



# **Estimation of reducing sugars by dinitrosalicylic acid method**

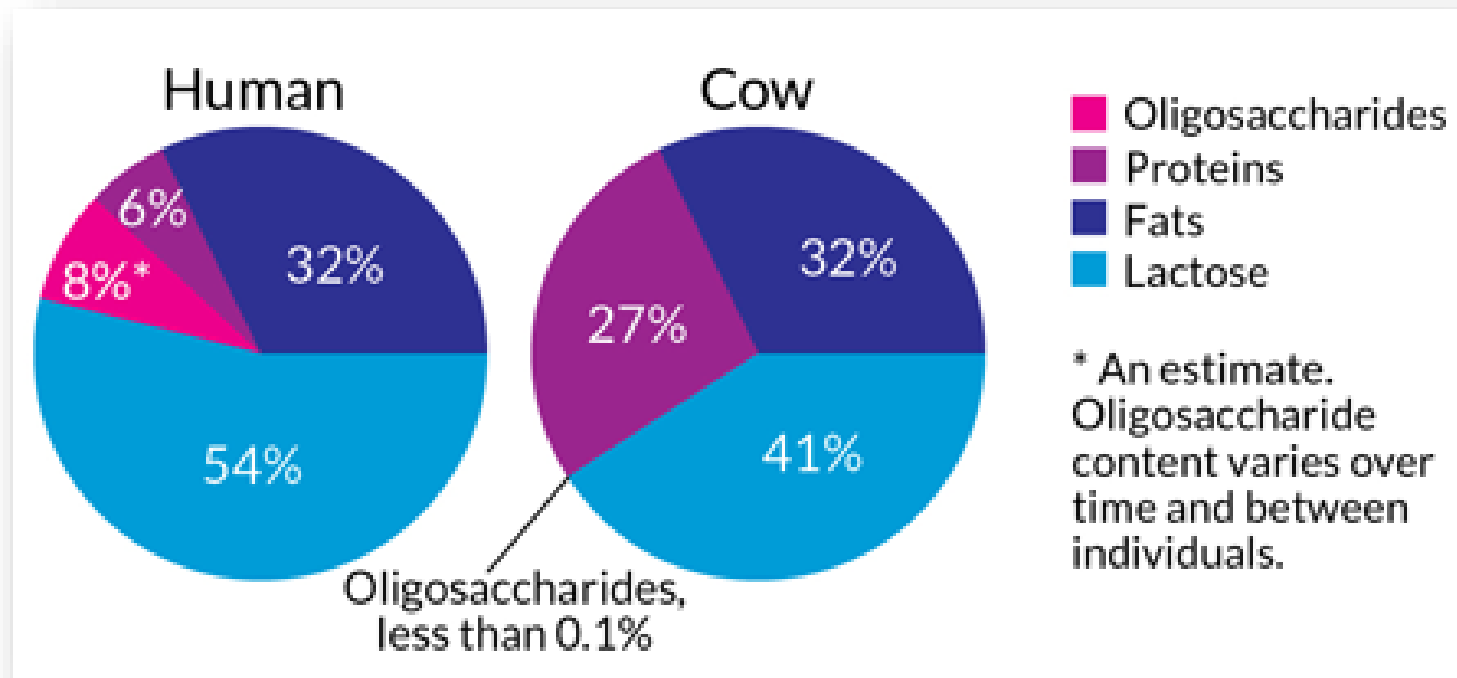
# Carbohydrate in milk:

- The major constituents of milk are **lactose**, **fats** and **proteins** .
- Other **free** carbohydrates found in milk but at **low concentrations**, including **glucose** , **galactose** and **others**.
- As **lactose** is the main carbohydrate in commercial milk, its determination is a basic indicator of **quality control and detection of abnormal milk**.



