Sucrose/D-Fructose/D-Glucose, UV method

Catalogue number: AK00201, 100 tests of each analyte

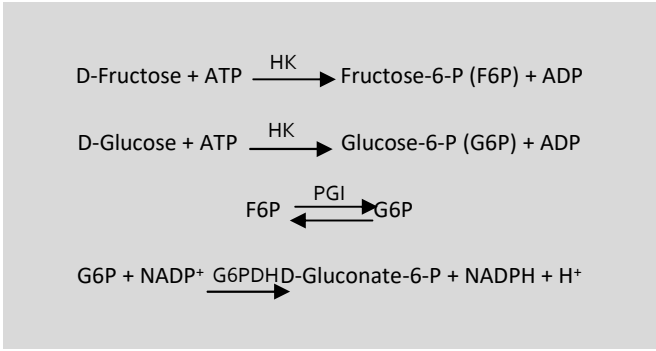
Application

This rapid and simple specific enzymatic method is used for the simultaneous determination of sucrose, D-fructose and D-glucose in foodstuffs, pharmaceuticals, cosmetics and biological samples.

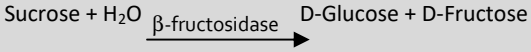
Introduction

Sucrose, D-fructose and D-glucose occur widely in plant organisms. In foods, they occur mainly in honey, wine and beer, and a range of solid foodstuffs such as bread and pastries, chocolate and candies. In the wine industry, the addition of sucrose is only allowed in few situations, such as champagne production.

Principles



Hydrolysis of sucrose (at pH 4.6)



The D-glucose concentration is determined before and after hydrolysis of sucrose by β-fructosidase. The sucrose content is calculated form the difference in D-glucose concentrations before and after hydrolysis by β-fructosidase. The D-fructose content in the sample is determined succeeding to the determination of D-glucose, after isomerization by phosphoglucose isomerase (PGI).

The amount of NADPH formed through the combined action of hexokinase (HK), phosphoglucose isomerase (PGI) and glucose-6-P dehydrogenase (G6PDH), measured at 340 nm, is stoichiometric with the amount of D-fructose, D-glucose and sucrose in sample volume.

Specificity

This method is specific for D-fructose and D-glucose. Since β-fructosidase also hydrolyses low molecular weight fructans (*e.g.* kestose) this method, as all others, is not totally specific for sucrose.

Some indication of the presence of fructo-oligosaccharides will be given by the ratio of D-glucose to D-fructose in the determination after hydrolysis by β-fructosidase. Deviation from 1:1 (increasing proportion of D-fructose) would indicate the presence of fructan. This can be tested by measurement of D-fructose in the "sucrose sample" subsequent to the determination of total D-glucose. Sufficient PGI is provided in the kit to allow this further analysis, if desired.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.010 AU and a sample volume of 1.00 mL. This corresponds to a D-fructose and D-glucose concentration of 0.68 mg/L sample solution when measured at 340 nm. The detection limit of 1.38 mg/L is derived from the absorbance difference of 0.020 (340 nm) and a maximum sample volume of 1.00 mL.

Linearity and precision

Linearity of the determination exists from 2 to 80 µg D-fructose, D-glucose or sucrose per assay (v = 1.00 mL). In a double assay using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur, which corresponds to a D-glucose concentration of approx. 0.35 to 0.68 mg/L (v = 1.00 mL).

Analysis of commercial sucrose should yield recoveries of approximately 100%. However, values below 100% will be obtained for D-glucose monohydrate and D-fructose due to moisture absorption by these sugars.

To confirm that sucrose is completely hydrolysed by β-fructosidase, perform incubation for the suggested time and twice this time. The final determined values for D-fructose and D-glucose should be identical.

Kit composition

Solution 1. Buffer (25 mL, pH 7.6). Stable for 2 years at 4 °C.

Solution 2. $NADP^+$ plus ATP and PVP. Stable for 2 years at -20 °C.

Dissolve in 22 mL of distilled water, divide into appropriately sized aliquots and store in PP tubes at -20 °C between use (stable for 2 years) and keep cool during use.

Suspension 3. Hexokinase plus glucose-6-P dehydrogenase (4.1 mL). Stable for 2 years at 4 °C. Swirl bottle before use.

Suspension 4. Phosphoglucose isomerase (2.25 mL). Stable for 2 years at 4 °C. Swirl bottle before use.

Solution 5. D-Fructose/D-glucose standard solution (5 mL, 0.20 mg/mL of each sugar). Stable for 2 years at room temperature. This standard solution can be used when there is some doubt about the method accuracy.

