[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

THE PREPARATION AND PROPERTIES OF AMYLASE FROM GERMINATED WHEAT AND RYE

BY N. M. NAYLOR, MABLE SPENCER AND MARGARET HOUSE

RECEIVED AUGUST 10, 1925 PUBLISHED DECEMBER 12, 1925

The source of a vegetable amylase is usually cereal grain, legume or mold. References are innumerable to the use of malted barley as a material from which to obtain active amylase. Baker and Hulton[1] have studied the end-products of digestion of starch by amylase of rye. Chrzaszcz[2] reports an extended piece of research on the properties of the amylase of barley, wheat, rye, oats and millet. Klempin[3] determined the optimum and lethal temperatures of the amylase obtained from an extract of ground oats.

These researches describe the activity of amylase only as tested on water or glycerol extracts of the germinated grain or on a precipitate obtained directly from this extract. The work of Osborne[4] and that of Sherman and Schlesinger[5] indicate the desirability of studying enzyme materials as solid precipitates obtained as free as possible from the salts and proteins with which they occur in the barley extracts. In the investigation covered by this paper it was desired to make a study of the amylase of wheat and rye which would determine whether the same methods of purification as described by Osborne and by Sherman and Schlesinger for barley, would give an active amylase from these grains, and whether the products obtained would have properties similar to those of malt amylase.

Experimental Part

The experiments were planned to cover two lines of procedure, the preparation of germinated wheat and rye and of amylase from these grains, and the determination of the properties of the amylase precipitates.

Method of Preparation. Germinated Grain.—The grain was allowed to steep for 12 hours in a nutrient solution prepared as described by Bakke and Erdman.[6] It was then spread in a thin layer on a paraffined cheese cloth stretched tightly over a shallow enameled pan, with the nutrient solution just seeping through the cloth. The grain was kept at a low temperature, 16° to 18°, and with access to air in order to prevent the development of mold. Germination was continued in contact with this nutrient solution for a period of three days, since previous experiments had demonstrated that beyond this time no further amylolytic power was developed. At the end of the

[1] Baker and Hulton, *J. Chem. Soc.*, **119**, 805 (1921).

[2] Chrzaszcz, *Biochem. Z.*, **142**, 417 (1923).

[3] Klempin, *ibid.*, **9**, 204 (1908).

[4] Osborne, THIS JOURNAL, **17**, 587 (1895). Osborne and Campbell, *ibid.*, **18**, 536 (1896).

[5] (a) Sherman and Schlesinger, *ibid.*, **35**, 1617 (1913); (b) **37**, 643, (c) 1305 (1915).

[6] Bakke and Erdman, *Am. J. Botany*, **10**, 8 (1923).

germination period the grain was washed to remove adhering salts, air-dried and ground to a fine flour.

Amylase.—The method outlined here is based on the work described by Sherman and Schlesinger[5a,5b] for the preparation of amylase from malt. A definite weight of ground grain was soaked for three hours in 2.5 times its weight of cold distilled water. The entire mass was placed in collodion bags and dialyzed against running tap water for 24 hours and then filtered. The filtrate was treated with solid ammonium sulfate (42 g. per 100 cc.) and the precipitate, which contains the active amylase, was separated by means of a centrifuge, dissolved in a small quantity of distilled water, dialyzed against running tap water and finally against distilled water until free from sulfate. This solution was centrifuged to remove any sac precipitate, placed in fresh collodion bags and concentrated by pervaporation[7] until reduced in volume by one-half. (The rye amylase lost activity in this pervaporation process, and therefore this step was omitted in the preparation of amylase from that grain.) Cold absolute alcohol was added to this solution to a volume of 60% and an inactive precipitate was separated by means of a centrifuge. The filtrate, made up to 80% alcohol, gives an active amylase precipitate.

