

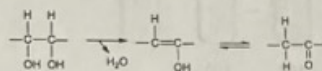
Phenol-Sulphuric Acid Assay (Total Carbohydrate Analysis)

Intro:

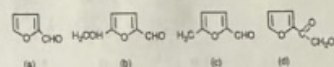
Introduction

Carbohydrates are one of the most important ingredients in foods and raw materials and it is important to know how much carbohydrates are present in the food. In this experiment we use phenol sulphuric assay to determine the carbohydrate concentration.

In the presence of strong acids and heat, carbohydrates get dehydrated to form furan derivatives such as furfural and hydroxymethyl furaldehyde.



Dehydration reaction.



Those give a green coloured product with phenol and has absorption maximum at 480-490nm in UV-VI spectrum.

The phenol-sulphuric method can be used on lipid-free extracts from cereals, seeds, and plants, provided the sample is in solution, and is appropriate for both reducing and non-reducing sugars. It is advantageous in that the reagents are low cost and readily available, the required equipment is minimal, and the assay is simple. Additionally, it can be used to quantify mono-saccharides, oligosaccharides, and polysaccharides. Absorption curves are characteristic for different sugars; therefore, this method provides the most accurate results when applied to samples containing only one type of carbohydrate. This assay has been used to quantify total sugars in a sample containing more than one type of carbohydrate. In this case, glucose is often used to construct the calibration curve and the results are then approximate only and should be stated as glucose equivalents.

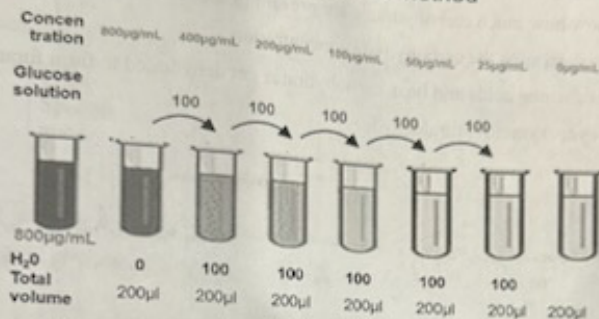
Materials

Test Tubes
96-wellplate
Pipettes (100 μ l) (10ml)
Vortex
Spectrophotometer/plate reader
Glucose (800 μ g/mL stock)
H₂SO₄ (98%)
Phenol 5%(W/V)

Method

1. Use prepared serial dilutions from 800µg/mL glucose stock solution to make standards of 100µL in total volumes according to following concentrations. The blank sample (0µg/mL) contains only H₂O without the glucose solution.

Phenol-Sulfuric Acid Method



Concentration	0	25	50	100	200	400	800
	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL
Abs (Average) 490nm	A1	A2	A3	A4	A5	A6	A7

2. Dilute wine by adding 0.02mL stock juice to 9.98mL water (500 x Dilution)

	A490nm	concentration
Wine 1		-
Wine 2		-
Wine 3		-
Wine Average		

3. When ready, pipette 50 µL of standards and each unknown sample into a microplate well.
4. Add 150 µL of concentrated sulphuric acid (98%).
5. Immediately add 30 µL of 5% phenol.
6. Place plate into the incubator for 20 min at 30°C.
7. Then measure the absorbance at 490 nm.
8. Use standard curve (trend line equation) to calculate unknowns' carbohydrate concentration.

0-Blank	25 ^g	50 ^g	100 ^g	200 ^g	400 ^g	800 ^g	A6	A6	A6	A11	A12
0-Blank	25 ^g	50 ^g	100 ^g	200 ^g	400 ^g	800 ^g	B6	B6	B6	B11	B12
wine/blank	Wine 1	C3	C4	C5	C6	C7	C8	C8	C8	C10	C11
wine/blank	Wine 2	D3	D4	D5	D6	D7	D8	D8	D8	D10	D11
wine/blank	Wine 3	E3	E4	E5	E6	E7	E8	E8	E8	E10	E11
F1	F2	F3	F4	F5	F6	F7	F8	F8	F8	F10	F11
G1	G2	G3	G4	G5	G6	G7	G8	G8	G8	G10	G11
H1	H2	H3	H4	H5	H6	H7	H8	H8	H8	H10	H11

For results

Subtract all the absorbance value with your blank absorbance.

- Plot Y against X (concentration) and get the marked scatter plot.
- Click on the data point and select add trend line.
- Under options make the intercept to zero. Obtain the equation.
- Using the equation calculate the total sugar concentration of wine sample.

Calculate total carbohydrate.

Plot glucose standard curve and calculate total carbohydrate concentrations (g/mL) and percentage (w/v%) g/100mL. Remember the volume used in test is 50 μ L. follow the dilution factors for correct conversion calculations (show).

So, for calculation,

Your Concentration should be X g/mL,

Total volume = 50 μ l = 50×10^{-3} ml

Total weight = concentration x total volume

Sample volume = 20 μ l = 20×10^{-3} ml

$$y = \text{absorbance of wine}$$

Example:

If, your concentration is 221.5 μ g/mL = 221.5×10^{-6} g/mL

w/v % = $(221.5 \times 10^{-6} \text{ g/ml} \times 20 \times 10^{-3} \text{ ml}) \times 100 / (1 \text{ mL} \times 50 \times 10^{-3} \text{ ml}) = 0.00886\%$

Multiply by 500 = 4.43%

Wine Analysis - Result

pH: 2.5-4.5 approx., measured by a pH paper.

S.G.: 0.995 - 1.000, measured by hydrometer.

Sweetness: Dry- Sweet

Degrees Brix: It is a measure of total soluble solids. Measured by brix refractometer.

Alcohol: 5%-21% (mainly 12-15%), measured by hydrometer.

Water activity (aw): Measured by Dew Point water activity meter.

Total Carbs: Phenol-Sulphuric Acid Assay

Temp: 22.29°C

Please add this chart under the results portion of your lab report

pH Value	Brix Value	Initial Specific gravity	Final Specific gravity	% Initial potential Alcohol	% Final potential Alcohol	Aw	Taste	%Total Carbs
3.5	8	1.100	0.990	13.20%	-3	1.0101	bitter, dry, not sweet	

How to calculate the % of Alcohol in Wine: $13.2 + 3 = 16.2$

% Real alcohol in wine = % initial potential alcohol - % final potential alcohol

accuScan FC



Raw data A1: UN1 Filter 1: 490nm Protocol: phennl-sulphuri 20.02.2024 14:08:54

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.133	0.156	0.189	0.710	0.502	8.690	1.251	0.186	0.178	0.126	1.073	0.132	
B	0.140	0.392	1.130	0.715	3.938	4.490	4.320	0.125	0.127	0.126	0.125	0.131	
C	0.126	0.158	0.129	0.128	0.126	0.126	0.127	0.126	0.126	0.125	0.128	0.130	
D	0.128	0.163	0.128	0.130	0.128	0.127	0.126	0.132	0.127	0.128	0.127	0.125	0.131
E	0.136	0.166	0.129	0.127	0.127	0.126	0.127	0.127	0.126	0.128	0.125	0.127	
F	0.126	0.345	0.127	0.130	0.127	0.126	0.127	0.127	0.126	0.126	0.132	0.127	
G	0.125	0.398	0.128	0.138	0.172	0.127	0.127	0.125	0.126	0.126	0.132	0.127	
H	0.128	0.165	0.141	0.133	0.130	0.126	0.125	0.128	0.126	0.128	0.125	0.127	

Navigation buttons: Move, OK>Edit, Close, Menu, File, Help, F1, F2, F3