SD6028: TOTAL SUGAR

ENZYMATIC – β-F/HK/PGI/G6PDH

PURPOSE OF THE TEST

In addition to monomeric hexoses (glucose and fructose), the must contains small amounts of the disaccharide sucrose, which is hydrolyzed into fructose and glucose by the enzyme β-Fructosidase (β-F). Although the natural content of sucrose in the must is relatively low and zero in finished wine, addition of sucrose is used specifically in the production of sparkling wines (second fermentation) and chaptalization (to increase the alcoholic degree artificially). The determination of the total sugar content, including that derived from the hydrolysis of sucrose, allows the monitoring of the process both at the beginning of the fermentation and at its end (residual sugars), improving the general control of it.

METHOD

β-fructosidase catalyzes the hydrolysis of sucrose in D-glucose and D-fructose.

$$\text{Sucrose} \xrightarrow{B-F} Glucose + Fructose$$

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

$$D\text{-}Glucose + ATP \xrightarrow{HK} Glucose\text{-}6\text{-}phosphate + ADP$$
$$D\text{-}Fructose + ATP \xrightarrow{HK} Fructose\text{-}6\text{-}phosphate + ADP$$

The fructose-6-phosphate is converted to glucose-phosphate by the phosphoglucose isomerase (PGI).

$$Fructose\text{-}6\text{-}phosphate \xrightarrow{PGI} Glucose\text{-}6\text{-}phosphate$$

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

$$Glucose\text{-}6\text{-}phosphate + NADP^+ \xrightarrow{PGI} Glucose\text{-}6\text{-}phosphate + NADPH + H^+$$

The increase in absorbance at 340 nm associated with the formation of NADPH is directly proportional to the concentration of D-glucose and D-fructose in the sample.

CONTENT

R1A	2 x 30 mL	TRIS buffer 100 mM, ATP 4 mM, NADP 3 mM, pH 7.6
R1B	2 x 1 mL	β-Fructosidase (suspension)
R2	1 x 15 mL	HK (>0,5 UI/L), G6PDH (>1,8 UI/L), PGI (>8 UI/L)
CTRL	1 x 3 mL	Sucrose 3 g/L (2,55 – 3,45 g/L)

REAGENT PREPARATION

Reagent 1: Prepare Reagent 1 by pouring 1 vial of R1B into a vial of R1A. Mix gently, avoiding foaming. The working reagent is stable up to 20 days if stored at 2-8 ° C avoiding contamination. Do not freeze.

Reagent 2 is ready to use.

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

SAMPLES

For use with wine and must samples. Must samples should be diluted 1:40 or higher at sample request.

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO_2 introduces instability in the measure. Samples containing CO_2 must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpolypyrrolidone (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. 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 as per the scheme:

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

Mix, incubate at 37ºC for 1 minutes and read absorbance at 340 nm (A_1). Then add into the cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 2	180 µL	180 µL

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

Concentration of total sugar is calculated as:

$$Total\ sugar\ (Suc + Glu + Fru) = \frac{(A_2 - 0.80xA_1)_{\text{sample}} - (A_2 - 0.80xA_1)_{\text{blank}}}{(A_2 - 0.80xA_1)_{\text{standard}} - (A_2 - 0.80xA_1)_{\text{blank}}}\ x\ C\ \text{g/L}$$

Factor 0.80 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the label for total sugar (glucose+fructose) of the STD.

ASSAY PARAMETERS FOR ANALYZERS DIONYSOS®

Dionysos model	**150**	**240**
Name	TOT.SUGAR	
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]	21 - 22	31 - 32
Units	g/L	
Decimals	0.00	
Measure range	0.04 ~ 6.00	
R1 Lim. Abs	3000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 6 g/L of total sugar or 4 g/L of sucrose. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of quantification: 0.04 g/L
Limit of linearity: 6 g/L (4 g/L sucrose)

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

1. Compendium of International methods of analysis – OIV, Vol1&2 (2008)
2. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY. (1974).