the fact that the conductivity of the material changed but slightly on standing at 110° shows that the impurities had little effect on the conductivity. The conductivity at 110°, after half an hour, may thus be assumed not in error by more than 10%. The results are given in Table I. From No. 1 we calculate $\kappa_{110°} = 0.0128$ mho. The density of the fused salt at 110° was found to be 1.3615 g./cc. From these data we calculate $\Lambda_{110°} = 2.56$.

Summary

*iso*Butoxymethyl-trimethylammonium iodide and hydroxide have been prepared. The electrical conductivity of fused ethoxymethyldiethyl-methylammonium iodide has been measured at 110°.

The decompositions of ethoxymethyldiethyl-methylammonium hydroxide and *iso*butoxymethyl-trimethylammonium hydroxide, on dehydration of the solution at room temperature, have been studied and compared with those of simple quaternary ammonium hydroxides under the same conditions. Ethoxymethyldiethyl-methylammonium hydroxide yields 18.5 mole % of diethylmethylamine and 81.5 mole % of ethylmethylamine, with a corresponding amount of ethylene; *iso*butoxymethyl-trimethyl-ammonium hydroxide yields only trimethylamine. Above 100° the former base yields only diethylmethylamine, upon decomposition. A mechanism similar to that in the decomposition of the simple quaternary ammonium hydroxides is involved. The theory of such decompositions is discussed.

Attempts to prepare crystalline hydrates and anhydrous forms of the above hydroxides failed.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF TRINITY COLLEGE]

EXTRACTION OF MALTASE FROM YEAST[1]

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RECEIVED NOVEMBER 12, 1926 PUBLISHED JULY 5, 1927

Some years ago we were anxious to prepare an active maltase solution to repeat the experiments of Hill[2] on the action of maltase on a strong glucose solution. As we never succeeded in obtaining a very active maltase solution by the methods given by Hill, we decided to learn more about the extraction of maltase.

We were handicapped by not being able to obtain fresh bottom yeast which is richer in maltase than top yeast. We are, however, very much indebted to the Fleischmann Yeast Company for supplying us at all

[1] Read before the Organic Section of the American Chemical Society in April, 1924, at Washington, D. C.

[2] Hill, *J. Chem. Soc.*, **73**, 634 (1898).

times with their baking yeast, a top yeast—in fact, without their assistance this research would have been impossible.

We soon learned that the heating and fine grinding recommended by Hill did not assist in the extraction of the enzyme. Further experiments showed that previous investigators had not recognized the marked effect of temperature, hydrogen-ion concentration and time on the rates of extraction. Neither had the difference between the rates of extraction from fresh yeast and dried yeast been recognized. When full allowance is made for these factors, many of the contradictory statements in the literature in regard to the content and extraction of maltase from fresh and dry yeast become intelligible.

Experimental Part

Preparation of the Yeast.—The pressed yeast consisting of pure yeast cells and containing about 70% of water was either extracted directly or else dried by the following method. The yeast was forced through a 40- or 60-mesh sieve with a pestle and the resulting threads of yeast were spread on glass plates in front of an electric fan operated at a moderate speed for 18 to 24 hours. The yeast at this stage is crisp and brittle and varies in color from light yellow to brown. It was then ground to a powder and put in a vacuum desiccator over phosphorus pentoxide until constant in weight. Yeast dried in this fashion retained its original maltase activity even after nine months.

Extraction of the Yeast.—Three extraction media were used, namely, 4.6 g. of disodium phosphate, $Na_2HPO_4.2H_2O$, 2 g. of magnesium carbonate and 4.6 g. of $Na_2HPO_4.2H_2O$ + 6 cc. of *N* sodium hydroxide solution for 80 cc. of solution in each case. These media gave a P_H, after the yeast was added, of 8.3 to 8.5, 10 to 10.5 and 11.5 to 12, respectively. In each case, the yeast was ground in a mortar with some water and the substance to be used for extraction. It was then rinsed into a flask and diluted to make 80 cc.; 1 cc. of toluene was added and the flask put into a thermostat. At intervals the flask was thoroughly shaken and a sample removed.[3]

[3] Some experiments were carried out to determine the effect of shaking the yeast in the extraction medium in a shaking machine operated at a slow rate. We used dry yeast and a solution of disodium phosphate for extraction. A parallel set of experiments was carried out where the flasks containing the yeast and phosphate solution were hung in the same thermostats with the following results.

