chloric, nitric, sulfuric), and the acid phosphates of sodium and potassium, all showed optimum activation in those concentrations which have essentially the same actual acidity. This optimum hydrogen-ion concentration, as determined by the electrometric method and expressed by Sörensen's exponent, was found in each case between the limits p_H^+ 4.2 to 4.6.

Additions of free acid in concentrations greater than the optimum have a marked depressing influence upon the activity of the enzyme which is naturally more striking in the case of the stronger acids. Acetic and propionic acids in quantities ten times the optimum decrease the activity about one-half; hydrochloric, nitric and sulfuric acids reduce the activity more than one-half when present in concentrations two and one-half times the optimum, while in presence of five to eight times the optimum of these strong acids the enzyme action was almost entirely destroyed. In determining the diastatic power of malt preparations, acid phosphate may conveniently be used to ensure activation with little danger of excessive acidity.

Whether the activating agent be an acid or a salt, the amyloclastic action, as measured by the Wohlgemuth method, reaches an optimum at a concentration of the activating agent much below that which gives optimum saccharogenic action. Those concentrations which give the optimum saccharogenic activity are so far above the optimum for amyloclastic action (Wohlgemuth method) as to show a distinct inhibitory influence.

We desire to express our indebtedness to the Carnegie Institution of Washington for use of malt amylase preparations which had been purified in connection with investigations conducted by aid of its grants and described in other papers from this laboratory.

For the data given in Tables X and XVI we are indebted to our former associate, Dr. C. F. Hinck.

LABORATORY OF FOOD CHEMISTRY, COLUMBIA UNIVERSITY,
NEW YORK CITY.

STUDIES ON AMYLASES. IX. FURTHER EXPERIMENTS UPON THE PURIFICATION OF MALT AMYLASE.

BY H. C. SHERMAN AND M. D. SCHLESINGER.

In continuing our experiments upon the purification of malt amylase[1] the operations of precipitation, solution, and dialysis which are involved in the purification process have been studied in some detail, with a view to determining quantitatively the loss of diastatic power at each step and,

[1] THIS JOURNAL, 35, 1617–23 (1913).

if possible, finding means to control these losses so as to secure a definite product of maximum activity.

In fractional precipitation with alcohol the greatest destruction of enzyme usually occurs in the first precipitation. Losses in the final precipitation with alcohol and subsequent drying in partial vacuum were found to be less serious than might be expected from the statements of some previous writers. In general, the largest losses of diastatic power occurred during dialysis, but since dialysis is the best available means of separating certain impurities from the enzyme it has not been found practicable to eliminate this step from our purification process. That the loss of activity which occurs during dialysis is not due in any large measure to the passage of the enzyme as such through the dialyzing membrane is shown by examination of the dialysate for diastatic power.[1]

Osborne's theory of the chemical nature of the enzyme[2] affords a possible explanation. If, as Osborne suggested and as our experiments also indicate, the enzyme is a compound of an albumin with a proteose or peptone, which compound is hydrolyzed on heating in water, it would seem probable that a fraction of the enzyme would be hydrolyzed in water solution even at low temperatures. The removal, then, of one of the products of hydrolysis (i. e., the proteose or peptone) by dialysis would result in a further hydrolysis of the enzyme and loss of diastatic power in the solution undergoing dialysis without any accumulation of enzyme in the dialysate. Quantitative experiments upon the loss of activity in dialyzed solutions of the purified enzyme, as contrasted with duplicate solutions kept at the same temperature but not submitted to dialysis, will be described in our next paper.

From the temperature coefficient of such hydrolytic reactions and the decreasing ionization of water on cooling, the hypothesis that the loss of activity during dialysis is chiefly due to hydrolytic destruction of the enzyme leads one to expect, what has now been fully established in our experience, that when the dialysis is conducted at ice-box temperature the loss of diastatic power is much diminished while the removal of impurities through the membrane is but little retarded.

