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THE OPTIMUM PH FOR DIASTASE OF MALT ACTIVITY

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The researches of Sherman et al. (1919) are usually referred to (Meyer and and Anderson, 1952) whenever the optimum hydrogen ion concentration for diastase is considered. By using diastase which had been extracted and purified in the laboratory, Sherman et al. found the optimum pH to be about 4.5. In our experiments with diastase of malt powder, which was secured through a supply house, we obtained a hydrogen ion activity curve markedly different from the one reported by Sherman et al. for amylase of malt. One is led to believe that Sherman et al. actually obtained a curve for β-amylase rather than diastase, inasmuch as the curves resulting from the use of β-amylase are similar to the curves presented by Sherman et al. Therefore, it would seem desirable to report the hydrogen ion activity curve of commercially available diastase of malt. In addition, hydrogen ion activity curves determined for α-amylase and β-amylase are presented herewith.

METHODS AND MATERIALS

Diastase of malt U.S.P. IX was used in all experiments involving diastase. A one-half percent solution of diastase was prepared in distilled water and allowed to stand for one hour. Next, it was suction-filtered through a gooch filter lined with acid-washed gooch asbestos. The filtration removed starch grains present in the crude diastase of malt preparation, thereby adding to the reliability of the tests used to determine the amount and rate of diastase activity. A fresh solution of diastase was prepared each day.

Alpha-amylase and β-amylase were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, and were used in stock solutions of varying concentrations. These stock solutions were made up fresh each day and, unlike the one-half percent diastase of malt stock solutions, were not filtered.

The soluble starch (C. P., powder) was purchased from The Coleman and Bell Co., Norwood, Ohio. It was prepared in one percent concentration by adding one gm to 100 ml of boiling distilled water and stirring until completely dissolved.

Two methods were used to determine the rate and amount of diastase activity: (1) Munson-Walker values as described in Lange's Handbook of Chemistry (1939, page 1012), which involved a determination of the maltose produced as the result of the diastase activity; (2) a determination of the time required for starch to reach the colorless stage with I_2-KI (Eyster, 1953).

The pH was regulated by the use of MacIlvaine buffers, and the pH of all buffered digests was checked on the Beckman Glass Electrode pH Meter. In digests of 100 ml, 40 ml of buffer at each desired pH were used. In digests of 50 ml, 20 ml of buffer were used. At 25°C the digests usually had a total volume of 100 ml but occasionally, where specified, the total volume was 50 ml. At higher temperatures it was more convenient to use 50-ml digests. The tem-

peratures were maintained within one degree of the desired temperature by means of a water bath.

All Munson-Walker determinations were accompanied by blank runs which contained identical amounts of soluble starch but to which an identical amount of diastase inactivated by boiling (one min) had been added. Net milligrams of maltose values were obtained by subtracting the average for the blank runs from the average for the experimental runs.

TABLE 1

Effect of pH on diastase activity

pH	Diastase activity†	
	Without NaCl min	With 0.05 N NaCl min
2.2	∞	∞
3.0	∞	∞
4.0	∞	∞
4.85	270	135
5.98	35	28
6.98	35	28
7.98	85	40
9.0	∞	∞

†Diastase activity measurements were made with I_2KI reagent, and denote the time in minutes required for diastase to convert starch to the colorless stage.

These digests were run at 25° C, and contained 25 ml 1% soluble starch, 1 and ½ ml ½% diastase, 40 ml buffer, and 33 and ½ ml water.

DATA AND RESULTS

Using a series of MacIlvaine buffered solutions the effective pH range of diastase was found to be from four to nine with an optimum at from six to seven (tables 1 and 2, and fig. 1). It can be observed that the temperature of the digests did not seem to affect the optimum pH value. Munson-Walker values secured from digests at 25°C were quite similar to Munson-Walker values secured from digests at 40°C. Diastase activity measurements by the two methods gave good agreement as can be seen by comparing the data in table 1 with the data in table 2. In the former, diastase activity at different pH levels was determined by measuring the time required for a known amount of added soluble starch to reach the colorless stage with I_2-KI, and in the latter method the measurements involved a determination of the maltose produced as a result of diastase activity at various pH levels.

