Qualitative tests of Carbohydrates-II-

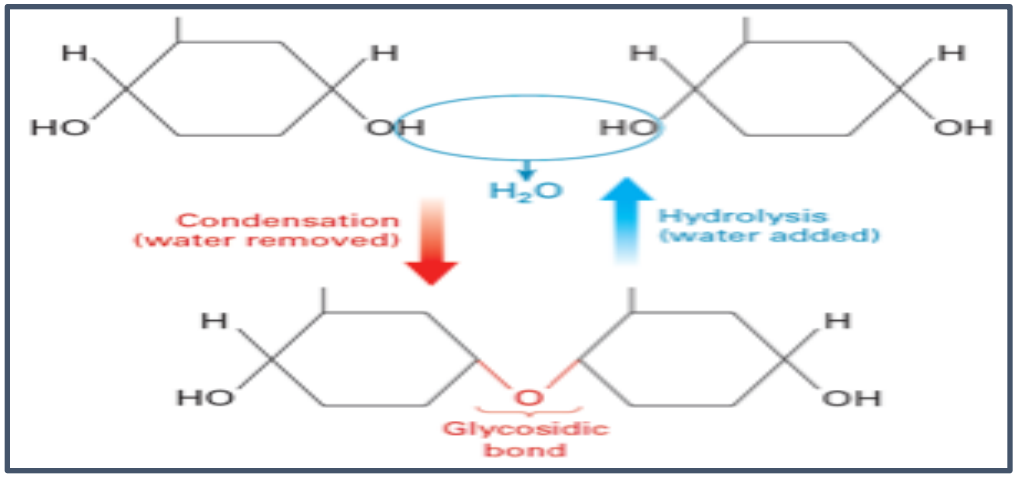
BCH302 [Practical]

Complex carbohydrate:

- **Complex sugars consist of <u>more than one unit</u> of monosaccharide, it could be:**

1. **Disaccharides:** contain **two** monosaccharide units.
2. **Oligosaccharides:** contain **3-9** monosaccharide units.
3. **Polysaccharides:** can contain **more than 9** monosaccharide units.

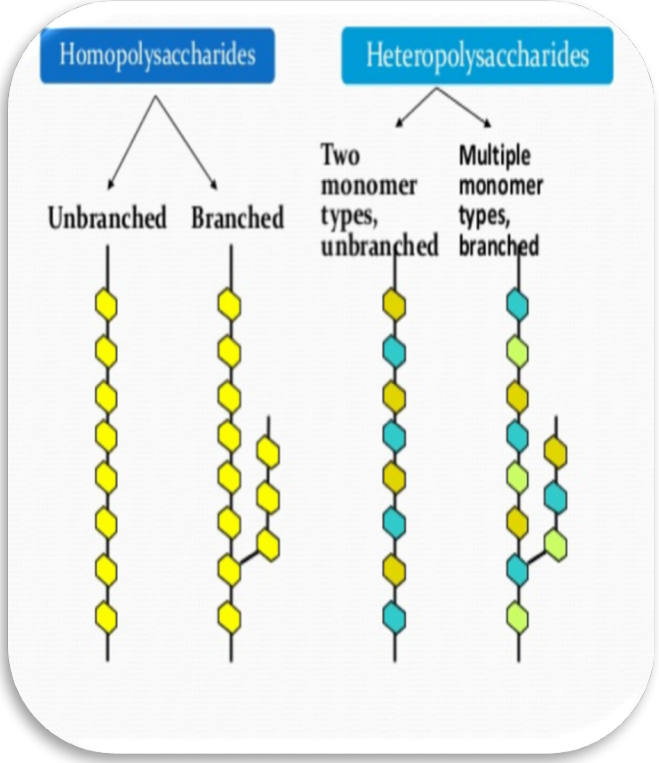
- Complex carbohydrates can be broken down into smaller sugar units through a process known as **<u>hydrolysis.</u>**



Polysaccharides :

- **Polysaccharides can either be :**

1. homopolymeric (same repeating monosaccharide unit).
2. heteropolymeric (mixture of monosaccharaides).



