Invertase

Invertase *) occurs in intestine, in bacteria, yeast and moulds, plants and cultivated land. It hydrolyses glycosides which contain β-glycosidically linked fructose (furanose form).

Determination in Food Chemistry

Hans Lück

Principle

Invertase catalyses the hydrolysis of cane sugar (sucrose):

(1) Sucrose + H_2O ⟶ fructose (β-D-fructofuranose) + glucose (α-D-glucopyranose)

The reducing sugars formed on hydrolysis are determined polarimetrically or chemically. Due to their transfructosylase[1,2] and transglucosidase activity[3] various invertases can form intermediate trisaccharides in equation (1).

Optimum Conditions for Measurements

The pH optimum of invertase is between 4 and 6. The invertase from honey has a pH optimum between 5.5 and 6.2[4], while the invertase from yeast has a pH optimum between 4.7 and 4.9[5,6]. With yeast invertase a maximum rate of hydrolysis is obtained with a sucrose concentration of 5 to 10% and then with higher concentrations the rate decreases[7]. The half maximum rate is obtained with a sucrose concentration of between 0.7 and 3%[8]. Generally, preservatives do not interfere with the reaction as long as their concentration does not exceed 0.25%[9].

Reagents

1. Sucrose, A. R.
2. Acetic acid, A. R., 1 N
3. Sodium hydroxide, A. R., 1 N

Only for the *iodometric determination* of the glucose formed:

4. Iodine, sublimed
5. Potassium iodide, A. R.
6. Sodium carbonate, crystalline, A. R., $Na_2CO_3 \cdot 10\ H_2O$
7. Sulphuric acid, A. R., 95 to 97% (w/w)
8. Sodium thiosulphate, A. R., $Na_2S_2O_3 \cdot 5\ H_2O$
9. Soluble starch
10. Mercuric iodide, HgI_2

---

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

1) *J. S. D. Bacon*, Biochem. J. *57*, 320 [1954].
2) *J. Edelman*, Biochem. J. *57*, 22 [1954].
3) *J. W. White* and *J. Maher*, Arch. Biochem. Biophysics *42*, 360 [1953].
4) *J. M. Nelson* and *D. J. Cohn*, J. biol. Chemistry *61*, 193 [1924].
5) *E. Hofmann*, Biochem. Z. *275*, 320 [1935].
6) *J. M. Nelson* and *G. Bloomfield*, J. Amer. chem. Soc. *46*, 1025 [1924].
7) *J. M. Nelson* and M. *P. Schubert*, J. Amer. chem. Soc. *50*, 2188 [1928].
8) *F. J. Bealing* and *J. S. D. Bacon*, Biochem. J. *53*, 277 [1953].
9) *C. F. Poe*, *M. Cooley* and *N. F. Witt*, Ind. Engng. Chem., analyt. Edit. *5*, 309 [1933].

Preparation of Solutions

I. Sucrose (*ca.* 0.3 M):
Dissolve 100 g. sucrose in distilled water and make up to 1000 ml. Boil for a short period.

II. Acetate buffer (0.2 M; pH 4.62):
Dilute 200 ml. 1 N acetic acid and 100 ml. 1 N NaOH to 1000 ml. with distilled water.

Only for the *iodometric determination* of the glucose formed:

III. Iodine-potassium iodide (0.02 N iodine):
Dissolve 1.27 g. sublimed iodine and 2 g. potassium iodide in 3 ml. distilled water and make up to 500 ml. with distilled water. Store in a brown bottle. Check the concentration against thiosulphate solution (VI).

IV. Sodium carbonate (0.1 N):
Dissolve 14.308 g. $Na_2CO_3 \cdot 10\ H_2O$ in distilled water and make up to 1000 ml.

V. Sulphuric acid (*ca.* 10% w/v):
Add 50 g. conc. H_2SO_4 in small portions to 300 ml. distilled water and make up to 500 ml. with distilled water.

VI. Sodium thiosulphate (0.01 N):
Dissolve 2.482 g. $Na_2S_2O_3 \cdot 5\ H_2O$ and 0.1 g. $Na_2CO_3 \cdot 10\ H_2O$ in distilled water and make up to 1000 ml.

VII. Indicator (starch):
Stir 1 g. soluble starch and 5 mg. HgI_2 into a paste with a little distilled water, add to 500 ml. boiling distilled water, allow to boil for a few minutes and then cool.