TABLE 2

Effect of pH on diastase activity

pH	Diastase activity 25° C 20 min	pH	Diastase activity 40° C 10 min
	mg maltose		mg maltose
5.23	57	5.0	43
6.13	118	5.81	96
6.99	109	6.71	106
7.97	64	7.68	42

Diastase activity measurements are Munson-Walker determinations and denote net mg of maltose formed. All measurements are averages of two determinations with the blanks averaging 9 mg maltose.

These digests contained 25 ml 1% soluble starch, 1 and ½ ml ½% diastase, 20 ml buffer and 3.5 ml distilled water.

In table 1 there is also a comparison of diastase activity at different pH levels with and without added chloride. Chloride ions were added as NaCl in a concentration of 0.05 M, the optimum for maximum stimulation of diastase activity at 25°C being 0.01 M (Eyster, 1953). The chloride anion produced a marked stimulation at all effective pH levels. However, the addition of chloride to diastase digests produced no change in either the enzyme's optimum pH or in its effective pH range.

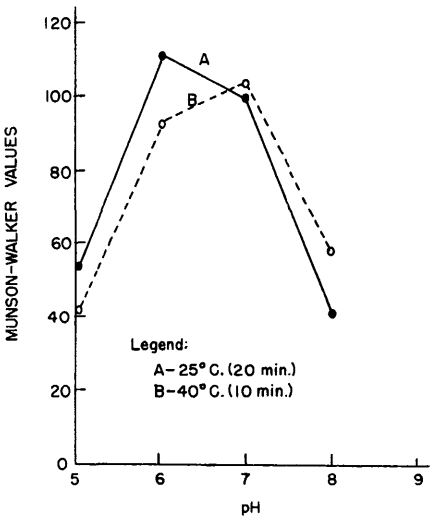

FIGURE 1. The effect of pH on diastase activity based on Munson-Walker determinations.

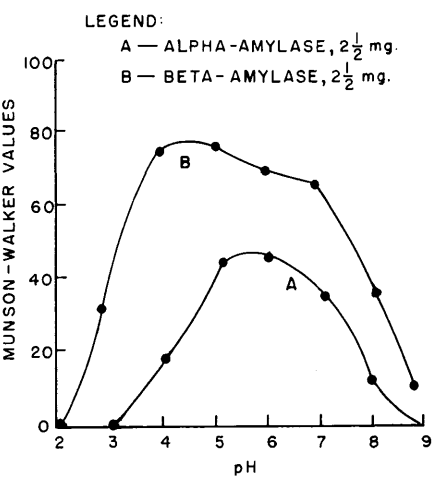

FIGURE 2. The effect of pH on alpha-amylase and beta-amylase activities based on Munson-Walker determinations of 15-min digests performed at 25° C.

Table 3 presents the effect of hydrogen ion concentration on β-amylase activity. These data are Munson-Walker determinations of the amount of maltose formed as a result of the action of β-amylase. The pH range was very broad extending from about pH 2 to about pH 9, and there was an optimum at about pH 5. The optimum hydrogen ion concentration was not very marked because there was considerable activity from as low as pH 4 to as high as pH 7.

The effect of hydrogen ion concentration on α-amylase activity is given in tables 4 and 5. In table 4 the measurements were made with I_2-KI reagent and denote the time in minutes required for α-amylase to convert starch to the colorless stage. In table 5 the amylase activity measurements were Munson-Walker determinations, and denote the net milligrams of maltose formed. The pH range was relatively narrow extending from about pH 4 to about pH 8. The greatest activity was from about pH 5 to about pH 7, being quite pronounced in table 4. The optimum pH was about six as indicated by the disappearance of starch, or about five and six as indicated by the amount of maltose formed. The pH activity range and the optimum pH for α-amylase were essentially the same as 40° C as at 25° C.

The activity rate of two and one-half mg β-amylase was found to exceed that of two and one-half mg α-amylase. Figure 2 presents the activity curves of 15-min digests performed at 25° C for both α-amylase and β-amylase over the entire effective pH range of each.

