UV method for approx. 16 assays each

For laboratory use only
Store between +2 and +8°C

The method is contained in the Austrian, Dutch, German, Swiss food laws. Recommended e. g. by IFU, AIJN, MEBAK, OICCC. Standardized by DIN, EN, GOST, NEN, NF.

Principle

Sucrose + H_2O ——— β-fructosidase ⟶ D-glucose + D-fructose
D-glucose + ATP ——— Hexokinase ⟶ G-6-P + ADP
D-fructose + ATP ——— Hexokinase ⟶ F-6-P + ADP
F-6-P ——— PGI ⟶ G-6-P
G-6-P + $NADP^+$ ——— G6P-DH ⟶ D-gluconate-6-P + NADPH + H^+

Ref.: Bergmeyer, H.U. & Bernt, E. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 3, pp. 1176-1179, Verlag Chemie Weinheim / Academic Press Inc., New York and London; Schmidt, F.H. (1961) Die enzymatische Bestimmung von Glucose und Fructose nebeneinander, Klinische Wochenschrift 39, 1244-1247

Assay performance

Wavelength: 340 nm (NADPH), ε = 6.3 l x $mmol^{-1}$ x cm^{-1}
Light path: 1.00 cm (glass or plastic cuvettes)
Temperature: +20 to +25°C
Assay volumes: 3.020 ml (sucrose, D-glucose)
3.040 ml (D-fructose)
Measurement: against air or against water
Sample solution: 4 to 150 µg sucrose + D-glucose + D-fructose in 0.100 to 1.800 ml (sucrose), resp. 2.000 ml (D-glucose, D-fructose) sample solution.

Reagents

S: Lyophilizate with citrate buffer, pH approx. 4.6, approx. 510 U β-fructosidase (for stability see pack label). *Dissolve contents of bottle # S with 7 ml redist. water.* The solution is stable for 1 month at +2 to +8°C, resp. for 2 months at -15 to -25°C.

1: Powder mixture with triethanolamine buffer, pH approx. 7.6, approx. 80 mg NADP, approx. 190 mg ATP, magnesium sulfate (for stability see pack label). *Dissolve contents of bottle # 1 with approx. 31 ml redist. water.* The solution is stable for 1 month at +2 to +8°C, resp. for 2 months at -15 to -25°C.

2: Approx. 0.7 ml hexokinase (HK) / glucose-6-phosphat dehydrogenase (G6P-DH) suspension (approx. 200 U / 100 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use.* Swirl bottle carefully before the suspension is pipetted.

F: Approx. 0.7 ml phosphoglucose isomerase (PGI) suspension (approx. 490 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use.* Swirl bottle carefully before the suspension is pipetted.

In addition (not contained in the kit):

Standard solution sucrose, ultrapure, 0.8 g/l for test control only; standard solution D-glucose, anhydrous, ultrapure, 0.5 g/l for test control only; standard solution D-fructose, ultrapure, 0.5 g/l for test control only.

The reagents for the determination of sucrose, D-glucose and D-fructose are not hazardous. The general safety rules for the work in chemical laboratories should be applied. After use the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Procedure

Pipette into cuvettes:	**Blank sucrose**	**Sucrose standard assay[1]**	**Sucrose sample assay[2]**	**Blank D-glucose D-fructose**	**D-glucose/ D-fructose sample assay**	**Assay with internal standard[3]**
Citrate buffer, β-fructosidase, solution # S	0.200 ml	0.200 ml	0.200 ml	-	-	0.200 ml
Sample solution[4] (e.g. 0.08 to 0.8 g sucrose/l)	**-**	**-**	**0.100 ml**	**-**	**0.100 ml**	**0.100 ml**
Standard solution[4] (e.g. 0.8 g sucrose/l)	-	0.100 ml	-	-	-	0.100 ml
Mix, e.g by gentle swirling of the cuvette. Incubate at +20 to +25°C (+37°C) for 15 min (5 min). Add:						
Tea buffer, NADP, ATP solution # 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
Redist. water	1.800 ml	1.700 ml	1.700 ml	2.000 ml	1.900 ml	1.600 ml
Mix[5], after approx. 3 min read the absorbances (A_1). Add:						
HK /G6P-DH suspension # 2	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix[5], after approx. 10 to 15 min read the absorbances (A_2). Repeat absorbance reading after another 2 min[6]. Add:						
PGI suspension # F	-	-	-	0.020 ml	0.020 ml	0.020 ml
Mix[5], after approx. 10 to 15 min read the absorbances (A_3).						

Please see notes on the next page

Calculation

$\Delta A_{D\text{-glucose}} = (A_2 - A_1)_{sample,\ resp.\ standard} - (A_2 - A_1)_{blank}$
$\Delta A_{D\text{-fructose}} = (A_3 - A_2)_{sample,\ resp.\ standard} - (A_3 - A_2)_{blank}$
$\Delta A_{sucrose} = [(A_2 - A_1)_{assay\ sucrose} - (A_2 - A_1)_{blank\ sucrose}] - [(A_2 - A_1)_{assay\ D\text{-glucose}} - (A_2 - A_1)_{blank\ D\text{-glucose}}]$

c = (V x MW x ΔA) / (ε □□x d x v x 1000) [g D-glucose, resp. D-fructose, resp. sucrose /l sample solution]
c = (3.020 x 180.16 x ΔA) / (6.3 x 1.00 x 0.100 x 1000) = **0.8636 x ΔA [g D-glucose/l sample solution]**
c = (3.040 x 180.16 x ΔA) / (6.3 x 1.00 x 0.100 x 1000) = **0.8693 x ΔA [g D-fructose/l sample solution]**
c = (3.020 x 342.3 x ΔA) / (6.3 x 1.00 x 0.100 x 1000) = **1.641 x ΔA [g sucrose/l sample solution]**

If the sample has been diluted during preparation, multiply the result with the dilution factor F.

