Tishk International University
Faculty of Applied Science
Nutrition and Dietetics Department

Nutritional Biochemistry I/ NUT 207
Pre-Lab (2): Qualitative Measurement of Carbohydrates
(Molisch Test)

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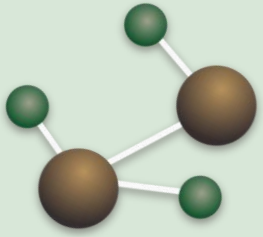
I'VE GOT
99 PROBLEMS
& THEY ALL
INVOLVE
CARBS.

Molisch test

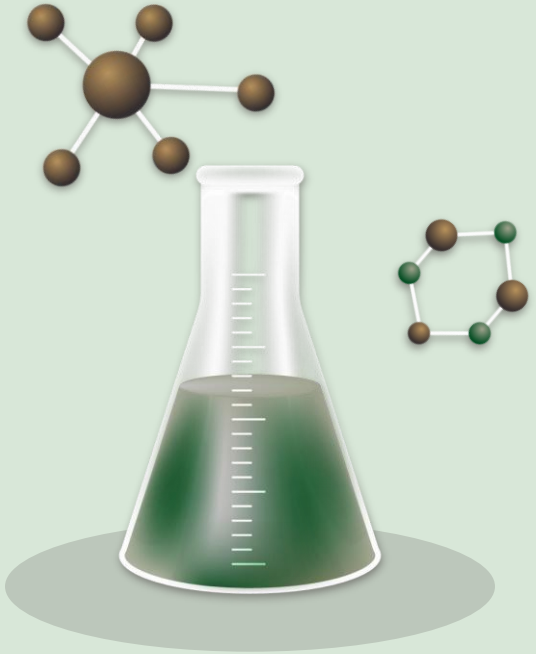
*Molisch test is a group test for all **carbohydrates**, either free or bound to proteins or lipids. It is a sensitive test that requires precision for the detection of carbohydrates.*

Carbohydrates are a group of naturally occurring carbonyl compounds (aldehydes or ketones) that also contain several hydroxyl groups.



Introduction to Carbohydrates

They are the most abundant organic molecules in nature and are also referred to as “***saccharides***”. The carbohydrates which are soluble in water and sweet in taste are called “***sugars***”.



Key aspects of biochemistry

Chemical processes

Organic molecules

Medicine applications

Important functions

Give energy

Form cell walls

Catalyze reactions

Protect organs

Cell production

Synthesis

Objectives of Molisch Test

To detect the presence of carbohydrates in a given sample.

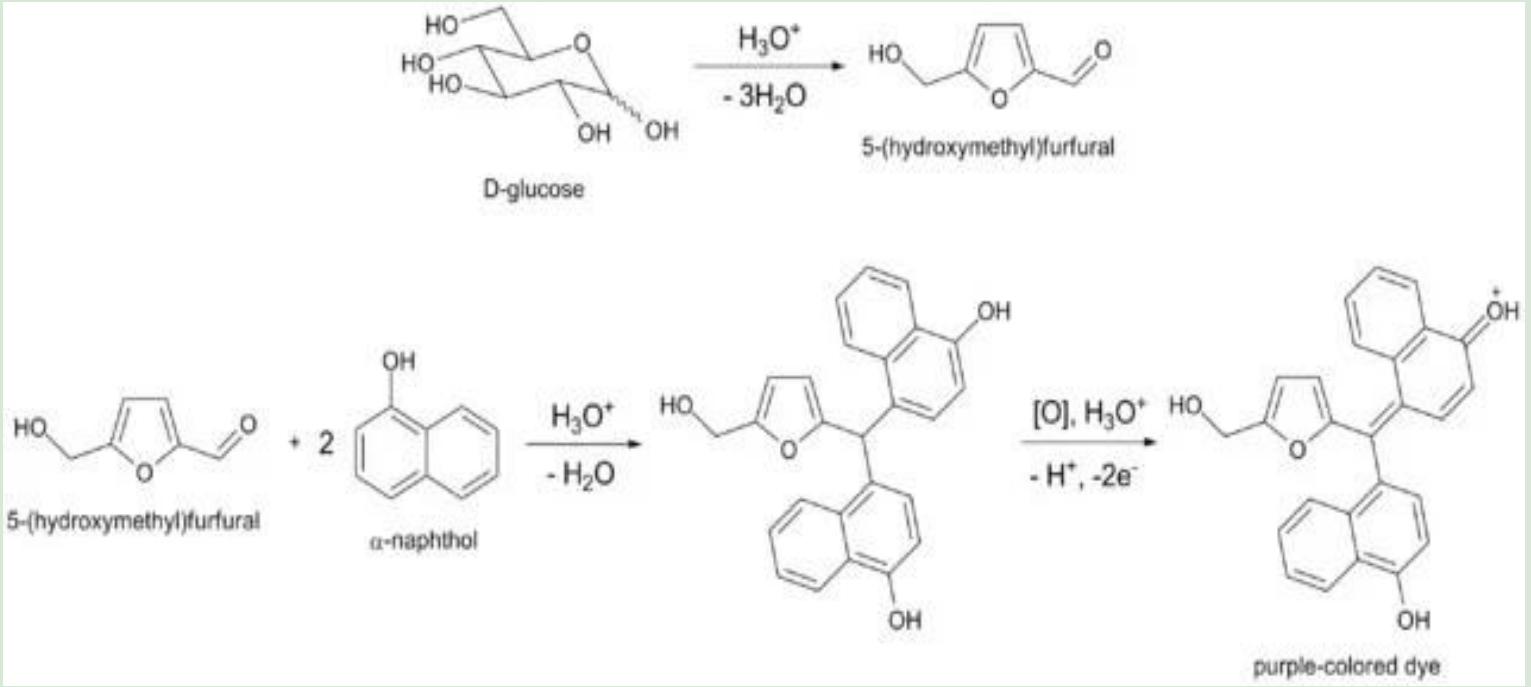
To distinguish carbohydrates from other biomolecules.