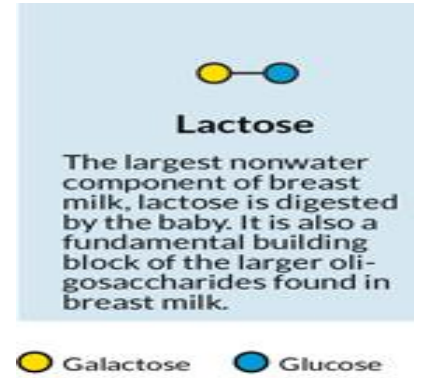
# Human vs cow milk:

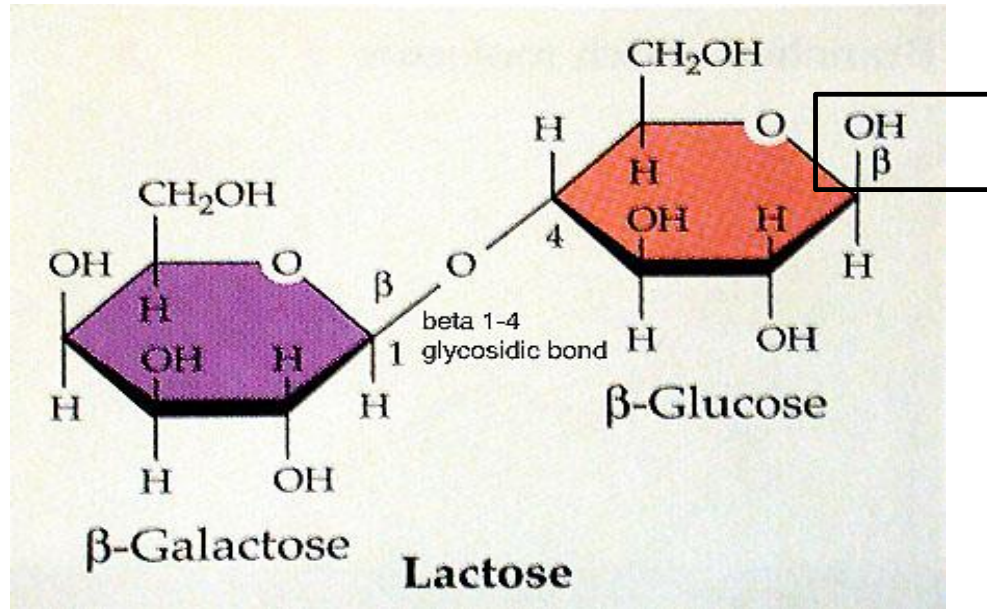
- Human milk contain more lactose than cow's milk.



# Lactose in milk:

- Lactose is a **disaccharide sugar** derived from **galactose** and **glucose**.
- It is a **reducing sugar (why?)**.
- Some of methods for lactose detection in milk are based on the assumption that lactose is the only reducing sugars in milk.
- In this experiment, **dinitrosalicylic acid (DNS)** method will be used, which based on the **detection of reducing sugar** ( which will give a general estimation for lactose not an accurate one, because in milk there are also other reducing sugars).





What is reducing sugar ?

# Practical Part

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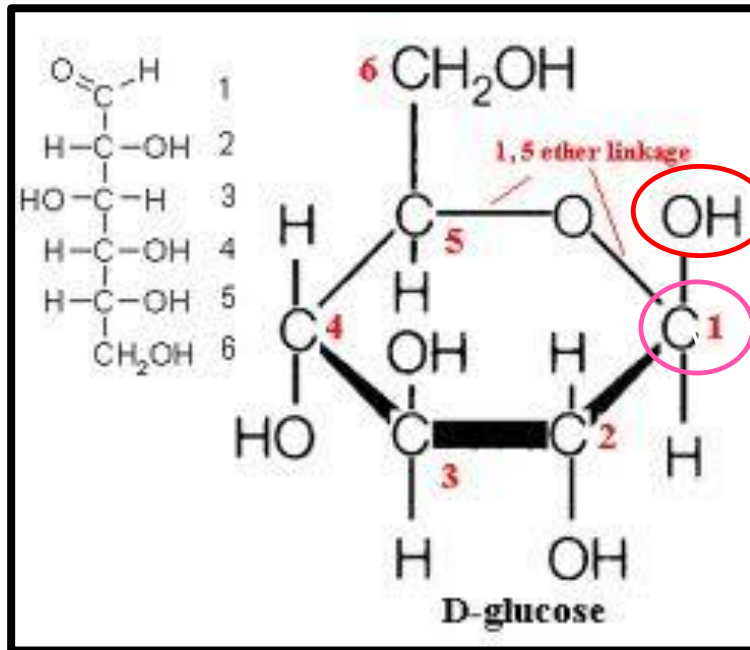
# Objective:

- Estimation of reducing sugars by dinitrosalicylic acid method in milk sample.

