I.—STUDIES ON INVERTASE. PART I. PREPARATION AND PURIFICATION OF THE ENZYME

By B. N. Sastri and Roland V. Norris.

The first attempt to prepare invertase dates back nearly forty years, when O'Sullivan and Thomson (*J.C.S.*, 1890, **57**, 834) tried to isolate the enzyme from yeast autolysates by precipitation with alcohol. The invertase was thrown out as a precipitate when the concentration of alcohol reached 47 per cent., and contained a large quantity of yeast-gum and protein matter. Repetition of the process after redissolving the precipitate in water gave a product which still contained much yeast-gum and protein. The activity of invertase preparations is now expressed by the "time value," defined by Willstätter and Kuhn (*Ber.*, 1923, **56**, 509) as the time required in minutes to reduce the rotation of a 20 per cent. sucrose solution to 0° using sodium light, when 0·05 gm. of an enzyme preparation dissolved in 5 c.c. of 0·5*N* mono-sodium phosphate solution is added to 20 c.c. of the sugar solution, the hydrolysis being carried out at 15·5°. This value is expressed as $\pm 0^\circ = t$ minutes. O'Sullivan and Thomson's most active preparation had a time value $\pm 0^\circ = 25{\cdot}1$ minutes and from a study of its properties they concluded that it contained both carbohydrate and albuminoid constituents. The preparation gave strong Millon and xanthoproteic reactions but not the biuret test. That the preparations obtainable by such methods always contain much yeast-gum is evidenced by the researches of Salkowsky (*Z. physiol. Chem.*, 1900, **31**, 305). Protein impurities are also present but are removable, as the researches of Osborne (*Z. physiol. Chem.*, 1899, **28**, 399) and Kolbe (*Z. physiol. Chem.*, 1900, **29**, 429) have shown. Osborne coagulated all the proteins of the yeast by means of alcohol, and, after decanting the alcohol, allowed the yeast to autolyse under chloroform water. The autolysate after filtration was treated with alcohol and the invertase precipitated thereby. This gave a preparation more or less free from proteins, but it contained almost the whole of the yeast-gum originally present in the raw material. O'Shima (*Z. physiol. Chem.*, 1902, **36**, 363) attempted to purify Osborne's preparations by precipitating with copper acetate the yeast-gum as the copper salt, but his method was not a sure one. Moreover, the activity of the enzyme was reduced, part of the enzyme being destroyed. The most difficult problem encountered in the preparation of pure enzymes is the removal of substances closely related to the enzyme itself and probably consisting of inactivated enzyme, the zymogens and decomposition products of the enzyme.

Lead acetate has been used by Nelson (*J. Amer. Chem. Soc.*, 1914, **36**, 363) for the precipitation of proteins from yeast autolysates, the

lead from the invertase-containing filtrates being subsequently removed by the use of sulphuretted hydrogen. The invertase is then precipitated by alcohol, but again is far from pure, still containing yeast-gum.

The above methods all suffer from one defect or another, and have therefore been abandoned in favour of adsorption. The latter may be applied in one of two ways, (1) adsorption of impurities in preference to the enzyme, the enzyme being then recovered from the filtrate, or (2) adsorption of the enzyme in preference to the impurities, the enzyme being subsequently eluted from the precipitate. The desired effect is obtained by the employment of suitable adsorbents and strict control of the experimental conditions, a careful and exhaustive study of the various factors affecting the adsorption being required. In some cases both types of adsorption may be utilised and the process used in the present research is such a one.

The following table gives some of the typical adsorption methods which have been employed for purifying invertase, and the activity of the preparation thus obtained :—

TABLE I.