Solution 6. Buffer (20 mL, pH 4.6). Stable for 2 years at 4 °C.

Suspension 7. β-Fructosidase (2.25 mL). Stable for 2 years at 4 °C. Swirl bottle before use.

Add 0.02 ml of Suspension 7 plus 0.180 ml of Solution 6, per assay, to a test tube and homogenise (Solution 6+7). This solution should be prepared for each assay day. Warm Solution 6+7 to 25-30 ºC before use.

Safety

The general safety measures that apply to all chemical substances should be followed. For more information regarding the safe usage of this kit please refer to MSDS available at www.nzytech.com.

Procedure (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~25 °C

Final volume: 2.42 mL (D-glucose); 2.44 mL (D-fructose)

Sample solution: 4-80 µg of total sucrose, D-glucose and D-fructose per cuvette (in 0.10-1.00 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes (mL)	Sucrose assay		D-Fructose/D-Glucose assay	
	Blank	**Sample**	**Blank**	**Sample**
Solution 6+7*	0.20	0.20	-	-
Sample	-	0.10	-	0.10
Mix. Incubate for 5 min. (Note: Warm *Solution 6+7* to 25-30ºC before pipetting)				
Then add:				
Distilled water (at ~25 °C)	2.00	1.90	2.20	2.10
Solution 1 (buffer)	0.10	0.10	0.10	0.10
Solution 2 ($NADP^+$+ATP)	0.10	0.10	0.10	0.10
Mix, measure the absorbance of the above solutions (A1) after approx. 3 min and start the reaction by adding:				
Suspension 3 (HK+G6PDH)	0.02	0.02	0.02	0.02
Mix, measure the absorbance of the above solutions (A2) at the end of the reaction (approx. 5 min)**				
Suspension 4 (PGI)	-	-	0.02	0.02
Mix, measure the absorbance of the above solutions (A3) at the end of the reaction (approx. 8-10 min)*				

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

Pipette both Solution 6+7 and sample into the bottom of the cuvette and mix by gentle swirling

** if necessary, continue to read the absorbance at 2 min intervals until the reaction ends.

If the absorbance continues to increase, it may be due to effects of colour compounds or enzymes in the sample. These interfering substances may be removed during sample preparation.

Calculation

Determine the absorbances difference for both blank and sample (A2-A1), in sucrose assay. Subtract the absorbances difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{\text{total D-glucose}}$.

Determine the absorbances difference for both blank and sample (A2-A1), in D-fructose/D-glucose assay. Subtract the absorbances difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{\text{D-glucose}}$.

The difference between $\Delta A_{\text{total D-glucose}}$ and $\Delta A_{\text{D-glucose}}$ yields $\Delta A_{\text{sucrose}}$

$$\Delta A_{Sucrose} = \Delta A_{Total\ D\text{-}Glucose} - \Delta A_{D\text{-}Glucose}$$

Determine the absorbance difference for both blank and sample (A3-A2). Subtract the absorbances difference of the blank from the absorbances difference of the sample, thereby obtaining $\Delta A_{\text{D-fructose}}$. The concentration of sucrose, D-fructose (g/L) and D-glucose (g/L), based on the Abs of NADPH at 340 nm (6300 $L \times mol^{-1} \times cm^{-1}$), are calculated as follows:

$$C(\text{D-Glucose}) = 0.6920 \times \Delta A_{\text{D-Glucose}} \quad [g/L]$$

$$C(\text{Sucrose}) = 1.315 \times \Delta A_{\text{Sucrose}} \quad [g/L]$$

$$C(\text{D-Fructose}) = 0.6978 \times \Delta A_{\text{D-Fructose}} \quad [g/L]$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

Interferences

If the concentration of D-glucose in the sample is much larger than D-fructose (e.g. 10x higher), then the precision of sucrose and D-fructose is compromised. In this situation, the content of D-glucose should be reduced using glucose oxidase/catalase reagent in the presence of oxygen (see *Sample Preparation*)

If the conversion of D-fructose and D-glucose completes within the time specified in the assay (approx. 10 min), it can be generally concluded that no interference has occurred. However, an internal standard should be included during sample analysis if the presence of interfering substances is suspected. A quantitative recovery of this standard should be expected. Identification of losses in sample handling and extraction may be identified by performing recovery experiments, i.e., by adding D-fructose and D-glucose to the sample in the initial extraction steps.

