CERTAIN CHARACTERISTICS OF THE SWEET POTATO AMYLASE SYSTEM

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INTRODUCTION

Many investigations have shown that the concentration of starch, total sugars and reducing sugars change in the sweet potato during curing, storing and baking. Amylase is considered to be an important enzyme that may cause some of these changes since this enzyme is known to be present in relatively large amounts in the root. In 1953 and 1954 the effects of curing, storing and baking on the carbohydrate changes in six varieties of sweet potatoes grown at four locations were studied by Ruth Reder. The data showed that carbohydrate interconversions occurred during these treatments. The objective of the experiments reported in this thesis was to study some of the characteristics of the amylase systems of certain varieties of sweet potatoes. It is hoped that the results will partially explain some of these carbohydrate changes.

LITERATURE REVIEW

AMYLASE CONTENT OF SWEET POTATO ROOTS

Gore in 1920 while working on the production of syrup found that the sweet potato root is rich in amylase (19, 20, 21). The amount of amylase present varied considerably in different varieties (12, 40). Gore (22) found that two widely grown varieties of sweet potatoes, Nancy Hall and Porto Rico, were rich in amylase. Culpepper and Magoon (12) observed that the maltose increase during cooking varied among varieties. After baking 35 varieties of sweet potatoes, they found that Triumph, Nancy Hall, Porto Rico, Yellow Strasburg, Purple 'Yam', Georgia, Miles and Mameyita were high in maltose. Nakamara et al. (40) also observed differences in amylase activity between varieties. They observed that the beta-amylase activity varied from 19.8 to 57.9 units per ml. of sample. The unit was the amount of beta-amylase which produces one mg. of maltose in one minute at 37°C. Johnstone (34) studied two varieties of sweet potatoes and found that the amylase activity of Porto Rico was greater than that of Triumph. According to Giri the amylase activity increased in passing from the skin to the interior of the potato, the innermost portion having three times the activity of the regions near the surface (16).

THE RESEMBLANCE BETWEEN SWEET POTATO BETA-AMYLASE AND CEREAL AMYLASES

Giri (16) in 1934 first observed that the amylase in sweet potatoes is largely saccharogenic (beta-amylase), resembling cereal amylases and differing from potato amylase and similar enzymes of animal origin in pH optimum, temperature stability, molecular weight, turnover number and other characteristics. Giri (18) also found that sweet potato beta-amylase behaved in the hydrolysis of starch like a pure beta-amylase from barley. The amylase selectively hydrolyzed a portion of the starch leaving a residual material which resembled erythrogranulose. Shukla (54) observed in studies of certain Indian roots that the amylase in white fleshed sweet potatoes resembled that of malt and is the beta-type. Crystalline beta-amylase from the sweet potato and from malt exhibited the same ratio of saccharifying to dextrinizing activity, indicating an identity in the enzyme specificity in these two amylases (38).

THE PRESENCE OF ALPHA-AMYLASE, INVERTASE AND MALTASE IN SWEET POTATO

Bois and Savary (10) reported the presence of alpha-amylase and invertase in addition to beta-amylase in the sweet potato. They did not find any evidence for a maltase. Balls, et al. (5) and Schwimmer (53) found large quantities of beta-amylase and maltase. Baba et al. (4) studied the

amylase in both sweet potato leaves and roots. They observed that expressed juice of sweet potato leaves, diluted with water and filtered, had a saccharifying activity on starch. They tested the starch-iodine color reaction and found that for the expressed juice of sweet potato leaves the starch-iodine color remained blue even after 120 minutes of digestion while the crude sweet potato root amylase converted the starch-iodine color from violet to red. The leaves of the sweet potato were presumed to contain only beta-amylase, whereas the roots contain a trace of alpha-amylase (4). Baba and Shimabayashi (3) also found in studies on a partially purified saccharogenic enzyme system of sweet potato leaves, that the enzyme system showed a higher saccharogenic activity than the crude enzyme or the crystalline beta-amylase from sweet potato roots. A comparison of the increase in reducing power and the decrease in substrate in experiments on the activity of this system, with maltose as a substrate, showed that maltase was present in the leaves (3).

THE CHANGE OF AMYLASE ACTIVITY DURING THE PERIODS OF SPROUTING, GROWTH AND STORAGE

Several workers reported that the amylase activity changed during the periods of sprouting, growth and storage (2, 24, 25, 39, 58). The liquefying power of amylase decreased throughout the period of sprouting, while the saccharifying power increased at the beginning of sprouting and then decreased gradually (58). Naka et al. (39)

observed that during the early stage of growth of the roots the pH of pressed juice was low. A general weakening of the amylase activity was observed during growth, while the pH of the pressed juice increased slightly. As the vigor of the shoots declined, at the termination of the growing season, the growth rate of the root progressively decreased. Under these conditions the starch content decreased slightly but the amylase activity remained high (39). The amylase activity of the pressed juice was low during early growth of the storage roots but increased rapidly when top growth stopped (25). Baba and Shimabayashi (2) observed that amylase activities in green leaves varied with the growth periods and varieties. They also found that the activity was higher in the middle leaves than in young or old leaves. Ikemiya and Yamada (26) studied the change of amylase activity of sweet potato roots during storage in 1950. Sweet potatoes were stored from November to June. The amylase activity increased rapidly to late December, remained at the maximum for one month, and then gradually decreased. After sprouting, the decrease in amylase activity was rapid. Tomita reported that during storage the amylase activity increased gradually (58).

THE ISOLATION, CRYSTALLIZATION AND CERTAIN CHARACTERISTICS OF SWEET POTATO BETA-AMYLASE

Early studies of the isolation and characteristics of amylase from sweet potatoes were made by Johnstone (34) and Giri (16, 17). In 1925 Johnstone (34) found that optimum

amylase activity occurred at a pH value of 7 to 9, with a marked decrease at pH 5. Giri (7) observed that the optimum pH range for the enzyme was 5.5 to 6.0. He also reported that the optimum temperature for the enzyme activity was 50 to 55°C. The process of heat inactivation followed a monomolecular course. The enzyme was most stable in the pH range corresponding to its optimum activity (16). Balls et al. (5) in 1948 crystallized beta-amylase from sweet potatoes. The beta-amylase was found to account for at least 60% of the starch breakdown. Spectroscopic examination gave no indication for the presence of an essential heavy metal or a recognized prosthetic group. It was found that the enzyme exhibits its maximum activity in the pH range of 4 to 5 (4). Nakayama and Kono (43) observed that the heat inactivation of sweet potato beta-amylase proceeded as a first order reaction with respect to time. The rate of the inactivation was increased with an increasing concentration of pH 5.4 acetate buffer from 0.01 M to 0.1 M as well as with a decreasing enzyme concentration. The enzyme preparation was stable at pH 5.0 to 5.8, with maximum stability against heat inactivation at pH 5.4 (43). There were substances present in some vegetable extracts which retard the heat inactivation of sweet potato beta-amylase (42). The beta-amylase activity of sweet potato roots was reduced to about 10% of its maximum by heating at 63°C for one hour, but about 90% of the activity remained when it was incubated with a homogenate from certain vegetables, such as the onion bulb, the leaves

of radish and turnip. This stabilizing factor was not found in sweet potato roots (42). Nakayama and Kono (44) studied the effects of inorganic and organic substances on heat-inactivation of sweet potato beta-amylase. They observed that inorganic ions, such as halogenides, cyanide, sulfate, phosphate and alkaline earths, more or less increased the rate of inactivation at 10^{-2} M. concentration. In the presence of organic acids, with the increase in number of carboxylic groups in the acid molecule there was an increase in the inactivation rate. Most monoamino-monocarboxylic acids and diamino-monocarboxylic acids had little or no effect upon heat-inactivation rate of the beta-amylase, except glycine which had a slight protective effect at 1 M. concentration. Monoamino-dicarboxylic acids at 5×10^{-3} M increased the rate of inactivation. Methyl and n-propylamine slightly increased the inactivation rate. They assumed that the heat-inactivation of sweet potato beta-amylase was stimulated by the charge (positive or negative) of ionic substances, which were added to the system.

THE CHARACTERISTICS OF SWEET POTATO BETA-AMYLASE

The characteristics of sweet potato amylase has been studied by Englard et al. (14, 15). The diffusion constant has been determined as $D^{20} = 5.77 \times 10^{-7}$ cm. per second. The molecular weight calculated from sedimentation and diffusion data was 152,000 $\pm$ 15,000. The isoelectric point, as determined by electrophoresis, was pH 4.74 to 4.79. The turnover number

has been calculated to be 250,000 glucosidic linkages hydrolyzed by one molecule of enzyme per minute at 30°C and pH 4.8 (14, 15). Unlike the animal alpha-amylase, sweet potato beta-amylase did not require Cl^- or any other anion for its activity. In contrast to malt alpha-amylase sweet potato beta-amylase was not activated by Ca^{++} (15).

