

SUBJECT: REVIEW METHODS OF DETECTING SUGAR IN A SAMPLE

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Abstract

The sugar is a simple type of carbohydrate and represents the main source of energy to power the biological and physical activities in the body. However, it has been reported that over weight gain, imperfect metabolic processes and heart diseases are mainly related to excess sugar intake, which causes diabetic complains. This has raised the demand of having reliable test methods for quantifying sugar amount in different samples to both monitor sugar levels and to study the biological effects of carbohydrates. This provides valuable information in various fields, including food science, biochemistry, and medicine. Several test methods have been applied to detect the presence of sugar in a biological sample. Conventional tests imply using special chemical reagents. Two well known tests are discussed briefly in this report, namely Molich's and Fehling's tests. The chemical reagents, test procedures, precaution as well as advantages and limitations were comprehensively discussed. A comparison between the two tests has been thoroughly addressed.

Keywords Carbohydrates, sugar quantification, diabetes, Molich's test, Fehling's Molich's.

INTRODUCTION

Objective:

The general test of detecting sugars of all kinds is free or linked, unilateral, bilateral or multiple, which distinguishes the sugars from other compounds.

- The solutions used

Glucose-fructose-water

Molich detector

Concentrated sulfuric acid

Applying Molisch's test involves slow addition of 2-3 drops of Molisch's reagent to a small quantity of the tested analyte and mixing thoroughly. [5]

Concentrated sulfuric acid is then added as few drops; one drop at a time down the sides of the test tube without mixing to enable development of a well-defined layer. [17]

If a purple ring is formed at the previous mentioned layer originated by the drop wise

addition of concentrated sulfuric acid, a positive indication of having carbohydrate is confirmed by Molisch's test.[24][15]

Otherwise, if no purple color emerges, the tested analyte does not include any carbohydrate component. [22]

- What is the Molisch's test?

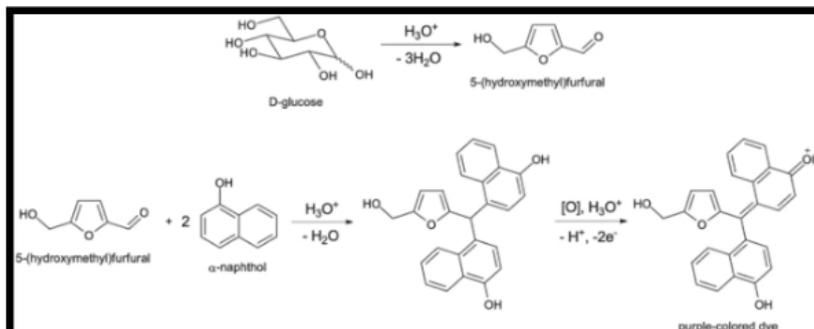
It is a well-known chemical test that confirms the presence of carbohydrates in a given analyte of molecular weight larger than tetroses. [6] Its name is derived from Austrian scientist; Hans Molisch, who was well-recognized with this test. [19][2]

The test involves dehydration of both pentoses and hexoses by concentrated[8][20] sulfuric acid, then furfural or hydroxyl methyl furfural are formed, respectively. [33]

Subsequently, a condensation reaction is occurred with α -naphthol and a purple product is clearly formed. [25][14]. The Molish test is based on adding the Molish detector (solution of α -naphthol in alcohol) to the test tube. This is followed by addition of a small amount of

concentrated sulfuric acid H₂SO₄ to the mixture.

The development of purple loop at the interface of H₂SO₄, and Molisch detector approves the existence of carbohydrates in the tested sample.



• Molisch test limits

Since the Molisch test was previously described as the most important test that is used to determine any sugars in an analyte. But the test has some limitations.

However, while all unilateral sugars, triple sugars and binary sugars are positive for the Molisch test, there are few exceptions. Trise and Tetrose will not react with a Molisch test. Therefore, even if the sample contains every trio and tetrose, but the test will result in negative results.

Molisch test applications

The Molisch test can be the primary test that must be done when one wants to know whether or not carbohydrates are present in the sample.