Author	Method of Preparation	Time value, ±0°
1. Euler, Lindberg and Melander (*Z. physiol. Chem.*, 1911, **69**, 152)	Precipitation of yeast autolysate by alcohol, extraction of precipitate by water; adsorption of impurities by kaolin; precipitation of invertase by alcohol	14 mins.
2. Euler and Kullberg (*Z. physiol. Chem.*, 1912, **73**, 335)	Similar	12 ,,
3. Euler and Svanberg (*Z. physiol. Chem.*, 1920, **112**, 282)	Fractional precipitation of yeast liquor by alcohol; adsorption of impurities by kaolin; precipitation of invertase by alcohol; dialysis	7·4 ,,
4. Euler and Svanberg (*Z. physiol. Chem.*, 1919, **107**, 294)	Similar	3·5 ,,
5. Svanberg (*Z. physiol. Chem.*, 1920, **109**, 65)	Similar	4·9 ,,
6. Willstätter and Racke (*Annalen*, 1921, **425**, 1)	Adsorption of invertase firstly by aluminium hydroxide and subsequently by kaolin	0·86 ,,
7. Willstätter and Racke (*Annalen*, 1922, **427**, 111)	Adsorption by aluminium hydroxide and then by kaolin	1·4 ,,
8. Willstätter, Graser and Kuhn (*Z. physiol. Chem.*, 1922, **123**, 1)	Precipitation of yeast liquor by lead acetate and purification by aluminium hydroxide	0·2 ,,
9. Euler and Josephson (*Ber.*, 1925, **56**, (B), 453)	Precipitation by alcohol; extraction of precipitate by water; adsorption on aluminium hydroxide.	0·3 ,,
10. Josephson (*Arkiv Kemi. Mineral Geol.*, 1923, **8**, 21)	Adsorption on aluminium hydroxide; subsequent adsorption on kaolin; repetition of above	...

The table shows that the methods often yield invertase of high time value indicating low purity. In only one case is there a preparation of time value $\pm 0^{\circ} = 0{\cdot}20$ min., this being the highest degree of purity yet attained.

A repetition of some of the above methods has been made in this laboratory with variable results. A method was subsequently developed which gave preparations of very low time value, sometimes as low as those of Willstätter. This method is reliable and yet very simple and easy to conduct.

Autolysis of Yeast.

As usual the starting material was brewers' pressed yeast, 50 lbs. of which were obtained from a local brewery. The yeast was thoroughly washed three times with tap water, a process of considerable difficulty, but on stirring the yeast with large quantities of water and transferring to tall, wide-mouthed cylinders, the yeast settled less slowly and the supernatant liquid could be syphoned. Addition of a small quantity of taka-diastase hastened the process of settling. The washed product was mixed with twice its weight of water and about 500 c.c. of toluene and set aside to autolyse at room-temperature in enamelled cast-iron tanks. That toluene is a very suitable autolysing agent has been shown by Hudson (*J. Amer. Chem. Soc.*, 1914, **36**, 1566) and also by Willstätter (*Annalen*, 1921, **425**, 1). At first a strong fermentation takes place with evolution of carbon dioxide in large amount followed by liquefaction. The whole bath was stirred every day by wooden paddles and fresh toluene added every now and then to replace the losses due to evaporation. Samples of autolysate were removed every day after the fifteenth day, centrifuged at 3,000 revolutions per minute for 10 minutes, and the time value of the clear liquid determined. It is found that the time value gradually falls and reaches a steady figure after about 34 days when the value is $\pm 0^{\circ} = 430$ mins., remaining practically constant even after one year. The autolysate contains 1·7–1·9 per cent. of yeast-gum calculated on the dry weight, the gum being determined by precipitation as copper salt by adding Fehling's solution (Salkowsky, *Z. physiol. Chem.*, 1919, **61**, 124). There is also present a large quantity of amino-acids set free by the hydrolysis of proteins by the yeast endo-tryptase (*Annalen*, 1921, **425**, 1), a large quantity of unhydrolysed proteins, and mineral salts consisting principally of phosphates. Several types of filter-presses and centrifuges were tried for filtering the large amounts of liquid employed but they were found unsuitable; finally, large folded filter-papers were used. The liquid after filtration was clear and yellowish, but became dark on standing; it was preserved under toluene. Throughout this work autolysates more than eight months old have alone been used. The time value of the liquor was $\pm 0^{\circ} = 430$ mins.

Method of Determining the Time Value.