Table 6 shows what happened when α-amylase and β-amylase were mixed in a starch digest. Experiments were run at 25° C. As measured by the I_2-KI starch disappearance test, the pH range did not vary. The pH optimum was con-

TABLE 3

Effect of pH on β-amylase activity

pH	Amylase activity 25° C for 15 min
	mg maltose
2.15	0.2
2.9	26.7
4.0	74.3
5.0	77.6
6.0	70.9
6.9	67.6
8.0	37.5
8.8	12.5

Amylase activity measurements are Munson-Walker determinations, and denote net mg maltose formed. All measurements are averages of two determinations.

The digests contained 25 ml 1% soluble starch, 5 ml β-amylase from 50 mg β-amylase per 100 ml stock solution, and 20 ml buffer. Blanks with β-amylase boiled 1 min averaged 5.4 mg maltose.

sistently at pH 6, and amylase activity was always slightly greater at pH 5 than at pH 7.

Results of additional experiments conducted were as follows (data not given): (1) Diastase purified by the method given in Meyer and Anderson's plant physiology laboratory manual (1941, page 67) gave the same pH curve of activity as the original commercial material. (2) Clark and Lub buffers furnished the same pH activity curve as when MacIlvaine buffers were used. (3) The addition of chloride did not stimulate the activity of β-amylase. Zero, five, 25, 50, and 100 mg NaCl were added to successive digests of 50 ml each at 25° C. These measurements were made by the Munson-Walker method. The amount of chloride and other anions present in the β-amylase should have been determined as there may have been a sufficient quantity of anions in the commercial preparation to give maximum activity. (4) The activity of α-amylase was stimulated much less by the addition of chloride than was the activity of diastase. Chloride measurements revealed that each 0.5 gm of α-amylase contained chlorides equivalent to 25 mg NaCl as compared to 9.7 mg NaCl for each 0.5 gm of diastase of malt. Hence, it appears that the comparatively small chloride stimulation of the α-amylase

TABLE 4

Effect of pH on α-amylase activity

pH	Amylase activity 25° C min	pH	Amylase activity 24.5° C min
2.2	∞	2.2	∞
3.0	∞	3.0	∞
3.99	∞	3.95	∞
4.98	155	5.00	116
5.95	120	6.00	106
6.82	160	6.90	150
7.85	∞	7.9	∞
8.8	∞	8.8	∞

Amylase activity measurements were made with I_2KI reagent and denote the time in minutes required for α-amylase to convert starch to the colorless stage.

The digests contained 50 ml 1% soluble starch, 2 ml α-amylase solution from 50 mg α-amylase per 100 ml stock solution, 40 ml buffer, and 8 ml water.

TABLE 5

Effect of pH on α-amylase activity

pH*	Amylase activity		
	2½ mg	5 mg 40° C	25 mg 40° C
	mg maltose	mg maltose	mg maltose
2.15	0.8		
3.0	0.5		
4.05	17.5	40.9	52.6
5.15	42.6	67.4	86.2
6.12	43.4	66.6	85.7
7.0	35.1	61.0	78.5
8.0	11.7	39.8	52.6
8.9	−2.3		

Amylase activity measurements are Munson-Walker determinations and denote net mg maltose formed in 15-min digests. All measurements are averages of two determinations.

*pH of digests at 40° C (5 mg) were 4.15, 5.15, 6.1, 7.0, and 7.9. pH of digests at 40° C (25 mg) were 4.0, 5.1, 5.95, 7.05 and 8.0.