When analyzing samples which are weighed out for sample preparation, calculate the content from the amount weighed:

$$Content_{sucrose/D\text{-glucose/D-fructose}} = \frac{C_{sucrose/D\text{-glucose /D-fructose}}\ [g/l\ sample\ solution]}{weight_{sample}\ [in\ g/l\ sample\ solution]} \times 100\ [g/100\ g]$$

Sample preparation

If the sample has one of the characteristics below, which hamper the test, please follow the corresponding sample preparation procedure.

1. Dilute *clear, colourless and almost neutral liquid samples* to get a sample solution with 0.08 to 0.8 g sucrose + D-glucose + D-fructose/l.
2. Filter or centrifuge *turbid solutions*, dilute (see pt. 1).
3. Degas *samples containing carbon dioxide*, e.g. by filtration, or add $NaHCO_3$ until the solution is slightly alkaline, dilute (see pt. 1).
4. Crush (corn size < 0.3 mm) or homogenize *solid or semi-solid (pasty) samples*, extract with water or dissolve in water, filter and dilute (see pt. 1) if necessary.
5. Extract *fat containing samples* with hot water at a temperature above the melting point of fat, e.g. in a 100 ml volumetric flask. Adjust to +20°C, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter. Alternatively, clarify with Carrez reagents (which can be recommended).
6. Clarify *samples containing protein* with Carrez reagents
   Weigh sufficient quantity of solid or pasty sample into 100 ml volumetric flask, add approx. 60 ml water. Or pipette liquid sample into 100 ml volumetric flask containing approx. 60 ml water. Add, and mix after each addition, 5 ml Carrez-I-solution (3.60 g $K_4[Fe(CN)_6]$ x 3 H_2O = potassium hexacyanoferrate(II)/100 ml), 5 ml Carrez-II-solution (7.20 g $ZnSO_4$ x 7 H_2O = zinc sulfate hepta-hydrate/100 ml). Adjust to pH 7.5 to 8.5 by the addition of e.g. 10 ml NaOH (0.1 M). Fill the flask to the mark, mix and filter.
7. Do not deproteinize samples with perchloric acid because sucrose is hydrolyzed.

Assay characteristics

1. *Specificity:* Specific for D-glucose and D-fructose. Relatively specific for sucrose in the absence of 2-β-fructosans. (2-β-Fructosans, if present in the sample, react slower than sucrose.) In the analysis of commercial sucrose, results of 100 % have to be expected. In the analysis of D-glucose and D-glucose monohydrate and of D-fructose, results of < 100 % have to be expected because the materials absorb moisture.
2. *Sensitivity:* 0.2 mg D-glucose/l or D-fructose/l (ΔA = 0.005; v = 2.000 ml; V = 3.020 / 3.040 ml)
   1 mg sucrose/l (ΔA = 0.010; v = 1.800 ml; V = 3.020 ml)
3. *Detection limit*: 0.4 mg D-glucose/l or D-fructose/l (ΔA = 0.010; v = 2.000 ml; V = 3.020 / 3.040 ml)
   2 mg sucrose/l (ΔA = 0.020; v = 1.800 ml; V = 3.020 ml)
4. *Linearity*: from 4 µg sucrose + D-glucose + D-fructose /assay (v = 1.800 ml; V = 3.020 / 3.040 ml)
   to 150 µg sucrose + D-glucose + D-fructose /assay (v = 0.100 ml; V = 3.020 / 3.040 ml)
5. *Precision:* ΔA = +/- 0.005 absorbance units (D-glucose, D-fructose)
   ΔA = +/- 0.010 absorbance units (sucrose)
   CV = approx. 1 to 2 % (D-glucose, D-fructose)
   CV = approx. 1 to 3% (sucrose)
   Fruit juice:
   $r = 1.9 + 0.033 \times c_{sucrose}$ [g/l]
   $R = 3.3 + 0.061 \times c_{sucrose}$ [g/l]
   $r = 0.42 + 0.027 \times c_{D\text{-glucose}}$ [g/l]
   $R = 1.0 + 0.042 \times c_{D\text{-glucose}}$ [g/l
   $r = 0.15 + 0.033 \times c_{D\text{-fructose}}$ [g/l]
   $R = 1.05 + 0.045 \times c_{D\text{-fructose}}$ [g/l
6. *Interferences*: none known

Notes

1 Run a "standard" to see "accidents" in analysis. The measurement of the standard is not necessary for calculating results.
2 This assay together with the blank is a single determination. In the case of a double determination, run two assays with different sample volumes. The absorbance differences measured have to be proportional to the sample volumes (calculate with resp. volumes v).
3 Recovery = $[(\Delta A_{sample + standard} - \Delta A_{sample}) / \Delta A_{standard}] \times 100$ [%].
4 Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
5 e.g. with a plastic spatula, or after closing the cuvette with Parafilm (trademark of American Can Co., Greenwich Ct., USA).
6 The reaction has stopped when the absorbance is constant. If the reaction has not stopped, continue to read absorbances until they increase constantly over 2 min. Extrapolate absorbance to the time of the addition of HK/G6P-DH (suspension # 2).