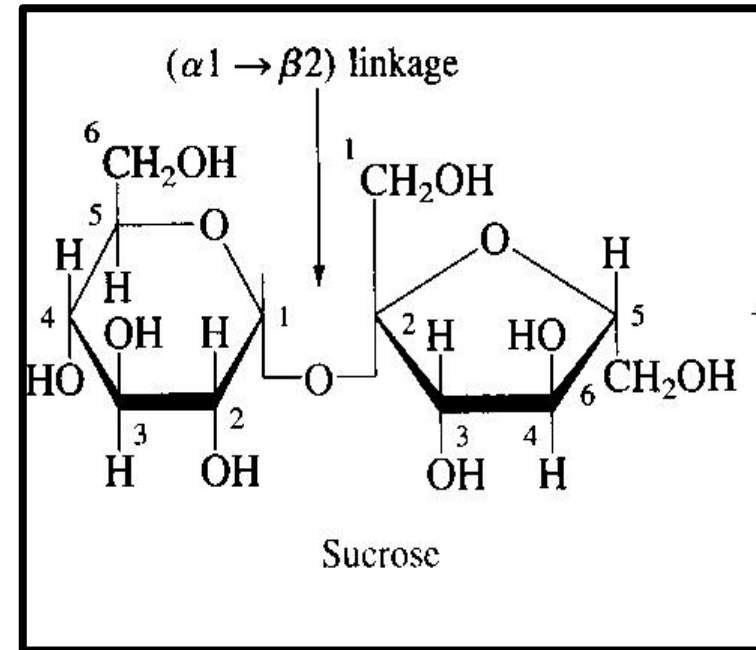
# DNS method:

- The dinitrosalicylic acid (DNS) method for estimating the **concentration** of **reducing sugars** in a sample.
- Not specific.
- Reducing sugars contain free carbonyl group, have the property to **reduce** many of the reagents.
- **All** monosaccharide and **some** disaccharide are reducing sugars (sucrose?).





Free  
carbonyl  
group

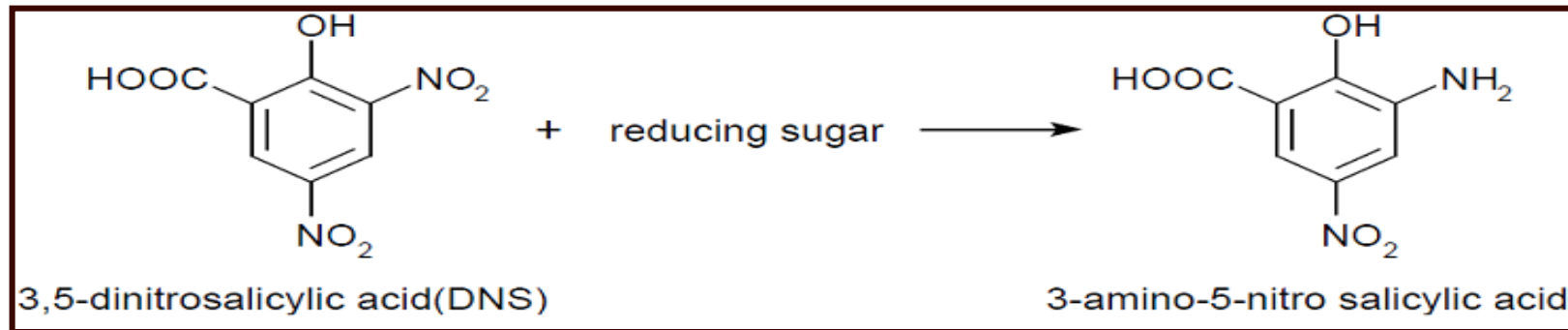


Reducing

Non-reducing

# Principle:

- When **alkaline** solution of 3,5-dinitrosalicylic acid reacts with reducing sugars (eg. Glucose, lactose..) it is converted into **3-amino-5-nitrosalicylic** acid with **orange** color.