- Plants and animals store glucose in the form of very large polysaccharide glucose homopolymers.

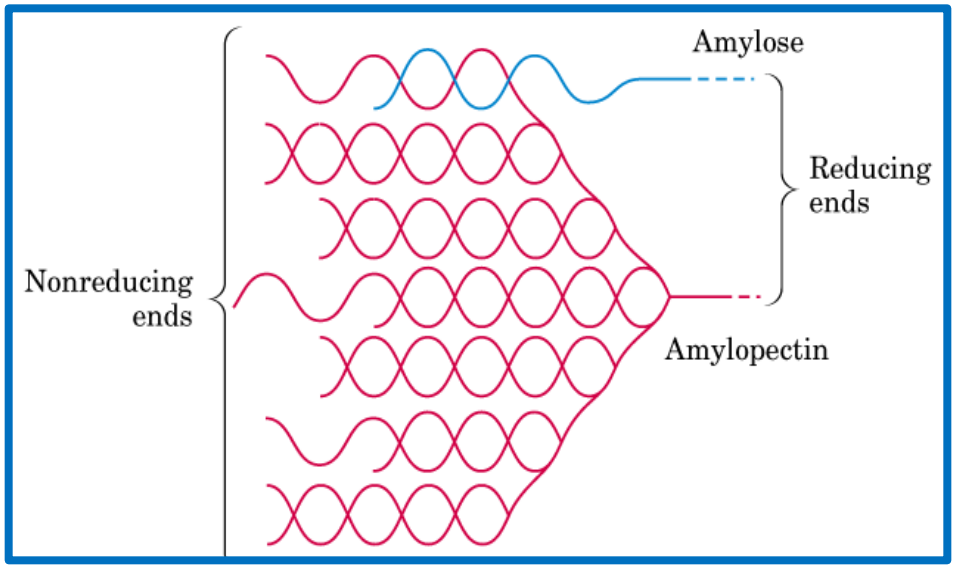
- The glucose homopolymer present in **plants** to store glucose is called **starch**.
- While the glucose homopolymer present in **animal** cells is called **glycogen.**

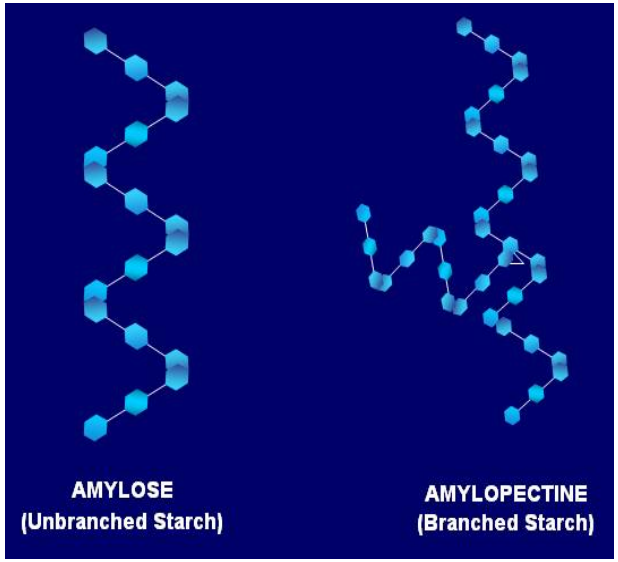
Starch:

