditions. The experimental results obtained in testing the amyloclastic and saccharogenic activity of the saccharogenic enzyme are shown in Tables VI to IX. All results are summarized in Table X.

TABLE VI

AMYLOCLASTIC ACTIVITY OF SACCHAROGENI

Volume of Enzyme in Cubic Cen

Number of Preparation	Type of Solution	0.1	0.2	0.3	0.4	0.5
	Diluted Standard	Blue	Blue	Blue	Blue	Violet-* blue
1	Saccb. Soln.(8cc H Cl)	Blue	Blue	Blue	Blue	Blue
	Diluted Standard	Blue	Blue	Blue	Violet-* blue	Blue-violet
2	Saccb.Soln.(8cc HCl)	Blue	Blue	Blue	Blue	Blue
	Diluted Standard	Blue	Blue	Blue	Blue	Violet-* blue
3	Saccb.Soln.(8cc HCl)					Blue
	Diluted Standard	Blue	Blue	Blue	Blue	Violet-* blue
4	Saccb.Soln.(8cc HCl)					
	Diluted Standard	Blue	Blue	Blue	Blue	Violet-* blue
5	Saccb.Soln.(8cc HCl)					Blue
	Diluted Standard	Blue	Blue	Blue	Violet-* blue	Blue-violet
6	Saccb.Soln.(8cc HCl)					Blue
	Diluted Standard	Blue	Blue	Blue	Violet-* blue	Blue-violet
7	Saccb.Soln.(8cc HCl)					Blue
	Diluted Standard	Blue	Blue	Blue	Violet-* blue	Blue-violet
8	Saccb.Soln.(8cc HCl)					Blue
	Diluted Standard	Blue	Blue	Blue	Blue	Violet-* blue
9	Saccb.Soln.(8cc HCl)					Violet-* blue

TABLE VI

TIVITY OF SACCHAROGENIC LIQUID EXTRACT.

of Enzyme in Cubic Centimeters

.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
ue	Blue	Violet-blue	Blue-violet	Violet	Red-violet	Violet-red	Red		
ue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Violet blu
ue	Violet-blue	Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red		
ue	Blue	Blue	Blue	Blue	Violet-blue	Blue-violet	Violet	Red-violet	Violet red
ue	Blue	Violet-blue	Blue-violet	Violet	Red-violet	Violet-red	Red		
		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
ue	Blue	Violet-blue	Blue-violet	Violet	Red-violet	Red	Orange		
							Blue	Blue	Blue
ue	Blue	Violet-blue	Blue-violet	Violet	Red-violet	Violet-red	Red		
		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	Violet-blue	Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red		
ue								Violet-blue	Blue-viole
	Violet-blue	Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red		
ue		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	Violet-blue	Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red		
ue		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
ue	Blue	Violet-blue	Violet						
		Violet-blue	Violet	Red-orange	Orange	Orange	Orange	Orange	Orang

1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
Violet-* blue	Blue-violet	Violet	Red-violet	Violet-red	Red			Orange-red
Violet-red	Violet	Red	Red	Red	Orange-red	Orange-red	Red-orange	Red-orange
Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Blue	Blue	Blue	Blue	Blue	Blue	Violet-* blue	Blue-violet	Violet
Blue	Blue	Blue	Blue	Violet-* blue	Blue-violet	Violet	Red-violet	Violet-red
Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red	Orange-red	Red-orange	Red-orange
Blue	Blue	Violet-blue*	Blue-violet	Violet	Red-violet	Violet-red	Red	Orange-red
Blue	Violet-* blue							
Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange

1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	Per Cent Loss in Activity
	Orange-red					Orange-red		56.33
d-range	Red-orange	Red-orange	Red-orange	Orange	Orange	Orange		50.00
Blue	Blue	Blue	Blue	Blue	Blue	Violet-blue	Red-violet	80.00
ue-iolet	Violet	Red-violet	Violet-red	Red	Red			72.23
d-iolet	Violet-red	Red	Orange-red	Red-orange	Red-orange			68.75
d-range	Red-orange	Red-orange	Red-orange	Red-orange	Red-orange			63.64
Red	Orange-red	Red-orange	Red-orange	Red-orange	Red-orange			71.43
								69.24
range	Orange							20.00

Table VI Continued

Number of Preparation	Type of Solution	0.1	0.2	0.3	0.4	0.5
	Diluted Standard	Blue	Blue	Blue	Violet-* blue	Blue-vio
10	Sacch.Soln.(5^{cc} HCl)					Blu
	Diluted Standard	Blue	Blue	Blue	Violet-* blue	Blue-vio
11	Sacch.Soln.(5^{cc} HCl)	Blue	Blue	Blue	Violet-* blue	Blue-vio
	Diluted Standard	Blue	Violet-* blue	Blue-violet	Violet	Red-vio
12	Sacch.Soln.(5^{cc} HCl)	Blue	Blue	Blue	Blue	Blu
	Diluted Standard	Blue	Violet-* blue	Blue-violet	Violet	Red-vio
13	Sacch.Soln.(5^{cc} NaOH)	Blue	Blue	Violet-* blue	Blue-violet	Vio
	Diluted Standard	Blue	Violet-* blue	Blue-violet	Violet	Red-vio
14	Sacch.Soln.(5^{cc} NaOH)	Blue	Blue	Blue	Blue	Blu
	Diluted Standard	Blue	Violet-* blue	Blue-violet	Violet	Red-vio
15	Sacch.Soln.(5^{cc} NaOH)	Blue	Blue	Violet-* blue	Blue-violet	Vio
	Diluted Standard	Blue	Violet-* blue	Blue-violet	Violet	Red-vio
16	Sacch.Soln.(5^{cc} HCl)	Blue	Blue	Blue	Violet-* blue	Blue-vio
	Diluted Standard	Blue	Violet-* blue	Blue-violet	Violet	Red-vio
17	Sacch.Soln.(5^{cc} HCl)	Blue	Blue	Blue	Violet-* blue	Blue-vio
	Diluted Standard	Blue	Violet-* blue	Blue-violet	Violet	Red-vio
18	Sacch.Soln.(5^{cc} HCl)	Blue	Blue	Blue	Violet-* blue	Blue-vio

(* indicates the end point tube)

