CXCIII. DETERMINATION OF MALTOSE IN PLANT EXTRACTS BY MALTASE.

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(*Received October 27th, 1930.*)

Davis and Daish [1912–13] have shown that during the acid hydrolysis of a mixture of maltose and sucrose, appreciable quantities of the resulting laevulose and smaller quantities of dextrose are destroyed, thereby rendering an estimation of these sugars by acid hydrolysis erroneous. Under conditions recommended by Brown and Morris [1893] for hydrolysing maltose in presence of sucrose, about 30 % of the laevulose is destroyed. A double concentration of the acid, other conditions being the same, destroys nearly 90 % of laevulose and about 6 % of dextrose. Experiments on 1 % maltose solution showed that the hydrolysis of maltose (according to the method of Brown and Morris) had proceeded to the extent of 97 % at the end of 1 hour, increasing to 98 % at the end of 2 hours, but showing a lower value at the end of 3 hours, owing probably to the destruction of dextrose. Prolonged hydrolysis for 24 hours at a lower temperature (70°) accounted for only 94 % of maltose. These authors therefore resorted to fermentation methods, involving a long incubation period of 3–4 weeks, which from many points of view are disadvantageous. It therefore appeared desirable to use maltase for the estimation of maltose, as methods of preparing the enzyme in an active state had been worked out in great detail by Krieble *et al.* [1927].

Experimental.

The enzyme extract was prepared from finely ground brewer's yeast desiccated over phosphorus pentoxide by treating the material with 10 parts of a 5·8 % solution of sodium phosphate at 20° for 14 hours. The extract was centrifuged for 10 minutes and the clear centrifugate, after neutralisation to litmus with a 4 % KH_2PO_4 solution, was preserved at 0°.

A comparative study of the acid and enzymic hydrolysis of a solution of maltose (0·572 g. of pure maltose in 100 cc. of water) was made. Acid hydrolysis, under conditions recommended by Brown and Morris [1893], accounts for 97·5 % of maltose while enzymic hydrolysis accounts for 99·7 % of the sugar. The enzyme extract was used as the source of invertase for the hydrolysis of a 1 % solution of sucrose and an estimation of the invert sugar in

the hydrolysate accounted for 99·8 % of sucrose, thereby establishing the efficiency of the enzyme extract as a rich source of invertase as well.

Experiments were then conducted on a mixture containing 0·5 g. of maltose and 0·5 g. of sucrose in 100 cc. of water. A fresh enzyme extract as well as an old enzyme extract stored at 0° for 7 days were used for the hydrolysis experiments. The temperature and the quantity of the enzyme used for the hydrolysis were varied. In all cases, the hydrolysis was allowed to proceed for 36 hours after which the hydrolysate was clarified with 5 cc. of "dialysed iron," filtered, and the filtrate was made up to 50 cc., 20 cc. aliquots of which were employed for the estimation of the reducing sugar by Bertrand's method.

Another 20 cc. portion of the original sugar mixture was hydrolysed with 2 g. of citric acid for 10 minutes at 97° and after neutralisation with alkali to phenolphthalein, the reducing sugars were similarly determined.

Calculation. The reducing power of the citric acid hydrolysate corresponding to 5 cc. of the solution in mg. of copper is 77·1. The direct reducing power of 5 cc. of the solution in mg. of copper is 27·3. The difference between the two values is that due to invert sugar and, calculating the sucrose content from this, 99·6 % of the sucrose is accounted for.

If A is the reducing power of maltose in mg. of copper of a certain quantity of the solution, and Z and Y, the copper equivalents in mg. of the maltase hydrolysate and citric acid hydrolysate respectively, then

$$A = (Z - Y) \times \frac{212{\cdot}04}{147{\cdot}96}.$$

From this equation A can be calculated, and from the sugar tables, the corresponding quantity of maltose.

Table I.

			Enzyme experiment		
Hydrolysis	HCl 2 hrs.	HCl 3 hrs.	2·5 cc. 25°*	5 cc. 25°	5 cc. 37°
Maltose found %	73·6	62·8	98·8	99·6	94·4

During acid hydrolysis the value of Z decreases largely due to the destruction of the laevulose portion of the invert sugar and also to the slight destruction of the dextrose, thereby reducing the value of maltose determined. It is clear from the above table that while acid hydrolysis is fatal in the estimation of sugar mixtures containing sucrose, the freshly prepared maltase extract is capable of hydrolysing quantitatively maltose and sucrose without destroying the products of hydrolysis. The sucrose in the mixture can be selectively hydrolysed by maltase-free invertase to obtain the value Y, while Z can be obtained by hydrolysing the mixture by the maltase extract which has been shown to be a rich source of invertase.

Estimation of added maltose in plant extracts by the enzyme method. Maltose (0·5291 g.) was added to 50 cc. of tissue fluid from healthy sandal leaves, after clarification with "dialysed iron" to eliminate tannins, proteins etc. 10 cc. portions of this solution containing the added maltose were hydrolysed with

3 cc. of the enzyme extract for 36 hours. Another 10 cc. portion of the clarified tissue fluid without added maltose was hydrolysed under identical conditions and sugar was estimated in 20 cc. of the total solution.

The difference between the above two sets of results gives the reducing power of the added maltose after hydrolysis and in this experiment 96·22 % of the added maltose was found.

Summary.

A reliable method for the estimation of maltose in mixtures of sugars occurring in plant extracts and tissue fluids has been described wherein an enzyme extract rich in both maltase and invertase has been used as the hydrolysing agent. The method is selective in its action and completely eliminates the errors inherent in the method of acid hydrolysis.

REFERENCES.

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