Stability of the solutions

The solutions are stable virtually indefinitely, providing that bacterial contamination is avoided. The sucrose solution should be prepared freshly more often and be stored in a refrigerator. It should be occasionally boiled for a short period just before use.

Procedure

Enzymatic reaction

If the enzyme solution to be assayed contains large amounts of sugar, dialyse for several hours against running water[10].

Pipette successively into an Erlenmeyer flask:

50 ml. sucrose solution (I)
40 ml. distilled water
5 ml. acetate buffer (solution II).

Warm to 30°C in a thermostat, add

5 ml. sample

and note the time. With low enzyme activity use more of the sample and correspondingly less water. Dilute enzyme solutions which are too concentrated. Add 10 or more ml. acetate buffer (solution II) to strongly buffered samples. After 30, 60, 90 and 120 min. incubation, remove 20 ml. samples and determine the invert sugar content iodometrically or polarimetrically.

---

[10] *F. Kiermeier* and *W. Köberlein*, Z. Lebensmittel-Unters. u. -Forsch. *98*, 329 [1954].

Polarimetric determination

Cool the samples to 20°C and determine the optical rotation in a 20 cm. tube at 20°C with a polarimeter. Clarify turbid or protein-containing solutions by the addition of lead acetate solution *) (5 ml./100 ml.) and then filter. Other clarifying agents are charcoal, ferric hydroxide, aluminium hydroxide and kieselgur. Do not use more of these clarifying agents than is *absolutely* necessary, since they all adsorb some sugar.

The specific rotation $[\alpha]_D^{20}$ [deflection of the plane of polarization (in degrees) by a 10 cm. layer of a solution containing 100 g. of the substance/100 ml.] for sucrose is +66.45° [the value is practically independent of temperature (13–22°C), addition of alcohol and sugar concentration]. The $[\alpha]_D^{20}$ of invert sugar is −20.59° in 10% solution (the value is dependent on temperature, alcohol and acid content, and the sugar concentration). From this it follows that the amount of cane sugar hydrolysed (Δc) in g./100 ml. at 20°C (for an initial cane sugar concentration of 5%) is:

(2) $$\Delta c = 0.57\,([\alpha_S]_D^{20} - 1.05\,[\alpha_I]_D^{20})$$

$[\alpha_S]_D^{20}$ and $[\alpha_I]_D^{20}$ are the optical rotations before and after the incubation with invertase (20 cm. tube). Δc is used for the calculations.

Iodometric determination

This method is especially suitable for the measurement of low enzyme activities. If after incubation with invertase the sample does not contain more than 0.1% glucose (fructose does not react), the glucose can be oxidized directly with iodine solution and the excess iodine back-titrated with thiosulphate.

Instead of the direct iodometric determination the glucose can also be determined by oxidation with *Fehling*'s or *Luff*'s solution and iodometric titration of the unreduced copper.

Pipette successively into an Erlenmeyer flask:

20 ml. sample (after 30, 60, 90 and 120 min. incubation in the enzymatic reaction)
25 ml. iodine solution (III)
20 ml. sodium carbonate solution (IV).

Allow to stand in the dark for 1/2 to 1 hour and then add

5 ml. 10% H_2SO_4 (solution V)
3 drops starch solution (VII).

Back-titrate with thiosulphate solution (VI).

Calculations

If the samples removed at different times are back-titrated with $Na_2S_2O_3$ solution, calculation of the absolute values for glucose is unnecessary. Only the difference of the ml. $Na_2S_2O_3$ solution consumed is used for the calculations. 1 ml. 0.01 N thiosulphate solution corresponds to 1 ml. 0.005 M glucose solution or 0.9005 mg. glucose or 1.7115 mg. hydrolysed sucrose. From the difference ΔV of the ml. thiosulphate solution consumed, the amount of cane sugar hydrolysed (Δc) in g./100 ml. is calculated as follows:

(3) $$\Delta c = 5 \times \Delta V \times 1.7115 \times 10^{-3} = 8.5575 \times 10^{-3} \times \Delta V$$

ΔV = difference in the ml. 0.01 N thiosulphate solution consumed.

*) Solution of basic lead acetate. Preparation: dissolve 3 g. $Pb(OAc)_2 \cdot 3\,H_2O$ in 10 ml. distilled water and dissolve 1 g. lead oxide (PbO) in this solution.