0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.
-*Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red			
Blue	Blue	Blue	Blue	Blue	Blue	Violet-*blue	Blue-violet	Vio
-*Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red			
-*Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red	Orange-red	Red	Oran r
t Red-violet	Violet-red	Violet-red	Red	Red-orange	Orange			
Blue	Violet-*blue	Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red	Oran r
t Red-violet	Violet-red	Violet-red	Red	Red-orange	Orange			
t Violet	Red-violet	Violet-red	Violet-red	Red	Red-orange	Orange		
t Red-violet	Violet-red	Violet-red	Red	Red-orange	Orange			
Blue	Violet-*blue	Blue-violet	Violet	Red-violet	Violet-red	Violet-red		
t Red-violet	Violet-red	Violet-red	Red	Red-orange	Orange			
t Violet	Red-violet	Violet-red	Red	Red-orange	Orange			
t Red-violet	Violet-red	Violet-red	Red	Red-orange	Orange			
-*Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red			
t Red-violet	Violet-red	Violet-red	Red	Red-orange	Orange			
-*Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red			
t Red-violet	Violet-red	Violet-red	Red	Red-orange	Orange			
-*Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red			

1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	
Blue-violet	Violet	Red-violet	Violet-red	Violet-red	Red	Orange-red	Red-orange	Red-orange	R
Red	Orange-red	Red-orange	Red-orange	Red-orange	Red-orange	Red-orange	Orange	Orange	
Red	Orange-red	Red	Orange-red	Red-orange	Red-orange	Red-orange	Red-orange	Red-orange	

9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	Per Cent Loss in Activity
nge	Red-orange	Red-orange	Red-orange	Red-orange	Red-orange			63.64
nge	Orange							00.00
nge	Red-orange	Orange	Orange	Orange	Orange	Orange		66.67
								33.34
								66.67
								33.34
								50.00
								50.00
								50.00

TABLE VII

SACCHAROGENIC ACTIVITY OF SACCHAROGENIC LIQU

Number of Preparation	Volume of Enzyme in cc. 0.2 Sacch. Mg·Cu_2O	0.2 Standard Mg·Cu_2O	Per Cent Loss in Activity	Volume of 0.4 Sacch. Mg·Cu_2O
1	81.5	101.6	17.92	164.0
2	37.3	66.1	43.57	77.3
3	29.9	64.6	51.11	63.8
4	39.4	64.4	38.81	81.5
5	54.3	68.7	20.96	107.9
6	47.4	72.8	34.89	100.3
7	59.4	78.0	23.84	120.5
8	44.5	66.2	32.77	88.2
9	73.8	73.0	— —	146.6
10	50.9	74.7	21.86	99.6
11	91.9	91.7	— —	179.2
12	69.5	87.9	20.93	142.8
13	83.2	84.1	— —	169.4
14	66.2	85.5	22.57	132.6
15	89.1	88.6	— —	173.1
16	70.6	91.8	23.09	138.9
17	65.1	85.4	23.77	131.5
18	61.8	91.0	32.08	127.2

TABLE VII

GENIC ACTIVITY OF SACCHAROGENIC LIQUID EXTRACT

dard 0	Per Cent Loss in Activity	Volume of Enzyme in cc. 0.4 Sacch. Mg·Cu_2O	0.4 Standard Mg·Cu_2O	Per Cent Loss in Activity
	17.92	164.0	199.8	17.92
	43.57	77.3	129.2	40.17
	51.11	63.8	130.5	51.11
	38.81	81.5	129.3	36.96
	20.96	107.9	135.6	20.42
	34.89	100.3	146.3	31.44
	23.84	120.5	152.3	20.87
	32.77	88.2	136.3	35.28
	— —	146.6	148.9	— —
	21.86	99.6	143.7	30.68
	— —	179.2	177.0	— —
	20.93	142.8	173.3	17.59
	— —	169.4	169.2	— —
	22.57	132.6	168.6	21.35
	— —	173.1	173.3	— —
	23.09	138.9	184.2	24.59
	23.77	131.5	171.8	30.64
	32.08	127.2	176.0	27.72

TABLE VIII

AMYLOCLASTIC ACTIVITY OF SOLID

Weights of Enz
Milligrams

Number of Preparation	Type of Solution	0.15	0.30	0.45	0.60	0.75
	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue
1	85% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue
	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue
2	85% Sacch. Soln.					
	65% Sacch. Soln.					
3	85% Sacch. Soln.					
	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue
4	85% Sacch. Soln.					
	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue
5	85% Sacch. Soln.					
6	65% Sacch. Soln.					Blue
7	65% Sacch. Soln.					Blue
8	65% Sacch. Soln.					
9	65% Sacch. Soln.	Blue	Blue	Violet-* blue	Violet	Violet-red
10	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue
11	65% Sacch. Soln.	Blue	Blue	Violet*- blue	Violet	Violet-red
12	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue

LE VIII

SOLID SACCHAROGENIC ENZYME.

of Enzyme in
ligrams.

0.75	0.90	1.05	1.20	1.35	1.50	1.65	1.80	1.95	
Blue	Blue	Blue	Blue	Violet-blue	Violet	Violet-red	Red	Red	
Blue	Blue	Blue	Blue	Blue	Violet-blue	Violet	Violet-red	Red	
Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	
					Blue				
					Blue	Blue	Blue	Blue	
					Blue				
Blue	Blue	Blue	Violet-blue	Red	Red				
					Blue				
Blue	Blue	Violet-blue	Violet	Red	Red				
					Blue				
Blue									
Blue	Violet-blue	Violet	Red-violet	Red-violet	Violet-red	Red	Orange	Orange	
					Blue	Blue	Blue	Violet-blue	
Violet-red	Violet-red	Red	Red	Red-orange	Orange	Orange	Orange	Orange	
Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	
Violet-red	Violet-red	Violet-red	Red	Red	Red-orange	Orange	Orange	Orange	
Blue	Blue	Violet-blue	Violet	Violet-red	Violet-red	Red	Red	Red	

1.80	1.95	2.10	2.25	2.40	2.55	2.70	2.85	3.00	3.
Red	Red	Red	Red	Red	Red			Red-orange	
olet-red	Red	Red	Red	Red	Red			Orange	
Blue	Blue	Blue	Blue	Blue	Violet-blue	Violet	Red	Red	
								Blue	
Blue	Blue	Blue	Violet-blue	Violet	Red	Red	Red	Red	
								Blue	
								Red	
								Blue	
								Blue	
range	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Ora
Blue	Violet-blue	Violet	Red-violet	Violet-red	Violet-red	Red	Red		
range	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Ora
Blue	Blue	Blue	Blue	Blue	Blue	Blue	Violet-blue	Violet	Red-vio
range	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Ora
Red	Red	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Ora