Thus, notwithstanding the relative stability of malt amylase solutions in ordinary conditions at room temperature, it is important that the dialysis of such solutions for the removal of impurities be conducted at lower temperature.

As starting material in many of our recent purification experiments we

---

[1] In a considerable proportion of the cases tested, the dialysate shows some diastatic activity. As this is not only small in comparison with the total loss during dialysis but also very irregular, it is uncertain whether it should be attributed to anything more than imperceptible defects in the membrane.

[2] THIS JOURNAL, **17**, 587–603 (1895); **18**, 536–542 (1896).

have used a concentrated malt extract made[1] by evaporating an infusion of pale malt *in vacuo* on a commercial scale, with stirring to prevent the solution from being heated above 40° in contact with the warming surface. Two such products were used.

Extract No. 1 had a specific gravity of 1.27 and contained 7% of alcohol as a preservative. This concentrated extract had been found capable of forming 17.6 times its weight of maltose from a surplus of 3% arrowroot starch paste in 30 minutes at 37.5°.

Extract No. 2 was from the same malt infusion as the above but was still further concentrated *in vacuo* to a specific gravity of 1.39. It contained only 4% of alcohol as preservative and had been found able to produce 23 times its weight of maltose in 30 minutes under the conditions just given in describing Extract No. 1.

The following illustrates the purification method which we have usually employed when starting with such extracts: One liter of the concentrated malt extract is divided into twenty equal portions, each of which is placed in a collodion sac of 500 cc. capacity.   The twenty sacs are suspended in 30 liters of water in an ice-jacketed metal tank, the dialysate being maintained at a temperature of 5–10° and allowed to dialyze for 24 hours. In sufficiently cool weather the dialysate is slowly but constantly renewed by means of a current of tapwater running at the rate of 800 cc. per minute; when the tapwater is too warm to permit the use of a constant stream, the dialysate is changed two or three times during the twenty-four hours. At the end of this dialysis the sacs always contain a deposit of material which has precipitated with the removal of dialyzable substances from the solution.   This insoluble material, which is without diastatic power, is separated by decantation (with the aid of the centrifuge if necessary) and rejected.   To the clear solution from the sacs (which in this case had increased to about three times its original volume) crystallized ammonium sulfate is added in the proportion of 45 g. to 100 cc., the solution being kept in an ice-bath and stirred almost continuously until the crystals have dissolved (usually about 15 minutes); the precipitate produced by ammonium sulfate is then settled by means of the centrifuge, the liquid decanted and rejected, and the precipitate dissolved in 500 to 600 cc. cold water.   This solution is divided between four 500 cc. collodion sacs and dialyzed as before for about twenty hours, again decanted from sediment, and then treated with an equal volume of (cold) pure alcohol (99.8%) keeping the temperature below 15°.   The material precipitated by this amount of alcohol is separated by means of the centrifuge and rejected.[2] To the liquid enough more cold alcohol is added to make the alcohol

---

[1] This extract was kindly prepared for us by Dr. C. Von Egloffstein of the American Diamalt Company to whom, as also to Mr. Kaltenbach and Dr. Schulhof of the same company, we desire to express cordial thanks for courtesies and coöperation.

[2] This is the material which we have sometimes referred to as "50% precipitate."

content 65% (by volume). The precipitate thus obtained is separated by means of the centrifuge, and dried on a watch-glass in partial vacuum over sulfuric acid in the dark, at a temperature not exceeding 15°.

As will be seen from the abstract of experimental data below, this method of preparation has in several cases been changed for experimental purposes but without resulting in a better product.

The twenty-two preparations made essentially as above described showed diastatic powers ranging from 760 to 1570 (new scale) equivalent to about 1200 to 2350 on Lintner's scale. Thus both the minimum and the average powers are much higher than in the previous year's work; the maximum powers found during the past year are essentially the same as those recorded a year ago.