Determination of the time value as defined above was carried out according to the method of Willstätter. The invertase-containing solution was mixed with the buffer-containing sugar solution, the quantity of invertase being so adjusted that the time required to reach zero rotation was between 60–180 minutes under normal conditions. When the decomposition was between 50 and 75 per cent., 25 c.c. of the reaction mixture was pipetted into a flask containing 5 c.c. of 2-*N* soda, allowed to stand at room-temperature for at least 15 minutes (Vosburgh, *J. Amer. Chem. Soc.*, 1921, **43**, 1693) and examined for rotation in a decimeter tube. Three more readings were taken in the same way at noted intervals, and the time for zero rotation to be attained was ascertained by a graphical method. The time value could also be calculated by applying the formula:

$$k = \frac{1}{t} \log \frac{a}{a - x},$$

a being concentration of sucrose solution before, and $a - x$, the concentration after, time t, and k = the velocity constant, the null-rotation time being calculated by the relation

Reaction constant × null-rotation time = 0·597 (at 15·5°).

Concentration of Yeast Autolysates.

The liberation of invertase, which is an intra-cellular enzyme, takes place best under certain conditions of concentration of the yeast suspensions, and for that reason the yeast should be mixed with a fairly large amount of water for the autolysis. The liquor can subsequently be concentrated, and this is necessary for two main reasons: (1) it facilitates the precipitation of invertase by ammonium sulphate, the required amount of which is thus minimised and (2) the adsorption of impurities by kaolin, as described below, is greater from concentrated solution than from more dilute ones. This concentration, which is thus so important, has been carried out by the very simple freezing method described in the note following this paper.

For the problem on hand, it is sufficient to concentrate the yeast-liquor two-fold, and this is effected in one freezing operation. The time value of the concentrated liquor, ± 0° = 410 mins., is nearly the same as the original, because not much impurity is removed in the process and the weight of impurities increases in proportion to the invertase.

Adsorption by Kaolin.

When a limited quantity of kaolin is added to the yeast extract adsorption of the albuminoid matter occurs, but no appreciable amount of invertase is removed, and this important fact is applied in purifying

the yeast invertase. Another very important point revealed by the investigation was the selective character of the adsorption in very dilute solutions, relatively more invertase being taken up than when more concentrated solutions are employed. On the other hand, the impurities, which are adsorbed with more difficulty, are taken up in larger quantities when in concentrated solutions. Hence it is necessary to carry out this preliminary adsorption with concentrated yeast autolysates, the concentration being effected by the freezing method referred to above. It is of interest to note that Krant and Wenzel (*Z. physiol. Chem.*, 1925, **142**, 71) independently came to a similar conclusion.

Preparation of Kaolin.—Kaolin for adsorption purposes was prepared by treating pure kaolin with sufficient 20 per cent. hydrochloric acid to cover it in a beaker and heating the beaker on a water bath for one hour with constant stirring. The acid was then filtered on a Buchner funnel and the kaolin washed repeatedly with distilled water until the washings were free from acid as tested by litmus. The kaolin was then heated on a water bath for one hour with constant stirring with distilled water, again filtered, and washed. It was then dried at 110° and preserved in a stoppered bottle. The kaolin thus prepared served admirably for the purpose.

The adsorption of the impurities by kaolin is carried out at the ice-box temperature.

Concentration of Invertase and further Removal of Impurities.

The concentration of invertase is effected by a process of precipitation from the yeast autolysates.

Precipitation by Alcohol.—Attempts to precipitate invertase by alcohol, ether, and acetone prove unsuitable as the invertase is destroyed sooner or later when left in contact with the precipitants. Invertase is precipitated from the yeast extract on addition of 95 per cent. alcohol when the concentration of the latter reaches 47 per cent., but the resulting precipitate is found to be inactive or nearly so. It may, however, be noted that alcohol has the maximum inactivating power at that concentration (Hudson and Paine, *J. Amer. Chem. Soc.*, 1914, **36**, 1571). When once it was clear that alcohol inactivates the enzyme it was thought advisable to dispense with its use altogether for, as mentioned before, it is extremely difficult, if not impossible, to separate inactivated enzyme from the active enzyme.

