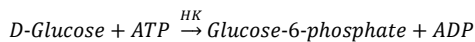


PURPOSE OF THE TEST

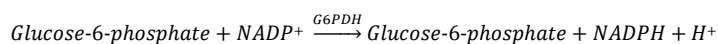
D-Glucose and D-Fructose are the main reducing sugars (fermentable) present in grapes and other fruits. The content of D-glucose and D-fructose in the grape is similar (in a relation between 0.74 and 1.12), with small variations depending on the state of maturity of the grape and its variety. Since D-glucose is fermented more rapidly by yeast, monitoring the relationship between D-glucose and D-fructose, in addition to its total sum, provides information about both the fermentation process and the final degree of sweetness expected. Fructose levels are calculated from the joint glucose and fructose assessment (code SY2404) by directly subtracting the glucose content.

METHOD

Hexokinase (HK) catalyzes the phosphorylation of D-Glucose (and D-Fructose) by Adenosine-5'-triphosphate (ATP).



The glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconate by reducing NADP⁺.



Increase of absorbance at 340 nm associated to NADPH formation is directly proportional to concentration of D-Glucose and D-Fructose of sample.

CONTENT

R1	2 x 30 mL	TEA buffer 100 mM, ATP 4 mM, NADP 3 mM, pH 7.6
R2	1 x 15 mL	HK (>0,5 UI/L), G6PDH (>1,8 UI/L)
CTRL	1 x 3 mL	Control Glucose 1,5 g/L (1,35-1,65 g/L)

REAGENT PREPARATION

Reagents are ready to use and are stable up to expiry date as supplied when stored at 2-8 °C. Do not freeze.

Discard if absorbance of blank is higher than 0.300 OD at 340 nm.

SAMPLES

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO₂ introduces instability in the measure. Samples containing CO₂ must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpyrrolidone (PVPP 0.1g for each 10 mL) to reduce the level of colour. Samples with concentration higher than the measurement range must be diluted accordingly with distilled water. Multiply the final result by the dilution factor.

PROCEDURE OVERVIEW

Treat standard, controls and samples as sample. Use distilled water as Blank.

Use WINECAL (code SY2100) or WINECALRTU (code SY2100RTU) as standard.

Volumes stated below can be adjusted to other analytical procedures. Expected performance can vary if those ratios S:R1:R2 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 1	720 µL	720 µL
Distilled water	9 µL	--
Sample/Standard	--	9 µL

Mix, incubate at 37°C for 1 minutes and read absorbance at 340 nm (A₁). Then add into the cuvette:

	Blank reaction	Test Reaction
Reagent 2	180 µL	180 µL

Mix, incubate for 10 minutes at 37°C and read absorbance at 340 nm (A₂).

Concentration of glucose is calculated as:

$$Glu = \frac{(A_2 - 0.80 \times A_1)_{\text{sample}} - (A_2 - 0.80 \times A_1)_{\text{blank}}}{(A_2 - 0.80 \times A_1)_{\text{standard}} - (A_2 - 0.80 \times A_1)_{\text{blank}}} \times C \text{ g/L}$$

Factor 0.80 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for glucose.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	150	240
Name	GLUCOSE	
Method	End Point A	
Direction	Increasing	
Main Wavelength	340	
Sec. Wavelength	--	
Sample	3	
Reagent 1	240	
Reagent 2	60	
Calibration	Linear	
Blank cycle [150 240]	3 - 4	3 - 4
Reading cycle [150 240]	20 - 21	31 - 32
Units	g/L	
Decimals	0.00	
Measure range	0.03 ~ 8.00	
R1 Lim. Abs	3000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 8 g/L. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of Quantification (LoQ): 0.03 g/L

Limit of linearity: 8.0 g/L

NOTES

It is recommended to use wine controls to verify quality of calibration. Each laboratory should establish its own quality criteria for acceptance, as well as proper corrective action procedures in case of rejection.

REFERENCES

- Compendium of International methods of analysis – OIV: OIV-MA-AS311-02
- Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY.
- Resolution OIV-OENO 600-2018. Determination of D-Glucose and D-Fructose in wines by automated enzymatic method. (2018)