Properties of Amylase from Wheat and Rye.—The activity of these enzyme precipitates was determined by digestion experiments as described by Sherman, Kendall and Clark.[8] The power reported signifies milligrams of maltose formed in the digestion of 2% Lintner soluble starch substrate for one-half hour at 40°, by 1 mg. of enzyme. The saccharogenic power of the best preparation obtained from rye extract was 150, and from wheat extract, 440. The lower activity of the rye amylase may be explained by the difficulty involved in the separation of the active enzyme from the excess of dextrins occurring in rye extracts.

Experiments were conducted to determine the hydrogen-ion concentration at which these enzyme materials show optimum activity. A series of substrates were prepared containing 0.06 *M* sodium dihydrogen phosphate (dry weight) and various amounts of hydrochloric acid and sodium hydroxide, and the enzyme was allowed to digest each starch substrate for one-half hour at 40°. A portion of each starch dispersion was used for electrometric determination of the hydrogen-ion concentration. The data obtained are shown in Table I.

TABLE I

INFLUENCE OF HYDROGEN-ION CONCENTRATION UPON ACTIVITY OF WHEAT AND RYE AMYLASES

				WHEAT AMYLASE				
P_H	3.49	3.81	4.17	4.51	5.17	5.74		
Power	304	338	407	438	434	333		
				RYE AMYLASE				
P_H	3.19	3.56	4.46	4.54	5.06	5.55	5.67	6.01
Power	62	146	152	148	141	121	101	96

These results indicate that wheat amylase exerts its optimum activity in a starch substrate at P_H 4.5 to 5.1 and in the presence of 0.06 *M* sodium

[7] Kober, THIS JOURNAL, **39**, 941 (1917).

[8] Sherman, Kendall and Clark, *ibid.*, **32**, 1073 (1910).

dihydrogen phosphate. The rye amylase showed a range of optimum activity between P_H 3.5 and 5.0 in the starch substrate as described.

Discussion of Results

It seems logical to consider that starch-digesting enzyme as obtained from various starch-storing grains might be the same substance. To throw light upon this hypothesis, enzyme material has been prepared from germinated wheat and rye by a method corresponding to that used for the preparation of purified enzyme from malt, and some of the properties of these amylase precipitates have been tested. The amylases from wheat and rye show the typical protein tests, as was indicated by the work of Sherman and Schlesinger for malt enzyme. The hydrogen-ion concentrations at which malt amylase exerts optimum activity has been determined by Sherman, Thomas and Baldwin[9] at P_H 4.4 to 4.6; the results obtained for optimum activity of wheat amylase at P_H 4.5 to 5.1 and of rye amylase at P_H 3.5 to 5.0 indicate a similarity in the activity of the amylases of these three cereal grains.

Summary

The amylases from germinated wheat and rye compare very favorably with amylase from malt described by Osborne and by Sherman and coworkers on the following points: (1) they are obtained by the same process of dialysis and fractional precipitation with alcohol; (2) they give the typical protein tests; and (3) they show optimum activity at a corresponding range of hydrogen-ion concentration in the starch substrate.

AMES, IOWA

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY OF JOHNS HOPKINS UNIVERSITY]

THE INFRA-RED ABSORPTION SPECTRA OF ORGANIC DERIVATIVES OF AMMONIA. II. ALPHA-NAPHTHYLAMINE AND SOME MONO- AND DIALKYL-ALPHA-NAPHTHYLAMINES

BY FREDERICK K. BELL

RECEIVED AUGUST 13, 1925 PUBLISHED DECEMBER 12, 1925

Introduction

In this, the second of a series of studies of the infra-red absorption of primary, secondary and tertiary amines, α-naphthylamine and some mono- and dialkyl-α-naphthylamines have been selected for investigation. Like the alkyl anilines, they represent mixed amines in which one aryl group is always present and one or two alkyl groups, corresponding to the secondary or tertiary amine, respectively, are introduced.

Coblentz'[1] measurements of naphthalene, dissolved in carbon tetra-

[9] Sherman, Thomas and Baldwin, THIS JOURNAL, **41**, 231 (1919).

[1] Coblentz, *Carnegie Inst. Publ.*, No. **35**, 127 (1905).