The quality of your life is dependent upon the quality of the life of your cells. If the bloodstream is filled with waste products, the resulting environment does not promote a strong, vibrant, healthy cell life- nor a biochemistry capable of creating a balanced emotional life for an individual.-

—Tony Robbins

Principle of Molisch Test

- The reaction is based on the fact that the concentrated acid catalyzes the dehydration of sugars to form furfural (from pentoses) or hydroxymethylfurfural (from hexoses).
- Either of these aldehydes condenses with two molecules of naphthol to form a purple or violet colored complex at the interface of the acid and test layer.
- If the carbohydrate is poly- or disaccharide, a glycoprotein or glycolipid, the acid first hydrolyses it into component ***monosaccharides***, which get dehydrated to form furfural or its derivatives.
- A green ring might be observed if any impurities are present in the reagent as they might interact with the α-naphthol and the acid.
- A rind ring is seen if a concentrated sugar solution is used. This might be due to the charring of the sugar due to the acid.


HO
HO
HO
O
OH
OH
D-glucose
H_3O^+
$-3H_2O$
HO
O
5-(hydroxymethyl)furfural
HO
O
5-(hydroxymethyl)furfural
+ 2
OH
α-naphthol
H_3O^+
$-H_2O$
HO
O
OH
OH
[O], H_3O^+
$-H^+, -2e^-$
HO
O
OH
OH
purple-colored dye

Requirements

1. Reagent

Molisch reagent: Dissolve 3.75 g of α-naphthol in 25 ml of Ethanol 99%. This reagent should be prepared fresh.
Concentrated sulphuric acid, Test sample

2. Materials required

Test tubes
Test tube stand
Pipette
Distilled water

Procedure of Molisch Test

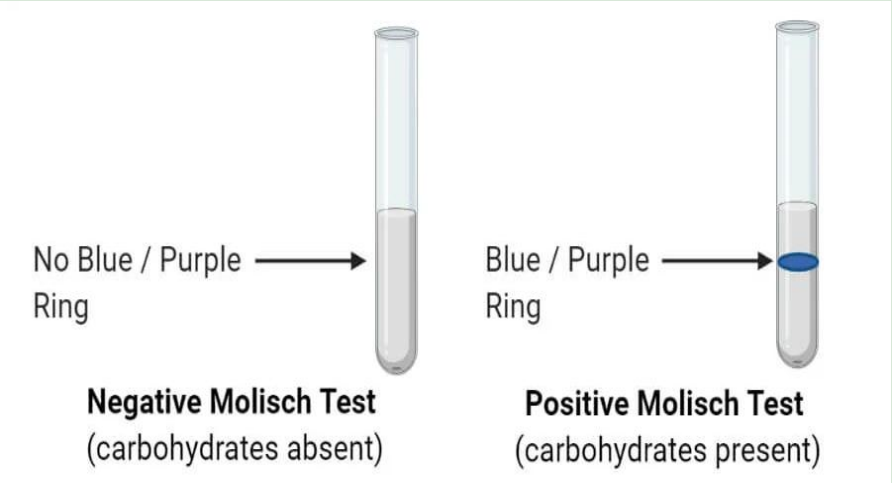
- Take 2 ml of each distilled water and test sugar solutions in four test tubes separately.
- Add two drops of Molisch reagent to each tube.

- Hold the test tube in an inclined position and gently add 1 ml concentrated H_2SO_4 along the wall of the test tube. Do not mix the acid with the solution. A black ring may form if concentrated acid is not added slowly as the heat generated from the reaction can char the carbohydrates.

- Observe the test tube for the formation of a purple-colored ring at the layer between the solution and the acid.

Result and Interpretation

- The formation of the purple-colored ring occurs at the interface between the sulphuric acid and the test solution.
- The sulphuric acid remains above the test solution as the acid is denser than the test solution.
- The absence of color indicates a negative result.



Uses of Molisch Test

- Molisch test is used to detect the presence of carbohydrates in different samples.

- It can be used to detect the formation of carbohydrates as a by-product in different reactions and distinguish it from other biomolecules.

Limitations of Molisch Test

- Trioses and tetroses do not have the necessary five carbon atoms for furfural formation, so they do not give a positive result for this reaction.

- Molisch test is not a specific test for carbohydrates. Furfurals as such or furfural yielding substance, some organic acids like citric acids, lactic acid, oxalic acid, formic acid, etc. can give a positive result.

QUESTIONS!