Time of extraction, hrs.	3/4	1 1/2	2 1/4	3	4	26
Standing	0.42	0.56	0.67	0.67	0.84	0.71
Shaking	.43	.56	.65	.72	.78	.62

Shaking, therefore, does not appear to help very much and, in fact, is somewhat harmful if continued for a number of hours. The values given above, as well as in all other tables, represent decrease in rotation in degrees on a 2% maltase solution determined as described later.

The rapid and complete removal of the cells from the extract proved somewhat difficult. We tried at first to filter through a Büchner funnel and then through a Mandler filter. This is essentially Hill's method and removes the cells but requires large amounts of extract and a good many filter candles. It was found that filtering through a filter plate covered with a dense damp paper worked equally well. Occasionally, the filtrate had to be filtered a second time. By this method both the time of filtration and the amount of media necessary were cut down. The following table gives a comparison of the two methods.

	A	B	C	D
Plate filtration	0.42	0.56	0.71	1.31
Mandler filtration	.42	.58	.69	1.25

Plate filtration was used in nearly all of the experiments reported. The only difficulty encountered was in the final stages of autolysis when the filter clogged badly, thus delaying the filtration.

Later on, we had the use of a large centrifuge and carried out a few experiments using the same yeast, extraction medium, temperature and time of extraction, but freed half of the extract from cells by the centrifuge and the rest by the method already described, with the following results.

Time of extraction, hrs.	14	17.5	19.5	26
Centrifuge	0.92	0.96	0.94	0.62
Usual filtration	.91	.93	.86	.63

The centrifuge does not remove all the yeast cells, but apparently the few that are left have small influence so far as the apparent maltase hydrolysis is concerned.

The Activity of the Extract.—A definite amount of the filtrate, which was always slightly alkaline, was neutralized to litmus with a 4% solution of potassium dihydrogen phosphate. One cc. of this neutralized extract was added to 20 cc. of either a 2 or 5% solution of maltase hydrate, buffered with phosphates to a P_H of —6.5 to —6.7, previously warmed to 30°. The hydrolysis was allowed to continue for 40 minutes at that temperature. Ten drops of glacial acetic acid were then added to stop the action, and one cc. of aluminum cream to aid in filtration. The volume was made up to 25 cc. and the solution immediately filtered. The extent of the hydrolysis was determined polarimetrically, using a 2-dcm. tube. Blanks were run in all cases.

As just stated, 1 cc. of the neutralized extract was used for hydrolysis but all the results in the following curves have been corrected for 1 cc. of the unneutralized extract which corresponds to the maltase taken from 0.1 g. of dry yeast. In making this correction, which was small, it was assumed that the amount of hydrolysis was directly proportional to the

amount of enzyme present—a rule found to hold for small changes in enzyme concentration when the amount of substrate is large.[4] The ordinates on all of the following curves represent the decrease in rotation of a maltase solution caused by the enzyme extracted from 0.1 g. of yeast when it was allowed to act on the sugar solution for 40 minutes at 30°.

Effect of Temperature on the Extraction of Maltase from Dry Yeast.—Fig. 1 represents what happens when dry yeast is extracted with sodium phosphate at various temperatures. It is obvious that the rate of extracting the enzyme from the cell increases with temperature. The amount of active enzyme in the extraction medium reaches a maximum, different at each temperature, and then falls off. Apparently, the best conditions for its extraction are not the best for its preservation. So long as the rate of extraction of maltase exceeds the rate of inactivation,

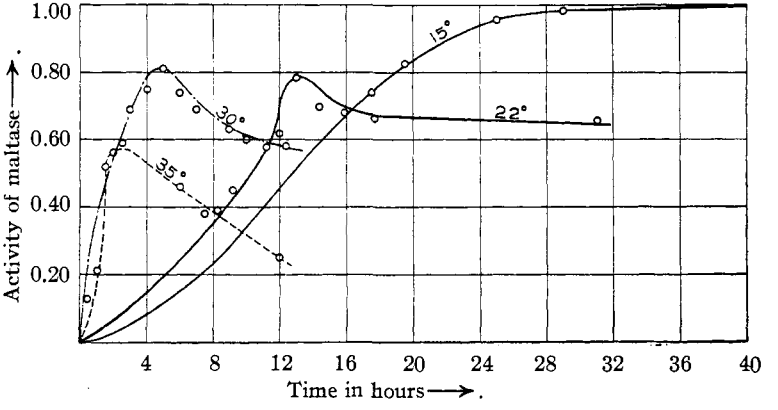

Fig. 1.—Na_2HPO_4 extraction of dry yeast at various temperatures.

the activity increases but when the rates are reversed, the activity falls. It is much easier to obtain a strong maltase solution at a lower temperature as the destruction is slower; furthermore, it is easier to stop extraction at the maximum activity. Similar results were obtained with the other two extraction media.