.40	2.55	2.70	2.85	3.00	3.15	3.30	3.45	6.00	7.
ed	Red			Red-orange				Red-orange	Ora
ed	Red			Orange				Color-less	Colo le
lue	Violet-blue	Violet	Red	Red				Orange	Colo le
				Blue				Blue	Bl
let	Red	Red	Red	Red				Orange	Ora
				Blue				Blue	Bl
				Red				Orange	Ora
				Blue				Blue	Bl
				Blue				Red	Red
							Violet-blue		
nge	Orange	Orange	Orange	Orange	Orange	Orange	Orange		
et-d	Violet-red	Red	Red						
nge	Orange	Orange	Orange	Orange	Orange	Orange	Orange		
ue	Blue	Blue	Violet-blue	Violet	Red-violet	Violet-red	Violet-red		
nge	Orange	Orange	Orange	Orange	Orange	Orange	Orange		
nge	Orange	Orange	Orange	Orange	Orange	Orange	Orange		

5	6.00	7.50	9.00	10.50	12.00	13.50	15.00	Amylo-clastic Power
	Red-orange	Orange	Color-less	Color-less	Color-less	Color-less	Color-less	3.703
	Color-less	Color-less	Color-less	Color-less	Color-less	Color-less	Color-less	3.333
	Orange	Color-less	Color-less	Color-less	Color-less	Color-less	Color-less	1.960
	Blue	Blue	Blue	Blue	Blue	Blue	Blue	- ---
	Orange	Orange	Orange	Color-less	Color-less	Color-less	Color-less	2.222
	Blue	Blue	Blue	Blue	Blue	Blue	Blue	- ---
	Orange	Orange						4.166
	Blue	Blue	Blue	Blue	Violet-blue	Red	Red	444
								z 4.761
	Red	Red	Red	Red	Orange	Orange	Orange	63
t-								
e								1.449
ge								5.555
								2.564
ge								11.111
t-								1. 754
ge								11.111
ge								4.761

Table VIII Continued

Number of Preparation	Type of Solution	0.15	0.30	0.45	0.60	0.75
13	65% Sacch. Soln.	Blue	Blue	Violet-blue*	Violet	Red
14	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue
15	65% Sacch. Soln.	Blue	Blue	Violet-blue*	Violet	Red
16	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Violet-blue
17	65% Sacch. Soln.	Blue	Blue	Violet-blue*	Violet	Red
18	65% Sacch. Soln.	Blue	Blue	Blue	Blue	Blue

(* indicates the end point tube)

60	0.75	0.90	1.05	1.20	1.35	1.50	1.65	1.80	1.9
let	Red	Red	Orange	Orange	Orange	Orange	Orange	Orange	Oran
ue	Blue	Blue	Violet-* blue	Violet	Violet-red	Violet-red	Red	Red	Red
let	Red	Red	Orange	Orange	Orange	Orange	Orange	Orange	Oran
ue	Violet-* blue	Violet	Red	Red	Orange	Orange			
let	Red	Red	Orange	Orange	Orange	Orange			
ue	Blue	Violet-* blue	Violet	Red-violet	Violet-red	Red			

1.65	1.80	1.95	2.10	2.25	2.40	2.55	2170	2.85
Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
Red	Red	Red	Orange	Orange	Orange	Orange	Orange	Orange
Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange

2170	2.85	3.00	3.15	3.30	3.45	6.00	7.50	9.00	1(
Orange	Orange	Orange	Orange	Orange	Orange				
Orange	Orange	Orange	Orange	Orange	Orange				
Orange	Orange	Orange	Orange	Orange	Orange				

.45	6.00	7.50	9.00	10.50	12.00	13.50	15.00	Amylo-clastic Power
nge								11.111
nge								4.761
nge								11.111
								6.666
								11.111
								5.555

TABLE IX

SACCHAROGENIC ACTIVITY OF SOLID SA

Weights of Enzyme in M

Number of Preparation	0.3 Saccharogen. Soln. (65%)		0.6 Saccharogen. Soln. (65%)		0.3 Saccharogen. Soln. (85%)		0.6 S
	Mg·Cu_2O	Power	Mg·Cu_2O	Power	Mg·Cu_2O	Power	Mg·Cu
1	172.6	468.66	335.3	460.80	180.5	491.00	340.6
2	42.8	116.00	86.7	116.50	6.5	17.42	12.0
3	40.7	109.00	80.4	107.83			
4	99.0	267.00	192.4	261.83	12.2	32.66	
5	139.4	377.33	268.9	368.66	21.2	17.04	
6	5.8	14.20	11.0	14.73	2.1	5.60	
7	90.5	245.00	179.1	243.00	7.0	18.73	
8	38.0	101.66	79.2	107.33	2.7	7.23	
9	98.4	264.66	203.5	276.66	2.7	7.23	
10	4.5	12.03	4.5	12.03			
11	94.7	255.00	188.8	256.83			
12	60.3	162.00	111.8	150.5			
13	102.2	277.00	197.3	251.33			
14	151.9	412.00	299.0	411.38			
15	122.2	330.66	248.8	340.83			
16	38.9	96.66					
17	80.5	220.66	109.1	146.83			
18	25.4	67.50	34.5	45.83			

TABLE IX

VITY OF SOLID SACCHAROGENIC ENZYME.

of Enzyme in Milligrams

Soln. (5) Power	0.6 Sacch. Soln. (85%) Mg. Cu_2O	Power	1.5 Sacch. Soln. (85%) Mg. Cu_2O	Power	7.5 Sacch. Soln. (85%) Mg. Cu_2O	Power
491.00	340.6	468.21				
17.42	12.0	16.06				
			9.5	4.82	47.2	15.73
32.66					207.0	22.54
17.04						
5.60					33.8	3.66
18.73					178.1	19.34
7.23					63.6	6.66
7.23					106.1	11.45

Table X

Summary of Work on Saccharogenic Enzyme

Number of Preparation	Per Cent Loss in Saccharogenic Activity of Saccharogenic Liquid Extract.	Per Cent Loss in Amyloclastic Activity of Saccharogenic Liquid Extract.	Saccharogenic Power of Solid Saccharogenic Enzyme.	Amyloclastic Power of Solid Saccharogenic Enzyme.
1	19.67	53.34	468.66	3,703
2	40.17	50.00	116.00	1,960
3	51.11	80.00	109.00	2,222
4	36.96	72.23	267.00	4,166
5	20.42	68.75	377.33	4,761
6	31.44	63.64	14.93	1,449
7	20.87	71.43	245.00	5,555
8	32.77	69.24	101.66	2,564
9	no loss	20.00	264.66	11,111
10	21.86	63.64	12.03	1,754
11	no loss	no loss	255.00	11,111
12	20.93	66.67	162.00	4,761
13	no loss	33,34	277.00	11,111
14	22.57	66.67	412.00	4,761
15	no loss	33.34	330.66	11,111
16	23.09	50.00	96.66	6,666
17	23.77	50.00	220.66	11,111
18	32.08	50.00	67.50	5,555