That a marked advance in the average result did not lead to any increase in the maximum previously observed suggests that the six most active preparations, having powers of 1430 to 1570 (new scale) equivalent to 2150 to 2350 on Lintner's scale, may approximate the maximum activity obtainable by this method of purification. It may also be noted that these products were obtained not only at intervals of several months but also from different materials; 3 of the preparations from kiln-dried pale malt, 1 from green malt, and 2 from the malt extracts described above. Nevertheless it appears probable, for reasons given in this and the following paper, that all the preparations contain inactive albumin in addition to the enzyme itself.

Brief descriptions of the various preparations made during the past year will be given at the end of the paper.

Our most active preparations, made as above described, contain 15.1–15.3% of nitrogen in the dry ash-free substance. Osborne's most active preparation, showing diastatic power of 600 Lintner, contained 16.1% nitrogen. His other preparations varied considerably both in power and in nitrogen content.[1] On account of the fact that our determinations of nitrogen were necessarily made upon very small amounts of material, and in view of the differences in nitrogen content among the various preparations described by Osborne, we do not attach any exact quantitative significance to the difference in the apparent percentages of nitrogen, but conclude simply that our preparations have a slightly lower nitrogen content than Osborne's. The principal difference in the methods by which Osborne's product and our own were obtained is that Osborne dialyzed for several days, apparently at the temperature of the room or of tap-water, while we dialyzed for not over forty-four hours in all and at a temperature below 10°. On following Osborne's general method, we obtained a preparation having practically the same nitrogen content and diastatic power as Osborne's most active product. (Our preparation

[1] THIS JOURNAL, **17**, 591–99 (1895).

124B with 16.0% of nitrogen and a diastatic power of 660 Lintner.) We attribute this increase of nitrogen content and decrease of power to the prolonged dialysis. In accordance with the theory of the constitution of the enzyme and its hydrolysis in solution outlined above, the higher nitrogen and lower power of the products obtained when dialysis is prolonged are explainable as due to the hydrolysis of the enzyme and the removal of the dialyzable constituent. This dialyzable constituent (proteose or peptone) being a hydrolytic product may be expected to contain a lower percentage of nitrogen than the enzyme as a whole. The corresponding albumin-fraction would thus contain more nitrogen than the enzyme as a whole.

In the case of our preparation IIIB the coagulum contained 16.1% nitrogen and the uncoagulable matter 13.7% nitrogen. While Osborne did not analyze the coagulated albumin obtained on boiling the solution of his enzyme, he has elsewhere[1] given an analysis of malt albumin which shows 16.71% of nitrogen.

It therefore seems probable that the lower nitrogen content and higher diastatic power of our best preparations are both due to the fact that the loss of the peptone or proteose fraction of the enzyme has been minimized by restricting the time and lowering the temperature of the dialysis.

It should be stated, however, that on examining one of our most active preparations (IIIB) we found it to contain slightly more coagulable albumin than did Osborne's most active preparation. It is possible that our methods of purification, while minimizing deterioration and yielding a more active product, may be less efficient than Osborne's in freeing the enzyme from some albumin which accompanies it in the malt. The problem of the chemical nature of the enzyme is discussed further in our next paper.

Abstract of Experimental Data.

Numbers 74[2]–76 were preliminary experiments upon a sample of concentrated extract.

Numbers 77–95 were experiments devoted to the study of losses of diastatic power at different steps in purification.

Preparation 96: Malt was extracted with $2^1/_2$ times its weight of cold water for one-half hour, the extract pressed out and filtered, then precipitated with ammonium sulfate and centrifuged, the precipitate dissolved in cold water and dialyzed in collodion sacs against cold distilled water, the solution then filtered and precipitated with alcohol as described above. Power 1170 (new scale).

Preparations 97, 98, 99[3] and 101 made in practically the same manner as the preceding, showed powers of 830, 762, 1140, 780, respectively (new scale).