- Intensity of the colour is an index of **reducing sugar**.



# Method:

	Glucose solution (100mg/dl) (ml)	Sample (ml)	Water (ml)	DNS reagent (ml)		Sodium potassium tartrate (ml)
B	--	--	1	3	Cover the tubes (with aluminum foil) And heat for 5 min. in a boiling water bath	1
1	0.1	--	0.9	3		1
2	0.2	--	0.8	3		1
3	0.3	--	0.7	3		1
4	0.4	--	0.6	3		1
5	0.5	--	0.5	3		1
6	0.6	--	0.4	3		1
7	0.7	--	0.3	3		1
8	0.8	--	0.2	3		1
9	0.9	--	0.1	3		1
10	1	--	--	3		1
S1	--	1	---	3		1
S2	--	0.6	0.4	3		1

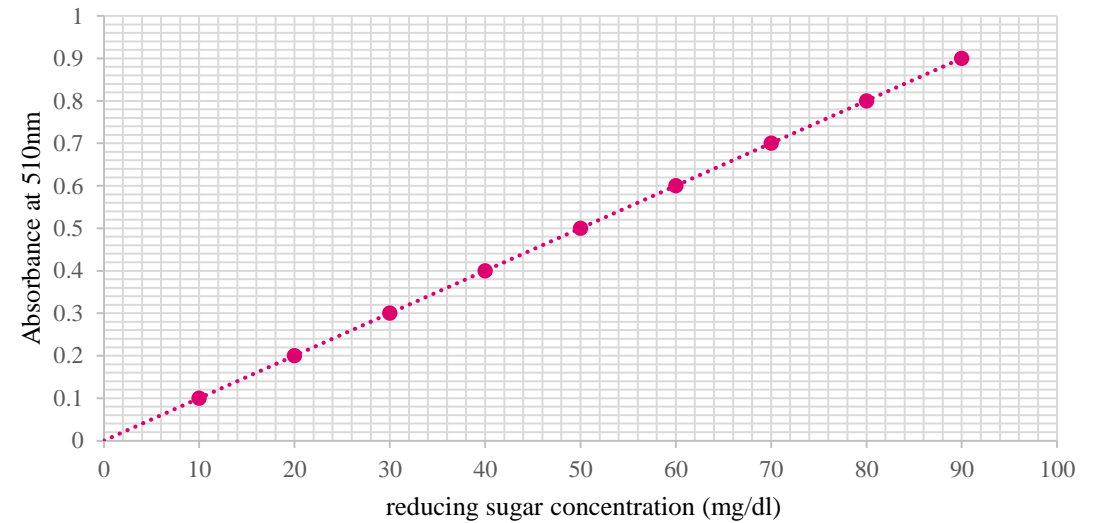
# Method:

- Mix the contents.
- Cool by immersing in cold water and read at **510 nm**.
- Plot the standard curve and calculate the amount in the sample from standard curve and calculate the contents.

# Results:

Tube	Absorbance at 510nm	CHO content (mg/dl)
B	--	--
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
S1		
S2		

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# Calculations:

- Dilution factor (DF) = final volume / aliquot volume
- The amount of carbohydrate in the sample = .....mg/dl x DF

# Home work:

- Is there any other more specific method? If yes name one?

# References:

- Meurant G. Handbook of Milk Composition. Academic Press, 1995.
- Leo M.L. Nollet, Toldra F. Handbook of Dairy Foods Analysis. CRC Press, 2009.
- Allan C. Somersall. The Healing Power of 8 Sugars: An Amazing Breakthrough in Nutrition, Sciences and Medicine. Natural Wellness Group, 2005.
- BCH 445- practical note