II. THE OPTIMUM HYDROGEN ION CONCENTRATION FOR WHEAT AMYLASE

A. Introduction

Although the optimum hydrogen ion concentration for the saccharogenic activity of malt has been very carefully determined (44) no record has been found which gives the corresponding conditions for amyloclastic activity. Whenever such determinations were needed, it was assumed that the optimum conditions used for saccharogenic sets could be used also for amyloclastic sets. Moreover, recent work by Sherman and Adams (45) and Sherman and Dale (46) have indicated that hydrogen ion adjustments may be accurately made with primary and secondary phosphates keeping the phosphate concentration constant. This seemed a better method of adjustment than that used in any previous work on hydrogen ion determinations. Therefore, the purpose of the experiments described in this part of the thesis was to determine as precisely as possible the hydrogen ion concentration which induces optimum activity of wheat amylase.

B. Methods and Data

The experiments were planned so as to make a systematic series of determinations of amyloclastic and saccharogenic activity, through the adjustment of hydrogen ion concentration with mono and di sodium phosphates, until the optimum

activity was exceeded and a distinct deleterious effect of the hydrogen ion concentration was observed. The hydrogen ion concentrations were determined by a glass electrode of the MacInnes type (26).

The following method was used in the preparation of the starch dispersions. To definite volumes of one and two per cent starch solutions sufficient primary and secondary sodium phosphates were added (Tables XI, XII, and XIII) to give a final concentration of 0.02 or 0.06 molar total phosphate when this starch was diluted to one or two per cent. One per cent starch dispersions were used for amyloclastic activity and two per cent starch dispersions for saccharogenic activity. The total phosphate concentration was held constant but the relative amounts of the two phosphates were varied in order to obtain the desired hydrogen ion concentration. In this way it was possible to obtain a series of one and two per cent starch solutions of varying hydrogen ion concentrations containing a constant concentration of phosphate.

For the amyloclastic determinations, one-half hour hydrolyses of five cubic centimeter portions of one per cent starch at 40°C. were carried out in the presence of 0.02 and 0.06 molar phosphate buffer mixtures at varying pH. The data show optimum amyloclastic activity at pH 4.6 to 6.3. In this range of pH no measurable differences in the amyloclastic activity could be noticed in solutions at 0.02 and 0.06 molar phosphate. This work determines accurately the optimum hydrogen ion con-

centration for amyloclastic activity of wheat amylase and indicates that phosphate has no activating effect upon the amylase in the concentrations used. This finding is consistent with results obtained on saccharogenic sets with pancreatic amylase

The data for amyloclastic activity are shown in Tables XI, XII, and XIII and Figure 1.

The optimum hydrogen ion concentration for saccharogenic activity was redetermined* using 0.02 molar total phosphate. The preparation of starch dispersions and the method of determination has been described. The data show optimum activity at pH 4.9 to 5.3. The entire range of hydrogen ion concentration studied is shown in Table XIV, and Figure 2.

A critical study of these results indicates that there is a more decided optimum for saccharogenic activity of wheat amylase than had been previously determined. The results show also that it is impossible to make the assumption that the range of hydrogen ion concentration for optimum amyloclastic and saccharogenic activity is the same. The results here obtained indicate a broad optimum at pH 4.6 to 6.3 for amyloclastic activity, and a narrow optimum at pH 4.9 to 5.3 for saccharogenic activity.

*Preliminary experiments on the optimum pH for wheat amylase were reported by Naylor, House and Spencer

Table XI

Optimum pH for Amyloclastic Activity of
Standard Wheat Amylase No.1
0.02 M Total Phosphate

Volume in Cubic Centimeters		pH	Amyloclastic Power
NaH_2PO_4 (0.2M)	Na_2HPO_4 (0.2M)		
10.00	0.00	3.71	12,195
9.80	0.20	4.10	20,833
9.70	0.30	4.47	33,333
9.68	0.32	4.60	33,333
9.66	0.34	4.68	41,666
9.64	0.36	4.78	41,666
9.62	0.38	4.85	41,666
9.60	0.40	4.89	41,666
9.50	0.50	5.17	41,666
9.40	0.60	5.35	41,666
9.30	0.70	5.46	41,666
9.20	0.80	5.58	41,666
9.10	0.90	5.67	41,666
9.00	1.00	5.88	41,666
8.00	2.00	6.24	41,666
7.80	2.20	6.21	41,666
7.60	2.40	6.26	41,666
7.40	2.60	6.30	41,666
7.20	2.80	6.34	33,333
7.00	3.00	6.46	33,333
6.00	4.00	6.60	33,333
5.00	5.00	6.77	33,333
4.00	6.00	6.93	27,777
3.00	7.00	7.06	27,777
2.00	8.00	7.23	23,809
1.00	9.00	7.46	18,518
0.00	10.00	7.79	13,888

Table XII

Optimum pH for Amyloclastic Activity of
Standard Wheat Amylase No.2
0.02 M Total Phosphate

Volume in Cubic Centimeters		pH	Amyloclastic Power
NaH_2PO_4 (0.2M)	Na_2HPO_4 (0.2M)		
9.80	0.20	4.14	41,666
9.75	0.25	4.19	41,666
9.70	0.30	4.49	41,666
9.68	0.32	4.55	41,666
9.66	0.34	4.68	55,555
9.64	0.36	4.75	55,555
9.62	0.38	4.88	55,555
9.60	0.40	4.95	55,555
8.00	2.00	6.15	55,555
7.80	2.20	6.20	55,555
7.60	2.40	6.29	55,555
7.40	2.60	6.35	55,555
7.20	2.80	6.39	41,666
7.00	3.00	6.44	41,666
6.00	4.00	6.57	41,666
5.00	5.00	6.74	41,666

Table XIII

Optimum pH for Amyloclastic Activity
of Standard Wheat Amylase
0.06 M Total Phosphate

Volume in Cubic Centimeters		pH	Amyloclastic Power
NaH_2PO_4 (0.2M)	Na_2HPO_4 (0.2M)		
29.0	1.0	3.63	11,904
28.5	1.5	4.02	16,666
28.0	2.0	4.76	41,666
27.0	3.0	5.40	41,666
26.0	4.0	5.66	41,666
23.0	7.0	6.07	41,666
21.0	9.0	6.24	41,666
19.0	11.0	6.39	33,333
17.0	13.0	6.53	33,333
16.0	14.0	6.59	33,333
15.0	15.0	6.64	27,777
14.0	16.0	6.70	27,777
13.0	17.0	6.75	23,809