- the required materials

- Ezoic
- Test tubes
- Test tube holder
- Picnic
- Molisch test actor

It will result from monoolic sugars and monolotherapy (except for trinity as well as quadruple) positive reactions, and sugary proteins as well as nucleic acids have a positive

reaction because all of these compounds decompose to monoolic sugars with strong mineral acids. Then the pentose is dried into Foreign, while the Hyksos is dried into 5-hydroxy methyl Forever. Any of these aldehyds, when they are, will be condensed with two molecules from the disintegration and the production of a purple compound, as shown in the example of glucose

•The principle of interaction

In Molisch’s test, adehydration reaction is occurred between the carbohydrate and concentrated hydrochloric or sulfuric acid and an aldehyde is produced.[1][7]

Subsequently, a condensation reaction is included between the aldehyde and α -naphthol compound in Molisch’sreagent, and a purple or reddish-purple colored complex is formed.[3][4]

• The method of work

Molisch’s reagent (a solution of α -naphthol in ethanol) is added to the tested analyte. Subsequently, a few drops of concentratedH₂SO₄ (sulfuric acid) is added gently to the mixture in drop wise mode.

If a purple or even a purple-reddishcircleis developed at the interface between the sulfuric acid, the tested analyte and Molisch’s reagent, a positive indication of the carbohydrates is

approved in the tested analyte.[39][38]

• Test results

Positive indication should be noticed when carbohydrates including mono saccharides, disaccharides, and polysaccharides as well as glycoproteins and nucleic acids. [44][41] Both trioses and tetroses are excluded—from this test

On the other hand, both pentoses and hexoses can be dehydrated to form furfural and 5-hydroxymethylfurfural, respectively. [49][40]

Both latter aldehydes, are subjected to condensation reaction with α -naphthol, and positive indication is demonstrated by the formation of a purple-colored circle.[39][19]

• Reserves:

Don't add much from the Molish detector.

Do not pour sulfuric acid directly into your solution. Otherwise, carbohydrates can occur with a black loop that may develop, which will give a negatively inaccurate negative test.

Pour the acid into your test tube, and drop it from the inside into the tube. The acid will not be able to interact quickly and the entire process remains

smooth and easy.

Things to take into account In order for the test to be considered successful,

Carbohydrates must have at least five carbon. This is because the result of Forforn derivatives, which contain 5 carbon atoms).

Imports appear in the detector a green color, indicating a false negative test.

Low sugar sugars and sugars are first divided into monoolic sugars using the acid, which gives a positive Molisch test.

Proteins and fats that contain attached carbohydrates may lead to a positive test.

- Precaution:
- Insure slow reactions by dropping the acid down the sides of the test tube very gently.
- Strong acid should be handled very carefully as they can cause severe chemical burns to skin.
- Do not shake the test tube as to avoid losing the purple ring once it is formed.



The formation of a purple ring is a positive indicator for Molisch's Test

Fehling's test

Fehling's solution is a basic, blue-colored organic

compound consisting mainly of a copper(II) ion and a tartaric ion, which stabilizes the copper(II)

ion in a basic solution.

It is a chemical reagent that can be applied to make clear distinction among functional chemical groups of water-soluble carbohydrate and ketones. It also helps distinguish between both reducing and non-reducing sugars. This test was first established by German chemist H.C. Von Fehling.

The goal:

Distinguishing reflux sugars (mono saccharides and some polysaccharides).

binary) for unreduced sugars (others of binary and polysaccharides)

{ Reference sugars are sugars that contain a free anomeric group }

Solutions used:

- Sugar solutions:

° Glucose - fructose - maltose [Reference]

° Sucrose

[unreferenced]

