Sucrose

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The determination of sucrose by measurements of the optical rotation after inversion [1] requires apparatus, which cannot be provided by every laboratory. In addition, the accuracy is low if the sample contains small amounts of sucrose and large amounts of optically active compounds. The reducing sugar formed on inversion can be determined chemically [2], if the sample contains virtually no other reducing substances. In comparison, the enzymatic determination of sucrose is simple to carry out on any type of sample and less than 10 μg. sucrose can be estimated.

Principle

Sucrose is hydrolysed by invertase *) to glucose and fructose (equation 1). The two hexoses are phosphorylated by ATP to the corresponding hexose-6-phosphates in the reaction catalysed by hexokinase (HK) (equation 2). Fructose-6-phosphate is isomerized to glucose-6-phosphate by phosphoglucose isomerase (PGI) (equation 3). Glucose-6-phosphate is oxidized by TPN and glucose-6-phosphate dehydrogenase (G6P-DH) to 6-phosphogluconate (equation 4).

(1) $$\text{Sucrose} \longrightarrow \text{glucose} + \text{fructose}$$

(2) $$\text{Glucose} + \text{fructose} + 2\,\text{ATP} \rightleftharpoons \text{glucose-6-phosphate} + \text{fructose-6-phosphate} + 2\,\text{ADP}$$

(3) $$\text{Fructose-6-phosphate} \rightleftharpoons \text{glucose-6-phosphate}$$

(4) $$2\,\text{Glucose-6-phosphate} + 2\,\text{TPN}^+ \rightleftharpoons 2\,\text{6-phosphogluconate} + 2\,\text{TPNH} + 2\,\text{H}^+$$

Therefore for each mole of sucrose 2 moles of TPNH are formed:

(5) $$\text{Sucrose} + 2\,\text{ATP} + 2\,\text{TPN}^+ \longrightarrow 2\,\text{6-phosphogluconate} + 2\,\text{ADP} + 2\,\text{TPNH} + 2\text{H}^+$$

The increase of optical density at 366 or 340 mμ due to the formation of TPNH is a measure of the over-all reaction.

The reactions proceed rapidly and quantitatively if the measurements are made at the pH optima: invertase reaction at pH 4.6; hexose determination at pH 7.6.

Reagents

1. Acetic acid, A. R., 1 N
2. Sodium hydroxide, A. R. 1 N
3. Invertase
   from yeast, dry powder or in solution. Commercial preparation, see p. 985.

In addition: reagents for the determination of fructose and glucose (see p. 156).

Purity of the enzyme preparation

Invertase must be free from hexoses. Commercial invertase solutions are usually stabilized with hexoses and must therefore be dialysed for 6 hours against distilled water before use. The enzyme must be free from melibiase, maltase, β-galactosidase and glucosidases.

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*) Synonyms: saccharase, sucrase, β-fructofuranosidase, β-fructosidase.

1) *A. Beythien:* Laboratoriumsbuch für den Nahrungsmittelchemiker. Th. Steinkopff, Dresden 1931, p. 278.

2) *E. Fischer* and *L. Kohtès*, Helv. chim. Acta *34*, 1123 [1951].

Preparation of Solutions

To avoid the growth of micro-organisms, sterilize the containers.

I. Acetate buffer (0.1 M; pH 4.6):
Dilute 100 ml. 1 N acetic acid and 50 ml. 1 N NaOH to 1000 ml. with distilled water and check the pH (glass electrode).

II. Invertase (*ca.* 2000 units*)/ml.):
Dissolve 20 mg. invertase** in 1 ml. acetate buffer (solution I) or dilute liquid commercial preparations correspondingly.

In addition: solutions for the determination of glucose and fructose (see p. 157).

Stability of the solutions

Store all the solutions, stoppered, in a refrigerator at 0 to 4° C. Prepare the invertase solution freshly each week.

Procedure

Preliminary treatment of the experimental material

Dilute soluble material (*e.g.* fruit juice, sugar beet syrup, artifical honey) with doubly distilled water, so that the solution contains about 0.1 % sucrose. Homogenize partly soluble material (*e.g.* sugar beet) with distilled water, filter off the insoluble residue and wash with distilled water. Dilute the filtrate as described above. Treat material containing soluble protein (*e.g.* ice cream powder, milk caramels) in a similar manner, deproteinize the filtrate with barium hydroxide-zinc sulphate (see p. 120). Deproteinization with acids, for example, with $HClO_4$, leads to partial hydrolysis of sucrose.

Assay

Prepare a blank to determine the glucose, fructose and the two hexose-6-phosphates contained in the sample.

Invertase reaction: Pipette successively in test tubes:

Experimental	*Blank*
5.00 ml. buffer (solution I)	5.00 ml. buffer (solution I)
1.00 ml. sample	1.00 ml. sample
0.20 ml. invertase solution (II)	0.20 ml. distilled water.

Incubate for *ca.* 10 min. in a water bath at 37° C. Adjust to pH 7.6 with 1 N NaOH, rinse out into a 10 ml. volumetric flask and dilute with distilled water to 10 ml. Determine the glucose + fructose in this solution.