The enzymatic hydrolysis of cane sugar is a zero order reaction under the conditions described here. Either the reaction rate constant k (equation 4) or the reciprocal of the time (= invertase units[11]) in which 50% of the sucrose in a 4.75% sucrose solution is hydrolysed (= 2.375 g. sucrose/100 ml.) (related to 1 g. enzyme preparation in 50 ml.) can be used as a measure of the activity:

(4) $$k = \frac{1}{E} \times \frac{\Delta c}{t}$$

where

E = g. enzyme preparation (dry weight) in the hydrolysis reaction mixture (100 ml.).
Δc = g. of sucrose hydrolysed/100 ml.
t = time in min.

Example

Determination of invertase activity in honey. The honey was dissolved in a little water and dialysed for 3 hours against running water. 5 ml. of the solution in the dialysis sac corresponded to 0.9 g. honey. In the iodometric determination of glucose the 20 ml. samples removed at 30, 60 and 90 min. required 4.03, 3.52 and 3.00 ml. of 0.01 N $Na_2S_2O_3$ solution respectively. The mean of the difference of the titration values ΔV is 0.515. According to equation (3)
$\Delta c = 8.5575 \times 10^{-3} \times 0.515 = 4.407 \times 10^{-3}$ g. sucrose hydrolysed/100 ml. hydrolysis reaction mixture.
According to equation (4):

$$k = \frac{1}{0.9} \times \frac{4.407 \times 10^{-3}}{30} = 1.632 \times 10^{-4}\ \text{min.}^{-1}.$$

If the hydrolysis continued so long, 2.375 g. sucrose would be hydrolysed in 1.46×10^4 min. The honey therefore contained

$$\frac{1}{2 \times 1.46 \times 10^4} = 3.42 \times 10^{-5}$$ invertase units according to[11].

(2 = conversion from 100 ml. to 50 ml.)
This relatively low invertase activity indicates that the honey had been heated more than is permissible.

Determination in Botanical and Agricultural Chemistry

Eduard Hofmann

Principle

See p. 901. However, in this case the invert sugar liberated is determined volumetrically with *Fehling*'s solution.

Reagents

For the enzymatic reaction:

1. Disodium hydrogen phosphate, $Na_2HPO_4 \cdot 12H_2O$
2. Acetic acid, A. R.
3. Toluene
4. Sucrose

For the sugar estimation:

5. Cupric sulphate, $CuSO_4 \cdot 5H_2O$
6. Potassium-sodium tartrate, $KNaC_4H_4O_6 \cdot 4H_2O$, Seignette salt

[11] *R. Weidenhagen*, Ergebn. Enzymforsch. *1*, 168 [1932].

7. Sodium hydroxide, A. R.
8. Sulphuric acid, 25% (w/v)
9. Potassium iodide, A. R.
10. Sodium thiosulphate, A. R., $Na_2S_2O_3 \cdot 5H_2O$
11. Sodium carbonate, A. R., $Na_2CO_3 \cdot 10H_2O$
12. Soluble starch
13. Sodium chloride, 25% (w/v)

Preparation of Solutions

For the enzymatic reaction:

I. Buffer (pH 5.5):
   a) Dissolve 358 g. $Na_2HPO_4 \cdot 12H_2O$ with warming in distilled water, make up to 1000 ml. and cool to 25°C.
   b) Dilute 60 ml. acetic acid to 1000 ml. with distilled water.
   Mix equal parts of solutions a) and b). Check the pH with a glass electrode and, if necessary, adjust with solution a) or b).

II. Sucrose (20% w/v):
   Dissolve 200 g. sucrose in distilled water and make up to 1000 ml.

For the sugar estimation:

III. *Fehling*'s solution:
   a) Dissolve 34.64 g. $CuSO_4 \cdot 5H_2O$ in distilled water and make up to 500 ml.
   b) Dissolve 173 g. Seignette salt and 50 g. NaOH in distilled water and make up to 500 ml.
   Immediately before use mix equal parts of solutions a) and b).

IV. Potassium iodide (33% w/v):
   Dissolve 3.3 g. KI in distilled water and make up to 10 ml.

V. Sodium thiosulphate (0.1 N):
   Dissolve 24.82 g. $Na_2S_2O_3 \cdot 5H_2O$ and 1 g. $Na_2CO_3 \cdot 10H_2O$ in distilled water and make up to 1000 ml.