Precipitation by Acetone.—The same is true of acetone as a precipitant. Acetone precipitates invertase when its concentration

reaches 45 per cent., but at that concentration invertase is almost completely destroyed in 16 hours. On the other hand, if the precipitate thrown down is removed immediately after the addition of acetone by a process of centrifuging, the precipitate is active. Thus in one experiment, 50 c.c. of yeast extract were mixed with 22 c.c. of acetone in the centrifuge tube and immediately centrifuged, the whole operation lasting 12 minutes. The precipitate was extracted with 10 c.c. of water, centrifuged and the activity of the clear liquor estimated. For this estimation one c.c. of the solution was treated with 20 c.c. of 10 per cent. cane-sugar at 25° for 30 minutes, when action was stopped by adding 5 c.c. of 0·2-*N* soda. The fall in rotation was determined after allowing the mixture to stand for 15 minutes at the room temperature, and is designated as 'activity' in this paper.

TABLE II.

Material	Activity = change in rotation.
Original yeast liquor	−2·02°
Centrifugate after addition of acetone ...	−0·06°
Precipitate after extraction with 10 c.c. of water	−4·82°

The table shows that the precipitate is active, but partial destruction of invertase is also evident. Experiments were then made to ascertain the minimum concentration of acetone requisite for complete precipitation. The results are tabulated below. The determination of activity was carried out as described above.

TABLE III.

Concentration of acetone, per cent.	Quantity of precipitate	Activity of precipitate	Activity of centrifugate
50·0	Large	−1·96°	−0·06°
44·5	,,	−1·96°	−0·06°
28·6	Scanty	−0·21°	−1·49°
16·7	,,	−0·06°	−1·83°

The activity of the original yeast liquor was −2·02°, the time taken for precipitation and centrifuging 20 minutes, and the precipitate in each case was extracted with 20 c.c. of distilled water and centrifuged.

There was in each case an inactive residue. It is thus advisable to use a process of fractional precipitation by first adding acetone to bring up the concentration to 15 per cent., centrifuging the solids, and then raising the concentration to about 45 per cent. A loss of invertase is, however, inevitable and such losses are often as high as 50 per cent. This method also was therefore abandoned.

Precipitation by Ammonium Sulphate.—50 c.c. of yeast-liquor of initial activity $-3{\cdot}08°$ were shaken up with 40 gms. of ammonium sulphate in a shaking-machine for 30 minutes and left in the ice-box overnight. The dirty white precipitate was separated on the centrifuge, washed by saturated ammonium sulphate solution, extracted with water, and the activity determined. The centrifugate was practically inactive, while almost the whole of the invertase was precipitated and the extract of this precipitate was very active. To ascertain the degree of saturation by ammonium sulphate necessary for complete precipitation, one c.c. of the concentrated yeast-liquor was placed in each of 10 similar test-tubes to which a saturated solution of ammonium sulphate was added in the order, 0 c.c., 1 c.c., 2 c.c., 3 c.c., 4 c.c., 5 c.c., 6 c.c., 7 c.c., 8 c.c. and 9 c.c., the total volume in each case was then made up to 10 c.c. by water. Immediate turbidity occurred with the 10th tube, while in tubes 6, 7, 8 and 9 slight opalescence appeared very slowly, and became detectable only after 30 minutes. The tubes were left overnight in the ice-chest and centrifuged, when it was found that the centrifugate in the 10th tube was practically inactive. The liquids in tubes, 6, 7, 8 and 9, did not clear even after long centrifuging at 2,500 revolutions per minute. The liquors in the other tubes were practically as active as that in 1. This shows that for rapid and complete precipitation a 90 per cent. saturation is necessary. It was further observed that more rapid precipitation occurred from concentrated yeast autolysates than from dilute ones.

Thirty c.c. of yeast-liquor, activity $-2{\cdot}05°$, were shaken with 15 gms. of ammonium sulphate for 30 minutes. Turbidity could not be removed by filtration or by continued centrifuging. On centrifuging, however, a minute quantity of a dirty white solid was thrown out, which gave an extract with very little inverting capacity on washing with a saturated solution of ammonium sulphate and extracting with 10 c.c. of distilled water; 1 c.c. of the extract had an activity of $-0{\cdot}43°$. The turbid liquid was allowed to stand overnight and then centrifuged. A greyish solid was again separated, which gave an extract of low activity when treated as above. The turbidity continued and the turbid liquid was allowed to stand in the ice-chest for another 24 hours. On centrifuging again, a white solid of high activity was thrown out leaving a clear liquor. The solid thus obtained was

almost completely soluble in water. The following table shows the results:—

TABLE IV.