Table XIV

Optimum pH for Saccharogenic Activity
of Standard Wheat Amylase
0.02 M Total Phosphate

Volume in Cubic Centimeters :		:	:	: Saccharogenic
NaH_2PO_4 (0.2M) : Na_2HPO_4 (0.2M) :	pH	: $MgCu_2O$	:	Power
10.0 : 0.0	: 3.64	: 163.2	:	221.38
9.9 : 0.1	: 3.80	: 170.6	:	231.38
9.8 : 0.2	: 3.99	: 171.3	:	232.36
9.7 : 0.3	: 4.23	: 188.5	:	256.16
9.6 : 0.4	: 4.71	: 207.7	:	282.95
9.5 : 0.5	: 4.98	: 212.8	:	290.05
9.4 : 0.6	: 5.21	: 216.8	:	295.50
9.3 : 0.7	: 5.38	: 210.4	:	286.61
9.2 : 0.8	: 5.42	: 206.6	:	281.33
9.1 : 0.9	: 5.58	: 203.2	:	276.60
9.0 : 1.0	: 5.64	: 203.1	:	276.46
8.8 : 1.2	: 5.81	: 201.8	:	274.60
8.6 : 1.4	: 5.92	: 200.5	:	272.76
8.4 : 1.6	: 6.06	: 197.0	:	268.00