The Effect of the Hydrogen-Ion Concentration on the Extraction of Maltase from Dry Yeast.—Hill[2] first pointed out that associated with the autolysis of the yeast cell and the extraction of the maltase, there is a production of acidity in the extraction medium. This has also been observed by Emmerling, Willstätter and others. Most of these investigators neutralized this acid with a solution of sodium hydroxide or ammonium hydroxide or else added a buffer to take care of it. No one, however, has made a systematic study of the effect of the reaction of the medium on the extraction. Fig. 2 gives the activity of maltase obtained

[4] Bayliss, "The Nature of Enzyme Action," Longmans, Green and Co., London, 1919, 4th ed., p. 107.

at 30° by three different extraction media, having a hydrogen-ion concentration varying from 8.3 to 12. Similar results were obtained at other temperatures. It is obvious that one needs a buffer near neutrality for obtaining the most active extract.

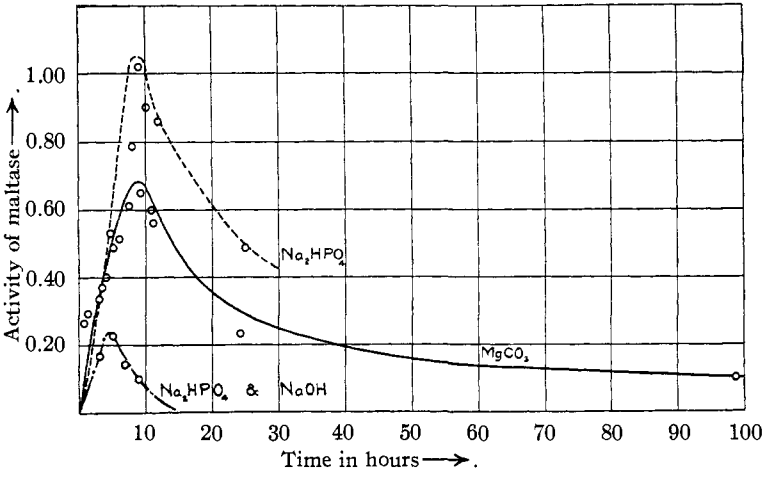

Fig. 2.—30° extraction of dry yeast at various P_H values.

The Effect of Hydrogen Ion, Temperature and Time on the Extraction of Maltase from Fresh Yeast.—Figs. 3, 4 and 5 show what happens when the extraction medium and temperature are varied, using fresh yeast. These experiments were all carried out on the same sample

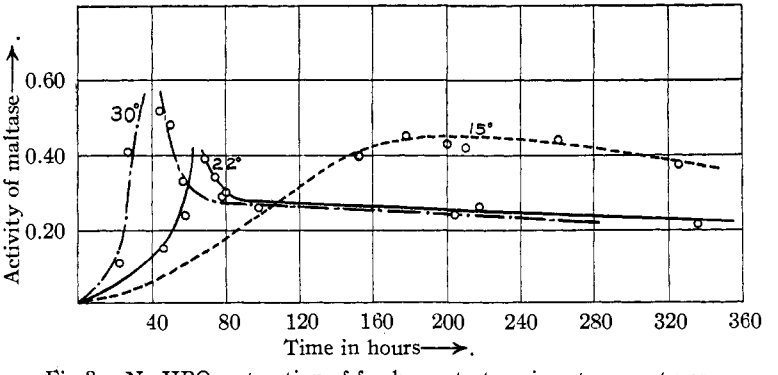

Fig 3.—Na_2HPO_4 extraction of fresh yeast at various temperatures.