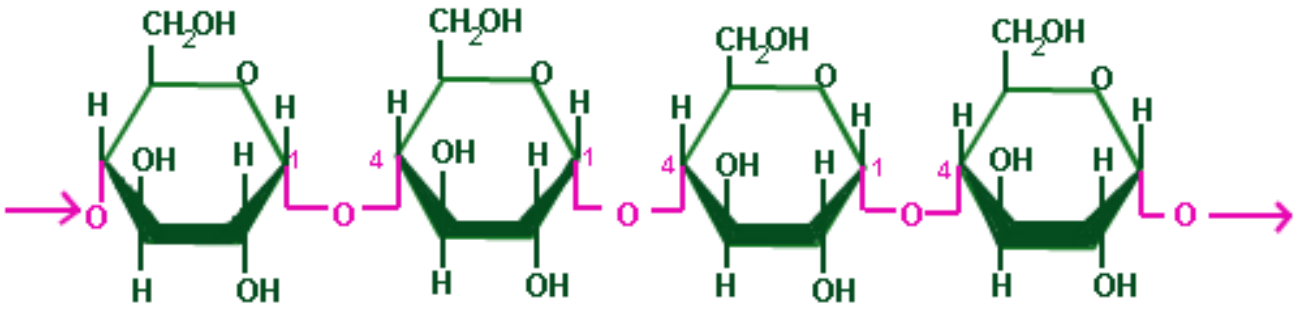
- **Starch consists of two forms:** amylose and amylopectin.

➔ Amylose is a Linear helical polymer of glucose linked by α-1,4 glycosidic bonds (100units).

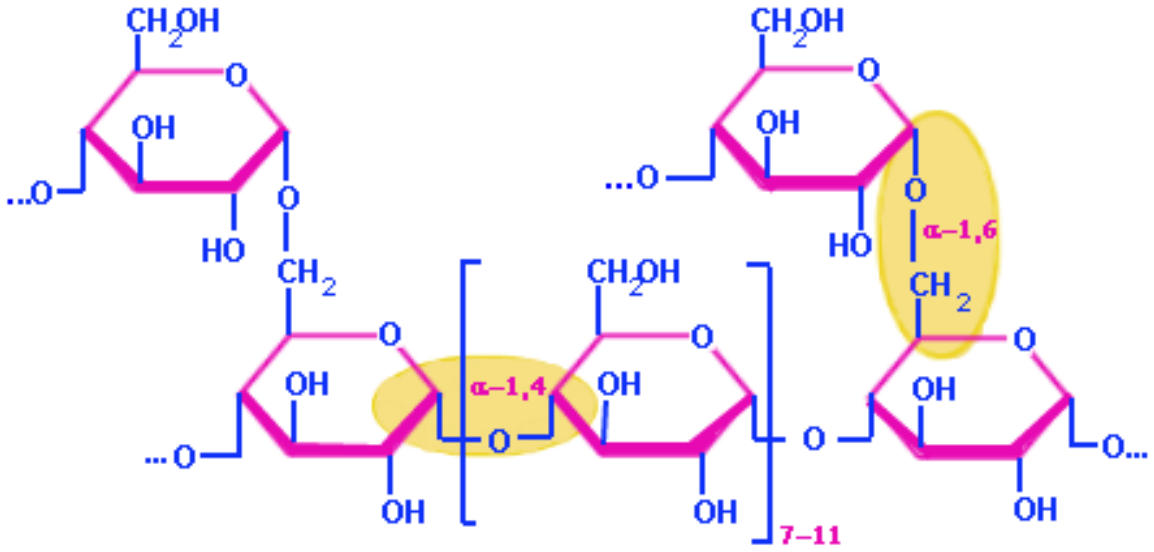
➔Amylopectin is a Branched polymer containing glucose linked by α-1,4 glycosidic bonds, branch points has α-1,6 glycosidic bonds, (100,000 units).