was due to its higher anion content. (5). The stimulation of diastase activity by nitrate and chloride anions was found to be additive. (6) The diastase accelerator in onion juice reported by Eyster (1948) was found to be due mostly to the chloride anion present. Since there were small amounts of additional anions present, it may be concluded that the total acceleration could be the result of the sum total of the stimulation produced by each of the anions present. (7) Variations in the optimum temperature for diastase of malt activity occurred with changes in the hydrogen ion concentration. At pH 8, the optimum temperature was approximately 50° C; at pH 6, approximately 40° C. These measurements were based on the rate of disappearance of starch using I_2-KI reagent. (8.) The optimum temperature for α-amylase activity was approximately 50° C at pH 5.95 as measured by the Munson-Walker method. (9) The optimum temperature for β-amylase activity ranged from 40 to 50° C at pH 5.0 for 5-min Munson-Walker digests. (10) Whereas 1 M glucose and 1 M sucrose retarded diastase activity about one-half (Eyster, 1942), only about 0.1 M maltose was required to produce a similar reduction in diastase activity. (11) Maltose retarded diastase activity as much at pH 7 as at pH 6.

DISCUSSION

Diastase is a mixture of enzymes which digests amylose (starch) to maltose. Diastase of malt consists of α-amylase, β-amylase, phosphatase, and numerous inorganic and other organic compounds. Beta-amylase is the enzyme which

TABLE 6

Digestion of soluble starch by mixtures of α- and β-amylases

pH	1 mg α	1 mg α + 1 mg β	1 mg α + 2 mg β	1 mg α + 3 mg β	1 mg α + 4 mg β	1 mg α + 8 mg β	2 mg α + 2 mg β
4.13	∞	∞	∞	∞	∞	∞	∞
4.95	116	98	79	72	75	75	31
6.0	106	80	68	63	60	60	23
7.15	150	113	107	105	105	103	37
8.1	∞	∞	∞	∞	∞	∞	∞

Amylase activity measurements were made with I_2KI reagent and denote the time in minutes required for the amylases to convert starch to the colorless stage.

The digests were run at 25° C and contained 50 ml 1% soluble starch, 40 ml buffer, and 10 ml of aqueous amylases and water.

converts amylose quantitatively to maltose and which is able to hydrolyze amylopectin to the residual dextrin. Dormant starchy seeds contain β-amylase, and as the seed germinates α-amylase appears. Alpha-amylase digests both amylose and amylopectin with the production of dextrins containing six or 12 glucose units, depending on whether there are one or two complete turns of the starch helix. The ability of starch to give a blue color with iodine is rapidly lost in the presence of α-amylase but is retained in the presence of β-amylase because of its inability to fully digest amylopectin. The general action of α-amylase is to produce progressively smaller and smaller particles from amylose and amylopectin, while β-amylase hydrolyzes amylose by successive removal of maltose units from the ends of the amylose chains inward. Beta-amylase also partially hydrolyzes amylopectin in a similar manner but the successive removal of maltose units from the ends stops at the first branch (Bonner, 1950). Hanes (1937) has shown that dextrins do not give an iodine color reaction if they contain six or fewer glucose units, that dextrins containing eight or 12 glucose units form a red complex, and that only the longer amylose chains give the typical blue iodine color. Phosphatase is the enzyme which liberates phosphate from phosphorylated sugars.

There is little or no diastase in chloroplasts, and the sugar to starch and starch to sugar reactions in chloroplasts are over pathways facilitated by phosphorylase. The study of diastase, however, has proven especially interesting and worthwhile because of the chloride ion and because of anions in general which accelerate not only diastase activity but also Hill reactions (Arnon and Whatley, 1949) and growth rates of *Chlorella pyrenoidosa* (Eyster, 1958).

SUMMARY

The effective pH range of diastase as determined by two different methods was found to be from four to nine with an optimum at from six to seven. Both the pH range and the pH optimum remained unchanged in digests at 40° C as compared with digests at 25° C. Although chloride anions markedly accelerated diastase activity, they altered neither the pH range nor the pH optimum.

The pH range for β-amylase extended from about pH 2 to about pH 9. Considerable activity occurred between pH 4 and pH 7, and a weak optimum appeared around pH 5. Alpha-amylase showed a pH range of about pH 4 to pH 8. Its greatest activity was exhibited from pH 5 to pH 7, and its optimum was around pH 5 and pH 6. Using equal amounts of α-amylase and β-amylase in separate digests, it was found that the activity rate of β-amylase exceeded that of α-amylase.

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