of yeast. The extraction in all cases is very much slower with fresh yeast than with dry yeast, as the time scale is different in Figs. 3, 4 and 5 from that used in Figs. 1 and 2. It is apparent that the hydrogen-ion concentration in the extraction medium is not nearly so important with fresh yeast as it is with dry yeast, as the maximum activity observed for a

given temperature is almost the same for the three hydrogen-ion concentrations studied. With dry yeast there was in some cases a four-fold difference. The effect of the temperature at which the extractions are made is also different between the fresh and the dry yeast; 15° seems to be the best for the dry yeast, whereas 30 or 35° is somewhat more

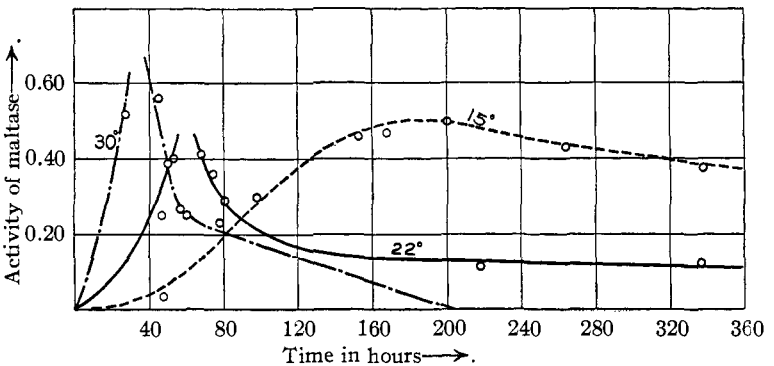

Fig. 4.—$MgCO_3$ extraction of fresh yeast at various temperatures.

favorable for fresh yeast. Fig. 6 also illustrates this point. This diagram also brings up another very interesting fact, namely, the great difference in samples of yeast. The greatest activity observed in Fig. 6 is nearly 100% higher than that observed in Fig. 3, a corresponding experiment on another sample. We found even greater differences at times between

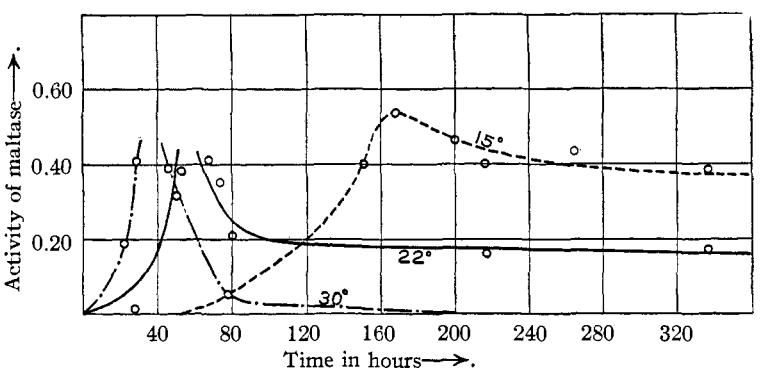

Fig. 5.—Na_2HPO_4 + NaOH extraction of fresh yeast at various temperatures.

samples which appeared to be perfectly fresh. Whether this is due to the age of the cells, the time interval during shipment, or to the media in which it was grown we are unable to say. It is not due to experimental error, for the results could be checked with the same sample of yeast within a few hundredths of a degree on the polariscope.

Willstätter,[5] describes a method to determine the amount of maltase

[5] Willstätter, *Z. physiol. Chem.*, **111**, 157 (1920).

in yeast quantitatively. We followed the method as given and found that the extract which corresponds with 0.256 g. of dry yeast caused a decrease of 0.64° in a 5% buffered solution of maltose hydrate. A part of this same sample of yeast was dried and extracted with disodium phosphate as already described. At the maximum of the extraction curve, 0.1 g. of dry yeast caused a decrease of 0.84° in a 5% buffered solution of maltase hydrate. As the maximum point on our curve can hardly represent the total amount of maltase present, it would appear that Willstätter's method cannot be considered as a quantitative method.