Hexose determination: Analyse 1 ml. of the experimental and blank mixtures according to p. 158, but use 0.98 ml. less of the buffer solution.

Calculations

The hexose determination gives the values for the glucose + glucose-6-phosphate and for fructose + fructose-6-phosphate. The free and phosphorylated sugars are not distinguished. If it is wished to estimate them separately, then proceed as described on p. 158.

*) A unit is the amount of enzyme which converts 1 μmole of substrate in 1 min. (refer to p. 32). For an example of an assay, see p. 902 under "Enzymatic reaction".

**) from the California Corp. for Biochemical Research, Los Angeles 63, Calif. USA.

According to p. 158:

$$E_2 - E_1 = \Delta E_{\text{glucose-6-phosphate}}$$

$$E_3 - E_2 = \Delta E_{\text{fructose-6-phosphate}}$$

The differences between the ΔE values for the experimental and blank reaction mixtures correspond to the amount of glucose and fructose liberated in the invertase reaction. If different values are obtained, use the smallest for the calculations (see under "Sources of Error"). Calculate the μmoles glucose (fructose) per assay mixture (cuvette) according to p. 158 and multiply by 10. This value gives the sucrose content per ml. of the diluted (deproteinized) sample. To calculate the mg. sucrose multiply by 0.342 (see "Example").

Example

Lemonade (1 g.) was diluted with distilled water to 100 ml. and 1.0 ml. (=10 mg. lemonade) of this solution was taken for the invertase reaction. Hexose determination: measurements at 366 mμ against 2.0 ml. buffer + 1 ml. sample.

Blank without invertase: $E_1 = 0.079$; $E_2 = 0.091$; $E_3 = 0.104$

$$\Delta E_{\text{glucose-6-phosphate}} = 0.012$$

$$\Delta E_{\text{fructose-6-phosphate}} = 0.013$$

Experimental: $E_1 = 0.078$; $E_2 = 0.348$; $E_3 = 0.668$

$$\Delta E_{\text{glucose-6-phosphate}} = 0.270$$

$$\Delta E_{\text{fructose-6-phosphate}} = 0.320$$

Differences between the experimental and blank measurements:

$$\Delta E_{\text{glucose-6-phosphate}} = 0.258$$

$$\Delta E_{\text{fructose-6-phosphate}} = 0.307$$

According to the general equation on p. 37:

$$\frac{0.258 \times 3.0}{3.3} = 0.235 \text{ μmoles glucose/assay mixture}$$

and $$\frac{0.307 \times 3.0}{3.3} = 0.279 \text{ μmoles fructose/assay mixture}$$

$0.235 \times 10 = 2.35$ μmoles glucose/ml. dilute sample or 235 μmoles glucose/ml. lemonade.
$235 \times 0.342 = 80.4$ mg. sucrose/g. lemonade or 8.04%.

Specificity and Sources of Error

Yeast invertase reacts with saccharides which contain an unsubstituted β-D-fructofuranosyl residue. Higher saccharides of the raffinose type are also hydrolysed, but the rate decreases with the number of galactose residues[3]. Gentianose, the trisaccharide formed from 2 moles of glucose and 1 mole of fructose, is hydrolysed[4] like the β-fructofuranosyl fructoses. There are a number of different views on the hydrolysis of inulin by invertase[5–9].

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[3] *C. B. Purves* and *C. S. Hudson*, J. Amer. chem. Soc. *56*, 702 [1934].
[4] *E. Bourquelot* and *H. Hérissey*, C. R. hebd. Séances Acad. Sci. *135*, 399 [1902].
[5] *C. Neuberg* and *I. Mandl* in *J. B. Sumner* and *K. Myrbäck:* The Enzymes. 1st Edition, Academic Press, New York 1950, Vol. I, part 1, p. 527.
[6] *G. Legrand*, Sucr. belge *70*, 229 [1951].
[7] *G. Legrand* and *C. Lewis*, C. R. hebd. Séances Acad. Sci. *232*, 186 [1956].
[8] *D. M. Mikhlin* and *B. O. Akhumbaera*, Biokhimiya *21*, 186 [1956].
[9] *H. Baumann* and *W. W. Pigman* in *W. W. Pigman:* The Carbohydrates. Academic Press, New York 1957, p. 591.

Of the naturally occurring sugars only sucrose is hydrolysed by invertase to give equal parts of glucose and fructose. If the invertase contains melibiase, then 1 mole of glucose and fructose are formed from raffinose. If it contains maltase, then 2 moles of glucose are formed from maltose.

With the use of pure invertase, an analytical result giving more fructose than glucose always indicates the presence of raffinose in the sample. Since equal parts of fructose and glucose are formed in the hydrolysis of sucrose by invertase the smallest value is taken for the calculations.

Invertase can also act as a transferase and so transfer β-fructofuranosyl residues to acceptors (alcohols). These products accumulate during the invertase reaction, reach a maximum (less than 10% of the total sugar[10]) and then decompose completely as the reaction proceeds. However, these side reactions do not interfere with the sucrose determination, because the samples are too dilute. The hexose content is constant up to at least 4 hours after the invertase reaction is complete.

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[10] *J. Edelman*, Biochem. J. *57*, 22 [1954].