Amylose

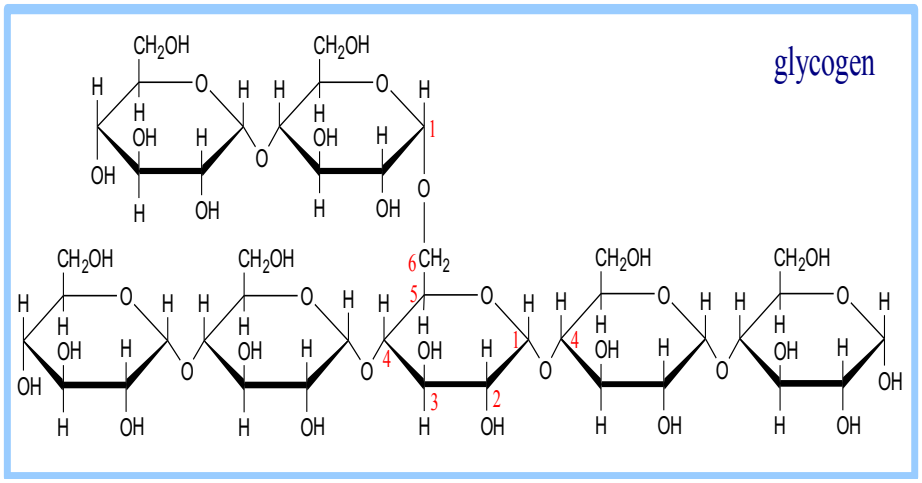


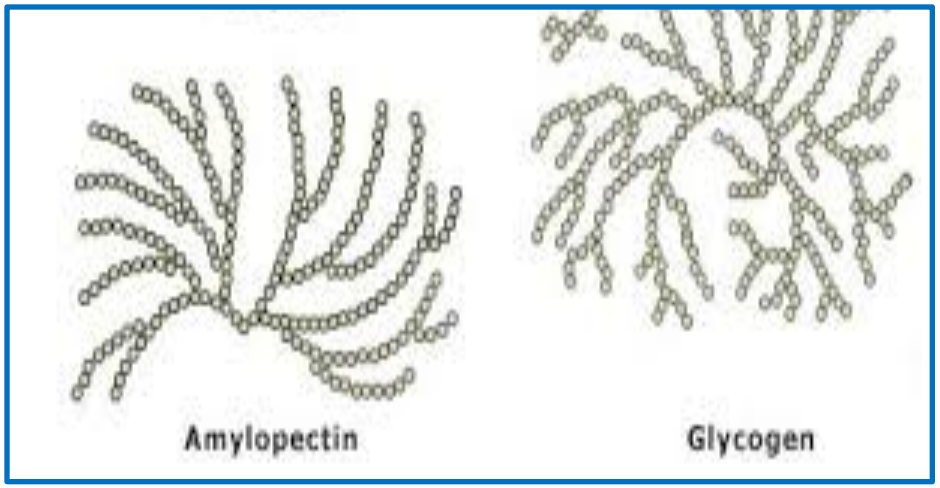
Amylopectin

Glycogen:

- **Glycogen**, is a branched polysacharide of D-glucose which contains both α(1 4) and α(1 6) is similar in structure to amylopectin.

➔ But glycogen has **more** α(1 6) **branches**.





Practical part

Qualitative tests of complex carbohydrates

1. Sucrose hydrolysis Test.
2. The Iodine/Potassium Iodide Test: for polysaccharides.
3. Hydrolysis of Starch.

Experiment 1 : Sucrose hydrolysis Test

- This test is used to convert sucrose (non-reducing disaccharide) to glucose and fructose (reducing monosaccharides).

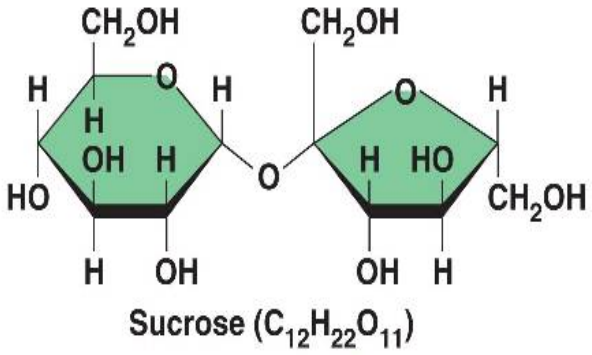
Objective:

- To identify the products of hydrolysis of sucrose (disaccharide).