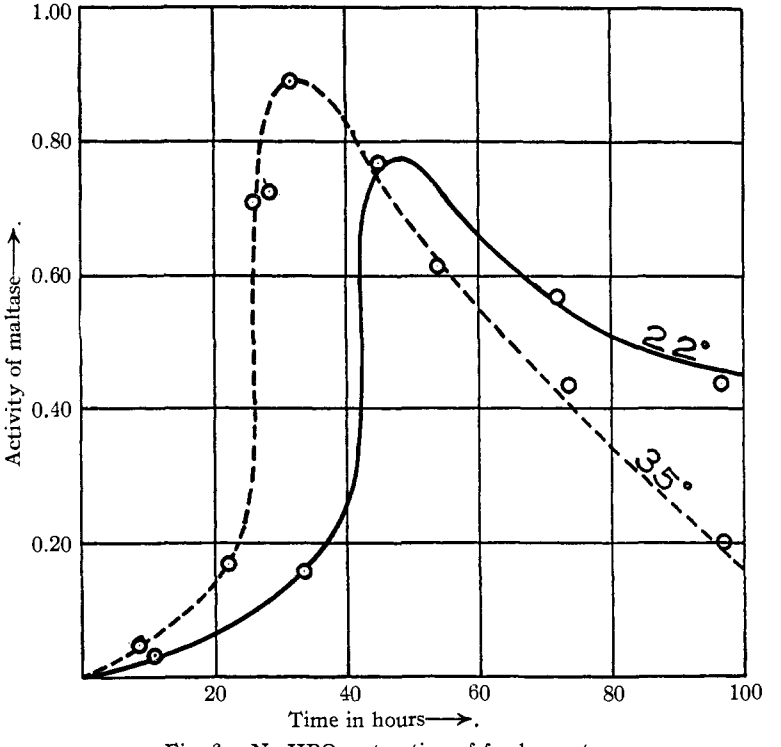

Fig. 6.—Na_2HPO_4 extraction of fresh yeast.

Summary

1. The rate of extraction of maltase from dry yeast varies with temperature. The lower the temperature, the slower the rate.

2. The maximum activity of the maltase extract obtainable from dry yeast varies with hydrogen-ion content of the extraction medium. The more nearly neutral the extraction medium, the more active the extract obtainable.

3. The rate of extraction of maltase from fresh yeast is always slower than from dry yeast.

4. The amount of alkali added to the extraction medium is not as important with fresh yeast as with dry yeast.

5. With dry yeast, 15° is a more satisfactory temperature for extraction than 30°, while with fresh yeast the reverse is true.

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[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY OF THE UNIVERSITY OF CHICAGO]

THE PREPARATION OF β-TRIPHENYLETHYLAMINE. REARRANGEMENT OF β-TRIPHENYLPROPIONHYDROXAMIC ACID

BY LESLIE HELLERMAN

RECEIVED DECEMBER 27, 1926 PUBLISHED JULY 5, 1927

The preparation of β-triphenylethylamine, $(C_6H_5)_3CCH_2NH_2$, was required in one of a series of researches now in progress in this Laboratory on the mechanism of the oxidation of compounds containing the primary amino group. In order to approach the problem of the exact nature of intermediate products formed when a primary amine of the type RCH_2NH_2, for example, is oxidized (for instance, in alkaline solution by halogen or by alkyl hypohalite) it was considered desirable to start with a substance of such structural properties that the intermediate products of its oxidation might reasonably be anticipated to possess considerable stability. A consideration of the structure of β-triphenylethylamine, where R is the triphenylmethyl group, from this point of view led to the selection of this substance as a decidedly promising starting product for investigation. It is to be noted, moreover, that this amine possesses potentially reactive hydrogen atoms only on the carbon atom adjacent to the amino group.

The synthesis of β-triphenylethylamine was first attempted by Elbs,[1] who reported that the amine resulted when triphenyl acetonitrile, $(C_6H_5)_3CC{\equiv}N$, was reduced in alcoholic solution with zinc and hydrochloric acid. Elbs gave a description of the compound, assigning a melting point of 116°, and also reported the preparation of the hydrochloride and the chloroplatinate. No melting point was given for the latter substance and no analyses whatsoever were reported. Later Biltz[2] reported that he was unable to reproduce the work of Elbs. An exhaustive study of the action of zinc upon the nitrile under varying conditions of solution, concentration and acidity failed to produce a method for the preparation of the amine. Biltz found, indeed, that the nitrile was reduced only[3]

[1] Elbs, *Ber.*, **17**, 700 (1884).

[2] Biltz, *Ann.*, **296**, 253 (1897).

[3] Compare the stability of triphenyl acetonitrile toward hydrolysis, E. and O. Fischer, *Ann.*, **194**, 262 (1878).