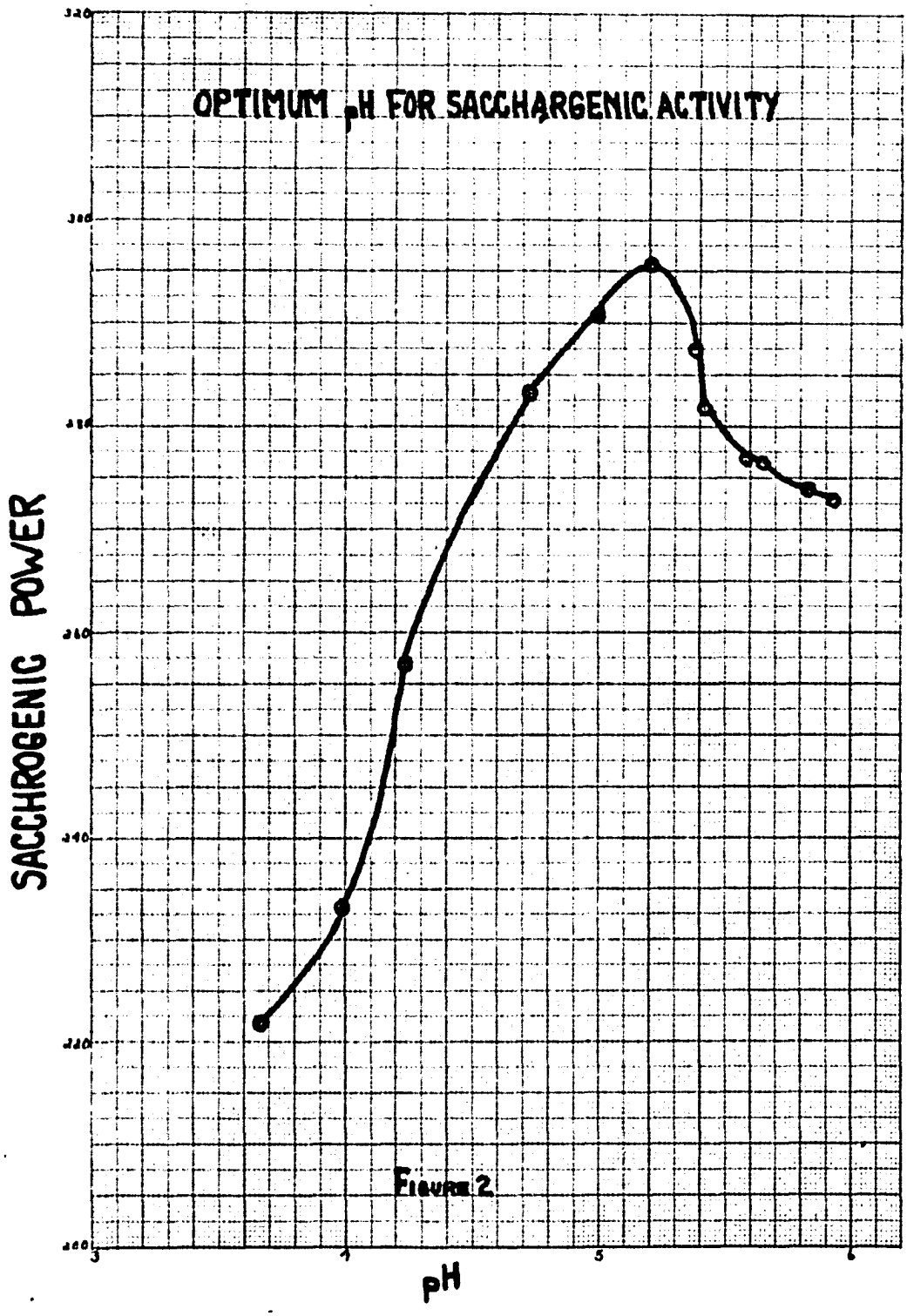
OPTIMUM pH FOR SACCHARGENIC ACTIVITY
SACCHROGENIC POWER
pH
5

FIGURE 2

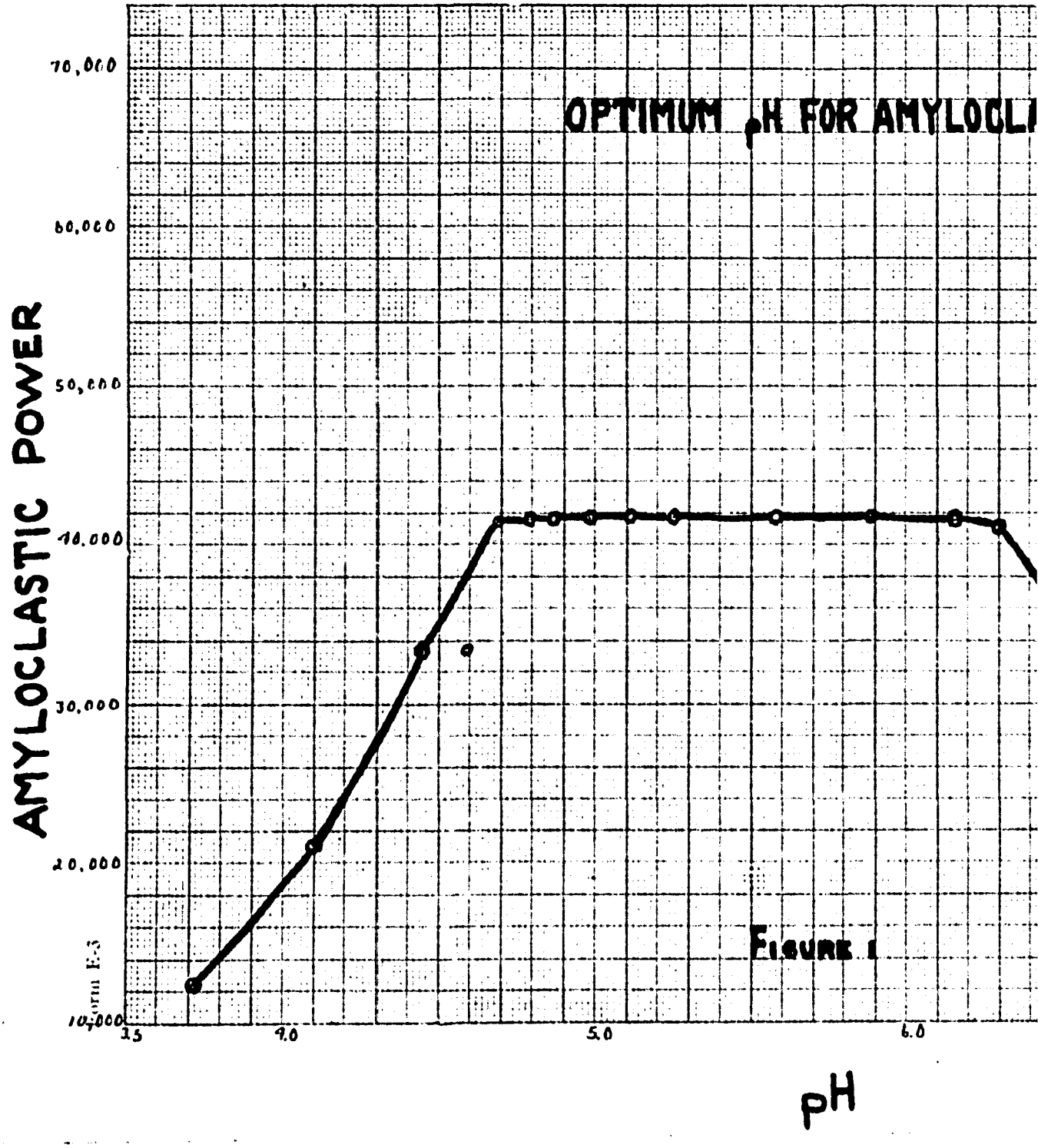
OPTIMUM pH FOR AMYLOCLA
AMYLOCLASTIC POWER
70,000
60,000
50,000
40,000
30,000
20,000
10,000
3.5
4.0
5.0
6.0
pH

FIGURE 1

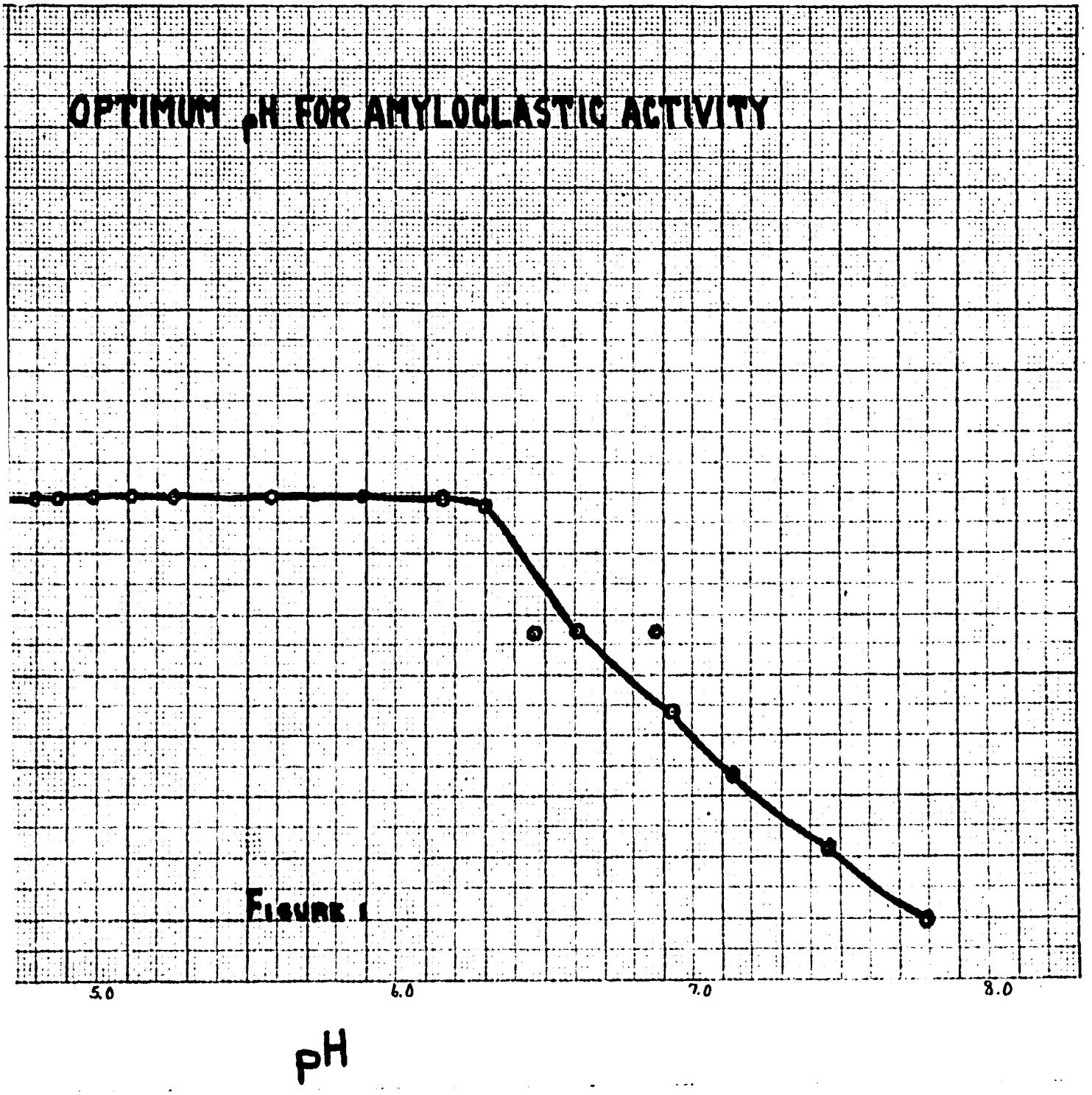

FIGURE 1

SUMMARY

1. An amyloclastic enzyme solution and a saccharogenic enzyme solution has been prepared from wheat extract by a method similar to that used by Ohlsson for malt extract.