Preparation 102: Malt was extracted with $2^1/_2$ parts of 10% ammonium sulfate for 1 to 2 hours, pressed and filtered, dialyzed for 23 hours against 10 volumes of cold distilled water with one change of dialysate; freed from sediment by means of the centri-

---

[1] THIS JOURNAL, 18, 556 (1896).

[2] Numbers 1–73 are outlined in THIS JOURNAL, 35, 1620–22 (1913).

[3] Preparation 100 was lost in drying.

fuge, then precipitated with ammonium sulfate and the preparation completed as described in the early part of this paper. Power 1572 (new scale).

Preparations 103 and 106 made in the same manner as preparation 102 showed powers 1340 and 1110, respectively (new scale).

In Experiments 104, 105, 107, 109 variations of method were tried without satisfactory result.

Preparation 108: Like 102 except that the original extraction was with 2% neutral lead acetate. Power 790.

Preparation 110: Like 102. Power 1010.

Preparation 111A was made from concentrated malt extract No. 1 as already described in the text. Power 1100; nitrogen in dry, ash-free substance, 15.2%. As the volume of solution became during dialysis too large to be handled as one preparation, a portion was set aside in the ice-box for several hours and then completed in the same way. This product, called 111B, had a power of 1470[1] and a nitrogen content (in dry, ash-free substance) of 15.2%. The yield of preparation 111 (A and B) was 2.23 g., obtained from 2 liters of the concentrated malt extract.

Preparation 112: 350 cc. of the same concentrated extract (No. 1) treated as in Preparation 111A yielded 0.8 g. with power of 900.

Preparations 113 and 114 were made from extract No. 2; otherwise practically duplicates of 111A; yields 0.22 and 0.23 g. per 100 cc. of original concentrated extract; powers 1020 and 1030, respectively.

Preparations 115 and 116 were made side by side from concentrated extracts Nos. 1 and 2, respectively. The first yielded 0.16 g. per 100 cc. with power of 800; the second, 0.24 g. per 100 cc. with power of 1050.

Preparations 117 and 118 were made from extract No. 2, duplicating the conditions which obtained in the case of preparations 111A and 111B, respectively. Yields per 100 cc., 0.14 and 0.24; powers 1455 and 1340, respectively.

Preparation 119: Like 117; yield 0.27 per 100 cc.; power 1185.

Preparations 120–122: Unsuccessful attempts at further purification by reprecipitation and repeated dialysis. Not only was most of the diastatic power destroyed but the material underwent changes which diminished its solubility both in water and in 50% alcohol. Preparation 122, which had been twice precipitated by ammonium sulfate as an additional precaution against retention of carbohydrate, contained 15.2% nitrogen in the dry, ash-free substance and showed a power of 470.

Preparation 123 was made from extract No. 2 in the same manner as Osborne's Precipitate XIII (THIS JOURNAL, 18, 539–40 (1896)), except that in dialyzing the temperature was kept below 15° and collodion sacs were used instead of parchment. Yield, 0.13 g. per 100 cc.; power, 105; nitrogen in dry ash-free substance, 15.1%.

Preparations 124A and 124B, made from extract No. 1, were obtained in essentially the same manner in which Osborne obtained his Precipitates III and IV (THIS JOURNAL, 17, 593 (1895)). The powers were 330 and 440 (equivalent to about 500 and 660 Lintner) and nitrogen contents (dry, ash-free basis) 15.9 and 16.0%, respectively, thus closely resembling Osborne's Preparation 15 (Ibid., p. 598) from his Precipitate IV.

Preparation 125 was intended as a repetition of 124 and gave products of practically the same power but the yield of the second fraction was too small to permit accurate determination of nitrogen.

LABORATORY OF FOOD CHEMISTRY, COLUMBIA UNIVERSITY,
NEW YORK CITY.

[1] Here, as in all cases, the diastatic power is expressed on the basis of air-dry material.