INHIBITORS OF SWEET POTATO BETA-AMYLASE AND THE MECHANISM OF INHIBITION

Reagents recognized as selective for -SH groups are powerful inhibitors of sweet potato beta-amylase (15); among those are p-chloromercuribenzoate, silver nitrate, mercuric chloride, o-iodosobenzoate, and copper sulfate. The inhibition by p-chloromercuribenzoate can be partially reversed by glutathione or by 1,2-dithiopropanol (14). Chemical alteration of the -SH groups of beta-amylase by specific reagents results in a complete loss of enzymatic activity. Inactivation by specific oxidation of -SH groups did not change the diffusion constant or the sedimentation velocity. Englard et al. (15) expresses the view that oxidation of the essential -SH groups of beta-amylase to the disulfide form proceeds by an intramolecular mechanism. Bessho (8) studied the inhibitory effect of ascorbic acid with copper salts on beta-amylase of sweet potato and found that the inhibition by Cu^+ is much higher than that by Cu^{++} but Cu^{++} strongly inhibits in the presence of L-ascorbic acid. This inhibition is ascribed to the reduction of Cu^{++} to Cu^+ by L-ascorbic

acid. Addition of 8-hydroxy-quinoline, which readily combines with Cu^{+}, reverses the inhibition produced by a mixture of copper sulfate and L-ascorbic acid. The inhibition is greatly decreased under conditions in which Cu^{+} is oxidized to Cu^{++}; the inhibition is also reversed by cysteine, thiourea, or sodium thiosulfate (8). Ito and Abe (28) express the view that reactivation with cysteine is due to its competition with the -SH groups of the enzyme for Cu^{+}. The inhibition at later stages of inactivation is not counteracted by cysteine. Studies on the mechanism of beta-amylase inhibition by ascorbic acid were made by Rao and Giri (48). They showed that the inhibition might be due to adsorption or complex formation of the ascorbic acid with the substrate, the modified substrate thus formed being less easily hydrolyzed than the free starch. Dehydroascorbic acid has no inhibition effect on beta-amylase. Hydrogen peroxide alone did not inhibit the hydrolysis of starch by amylase, but in the presence of copper it exerted a feeble inhibitory effect (48). The mechanism of inhibition by L-ascorbic acid and Cu^{++} has been proposed by Ito and Abe (27, 28, 29, 30, 31) to be as follows: (a) essential -SH groups of beta-amylase combine rapidly with Cu^{+} produced from Cu^{++} and L-ascorbic acid, to form a cuprous mercaptide; (b) as L-ascorbic acid decreases, cuprous mercaptides return partially to free -SH groups, and the enzyme recovers its activity. Other parts of mercaptides undergo an intramolecular rearrangements in the presence of oxygen to form an inactive disulfide form; the hydrogen

peroxide accumulated by autoxidation of L-ascorbic acid has a promoting effect on this reaction; (c) in later stages of the L-ascorbic acid autoxidation, the remaining -SH groups in beta-amylase are oxidized to disulfide directly by accumulated hydrogen peroxide. A slight inhibition caused by L-ascorbic acid alone can only account for the second step of the mechanism. The inhibition by cuprous mercaptide formation occurs instantaneously and is not affected by a change of temperature, while the subsequent oxidation of mercaptides by oxygen is slow and requires activation energy. At low pH values, the extent of cuprous mercaptide formation is presumed to decrease as the result of a lower affinity of Cu^{+} for -SH groups in beta-amylase, consequently the oxidation of mercaptide proceeds at a reduced rate. The features of the cuprous mercaptide formation are quite the same as those of the inhibition by Ag^{+} but differ in every respect from those of the inhibition by mercuric chloride and p-chloromercuribenzoate. Nakayama and Watanabe (41) in 1954 found that the filtrate of radish (daikon) juice contains a component which protects beta-amylase from the inhibiting action of ascorbic acid and Fe^{+++}. This component has little or no effect on the inhibiting action of Cu^{++} and Hg^{++}.

THE ACTION OF SWEET POTATO BETA-AMYLASE

The action pattern of beta-amylase has been established by Bernfeld and Burtle (7). The action of sweet potato beta-amylase followed a multichain mechanism (9, 10). Peat et al.

(47) observed that the action of crystalline sweet potato beta-amylase was different from that of an amorphous preparation since the crystalline beta-amylase converted potato starch into maltose to the extent of 70 per cent. Oshima et al. (46) found that the degradation of the amylose by purified beta-amylase reached an arrest point which lay between 35 and 60 per cent of the total amylose concentration. Evidence has been presented by Peat et al. (47) that in addition to beta-amylase an enzyme (Z-enzyme) supplements the beta-amylase in effecting the complete conversion of amylose to maltose.

THE STARCH OF SWEET POTATO

Sweet potato starch granules are similar in shape to corn starch granules, but the size is about one and one-half to two times that of the corn starch granules (56). Boswell et al. (11) reports that the starch content of sweet potatoes varies according to variety, location and time of growth. Varietal and environmental differences are reflected in the gelatinization temperature of the starch. It is assumed that varietal and environmental factors in some manner affect the structure of the starch granule. During the cure period there is a change in the nature of the starch granule which lowers the temperature at which gelatinization occurs (6). Weier and Stocking (59) find that sweet potato root starch gelatinizes at a lower temperature than that at which amylase is inactivated. Reichert (50) finds that before gelatiniza-

tion takes place a swelling of the starch grain begins between 45° and 55°C. Sweet potato starch grains in the initial stages of swelling show a rapid expansion of the inner lamellae. The surface layers are usually cracked by this internal pressure (56). The gelatinization temperature as determined by Reichert (51) and Sinoda and Kodera (55) is found to be 74°C. Doremus et al. (13) measured the amylose content of sweet potato starch and find that approximately 20 per cent was amylose. The varietal variation in amylose content is from 17 to 21 per cent of the total starch.

THE RELATIONSHIP OF AMYLASE AND CARBOHYDRATE CHANGES DURING BAKING

Changes in starch, total and reducing sugars during the curing, storage, cooking, dehydration and canning of the sweet potato are established (37). The study of the amylase and its relationship to the carbohydrate changes in the sweet potato during the baking process is mentioned. However, our knowledge of such a relationship during the period of curing and storage is still lacking. Gore in 1920-23 (19, 21) was apparently the first to recognize that the conversion of starch to dextrins and maltose at elevated temperature is due to amylase present in the sweet potato. Maltose formation in the initial stages of the enzyme action is extremely rapid. Culpepper and Magoon (12) in 1926 report that during cooking, the increase of maltose and dextrins is accompanied by a corresponding decrease in starch. A high dextrin

content is nearly always associated with high maltose content. The observation that maltose, and dextrins increase and starch decreases in sweet potato roots during baking is reported by several groups (12, 32, 33, 49). Jenkins and Gieger (33) observed that the conversion rate of starch to sugars is rapid between 20° and 65°C. The rate of enzymatic hydrolysis of starch is proportional to temperature.

MATERIALS AND METHODS

MATERIALS

Roots harvested at Vegetable Research Station at Bixby, Oklahoma in October, 1957 were cured at 85° for 6 days then stored at 55°F. Varieties were designated as L-3-77, L-180, L-3-79, Clemson 607, G-52-15-1, Allgold, Oklahoma 51, Porto Rico, B-6455, Oklahoma 54, G-50-30-2, HM 122, HM 120 and Redgold.

PREPARATION OF SAMPLES

Dry samples were prepared by cutting 1/2 inch square longitudinal sections of the root and freeze-drying to less than 10 per cent moisture. These freeze-dried samples were pulverized in a mortar and stored in screw-capped bottles at room temperature or in a refrigerator at 34°F.

One gram aliquots of the freeze-dried flour were extracted with a total of 200 ml. of distilled water. The supernatants of this extraction were used for the amylase determinations.

Ten grams of fresh sweet potato tissue, representing a sample from not less than six roots, were ground with 200 ml. of distilled water in a Waring blendor for ten minutes. The extracts were then diluted to 500 ml. with distilled water,

mixed well, and allowed to stand for a short period. The supernatants of these extracts were used for the amylase determinations.

EXPERIMENTAL METHODS

The method of Kneen, Sandstedt and Hollenbech (36, 52) and the method of Kneen and Sandstedt (35) were used for the determination of alpha- and beta-amylase activities respectively. The definitions of the alpha- and beta-amylase units were modified from that given by these authors as follows; a beta-amylase unit is that amount of enzyme which converts one gram of starch to maltose in one hour at 30°C; an alpha-amylase unit is that amount of enzyme which, under the influence of an excess of beta-amylase, will dextrinize one gram of starch in one hour at 30°C. The above unit values were calculated on a dry weight basis throughout.

The alpha-amylase method of Kneen, Sandstedt and Hollenbeck was a micromethod. Extract equivalent to 0.05 gram of material was allowed to act on 20 ml. of beta-amylase-treated 2 per cent 'Lintner starch' (alpha-amylodextrin) for 18 hours at 30°C. At the end of this period constant amounts of alpha-amylase source (Rhozyme 33) were added to the unknown and to an alpha-amylodextrin substrate and the dextrinization time measured. Any shortening of the blank dextrinization time was proportional to the degree of dextrinization which took place in the prolonged action of the unknown sample. This in turn was porportional to the amount of alpha-

amylase in the unknown sample. The alpha-amylase activity was such in the sweet potato that no correction was needed in the calculation of beta-amylase activity from the total determination.