2. A solid amyloclastic enzyme with low sugar-forming power has been prepared by heating wheat extract for fifteen minutes at 70° and fractionally precipitating with alcohol.

3. A solid saccharogenic enzyme has been prepared from wheat extract by bringing the hydrogen ion concentration to a pH of 3.3 and then adjusting it to a pH of 6.0, and finally precipitating with alcohol. The solid enzyme showed an unusually high sugar-forming power and low amyloclastic power.

4. The optimum hydrogen ion concentration for the amyloclastic activity of wheat amylase has been found to be at a pH 4.6 to 6.3. The range of hydrogen ion concentration has been obtained by varying the relative amounts of sodium dihydrogen phosphate and disodium hydrogen phosphate, thus keeping the total phosphate concentration constant. The optimum hydrogen ion concentration for saccharogenic activity has been determined by the method just indicated and found to be at a pH 4.9 to 5.3.

LITERATURE CITED

1. Bartholomew, E. T.
Concerning the presence of diastase in certain red algae. Botan. Gaz., 57:136-147. 1914.

2. Bertrand, G. and Compton, A.
Action de la chaleur sur l'emulsine. Compt. rend., 152:1518-1521. 1911.

3. Borchardt, H. and Pringsheim, H.
Über Malzamylase und Kartoffelamylase. Biochem. Z., 239:193-200. 1931.

4. Bourquelot, Em.
Sur les caractères de l'affaiblissement éprouvé par la diastase sous l'action de la chaleur. Compt. rend., 104:576-579. 1887.

5. Brown, H. T. and Morris, G. H.
XXX. Researches on the germination of some of the gramineae. J. Chem. Soc., 57:458-528. 1890.

6. Chittenden, R. H. and Smith, H. E.
VIII. The diastatic action of saliva, as modified by various conditions, studied quantitatively. Connecticut Acad. Sci. Trans., 6:343-376. 1882-1885.

Chittenden, R. H. and Cummins, G. W.
IV. The amyloclastic action of diastase of malt, as modified by various conditions; studied quantitatively. Connecticut Acad. Sci. Trans., 7:44-59. 1885-1888.

Chittenden, R. H. and Painter, H. M.
V. Influence of certain therapeutic and toxid agents on the amylolytic action of saliva. Connecticut Acad. Sci. Trans., 7:60-83. 1885-1888.

Chittenden, R. H. and Martin, W. E.
VIII. Influence of temperature on the relative amylolytic action of saliva and the diastase of malt. Connecticut Acad. Sci. Trans., 7:125-133. 1885-1888.

Chittenden, R. H. and Cummins, G. W.
IX. Influence of bile, bile salts and bile acids on amylolytic and proteolytic action. Connecticut Acad. Sci. Trans., 7:134-148. 1885-1888.

Chittenden, R. H. and Hutchinson, M. T.
XVII. Influence of uranium salts on the amylolytic action of saliva and the proteolytic action of pepsin and trypsin. Connecticut Acad. Sci. Trans., 7:261-273. 1885-1888.

7. Chrzaszcz, T.
Neue Untersuchungen über Diastase. Wochschr. Brau., 28:510. 1911.

Chrzaszcz, T.
Neue Untersuchungen über Diastase. Z. Spiritusind, 34: 545. 1911. Original not seen. Abstracted in Chem. Zentr., V.82, pt.2, p.1868. 1911.

8. Chrzaszcz, T. and Joscht, A.
Über die Verschiebung einzelner amylolytischer Kräfte der Malzamylase und deren Verhalten beim Aufbewahren in Gegenwart verschiedener Reagentien. Biochem. Z., 80: 211-241. 1917.

9. Chrzaszcz, T.
Einfluss der Temperatur auf die Wirkung der Amylase. Biochem. Z., 142:417-439. 1923.

10. Chrzaszcz, T.
Über die Malzamylase aus verschiedenen Getreidearten. Wochschr. Brau., 30:538-541, 545-549. 1913.

11. Defren, G.
The determination of reducing sugars in terms of cupric oxide. J. Am. Chem. Soc., 18:749-766. 1896.

12. Effront, J.
Sur les propriétés distinctives des amylases de différentes provenances. Compt. rend., 174:18-21. 1922.

13. Euler, H. V. and Svanberg, O.
Über die Charakterisierung von Amylaselösungen. Z. physiol. Chem. Hoppe-Seyler's, 112:193-230. 1920-1921.

14. Fränkel, V. S. and Hamburg, M.
Über Diastase. I. Versuche zur Herstellung von Reindiastase und deren Eigenschaften. Beitr. Chem. physiol. path., 8:389-398. 1906.

15. Fricke, R. and Kaja, P.
Über Inhomogenität und sonstige Eigenschaften von Malz-Diastase. Ber., 57B:313-316. 1924.

16. Grüss, J.
Über Zucker-und Stärkebildung in Gerste und Malz. Wochschr. Brau., 14:321-323. 1897.

17. Grützner, P.
Notizen über einige ungeformte Fermente des Säugethierorganismus. Arch. ges. Physiol. Pflügers, 12:285-307. 1876.

18. Guérin-Varry, R. T.
Memoire concernant l'action de la diastase sur l'amidon de pommes de terre, et les proprietes du sucre d'amidon. Compt. rend., 1:81-85. 1835.

19. Hizume, K.
Zur Kenntnis der Diastasen. Zugleich ein Beitrag zur Frage der Zweienzymtheorie. Biochem. Z., 146:52-71. 1924.

20. Holmbergh, O.
Einwirkung von Kaliumjodid auf die Verzuckerung der Stärke durch verschiedene Amylasen. Biochem. Z., 145: 244-248. 1924.

Holmbergh, O.
Studien über Leberamylase. Z. physiol. Chem. Hoppe-Seyler's, 134:68-96. 1924.

21. Kendall, E. C. and Sherman, H. C.
Studies on amylases. II. A study of the action of pancreatic amylase. J. Am. Chem. Soc., 32:1087-1105. 1910.

22. Kirchoff, G. S. C.
Über die Zuckerbildung beim Malzen des Getreides, und beim Bebrühen seines Mehls mit knochendem Wasser. J. Chem. Physik Schweigger's, 14:389-398. 1815. Original not seen. Abstracted in Walton, R. P. A comprehensive survey of starch chemistry. p.15 entry 140. The Chemical Catalog Co. 1928.