Time of standing after saturation with ammonium sulphate	Activity of precipitate extracted with 10 c.c. of water	Activity of centrifugate	Remarks
20 hours	— 0·43°	— 1·36°	Large quantity of invertase remains in centrifugates.
44 ,,	— 0·46°		
68 ,,	— 2·61°	— 0·56°	Large quantity of enzyme precipitated.

If, however, the yeast-liquor is more concentrated so that its activity is $-3{\cdot}0°$ or greater, precipitation is more rapid and the precipitate can be separated after allowing the liquid to stand in the ice-chest for 30 hours.

The centrifugate after precipitation of invertase by ammonium sulphate, contains almost the whole of the yeast-gum present in the original yeast-liquor, yeast-gum not being precipitated by saturation with ammonium sulphate, a fact which was shown before by Harden and Young (*J.C.S.*, 1912 **101**, 1928). The precipitated invertase is thus gum-free. The invertase in contact with ammonium sulphate is very stable. Thus one preparation left at the room temperature (23–28°) for 20 days showed practically unchanged activity, while a dialysed preparation, containing no ammonium sulphate, lost almost the whole of its activity in less than 2 days under similar conditions. Ammonium sulphate is thus an excellent precipitant for invertase.

Adsorption of Invertase by Aluminium Hydroxide.

Invertase from partially purified yeast liquor is easily adsorbed by ammonium hydroxide, ferric hydroxide and kaolin. The method of preparing the adsorbent seems to be of great importance in determining its efficiency. For instance, four methods of preparing aluminium hydroxide have been described by Willstätter (*Ber.*, 1923, **56 B**, 149, 1117; 1924, **56 B**, 58). The aluminium hydroxide used in these experiments was the 'aluminium hydroxide C' and was found to be best for the purpose.

Preparation of 'Aluminium Hydroxide C.'—A large enamelled cast-iron tank of 12–15 litres capacity was provided with a stirrer having nickel paddles connected with a hot-air motor. Four per cent. ammonia (7·5 litres) were raised by electric heaters to 60° and main-

tained at approximately that temperature in the tank. Aluminium sulphate (500 gms.) was dissolved in 1·5 litres of water, heated to 65°, and poured in very small quantities at a time into the vigorously stirred contents of the tank. When all was added the heating was stopped, but stirring was continued for 15 minutes. The hydroxide precipitate soon settled and the mother liquor could be syphoned. Distilled water (10 litres) was added, the contents of the bath stirred for some time and the mother liquor syphoned. This process was repeated five times. Four litres of 4 per cent. ammonia were then added, the contents heated to 70° and stirred vigorously for 15 minutes. The precipitate was again washed 10-12 times with distilled water by syphoning, after which the wash-water became turbid, and was free from ammonia (Nessler). The preparation is white, voluminous and extremely finely divided.

Aluminium hydroxide absorbs yeast-gum as recognised by Willstätter, but in our experiments the liquor treated with aluminium hydroxide was gum-free and hence no trouble arose. As before remarked, the adsorbent takes up varying quantities of impurities which require to be minimised. The preliminary removal of impurities by kaolin and subsequent precipitation of invertase by ammonium sulphate and dialysis gives a fairly pure invertase preparation and this treatment tends to purify it further. The absorption of impurities takes place in greater quantities in concentrated solutions than in more dilute ones, while, on the other hand, the adsorption of invertase, in preference to the impurities, takes place best in dilute solutions. Hence it is necessary to employ very dilute solutions for the purpose of adsorption. Further, the amount of adsorption is relatively greater from dilute solutions and falls off rapidly the greater the concentration, as can be anticipated from the expression of the adsorption law,

$$x/m = \beta\, p^{1/n}$$

where β and n are constants and x/m = concentration of solid phase; x represents the amount of substance adsorbed by m gms. of adsorbent and p the concentration of the liquid phase.