PROCEDURE FOR THE DETERMINATION OF ALPHA-AMYLASE ACTIVITY

The procedure for measurement of dextrinization time was as follows. For each determination, 20 ml. of buffered alpha-amylodextrin substrate was added to a 50 ml. Erlenmeyer flask with water and the enzyme extract added to bring the total volume of the reacting mixture to 30 ml. The flask was placed in a water bath for a few minutes to reach 30°C. The time of adding the enzyme to the substrate was recorded. At appropriate time intervals, one ml. of the digesting mixture was withdrawn and added to one of the iodine tubes, again noting the time. Color comparisons with a standard were made before a lightly screened 100-watt 'daylight' bulb comparitor. The exact time required to reach the match point was recorded. For accuracy in this analysis the amount of enzyme solution used was such that the time required to reach the endpoint was between 10 and 15 minutes.

PROCEDURE FOR THE DETERMINATION OF BETA-AMYLASE ACTIVITY

To 20 ml. of 2 per cent buffered soluble starch solution of pH 4.55 in a 200 ml. test tube, sufficient water was added so that the addition of the extract brought the total volume to 30 ml. When the flask contents had reached 30°C

the sweet potato extract was added, and the hydrolysis allowed to proceed for exactly 15 minutes when the reaction was stopped by the addition of 20 ml. of one per cent sulfuric acid from a fast-flowing pipette. As the acid was added the reaction mixture was agitated for rapid mixing. Shortly after the acid addition, a 5 ml. aliquot of the solution was transferred to a test tube containing 10 ml. of 0.10 N ferricyanide solution. The tubes were immersed in a vigorously boiling water bath for exactly 20 minutes. The contents were cooled immediately and rinsed into 125 ml. Erlenmeyer flasks with 25 ml. of an acetic acid-salt solution containing 20 per cent acetic acid, 7 per cent KCl, and 4 per cent $ZnSO_4 \cdot 7H_2O$. One ml. of soluble starch-potassium iodide solution was added and the contents titrated with 0.1 N sodium thiosulfate. The conversion was made from ferricyanide reduced to maltose equivalents.

In both the inhibition study and the study of sweet potato amylase digesting its own starch at different temperatures, one ml. of digestion aliquots were removed at different time intervals, and added to 10 ml. of 0.10 N ferricyanide solution. The same method of reducing sugar measurement was used. A constant digestion temperature was maintained in a Dubnoff shaker.

RESULTS AND DISCUSSION

LINEARITY TEST

The purpose of this experiment was to test the linearity relationship of per cent starch conversion and enzyme concentration. In this experiment different levels of enzyme concentration, 0.5, 1.0, 2.0, 3.0 ml. of 100 mg. per 100 ml. beta-amylase preparation of barley, were added to the prepared 'Lintner starch' substrates. Digestion of these mixtures and the method of reducing sugar determination followed the same procedure as previously described. The relationship between per cent starch conversion and mg. beta-amylase of barley as shown in Figure 1 was obtained in this manner. The relationship between beta-amylase concentration and conversion of the starch to maltose was linear up to the 35 per cent point. It was apparent that when determining the saccharifying activity of a barley beta-amylase preparation the enzyme concentration should be so regulated that the starch conversion did not exceed 35 per cent. This result compared favorably with the observation of Kneen and Sandstedt (35) who found that in ungerminated barley conversion of the starch to maltose was linear up to approximately 30 per cent. Balls et al. (5) and Schwimmer (53) using sweet potato juice have studied the relationship between the beta-

amylase concentration and the per cent starch conversion. They found that a linear relationship held to as high as 50 to 60 per cent of starch conversion. Therefore, it seemed safe to conclude that this assay method was satisfactory for the purpose of this investigation.

AMYLASE ACTIVITY IN SWEET POTATO VARIETIES

The purpose of this experiment was to study the amylase activity in certain sweet potato varieties. Variations in amylase activity with portions of the root and the effects of curing, storage and extraction method were also observed. Differences in beta-amylase activity of certain sweet potato varieties as presented in Table 1 were found. Those varieties listed from L-3-77 to Oklahoma 51 were higher in beta-amylase activity than those listed below. The amylase content of the root was probably the determining factor as to the amount of starch hydrolyzed and maltose and dextrins formed in the baking process. Thus the amylase activity might be related to the quality of baked sweet potato roots. Gore (21) suggested that differences in amylase activity between different varieties of sweet potatoes, and between sweet potatoes grown under different cultural conditions or kept under different conditions of storage, might be related to their baking quality. Thus amylase content might serve as a guide in the breeding of sweet potato varieties of superior quality.

The alpha- and beta-amylase activity of three varieties of sweet potato roots at harvest and after storage are presented in Table II. In comparison with the beta-amylase content, only a trace of alpha-amylase is found in the sweet potato roots. This observation confirms the results of Baba et al. (4) and Balls et al. (5) who found large quantities of beta-amylase and only traces of alpha-amylase in the sweet potato roots. The alpha-amylase activity is so low that no correction is needed in calculation of the beta-amylase activity from the total determination. In other words, the total saccharification in the baking process is in a large part due to beta-amylase. There is little variation in the beta-amylase activity from the surface to the interior portion of the sweet potato roots. This observation is contrary to that of Giri (16). Varietal differences in beta-amylase activity can also be seen in these three varieties. Allgold has a higher beta-amylase activity than either the Porto Rico or Georgia Red varieties. Table II also shows that the amylase activity of freeze-dried sweet potato flour decreased after storage for eight months at room temperature. The amylase activity decrease in the Georgia Red variety under these conditions is 41.9 units per gram which is greater than in Porto Rico or Allgold varieties.

Table III shows that higher values of beta-amylase activity were obtained when a finely ground, freeze-dried sweet potato flour is used for extraction instead of a Waring blendor extract of fresh roots. As indicated in this table

an average increase of 40.1 units per gram in beta-amylase activity is obtained for the finely ground freeze-dried material probably as a result of a more complete extraction. Thus, extraction of the fresh tissue by Waring blendor is less efficient than extraction of freeze-dried sweet potato flour.

The data in Table IV show that the beta-amylase activity decreases during curing and storing. This result is contrary to that reported by Tomita (58) who found that during storage the amylase activity increased gradually. The amylase activity is more stable in whole root tissue and in samples stored at a lower temperatures than in freeze-dried sweet potato flour. This is shown by a comparison of the activity difference in Table II and Table IV. The decrease in activity shown in Table IV where the whole sweet potato roots were stored at 34°F in a refrigerator is much less than that shown in Table II where the sweet potatoes were freeze-dried and stored at room temperature. It is thought that the amylase is less stable in dry sweet potato which was dehydrated below enzyme inactivation temperature than in whole root tissue.

INACTIVATION TEMPERATURE

The purpose of this experiment was to determine the inactivation temperature of sweet potato beta-amylase. Extracts of two varieties of sweet potatoes, Allgold and Porto Rico, were studied and compared with a barley beta-

amylase preparation. Aliquots of the fresh sweet potato extract (10 grams per 500 ml. water) were held at different temperatures for 15 minutes then the beta-amylase activity determined. The results, (Figure 2), showed the inactivation temperature of sweet potato beta-amylase when a 15 minutes treatment interval was used. It was found, (Figure 2), that the sweet potato started to lose beta-amylase activity at 60°C and the amylase was completely inactivated at 80-85°C during a 15 minutes treatment period. The change of activity per unit temperature showed a maximum at 71-74°C which meant that the most rapid inactivation occurred in this temperature range. Barley beta-amylase started to lose amylase activity at 40°C and was completely inactivated in 15 minutes at 60°C which was about 20° lower than the inactivation temperature of sweet potato beta-amylase. However an exact comparison of the inactivation temperature of these two beta-amylases still should not be made from these results since the sample of sweet potato beta-amylase was a crude extract while the barley beta-amylase was virtually free of alpha-amylase. The sweet potato beta-amylase probably might be stabilized by combining it with the substrate of the sample and inactivation retarded. The maximum change of barley beta-amylase activity occurred at 52°C.

OPTIMUM SACCHARIFICATION REMPERATURE

The optimum saccharification temperature of sweet potato beta-amylase was determined by the same procedure.

However, in this study the digestion temperature was changed from 30°C to other selected temperatures. The reducing sugar produced at different temperatures was assumed to be maltose. The per cent starch conversion was then calculated from the maltose value.