- Fehling's solution

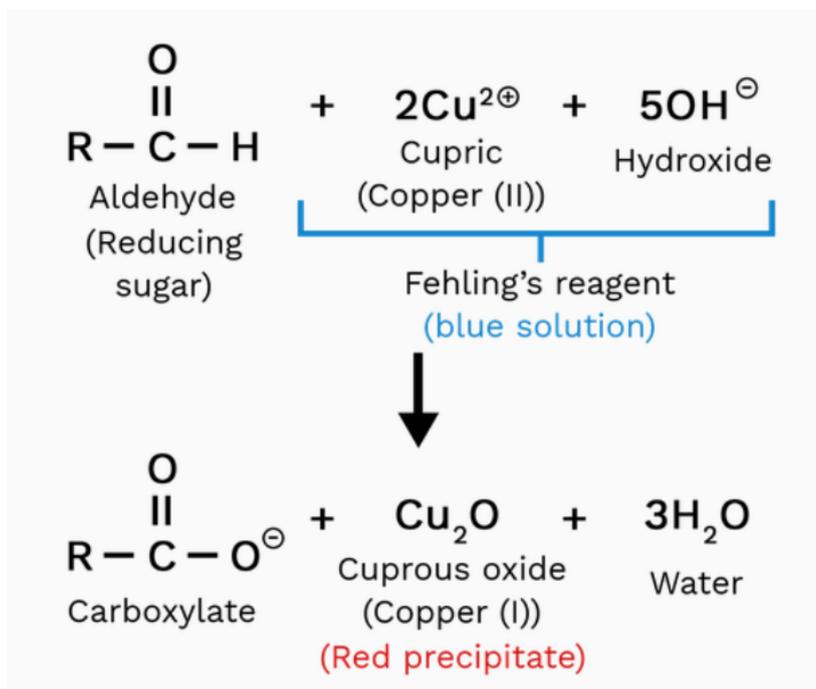
Detector components:

Fehling A, is an aqueous solution containing copper(II) sulfate. It is a blue colored solution

Fehling B, is an aqueous clear solution containing consisting of potassium sodium tartrate (Rochelle salt). A strong alkali with KOH is formed.

Each solution is stored separately. They can be mixed when needed for testing, considering that the copper (II) complex is unstable when both solutions are combined.

Copper(II) tartrate is the active reagent that plays an oxidizing factor and Tartrate acts as alkoxy of a double-bonding feature.



The method of work :

Test solutions:

* Sugar reference

* Unrefined sugar

*Fehling detector:

Solution A: CuSO₄.5H₂O, Solution B: Sodium Potassium Tartrate.

* water bath,

*Pipettes,

* Dry test tubes.

How to conduct the test:

