

University of Baghdad
College of Science for Women
Department of Chemistry



BIOCHEMISTRY LAB

(For Biology students/First class)

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2023-2024

3. Barfoed's Test:

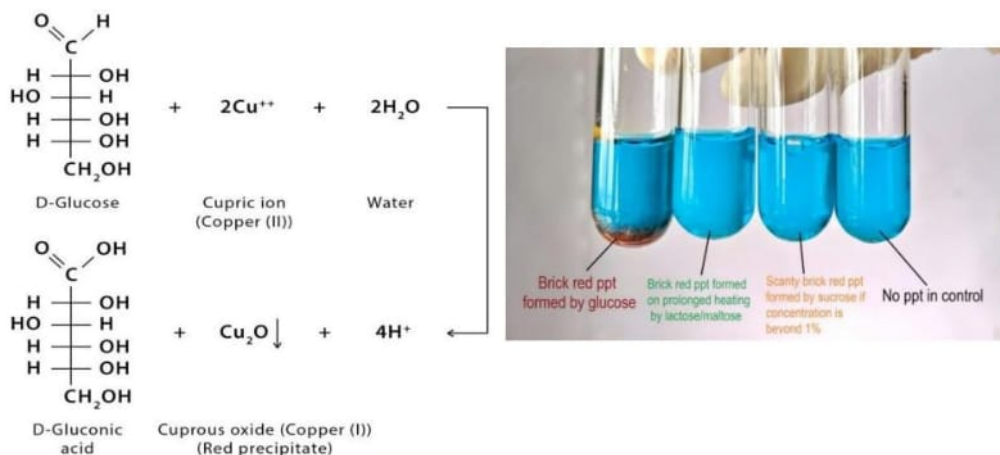
- Used to detect the presence of reducing monosaccharides in a sample, distinguishing them from reducing disaccharides.
- This reaction will detect reducing monosaccharides in the presence of disaccharides. Reagent uses copper ions to detect reducing sugars in an acidic solution. Barfoed's reagent is copper acetate in dilute acetic acid (pH 4.6)
- Reducing monosaccharides are oxidized by the copper ions in a weak acidic medium to form a carboxylic acid and a forms a brick-red precipitate of Cu₂O (cuprous oxide).
- Reducing disaccharides (lactose but not sucrose) undergo the same reaction but at slower rate. Therefore, boiling time is critical and positive test within (2min.) indicate the monosaccharides in case of disaccharides they are first converted into monosaccharide and then colored precipitate are formed.
- Therefore, the prolonged boiling may hydrolyses disaccharides to give a false positive test.

■ Method:

- 1 ml of the solution to be tested + 2 ml of freshly prepared Barfoed's reagent.
- Place test tubes into a boiling water bath and heat for 2 minutes.
- Remove the tubes from the bath and allow cooling.
- **Result:** Red precipitate within the first few minutes indicates the presence of reducing monosaccharides. A red precipitate after 3 minutes indicates the presence of reducing disaccharides Do not heat the tubes longer than 3 minutes, as a positive test can be obtained with disaccharides if they are heated long enough.

Limitation:

- It cannot be used to detect sugar in urine because urine contains chloride ions, which might interfere with the reaction.
- If a higher concentration of disaccharides is present in a sample, it might give a positive result, which could be misleading



4. Seliwanoff's Test:

Seliwanoff's test is a chemical test used to differentiate between aldoses and ketoses, which are types of sugars. Aldoses contain an aldehyde group, while ketoses contain a ketone group. The test is based on the dehydration reaction, which occurs more quickly in ketoses than in aldoses. When heated with acid and resorcinol, ketoses produce a deep cherry red color, while aldoses react more slowly and produce a light pink color.

The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural.

5-hydroxymethylfurfural further reacts with resorcinol present in the test reagent to produce a red product within two minutes.

Method:

- 1 ml of a sample + 2 ml of Seliwanoff's reagent (dissolving 50 mg of resorcinol in 33 ml of concentrated hydrochloric acid (HCl) and then dilute it to 100 ml with water) is added.
- The solution is then heated in a boiling water bath for two minutes.
- A positive test is indicated by the formation of a red product.