The results are shown in Figure 3. It may be seen from Figure 3 that the starch conversion power, or saccharification power, increases as temperatures increased from 20° to 60°C, this increase being almost a straight line function for this temperature range. The maximum saccharification power of beta-amylase is reached at 55-60°C and decreases rapidly thereafter. Saccharification power is completely lost at 85-90°C in 15 minutes. The results compare quite well for the two varieties studied. The increase of saccharification power as temperature increased from 20° to 60°C is a result of an increasing rate of enzymatic hydrolysis of starch. This observation confirms the results of Jenkins and Gieger (33) who found that the conversion rate of starch to sugars in the sweet potato is rapid between 20° and 65°C. They also report that the rate of enzymatic hydrolysis of starch was directly proportional to temperature. Above 60°C the rapid drop of saccharification power as the temperature increased is apparently a result of enzyme inactivation. The saccharification power is optimum at 55° to 60°C which is the same as the result of Ono and Takahara (45) who observed that the optimum saccharification was 55°C. This temperature is a little higher than the result of Giri

(16) who found that the optimum temperature for the enzyme was 50-55°C. The Allgold variety has a higher beta-amylase activity than the Porto Rico variety. By comparing the slopes of the curves for the two varieties in Figure 3, it may be seen that beta-amylase of Allgold shows a more rapid saccharification than Porto Rico amylase at temperatures lower than 60°C. The hydrolysis of starch is rapid from 20° to 50°C for the barley beta-amylase. The optimum saccharification temperature is 50°C. After 50°C the saccharification power decreases rapidly as temperature increases. At 70°C only a slight saccharification power remains.

OPTIMUM PH RANGE

In this experiment pH values in the range 3.4 to 6.8 were studied. Acetic acid-sodium acetate buffers were used. Extracts of fresh sweet potato tissue of the varieties Allgold and Porto Rico were digested with the soluble starch buffered solutions for 15 minutes and the reducing sugar produced was indicated by the amount of 0.10 N ferricyanide reduced.

The results are shown in Figure 4. The optimum pH for the amylase activity is in the wide range of 4.25 to 6.25. The amylase activity diminishes considerably at the pH to either side and ceases completely at a pH either higher than 6.80 or lower than 3.50. The pH optimum observed in this experiment is very close to the observation of Giri (16) who found that the optimum pH for the enzyme was 5.5 to

6.0, but this result is contrary to the report of Johnstone (34) who found that the optimum amylase activity occurs in a range of pH value from 7 to 9, with a marked decrease of activity at pH 5. Since the samples used in this experiment are crude extracts of sweet potato root tissue, trace amounts alpha-amylase, maltase and other enzymes may be present in the preparations. However, the concentrations of these other enzymes in the sweet potato are shown to be very low in Experiment I and also by the works of Baba et al. (4), Balls et al. (5) and Schwimmer (53). It is unlikely that these traces of enzymes would introduce a significant distortion in the pH optimum curve of the beta-amylase of sweet potato.

THE STUDY OF THE ACTION OF BETA-AMYLASE IN HYDROLYZING THE NATURAL OCCURRING SWEET POTATO STARCH

Freeze-dried sweet potato flour was digested in water at a constant temperature in the Dubnoff shaker. At certain intervals aliquots of the supernatants were removed for reducing sugar determinations. An ethanol-heat treated sample was digested in the same manner and used as an indication of the non-enzymatic hydrolysis of starch. The ethanol and heat treatment was as follows. A portion of sweet potato flour (0.5 or 1.0 gram) was treated with 15 ml. of 95 per cent ethanol and held at 78°C until the ethanol was completely evaporated.

Figure 5 shows the results of the digestion at 53°C of two different levels of sweet potato flour; one gram per 20

ml. of water, and 0.5 gram per 20 ml. of water. Saccharification takes place rapidly in the first half hour with the untreated samples, after that the rate of maltose production decreases. Only a small amount of maltose was produced from 3.5 to 5.5 hours incubation. This is probably due to lack of starch available for hydrolysis in the incubation flask. For the ethanol-heat treated sample only a slight increase of maltose is shown during the entire digestion period. It is of interest that sweet potato flour starch may be partially hydrolyzed by the action of the amylase present in the sweet potato though the suggested gelatinization temperature of the sweet potato starch has not been reached. Alsberg and Griffing (1) have shown that the starch granules may be injured in the grinding process, so that a part of the starch swells and disperses when water is added. Stamberg and Bailey (57) have also demonstrated that raw wheat starch which had been finely pulverized is easily hydrolyzed by both alpha- and beta-amylases. It is reasonable therefore to assume that the action on the sweet potato raw starch by the amylase in the sweet potato is partly due to the starch injured in the grinding process. It is also recognized that under 53°C large portions of the sweet potato starch are available for the enzymatic hydrolysis.

Figure 6 shows the different levels of maltose produced at different incubation temperatures. In this study one gram portions of each sample are incubated with 25 ml. of distilled water. Results of this experiment clearly show that the

amounts of maltose produced increases gradually as the incubation temperature increases from 50.5° to 58.5°C. It may also be seen from the slopes of these curves that the rate of starch hydrolysis by the amylase increases rapidly as the temperature is increased. These results confirm the observations of Jenkins and Gieger (33). When a comparison is made of the maltose produced by an ethanol-treated sample to the maltose produced by an untreated sample it is shown that 95 per cent ethanol has little effect on the amylase activity of the sweet potato. The ethanol in this treatment is removed under vacuum at a low temperature.

THE STUDY OF THE INHIBITORY EFFECTS OF SEVERAL COMPOUNDS ON THE BETA-AMYLASE OF THE SWEET POTATO

The general methods used were the same as in the study of the action of beta-amylase in hydrolyzing the natural occurring sweet potato starch. The finely ground freeze-dried sweet potato flour extracts of one gram per 25 ml. distilled water or with different inhibitory compounds were digested in a constant temperature Dubnoff shaker as was an ethanol-heat treated sample. At certain intervals one ml. aliquots of the supernatant were taken and tested for sugars as previously described. Three sweet potato varieties, All-gold, Porto Rico and L-3-79 were studied. The following chemical reagents were tested for their inhibitory effects: 10^{-5}M silver nitrate, 10^{-5}M mercuric chloride, 10^{-3}M copper sulfate, 10^{-3}M iodosobenzoic acid, 5×10^{-6}M p-chloromercur-

ibenzoic acid Na-salt, 10^{-3}M ascorbic acid.

The results are presented in Figures 7-10. It is shown in Figures 7 and 8 that for the Porto Rico and Allgold varieties the maltose produced is much less in the samples containing inhibitory compounds than in the untreated samples. It is known that 10^{-3}M copper sulfate; 10^{-3}M iodosobenzoic acid; 10^{-5}M silver nitrate and 10^{-5}M mercuric chloride have an inhibitory effect on the beta-amylase of the sweet potato roots. When compared with the level of maltose produced in the ethanol-heat treated samples, it is clearly shown that the beta-amylase is not completely inhibited by these reagents. Since the levels of the inhibition curves are not parallel for the two varieties studied, no conclusion is made as to which reagent has the greatest inhibitory effect on beta-amylase. However, this study does support the observation of Englard (14, 15) that copper sulfate, iodosobenzoic acid, silver nitrate and mercuric chloride are inhibitors of sweet potato beta-amylase. Figures 9 and 10 show the effects of p-chloromercuribenzoate and ascorbic acid on beta-amylase activity of the sweet potato. As shown in these two figures 5×10^{-6}M p-chloromercuribenzoate and 10^{-3}M ascorbic acid slightly inhibit the beta-amylase of Allgold and L-3-79 varieties. Since p-chloromercuribenzoate has also been recognized as a reagent selective for -SH groups, the slight inhibition observed by this reagent is possibly due to the low concentration of the compound in it. Ascorbic acid has very

little inhibitory effect on the beta-amylase of Porto Rico variety as shown in Figure 9. However, it may be concluded from this experiment that the compounds tested are inhibitors of sweet potato beta-amylase.

SUMMARY AND CONCLUSIONS

Some of the characteristics of beta-amylase in certain varieties of sweet potato were studied for the purpose of determining the relationship of amylase to carbohydrate changes that occur during curing, storing and baking.

During the baking process the transformation of starch to maltose and dextrins is by the action of amylase present in the sweet potato roots until the inactivation temperature of the enzyme has been reached. The amount of maltose and dextrin increase is determined by the starch content, degree of gelatinization of the starch, amylase activity, temperature and time of baking, pH of the root juice and certain other factors as yet undetermined.

Small amounts of maltose were produced by beta-amylase when the sweet potato flour was digested with water at 50.5°C for 5 hours. However, as the temperature increased gradually from 50.5° to 58.5°C large quantities of maltose were produced. It was found that a partial gelatinization of sweet potato flour starch occurred after the digestion temperature reached 50°C. During the first half hour the hydrolysis was rapid. Sweet potatoes lost a part of their beta-amylase activity when the extracts were held at a temperature of 60°C for 15 minutes. Beta-amylase was completely inactivated at 80-85°C

in 15 minutes. Maximum saccharification power was observed in the temperature range of 55° to 60°C and the pH range of 4.25 to 6.25 when beta-amylase hydrolyzed the gelatinized 'Lintner starch' in the 15 minutes tests. More maltose and dextrins were produced when the baking temperature was increased slowly. After the temperature passed 60°C the amount of starch hydrolysis decreased. After the temperature reached 80-85°C for 15 minutes, almost no enzymatic hydrolysis of starch occurred.

The following compounds were observed to have an inhibitory effect on the sweet potato beta-amylase activity: copper sulfate, silver nitrate, mercuric chloride, iodosobenzoic acid, p-chloromercuribenzoate and ascorbic acid. The inhibition caused by ascorbic acid was considerably lower than that of other compounds.