VI. Starch (indicator):
   Stir 0.5 g. soluble starch with a little water, pour into 100 ml. boiling 25% NaCl solution and boil for *ca.* 1 min.

Stability of the solutions

See p. 902.

Procedure

Experimental material

Soil: Spread out in the air (not in sunlight), dry for 24—28 hours at 25—35°C and sieve through a 2 mm. mesh sieve.

Plants: Plant material can be treated in the following ways:

a) Grind, mince or homogenize (see p. 49, 723). Analyse the resulting brei directly.

b) Ethanol-ether dried preparations: homogenize the plant material finely without the addition of water and with continuous stirring, introduce into about 10 volumes of a mixture of 4 parts by volume ethanol and 1 part by volume ether. Allow the plant material to settle, decant off

most of the supernatant ethanol-ether mixture and filter the residue on a Buchner funnel with gentle suction. Remove the alcohol by repeated washings with ether, and dry the residue (enzyme preparation) in a desiccator over $CaCl_2$. This preparation is stable for a long period and is usually free from interfering contaminants, especially sugars and chlorophyll. In most cases the blank determination can be dispensed with.

c) Glycerol extracts: finely grind 5 g. plant material, 2 ml. toluene, *ca.* 10 g. purified sea sand and a little 20% glycerol solution in a mortar and then wash out into a 100 ml. volumetric flask with glycerol solution. After the addition of 1 ml. toluene, shake for 1 hour, dilute to the mark with 20% glycerol solution, mix thoroughly and filter through a fluted filter paper. Store the filtrate (+1% toluene) in a refrigerator until required. It is considerably purer than the original plant material.

Enzymatic reaction

Soil: A blank is usually only necessary with peaty soil. Prepare the blank as for the sample, but substitute distilled water for the sucrose solution (II).

Plants: Prepare the blank as for the sample, but substitute distilled water for the sucrose solution (II).

As the activity of the samples varies considerably, the first determination serves as a trial assay. On repeating the analysis take sufficient of the sample so that about $^3/_4$ of the sucrose present is hydrolysed.

The pH optimum of invertase in seed corn is 5.0 (not 5.5). For accurate measurements in corn adjust the buffer solution (I) to pH 5.0 with acetic acid.

Add successively to 100 ml. volumetric flasks:

10 g. dried soil sample or
plant sample after trial assay
1.5 ml. *) toluene.

Mix thoroughly by shaking, after 15 min. pipette in

10 ml. buffer solution (I)
10 ml. sucrose solution (II)

and stopper the flasks with rubber bungs. Incubate for 23 hours in an incubator at 37° C, dilute to 100 ml. with

(tap) water at 37° C,

shake and continue incubation at 37° C. After exactly 24 hours analyse the supernatant fluid for reducing sugar.

Sugar estimation [1]

For each series of estimations prepare a reagent blank containing 10 ml. *Fehling*'s solution (III) + 20 ml. distilled water.

Pipette into 100 ml. Erlenmeyer flasks:

10 ml. *Fehling*'s solution (III)
20 ml. **) sample (from the enzymatic reaction).

*) With peaty soil take up to 5 ml. Sufficient toluene must be added so that after the dilution with water it forms a layer which floats on the surface.

**) Only use 5 or 10 ml. with peaty soil or soil which is well cultivated and make up to 20 ml. with distilled water. Allow for this in the calculations.

[1] *N. Schorl* and *A. Regenbogen*, Z. analyt. Chem. *56*, 191 [1917].

Heat for exactly 5 min. in a boiling water bath and quickly cool to $< 25°C$ under running tap water. Add

3 ml. KI solution (IV)
4 ml. 25% H_2SO_4,

mix and titrate with

$Na_2S_2O_3$ solution (V).

Towards the end of the titration add

0.5 ml. starch solution (VI)

and continue the titration until the end-point is reached.

Calculations

The difference in the amount of thiosulphate required (in ml. thiosulphate solution V) for the sample and for the reagent blank gives the invertase units. If the sample (peaty soil, plants) already contains reducing substances (sample blank), this value must be subtracted from the result of the unknown.

Evaluation

See p. 721, 722.