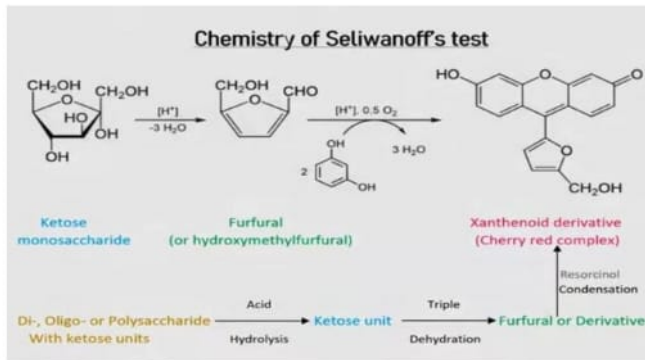
Note:

1. Aldohexoses react to form the same product, but do so more slowly.
2. In case of sucrose, avoid over-boiling because sucrose may be hydrolyzed to its

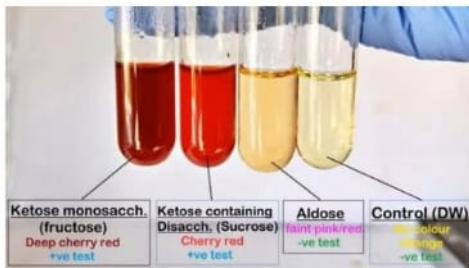
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component (glucose and fructose) and gives false positive result.

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Result: A deep cherry red color indicates the presence of ketoses after 1 minute. A light pink color indicates the presence of aldoses, but this can take longer to appear.



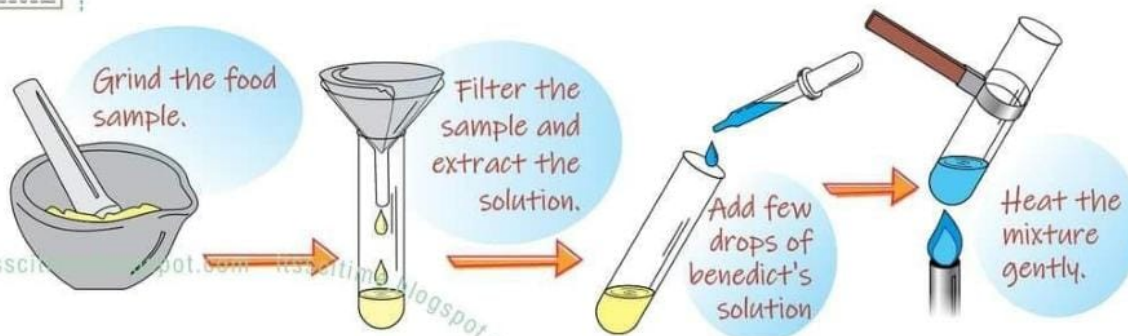
Limitations:

Although this test is able to adequately distinguish a ketose sugar from an aldose sugar, it is not very specific since an aldose may also form the complex. The difference is the time taken to do so.

This test also is a generalized test that does not differentiate the specific ketose present but rather illustrates that a ketose sugar is present. Specific ketose sugar identification must be performed by further testing.



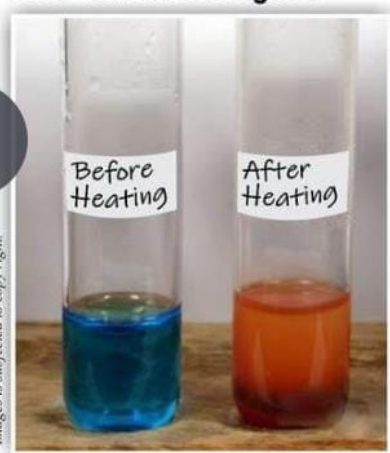
FOOD TESTS - Glucose Test (Benedict's Test)



Result : If glucose present in the food following colour change occurs.

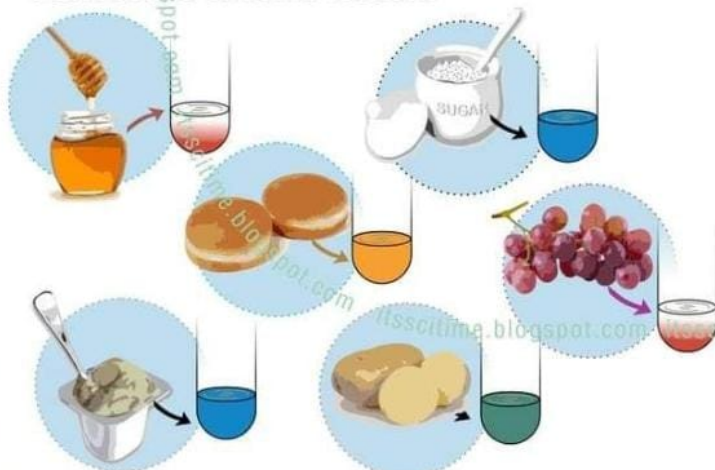


Real colour change...



* Images is subjected to copy right.

Test results of some foods...



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