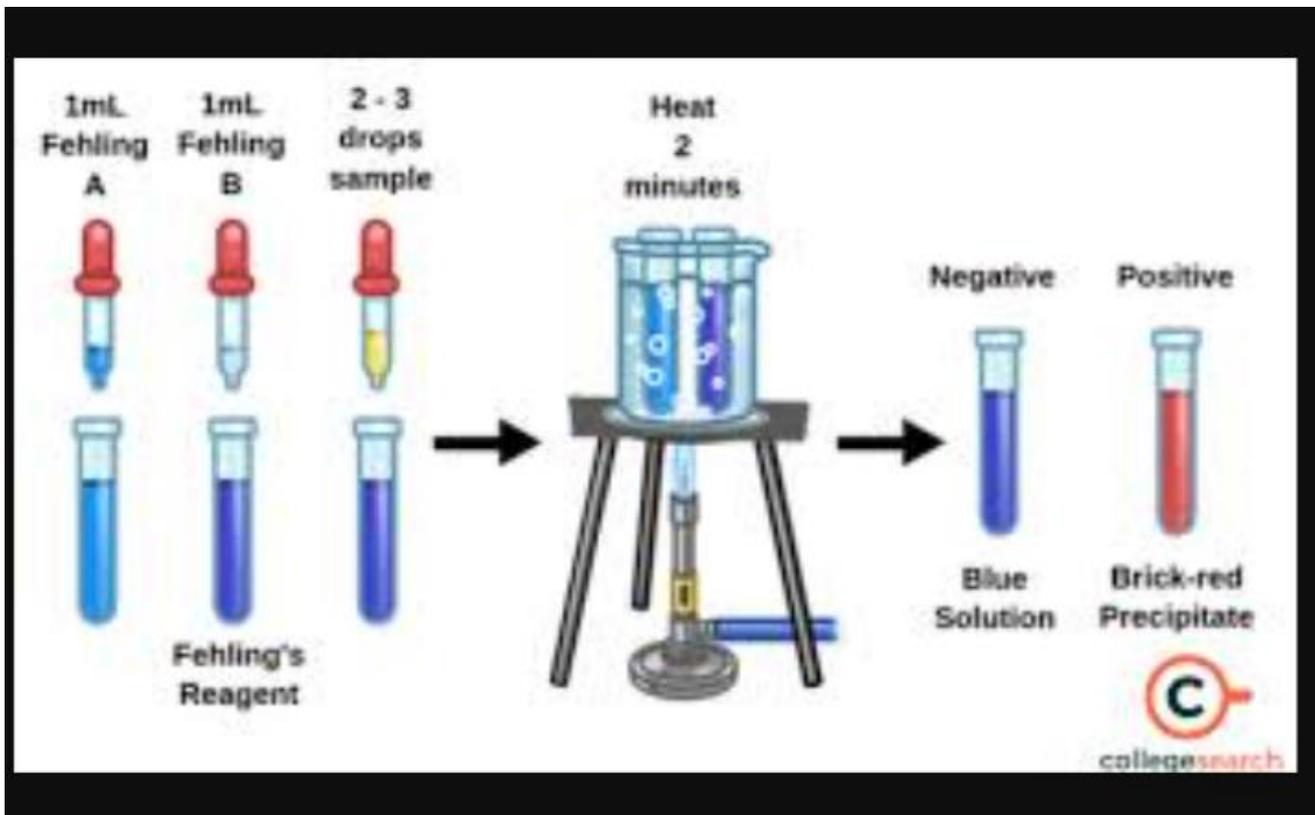
Add 1 ml of the tested solution to a test tube. Add 1

ml of distilled water to separate tube (as a control sample). Finally, pour 1 ml of Fehling's reagent A and B to both tubes, then heat them in a boiling water bath. A red material should be precipitated in the first tube.

The presence of a reddish-brown precipitate, as in the case of glucose, fructose, and lactose (positive Fehling test).

Absence of a reddish-brown precipitate as in sucrose and starch (negative Fehling test).

The result :



	<u>Molish</u>	Fehling
Objective	The general test of detecting sugars of all kinds is free or linked, unilateral, bilateral or multiple, which distinguishes the sugars from other compounds.	Distinguishing reflux sugars (monosaccharides and some polysaccharides). binary) for unreduced sugars (others of binary and polysaccharides)
The solutions used	Glucose-fructose-water Mulich detector Concentrated sulfuric acid	<ul style="list-style-type: none"> • Sugar solutions: ° Glucose - fructose - maltose [Reference] ° Sucrose [unreferenced] • Fehling's solution
•The principle of interaction	In Molisch's test, a dehydration reaction is occurred between the carbohydrate and concentrated hydrochloric or sulfuric acid and an aldehyde is produced Subsequently, a condensation reaction is included between the aldehyde and α -naphthol compound in Molisch's reagent, and a purple or reddish-purple colored complex is formed	Each solution is stored separately. They are mixed when needed for testing, considering that the copper (II) complex is unstable when both solutions are combined Copper(II) tartrate is the active reagent that plays an oxidizing factor and Tartrate acts as alkoxy of a double-bonding feature.
Test results		The presence of a reddish-brown precipitate, as in the case of glucose, fructose, and lactose (positive Fehling test). Absence of a reddish-brown precipitate

		as in sucrose and starch (negative Fehling test).
	<p>Positive indication should be noticed when carbohydrates including mono saccharides, disaccharides, and polysaccharides as well as glycoproteins and nucleic acids.. [44][41] Both trioses and tetroses are excluded—from this test</p> <p>On the other hand, both pentoses and hexoses can be dehydrated to form furfural and 5-hydroxymethylfurfural, respectively. [49][40]</p> <p>Both latter aldehydes, are subjected to condensation reaction with α-naphthol, and positive indication is demonstrated by the formation of a purple-colored circle [39]</p>	

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