23. Ling, A. R. and Nanji, D. R.
Studies on starch. Part I. The nature of polymerised amylose and of amylopectin. J. Chem. Soc., 123:2666-2688. 1923.

24. Lüers, H. and Sellner, E.
Beiträge zur Reinigung der Malzamhlase. Wochschr. Brau., 42:97-99, 103-105, 110-112. 1925.

25. Lüers, H. and Wasmund, W.
Über die Wirkungsweise der Amylase. Fermentforschung, 5:169-235. 1921-1922.

26. MacInnes, D. A. and Dole, M.
The behavior of glass electrodes of different compositions. J. Am. Chem. Soc., 52:29-36. 1930.

27. McGuigan, H.
The action of ptyalin. J. Biol. Chem., 39:273-284. 1919.

28. Maercker, M.
Standpunkt unserer Kenntniss über die diastatischen Vorgänge. Naturf.-Versamml. zu München, p.222. 1877. Original not seen. Abstracted in Chem. Zentr., 49: 559-560. 1878.

29. Maquenne, L. and Roux, Eug.
Sur la constitution, la saccharification et la rétrogradation des empois de fécule. Compt. rend., 140: 1303-1308. 1905.

Gatin-Gruzewska, Z.
Sur la composition du grain d'amidon. Compt. rend., 146:540-542. 1908.

30. Musculus, F.
Remarques sur la transformation de la matière amylacée en glucose et dextrine. Compt. rend., 50:785-787. 1860.

31. Naylor, N. M., Spencer, M. and House, M.
The preparation and properties of amylase from germinated wheat and rye. J. Am. Chem. Soc., 47:3037-3039. 1925.

32. Norris, R. V. and Viswanath, B.
Cholam (Andropogon sorghum) as a substitute for barley in malting operations. Agr. J. India, 18:362-373. 1923. Original not seen. Abstracted in C. A., 17: 3399. 1923.

33. Ohlsson, E.
Sur l'existence de deux ferments amylolytiques dans la diastase du malt. Compt. rend. soc. biol., 87:1183-1184. 1922.

34. Ohlsson, E.
Über die beiden Komponenten der Malzdiastase, besonders mit Rücksicht auf die Mutarotation der bei der Hydrolyse der Stärke gebildeten Produkte. Z. physiol. Chem. Hoppe-Seyler's, 189:17-63. 1930.

35. Olsson, U.
Nachtrag zu der vorausgehenden Mitteilung über Vergiftungserscheinungen an Amylasen. Z. physiol. Chem. Hoppe-Seyler's, 119:1-3. 1922.

36. Petit, A.
Note sur la transformation de l'amidon par l'action de la diastase, et production d'une nouvelle matiere sucree. Bull. soc. chim., 24:519-520. 1875.

37. Pottevin, M.
La Saccharification de l'Amidon. Ann. inst. Pasteur, 13 :665-688. 1899.

38. Pringsheim, H. and Fuchs, W.
Ein Komplement der Amylasen. Ber., 56B:1762-1768. 1923.

39. Pringsheim, H.
Über die Konstitution des Stärke, des Glykogens und der Flechtenstärke. (Beiträge zur Chemie der Stärke XII). Ber., 57B:1581-1598. 1924.

40. Prior, E.
Über neuere Maischverfahren. Allgem. Z. f. Bierbrauerei u. Malzfabrikat, 32:285-290. 1904. Original not seen. Abstracted in Chem. Zentr., V.75, pt.3, p.923. 1904.

41. Reinitzer, F.
Über die Enzyme des akaziengummis und einiger anderer Gummiarten. Z. physiol. Chem. Hoppe-Seyler's, 61:352-394. 1909.

42. Seyffert, H.
Untersuchungen über Gerstenmalzdiastase. Z. ges. Brauw., 21:195-197, 207-209, 221-223, 611-617, 633-636. 1898. Original not seen. Abstracted in Chem. Zentr. V.69, pt.2, p.73-74, 1224-1225, 1291. 1898.

43. Sherman, H. C. and Schlesinger, M. D.
Amylases VI. A comparison of amyloclastic and saccharogenic powers. J. Am. Chem. Soc., 35:1784-1790. 1913.

Sherman, H. C. and Baker, J. C.
Experiments upon starch as substrate for enzyme action. J. Am. Chem. Soc., 38:1885-1904. 1916.

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

Lüers , H. and Wasmund, W.
Über die Wirkungsweise der Amylase. Fermentforschung, 5:169-235. 1921-1922.

45. Sherman, H. C., Caldwell, M. L. and Adams, M.
A quantitative comparison of the influence of neutral salts on the activity of pancreatic amylase. J. Am. Chem. Soc., 50:2538-2543. 1928.

46. Sherman, H. C., Caldwell, M. L. and Dale, J. E.
A quantitative study of the influence of sodium acetate, sodium borate, sodium citrate and sodium phosphate upon the activity of pancreatic amylase. J. Am. Chem. Soc., 49:2596-2598. 1927.

47. Sjöberg, K.
Beiträge zur Kenntnis der Amylase in Pflanzen. II. Die Temperaturempfindlichkeit der Amylase von Phaseolus vulgaris. Biochem. Z., 133:294-321. 1922.

48. Sjöberg, K. and Eirksson, E.
Über Amylase. Z. physiol. Chem. Hoppe-Seyler's, 139: 118-139. 1924.

49. Teichek, R. F.
Studien über die Verteilung der diastatischen Enzyme des Grünmalzes. Chem. Ind., 27:270-275. 1904.

50. Tomioka, T.
Enzyme action and ion concentration. I. The destruction of amylase by acids. Japan. J. Gastroenterol., 1: 158-168. 1929. Original not seen. Abstracted in C. A., 24:386. 1924.

51. Van Klinkenberg, G. A.
Ueber die Trennung und die Wirkung der beiden Malzamylasen; ein Beitrag zur Kenntnis der Stärke und ihrer Beziehung zum Glycogen. Proc. Acad. Sci. Amsterdam, 34:893-905. 1931.

52. Wijsman, H. P.
La diastase considérée comme un mélange de maltase et de dextrinase. Rec. trav. chim., 9:1-13. 1890.

53. Windisch, W., Dietrich, W. and Beyer, A.
Untersuchungen über die stärkeverflüssigende Funktion der Malzdiastase. Wochschr. Brau., 40:49-50, 55-56, 61-63, 67-70. 1923.

54. Windisch, W.
Ueber die Malzamhlase. Wochschr. Brau., 42:276-278, 283-286, 290-293. 1925.