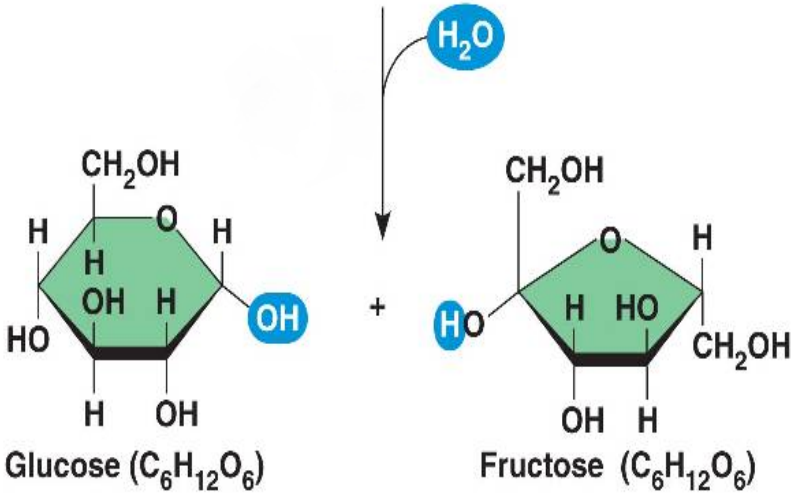
Principle:

- Sucrose is a non-reducing disaccharide so it does not reduce the Cu++ solution (Bendict's and Fehling's test) because the glycosidic bond is formed between the two hemiacetal bonds.
- So there is no free aldehydic or ketonic group to give positive reducing properties.
- When glycosidic bond **hydrolysed (in the presence of acid)** to glucose + fructose (which are reducing sugars) are then able to give positive reducing test.

Non-reducing disaccharide



-ve result with benedict



Reducing monosaccharides

+ve result with benedict

Experiment 1 : Sucrose hydrolysis Test

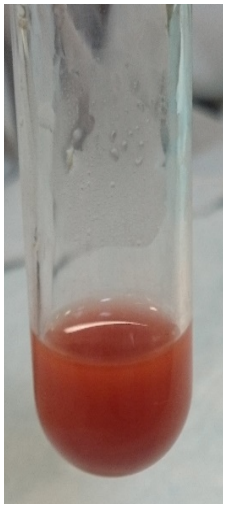
Method:

1. Set up two tubes, add to each one 4ml of a sucrose solution, label the tube: (Sucrose with HCL, Sucrose without HCl)
2. To only one tube add 7 drops of concentrated hydrochloric acid (HCl). CARFULLY
3. Heat both tubes in boiling water bath for 15 minutes.
4. Add 15 drops of concentrated NaOH to each tube (why?).
5. From the tube containing HCl take 2ml in two tube to do Benedict's test and Seliwanoff's test, label the tube (Benedict +HCl) and (Seliwanoff +HCl)
6. Add 2 ml of Benedict's reagent and 2.5 ml of Seliwanoff's reagent .WHAT do expect?
7. From the tube which contain only sucrose take 2 ml to do Benedict's test only (add 2 ml of Benedict's reagent). WHAT do expect?

Results:

Sucrose with HCL		Sucrose without HCL
Benedict's test	Seliwanoff's test	Benedict's test

Sucrose + HCl
(+) Seliwanoff's test



Sucrose + HCl
(+) Benedict's test

Sucrose only
(-) Benedict's test

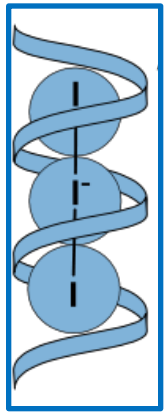
Experiment 2 : The Iodine/Potassium Iodide Test

Objective:

- This test used to distinguish between polysaccharides and mono or oligosaccharides.
- To detect the presence of starch in a sample.