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TABLE I

Varietal Differences of Beta-amylase Activity in Certain Varieties of Sweet Potatoes as Determined on Extracts of Fresh Roots at Harvest and Freeze-Dried Roots after Cure and Storage

Variety	Specific Activity of Beta-amylase	
	Fresh, At Harvest Units/gram (Dry Basis)	Freeze-Dried, After Storage Units/gram (Dry Basis)
L-3-77	100.9	98.3
L-180	97.9	118.1
L-3-79	84.1	135.9
Clemson 607	79.6	95.0
G-52-15-1	78.1	101.7
Allgold	73.7	119.9
Oklahoma 51	71.6	101.5
Porto Rico	59.3	70.0
B-6455	59.3	39.2
Oklahoma 54	59.3	70.7
G-50-30-2	56.9	----
HM 122	54.9	78.1
HM 120	49.3	44.1
Redgold	----	58.7

TABLE II

Alpha- and Beta-amylase Activity in Three Varieties of Sweet Potato Roots, Sampled at Harvest and After Storage, Extracted from Freeze-Dried Root Tissue.

Variety	Portion of Root	At Harvest		# After Storage Beta-amylase Activity	# Decrease of Beta-amylase Activity after Storage
		Alpha-amylase Activity	Beta-amylase Activity		
		Units/gram Dry Basis	Units/gram Dry Basis	Units/gram Dry Basis	Units/gram Dry Basis
Allgold	Center 1/3	0.22	93.7	56.4	
	Intermediate 1/3	0.24	92.0	52.6	
	Outer 1/3	0.20	74.4	44.0	
	Average	0.22	87.0	51.0	36.0
Porto Rico	Center 1/3	0.19	61.8	45.0	
	Intermediate 1/3	0.22	61.2	42.4	
	Outer 1/3	0.13	67.4	55.2	
	Average	0.18	63.5	47.6	15.9
Georgia Red	Center 1/3	0.12	64.2	22.2	
	Intermediate 1/3	----	----	----	
	Outer 1/3	0.12	64.6	22.7	
	Average	0.12	64.4	22.5	41.9

After eight months room temperature storage of the sweet potato flour

TABLE III

Beta-amylase Activity of Certain Sweet Potato Varieties as Determined on Extracts of Fresh and Freeze-Dried Tissue from Cured and Stored Roots

Variety	Specific Activity of Beta-amylase		Ratio Fresh/ Freeze-Dried
	Freeze-Dried Units/gram	Fresh Units/gram	
L-3-79	135.9	62.2	0.46
Allgold	119.9	72.2	0.60
L-180	118.1	74.3	0.63
Clemson 607	95.0	69.3	0.73
Porto Rico	70.0	35.4	0.51
Redgold	58.7	43.7	0.74
Average for six Varieties	99.6	59.5	0.60

TABLE IV

Beta-amylase Activity of Five Sweet Potato Varieties as Determined on Extracts of Fresh Root Tissue at Harvest, and on Cured and Stored Roots

Variety	Specific Beta-amylase Activity		Ratio Cured and Stored / At Harvest
	At Harvest Units/gram Dry Basis	Cured and Storage Units/gram Dry Basic	
L-3-79	84.1	62.2	0.74
Allgold	73.7	72.2	0.98
L-180	97.9	74.3	0.76
Clemson 607	79.6	69.3	0.87
Porto Rico	59.3	35.4	0.60
Average For Five Varieties	78.9	62.7	0.80

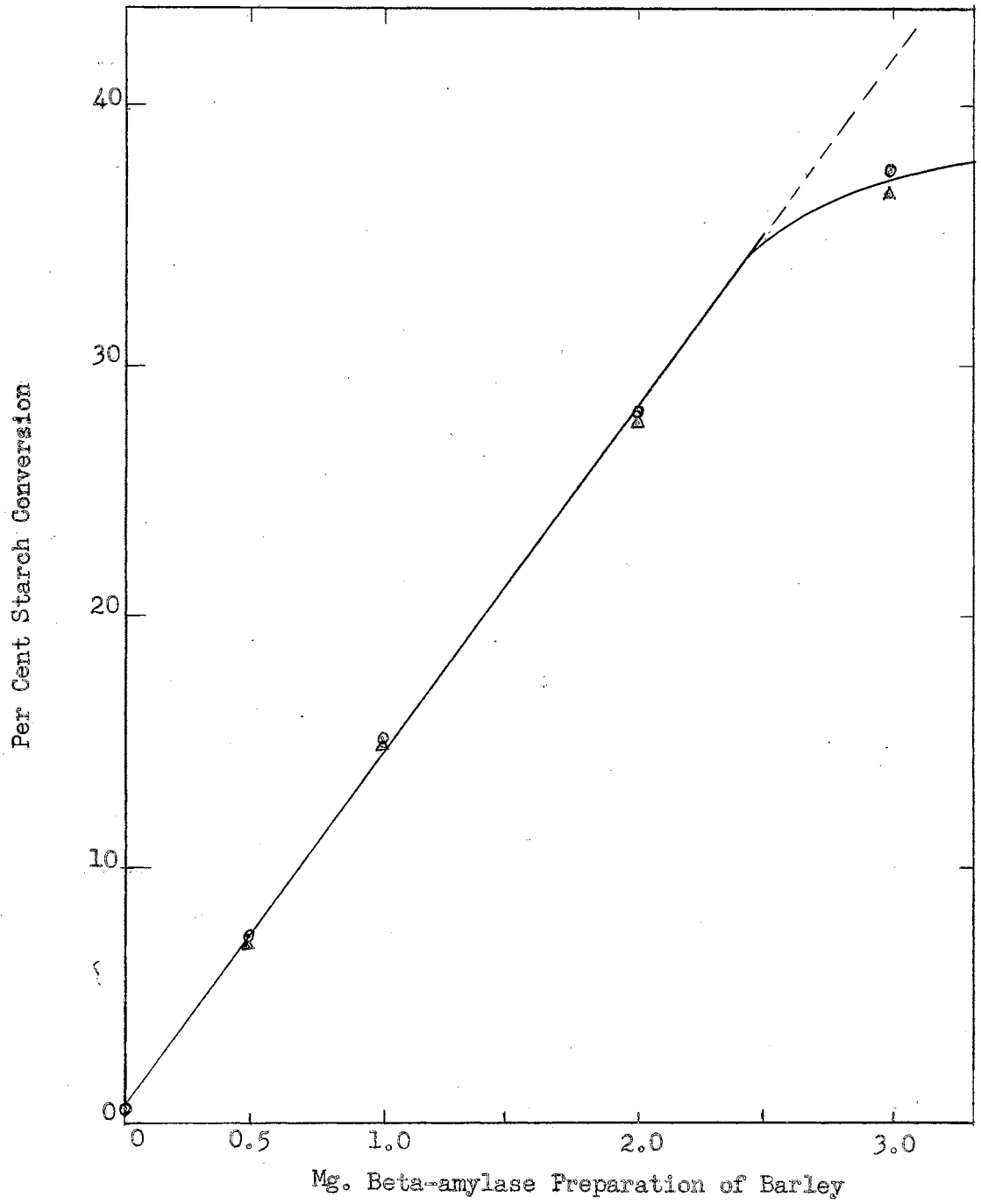

Figure 1. Relationship of Per Cent Starch Conversion and Concentration of Barley Beta-amylase