Influence of P_H *on adsorption.*—The adsorption by aluminium hydroxide takes place best in slightly acid solutions, as the following table shows:—

TABLE V.

Adjusted P_H by acetic acid or sodium hydroxide	4·4	4·7	5·2	5·6	7·9
Per cent. adsorbed by 10 c.c. of $Al(OH)_3$ suspension from 50 c.c. of solution	32	34	36·5	40	11·5

In practice the P_H of the solution will normally be slightly acid.

Temperature-coefficient of adsorption.—The temperature co-efficient of adsorption has also been studied. It is very small, very little difference being noticeable when the absorption was carried out at 0° and at 28°, other conditions being the same.

TABLE VI.

Aluminium hydroxide	Percentage adsorbed	
	0°	28°
2·5 gms.	62·7	62·5
3·5 ,,	71·0	70·0
5·0 ,,	89·0	88·0

Another important fact to be mentioned, which makes the use of aluminium hydroxide particularly advantageous, is that since small quantities of impurities accompany the enzyme during adsorption, the smaller the quantity of adsorbent used the better. Aluminium hydroxide C has a very high adsorbing capacity and is thus excellently suited for the purpose. Moreover, the whole of the invertase should not be adsorbed, as in the last stages less enzyme is adsorbed than impurities. In fact the adsorption should be stopped when 90 per cent. or even less of the enzyme has been assimilated.

ELUTION.

The removal of enzyme from the adsorbate is not accomplished by water, but by aqueous solutions of salts, usually the phosphates, citrates or arsenates of sodium, potassium or ammonium.

The following eluting agents have been tried :—Ammonia, 0·02 per cent., diammonium hydrogen phosphate, 0·1 *N*, potassium dihydrogen phosphate, 1 per cent., sodium carbonate, 1 per cent. and disodium hydrogen phosphate, 1 per cent. in aqueous glycerine (1 per cent.)

Of these ammonia did not function at all, a surprising fact in view of the satisfactory results obtained by Willstätter. The experiments were carried out with various concentrations of ammonia, and using various aluminium hydroxide preparations as adsorbent, all with

the same completely negative result. A one per cent. solution of disodium hydrogen phosphate in 1 per cent. aqueous glycerine gave the best results. The elution is then rapid.

Dialysis.

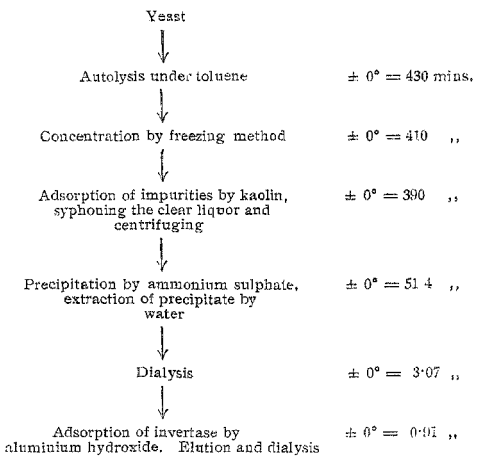
Dialysis is carried out in collodion thimbles prepared according to the method of Farmer (*J. Biol. Chem.*, 1917, **32**, 447). Experiments with parchment thimbles showed that almost the whole of the invertase passes out in about 70 hours. Even with collodion thimbles a loss of about 15 per cent. occurs in 3 days. The dialysis is conducted in a slow stream of distilled water at 0°.

Method of Preparing the Enzyme.