Principle:

- Starch forms deeply blue color complex with iodine.
- Starch contains α- amylose, a helical saccharide polymer and amylopectin. Iodine forms a large complex with α-amylose helix.
- This complex **absorbs light and reflects the blue light only**.
- Simple oligosaccharides and mono saccharides do not form this complex.



Note:

Other polysaccharides like glycogen may give other colors (red).

Experiment 2 : The Iodine/Potassium Iodide Test

Method:

1. Label two tubes A and B.
2. **In tube A:** add 2ml of starch and 2drops of iodine solution and one ml of water. Shake it well
3. A positive test is indicated by the formation of a blue-black complex.
4. Take a half of the tube A and heat it in boiling water bath for 3 min compare between the two tubes and write your observation. WHAT do expect?
5. **In tube B: :** add 2ml of glucose and 2drops of iodine solution. Record your results.

Results:

Tube	Observation
(Starch + Iodine) without heating	
(Starch + Iodine) after heating	
(Glucose+ Iodine)	

Glucose + iodine
(-ve)

Starch + iodine
(+ve)

Heating

Starch + iodine

Experiment 3 : Hydrolysis of Starch

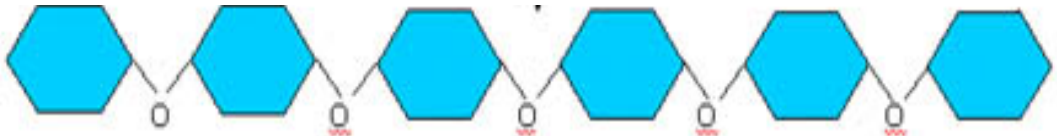
- This experiment illustrates the conversion of starch (non-reducing sugar) to glucose (reducing sugar) by the action of **hydrochloric acid at boiling point**. The longer the starch is exposed to the acid the further hydrolysis proceeds.

Objective:

- To establish the effect of concentrated HCl on a glycosidic bond in starch.

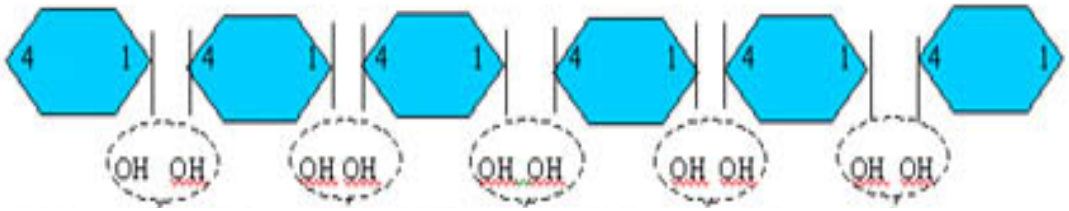
Principle:

- Although starch has free hemiacetal in the terminal glucose residue, it has no reducing properties, because the percentage between the free residues is very low in comparison to the whole molecule.
- **Heating starch solution in acid medium** hydrolysis the glycosidic bonds giving many free glucose residues ➔These glucose molecules give reducing properties to the hydrolysis product.



Non-reducing

Hydrolysis of glycosidic bond (HCl+heating)



Reducing

Experiment 3 : Hydrolysis of Starch

Method:

1. Two ml of starch in large tube.
2. Add 15 drops of Hydrochloric acid, heated in boiling water bath for 10 mints. then cold solution .
3. Add 25 drops of sodium hydroxide to become basic.
4. Divided in two tube (A,B)
5. In tube (A) add 1 ml of iodine solution and note the result. WHAT do you expect?
6. In tube (B) add 1 ml of Benedict reagent, mix and heated for 3 mint and record result. WHAT do you expect?

Results:

Starch with HCL	
Benedict's test	Iodine test

Starch with HCL
[After heating]

[-ve] Iodine test

[+] Benedict's test