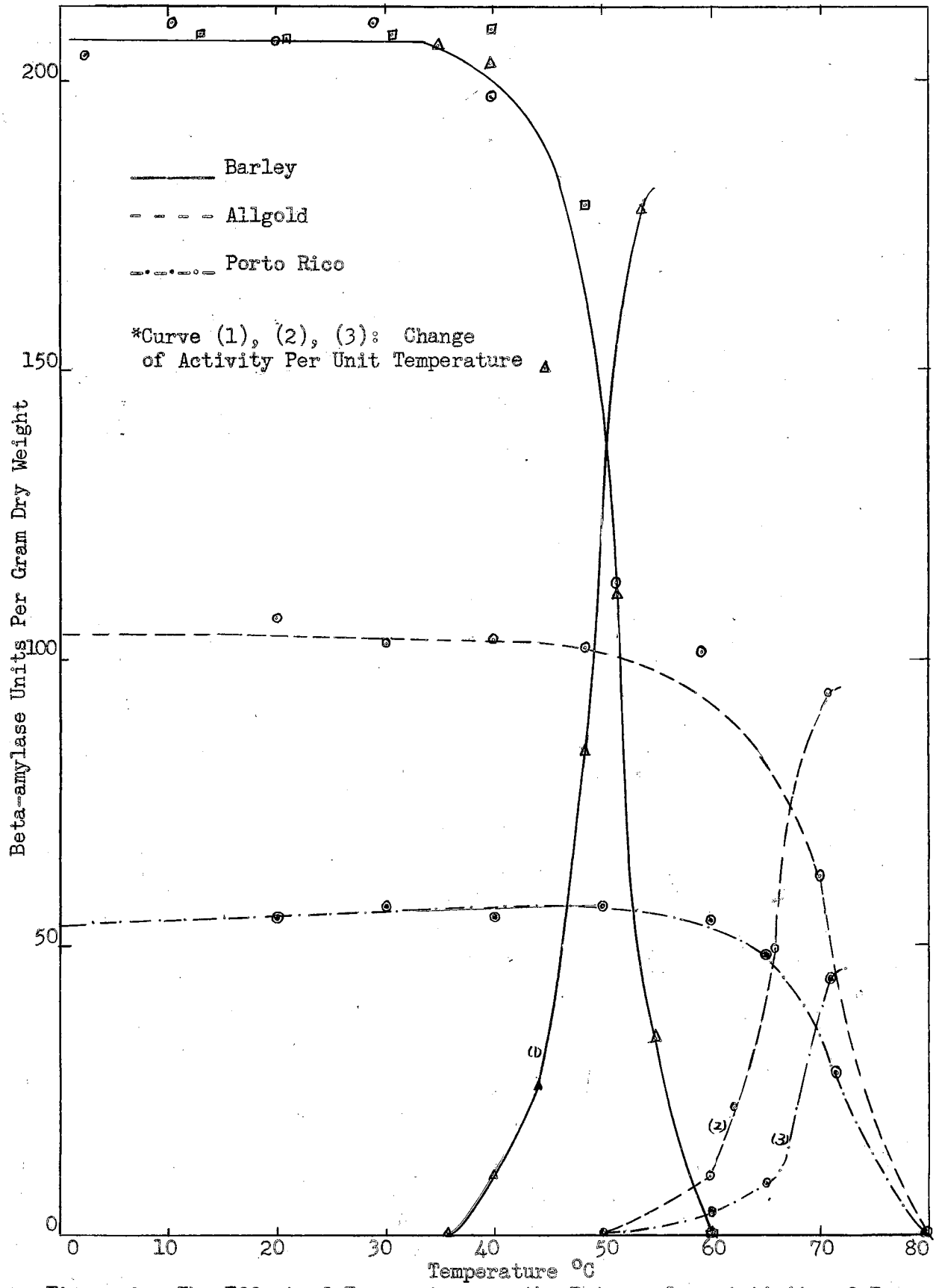

Figure 2. The Effect of Temperature on the Beta-amylase Activity of Two Varieties of Sweet Potatoes, Allgold and Porto Rico, and the Beta-amylase from Barley.

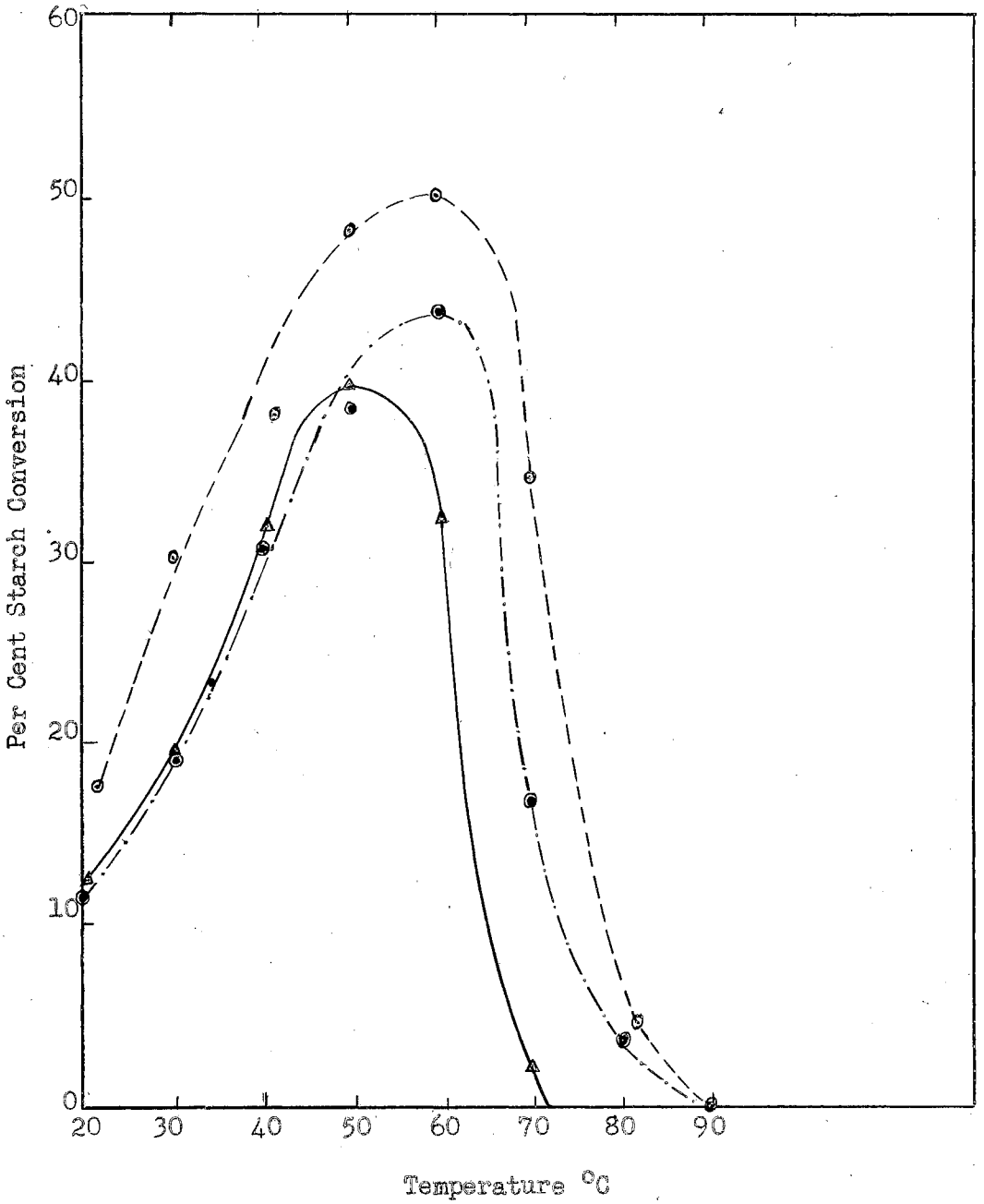

Figure 3. The Effect of Temperature on the Saccharification Power of Beta-amylase of Two Varieties of Sweet Potatoes, Allgold and Porto Rico, and the Beta-amylase from Barley.

——— Barley

– – – – Allgold

—·—·—·— Porto Rico

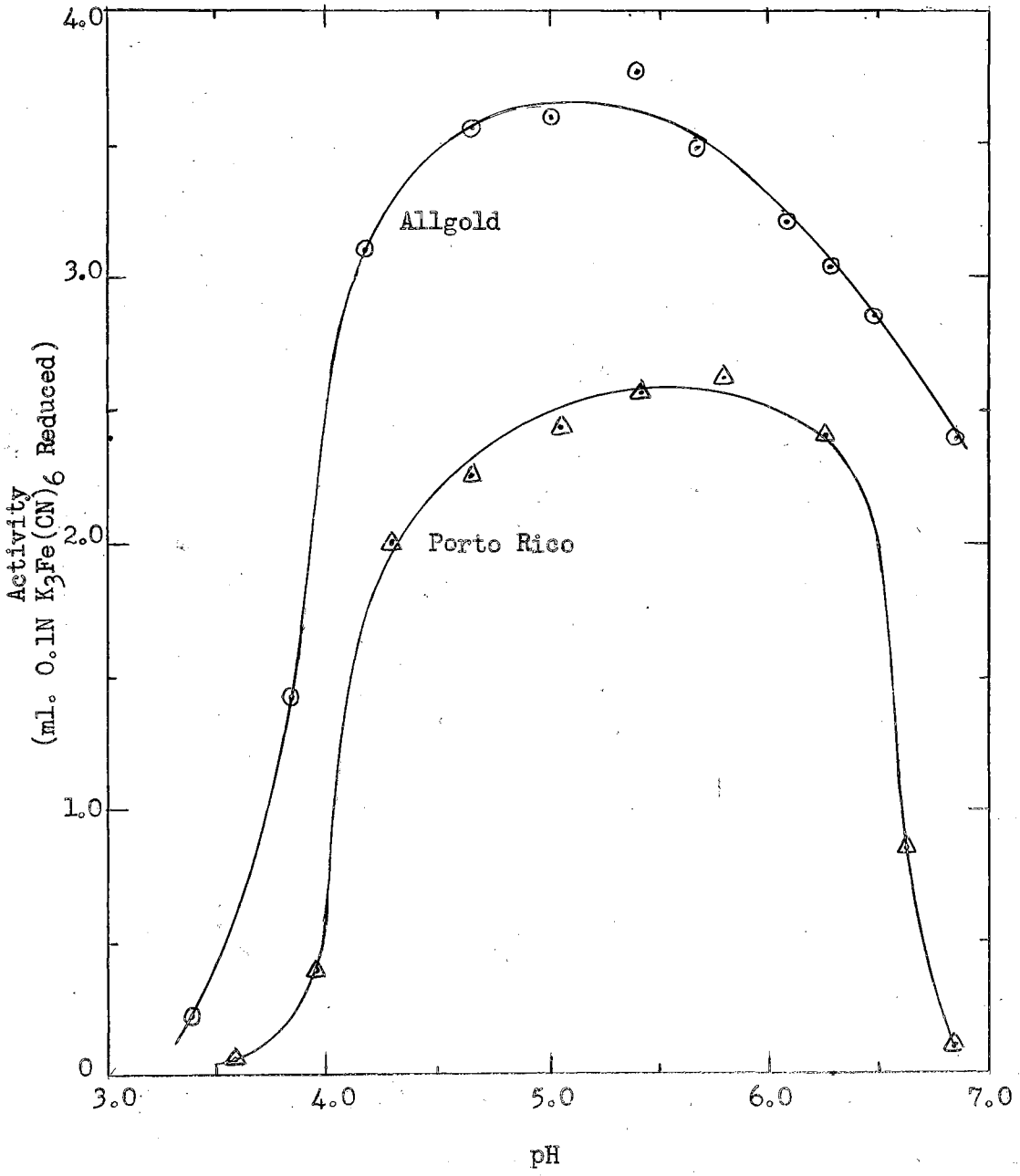

Figure 4. The Beta-amylase Activity of Two Varieties of Sweet Potatoes at Different pH Values.