General information on sample preparation

The total amount of sucrose, D-fructose and D-glucose present in the cuvette should range between 2 and 80 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield a sugar concentration between 20 and 800 mg/L. However, the sample volume can range from 0.10 to 1.00 mL, by replacing water (analytes range from 20 to 800 mg/L).

To implement this assay use clear, colourless and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (*e.g.* by filtration); adjust acid samples, which are used undiluted for the assay, to pH 7.6 by adding sodium or potassium hydroxide solution; adjust acid and weakly coloured samples to pH 7.6 and incubate for approx. 15 min; measure "coloured" samples (if necessary adjusted to pH 7.6) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with PVPP (ad 0.2 g of PVPP/10 mL sample, shake vigorously for 5 min and filter through Whatman nº1 filter paper); crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water.

Examples of sample preparation

Determination of sucrose, D-fructose and D-glucose in fruit juices

Filter turbid juices or clarify using Carrez reagents. Dilute to give a sugar concentration of approximately 0.1-1.5 g/L. Strong coloured samples should be treated with PVPP (see above). Slightly coloured samples can be assayed directly. Typically, for orange and apple juice, a dilution of 1:100 and a sample volume of 0.1 mL are satisfactory.

Determination of sucrose, D-fructose and D-glucose in beer

Remove carbon dioxide by stirring 5-10 mL of the beer for 1 min or filter through filter paper. Use the clear, slightly coloured solution directly for assay. Typically, no dilution is required and a sample volume of 0.1-0.2 mL is satisfactory.

Determination of sucrose, D-glucose and D-fructose in honey

After stirring the honey sample thoroughly with a spatula, transfer approx. 10 g of the viscous or crystalline material to a beaker and heat for 15 min at approx. 60 °C and stir (there is no need to stir liquid honey). After cooling, prepare 1% (w/v) honey solution: pour approx. 1 g of the liquid sample accurately weighed, into a 100 mL volumetric flask. Dissolve initially with only a small volume of distilled water and then dilute to the mark and mix.

Determination of D-fructose and D-glucose: typically, a dilution of 1:10 of the 1% (W/V) honey solution and a sample volume of 0.1 mL are satisfactory.

Determination of sucrose: if the estimated sucrose concentration of the honey lies between 5 and 10%, a dilution 1:3 of the 1% (W/V) honey solution and sample volume of 0.1 mL are satisfactory. If the estimated sucrose concentration of the honey lies below 5%, D-glucose should be removed (see below), otherwise the precision of the sucrose determination will be compromised.

Determination of sucrose, D-glucose and D-fructose in jam

Homogenise about 10 g of jam in a mixer. Pour approx. 0.5 g of the sample accurately weighed, into a 100 mL volumetric flask. Dissolve initially with only a small volume of distilled water (~50 mL) and then dilute to the mark, mix and filter. Discard the first 5 mL of filtrate. Typically, no dilution will be required and a sample volume of 0.1-0.2 mL is satisfactory.

Special sample preparation for the determination of sucrose and D-fructose in the presence of excess of D-glucose

Pipette to a 25 mL volumetric flask: 5 mL of buffer (300 mM sodium phosphate plus 5 mM $MgCl_2$, pH 7.6), 5 mL of sample solution and 0.2 mL of enzyme solution (glucose oxidase 600 U/mL plus catalase 15000 U/mL). Incubate at ~25 ºC and pass a current air (O_2) through the mixture for 1 h. After the reaction, inactivate the enzymes by incubation the flask in a boiling water bath for 10 min. After cooling, dilute the content to the mark with distilled water. Mix and filter. Use 0.5 mL of the clear solution for the determination of sucrose and D-fructose. Determine residual D-glucose as habitual.

References

Outlaw, W. H. & Mitchell, C. T. (1988). Sucrose. In Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol. VI, pp. 96-103, VCH Publishers (UK) Ltd., Cambridge, UK.

Beutler, H. -O. (1988). D-Fructose. In Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol. VI, pp. 321-327, VCH Publishers (UK) Ltd., Cambridge, UK.

Kunst, A., Draeger, B. & Ziegenhorn, J. (1988). D-Glucose. In: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol.VI, pp. 163-172, VCH Publishers (UK) Ltd., Cambridge, UK.

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Certificate of Analysis

Test	Criteria	Result
Test Performance	Reaction completed within time stated	Meets specification
	Target value for recommended standard material +/- 10%	Meets specification
Blank reaction absorbance	+/- 10% of the blank value	Meets specification

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only

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