Five litres of the concentrated yeast-liquor, having a time value of $\pm 0° = 410$ mins. was treated with 500 gms. of prepared kaolin, shaken thoroughly for about 30 minutes, and left in the ice-chest overnight. The kaolin having adsorbed large amounts of protein and other impurities, formed a sediment from which the clear supernatant liquid was syphoned and centrifuged; this liquor had a time value $\pm 0° = 390$ mins. The liquor was treated with pure powdered ammonium sulphate in the proportion of 400 gms. to 640 c.c., shaken in a shaking-machine for 30 minutes, and then left in the ice-chest for 30 hours. Complete precipitation was ascertained by testing the activity of the supernatant liquid after centrifuging. The precipitate was then separated from the mother liquor by centrifuging and washed twice with saturated ammonium sulphate on the centrifuge itself. The washed precipitate was then extracted successively with small quantities of water, 560 c.c. being found sufficient. Residue was separated on the centrifuge and consisted of insoluble matter not containing invertase. The slightly yellowish clear extract contained a large proportion of the invertase and had a time value $\pm 0° = 51{\cdot}5$ mins., this low activity being due to its containing a large quantity of ammonium sulphate which can be removed by dialysis, conducted in collodion bags of about 40–50 c.c. capacity in a very slow stream of distilled water for 3 days at 0°. This left a colourless solution with a time value $\pm 0° = 3{\cdot}07$ mins. The dialysed solution was next diluted with water to about one litre and treated with 30 c.c. of an aluminium hydroxide suspension containing 1 gm. of Al_2O_3. The aluminum hydroxide containing the enzyme was centrifuged from the mother liquor and washed twice with distilled water on the centrifuge. The wash-liquor was practically free from enzyme, about 90 per cent. being adsorbed in this way. The washed precipitate was then shaken with 180 c.c. of a 1 per cent. solution of disodium hydrogen phosphate in 1 per cent. aqueous glycerine for 30 minutes, after which the elution

liquid was filtered through a bed of previously ignited kieselguhr. A small quantity of enzyme was lost through adsorption on the kieselguhr, but this retains all the alumina, a part of which would otherwise pass into the filtrate.

About 70 per cent. of the enzyme was extracted by the phosphate mixture. The final dialysis was conducted exactly as before, in collodion bags under a continuous stream of distilled water at 0° for 72 hours. The dialysed liquid had a time value $\pm 0° = 0{\cdot}91$ min. Another preparation obtained by a repetition of the above process had a time value of $\pm 0° = 0{\cdot}80$ min. and a third preparation $\pm 0° = 1{\cdot}10$ min. The following is a diagramatic sketch of the various stages in the preparation.



PROPERTIES.

The preparation is white and free from yeast-gum. It is odourless and gives neither the Molisch test for carbohydrates nor the Millon test for proteins. It, however, gives a very faint biuret reaction and the xanthoproteic reaction. The nitrogen content is very low, as also that of ash. It is free from maltase, oxidase and reductase and all the other enzymes known to be contained in the yeast.

The following table shows the yields in the several stages in the course of the preparation. The various figures are calculated according to the method of Willstätter (*Annalen*, 1921, **425**, 1) :—

TABLE VII.

No.	Enzyme material	Quantity	Null-point time in minutes	Mass-time product M.Z.P. (1)	Mass-time quotient M.Z.Q. (2)	Yield per cent.
1	Brewery yeast ...	10 lbs. (4540 gms.)	112·7 mins. for 1·176 gms. of yeast	·0426	23·48	100
2	Yeast-extract filtered	9 litres	85·1 mins. for 5 c.c.	·0473	21·13	90
3	Yeast-extract concentrated by freezing	5 ,,	96 mins. for 2·5 c.c.	·0480	20·83	88·71
4	Precipitation by ammonium sulphate solution and dialysis of precipitate	560 c.c.	107·7 mins. for 1 c.c.	·192	5·196	22·13
5	Adsorption on aluminium hydroxide; elution and dialysis	180 c.c	108·1 mins. for 1 c.c.	·601	1·665	7·19

(1) The mass-time product is calculated as follows :—

$$\frac{112{\cdot}7 \times 1{\cdot}716}{4540} = 0{\cdot}0426.$$

(2) The mass-time quotient is the reciprocal of the mass-time product, and is a measure of the invertase content.

Further Work :—

Attempts are being made to obtain even higher purity by a second adsorption on kaolin. Another improvement under investigation is the employment of zirconium hydroxide as an adsorbent for invertase.

The line of work followed, and applied to invertase, is suggestive and may be applicable to a number of other enzymes as well. It is not, however, suggested that ammonium sulphate is necessarily the most suitable precipitating agent for all enzymes. Other precipitating agents might be employed and the adsorption methods applied to the already partially purified products.

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