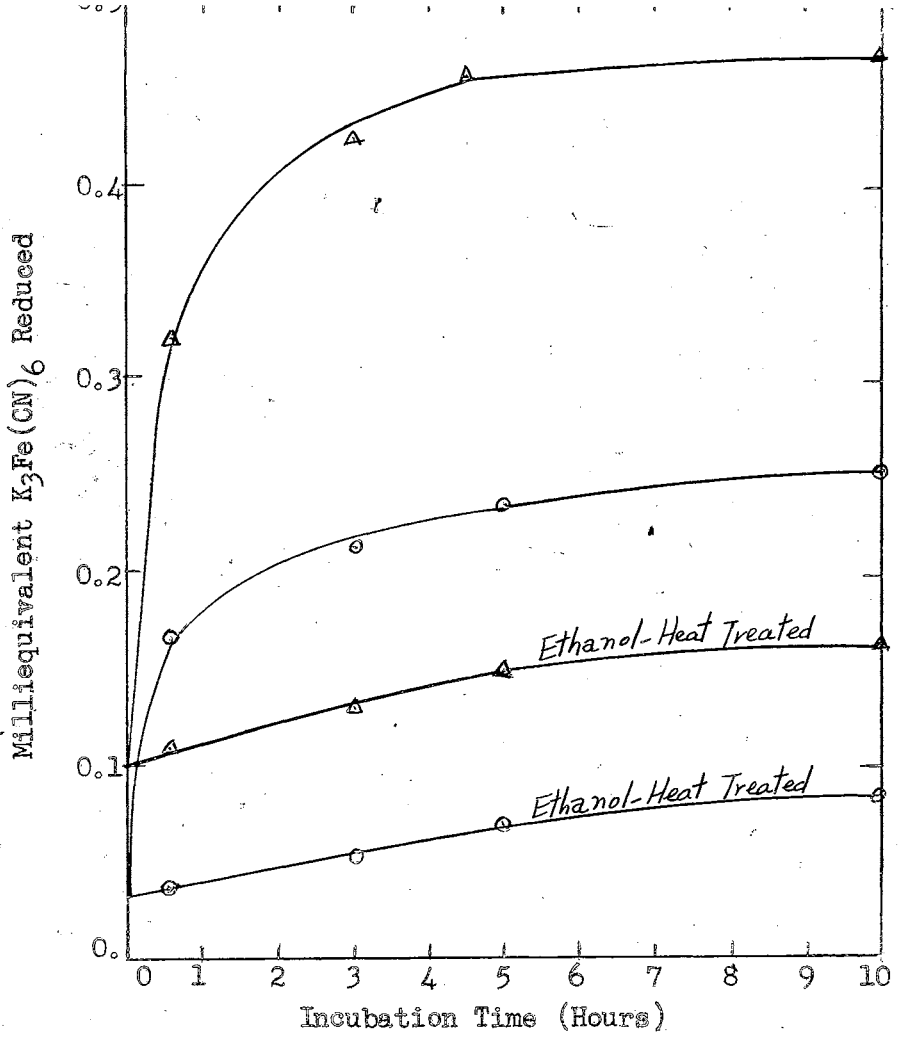

Figure 5. The Hydrolysis of Sweet Potato Starch by Beta-amylase of Allgold Variety at 53°C.

○ 0.5 Gram/20ml. level
△ 1 Gram/20ml. level

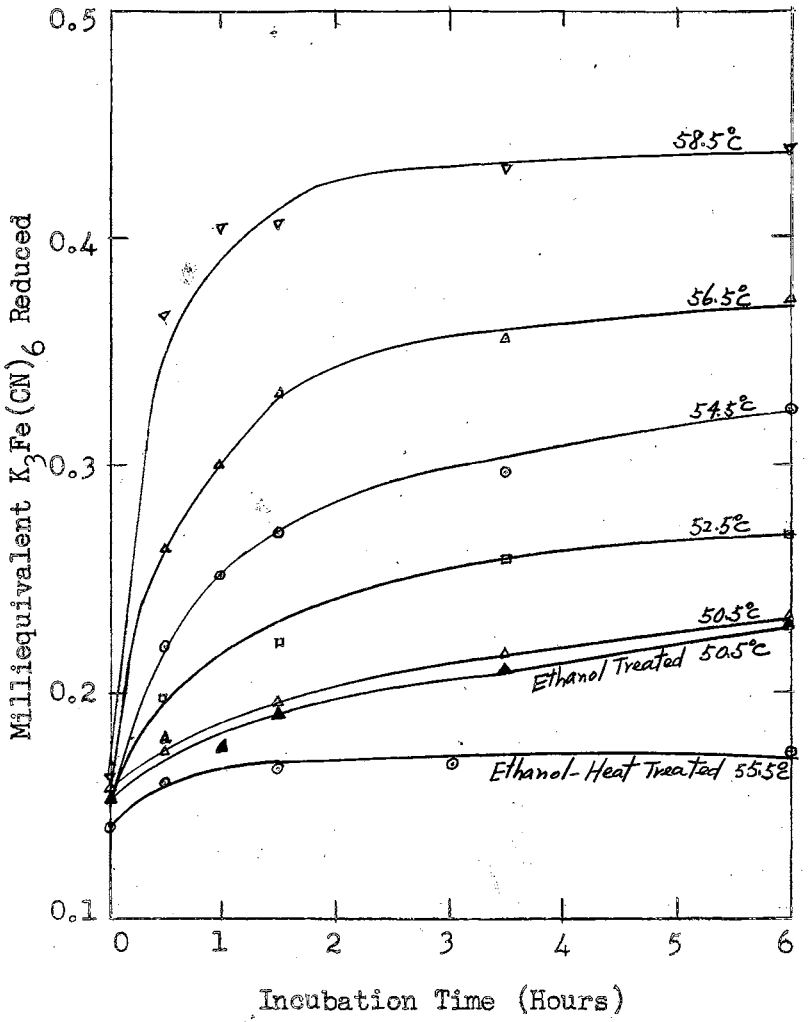

Figure 6. The Hydrolysis of Sweet Potato Starch by Beta-amylase of L-3-79 Variety at Different Temperatures

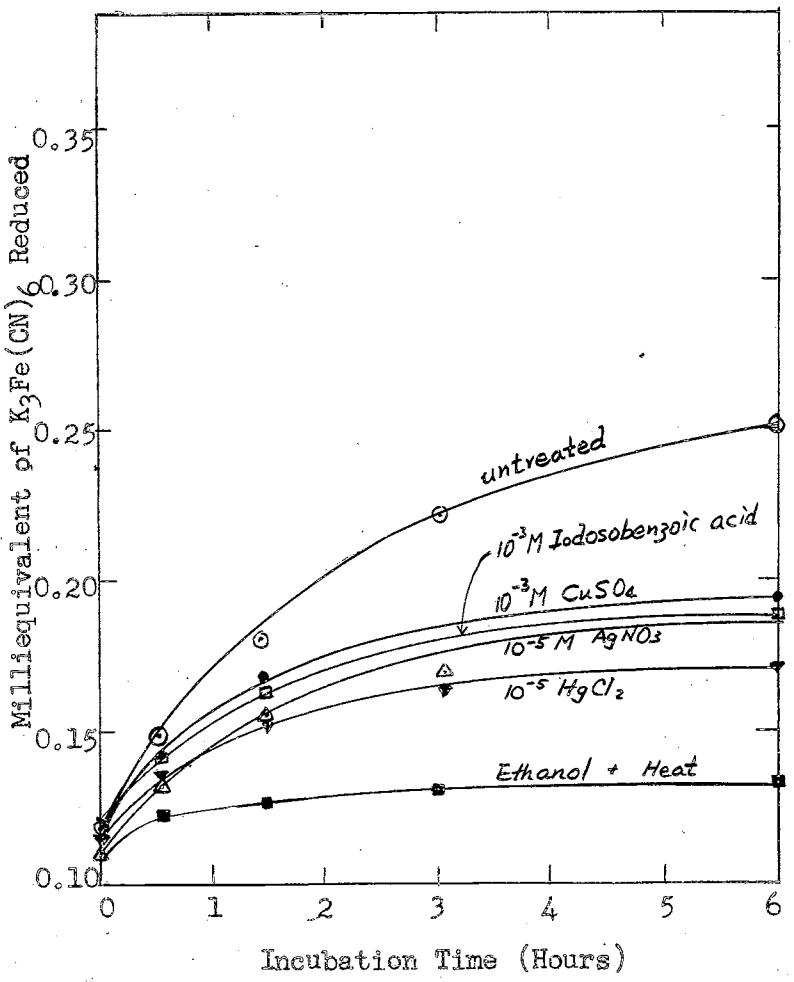

Figure 7. The Inhibitory Effect of Several Compounds on Beta-amylase of the Porto Rico Variety.

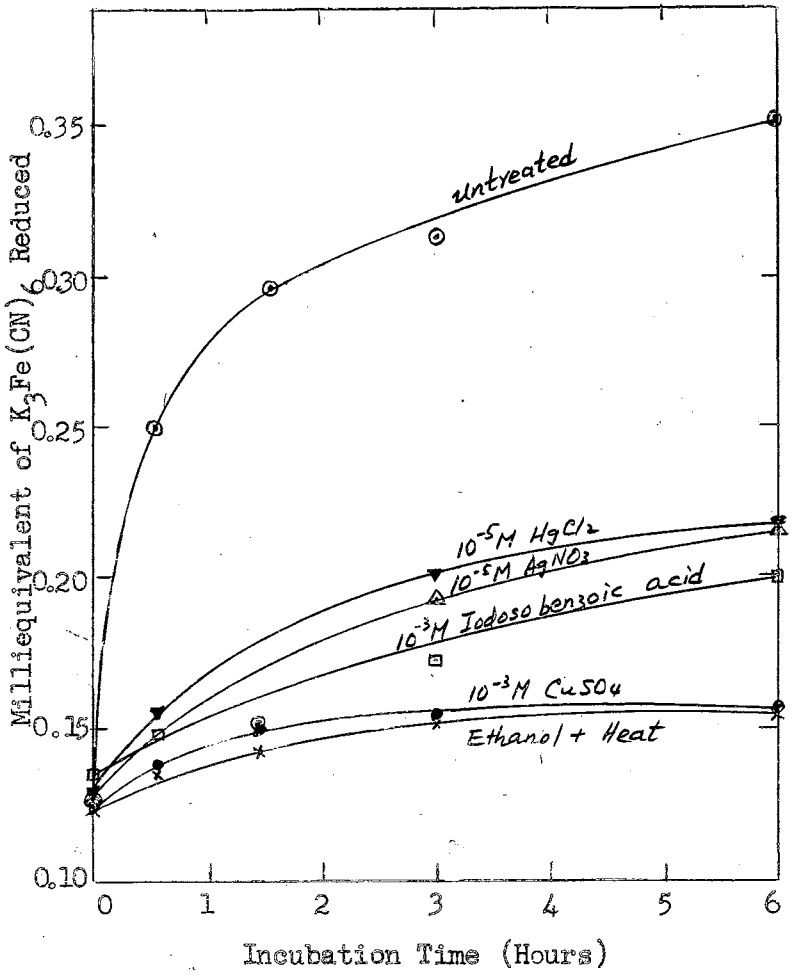

Figure 8. The Inhibitory Effect of Several Compounds on Beta-amylase of the Allgold Variety.

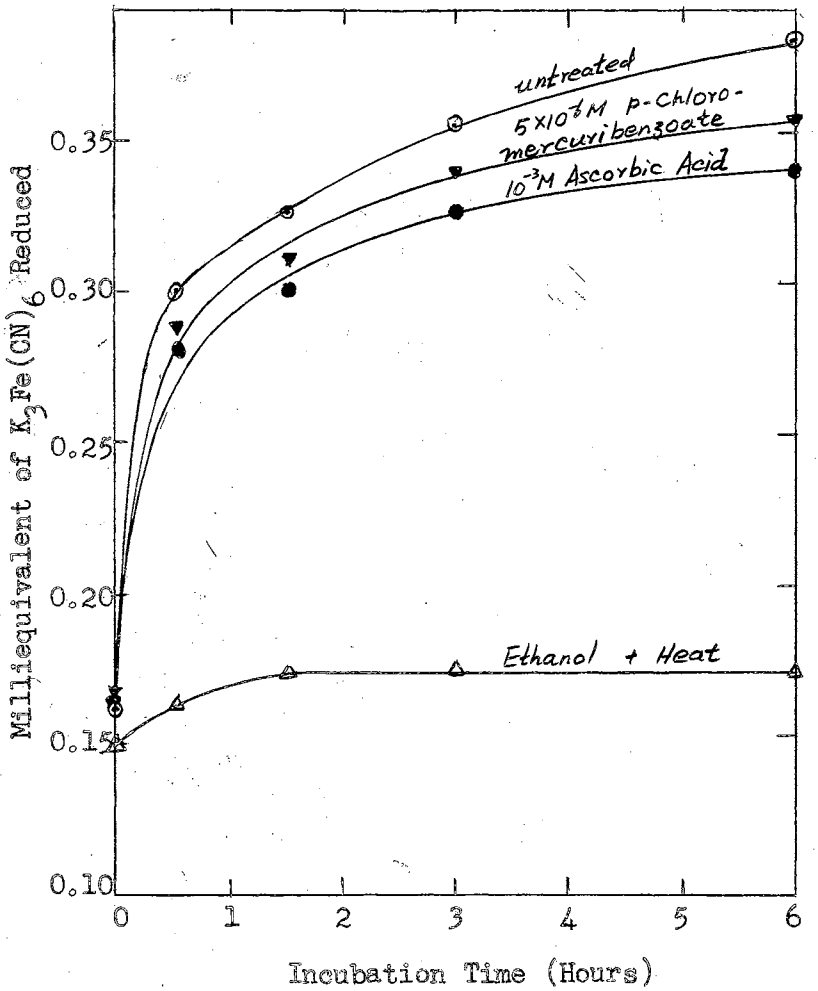

Figure 9. The Inhibitory Effect of p-chloro-mercuribenzoate and Ascorbic Acid on the Beta-amylase of the Porto Rico Variety.

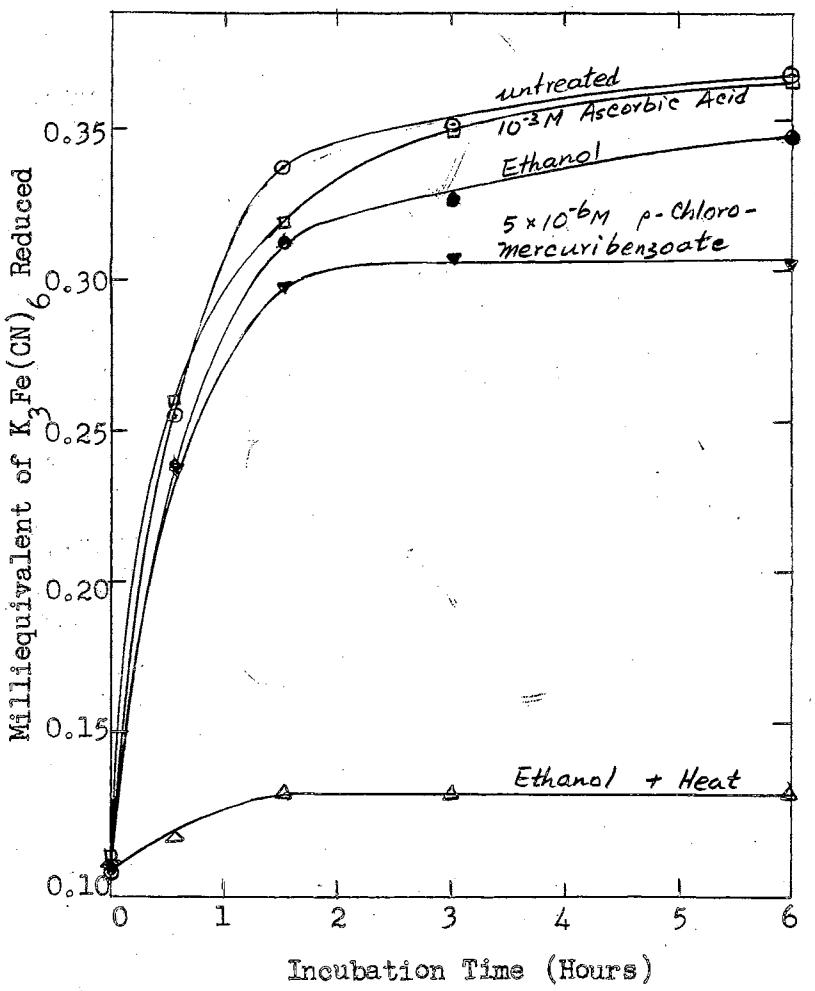

Figure 10. The Inhibitory Effect of p-chloromercuribenzoate and Ascorbic Acid on the Beta-amylase of L-3-79 Variety.

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