BCH 445 Biochemistry of Nutrition [Practical]
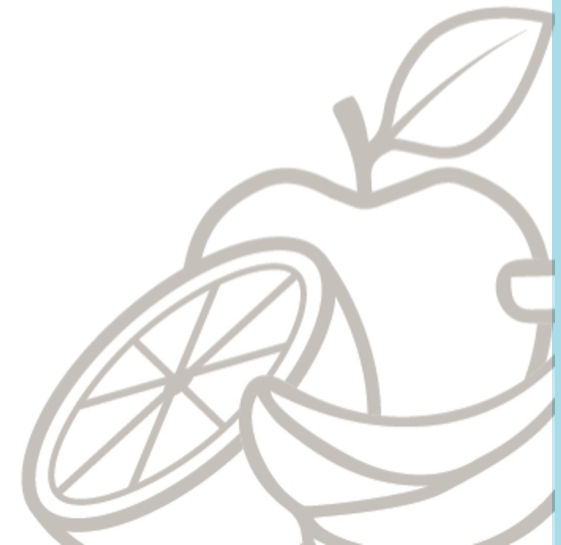
Estimation of reducing sugars by Dinitrosalicylic acid method

Carbohydrates in milk

- The major constituents of milk are carbohydrates, fats and proteins.

- The carbohydrate fraction of milk comprises a complex mixture of mono-, di-, and oligosaccharides of which lactose, is the major constituent in most mammalian species.

- Other free carbohydrates found in milk but at <u>low concentrations</u>, including glucose, galactose, amino sugars, sugar phosphates, neutral and acid oligosaccharides, and nucleotide sugars.

- As lactose is the main carbohydrate in commercial milk, its determination is a basic indicator of <u>quality control</u> and detection of <u>abnormal milk.</u>

Human vs cow milk

Human milk contains more lactose than cow's milk.

> ➤ However, cow milk contains higher content of protein.

Component	Human	Bovine
Protein (g/dL)[1]	0.9 to 1.2	3.3
Fat (g/dL)[1]	3.2 to 3.6	3.7
Lactose (g/dL)[1]	6.7 to 7.8	4.5
Oligosaccharides (g/dL)[1]	0.7 to 1.2	0.1
No. of identified oligosaccharides[2]	<200	approximately 40
% fucosylated[2]	35% to 50%	approximately 1%
% sialylated[2]	12% to 14%	Less than 25%

Sources:[1] (Ballard & Morrow, 2013), [2] (Totten et al., 2012).

Figure 1. Differences in human and cow milk composition.

Lactose in milk

- Lactose is a disaccharide sugar derived from galactose and glucose.

- It is a reducing sugar (why?).

- Some of the 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).

Practical Part

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, which have the property to **reduce** many of the reagents.

- **All** monosaccharides and **some** disaccharides are reducing sugars (sucrose?).
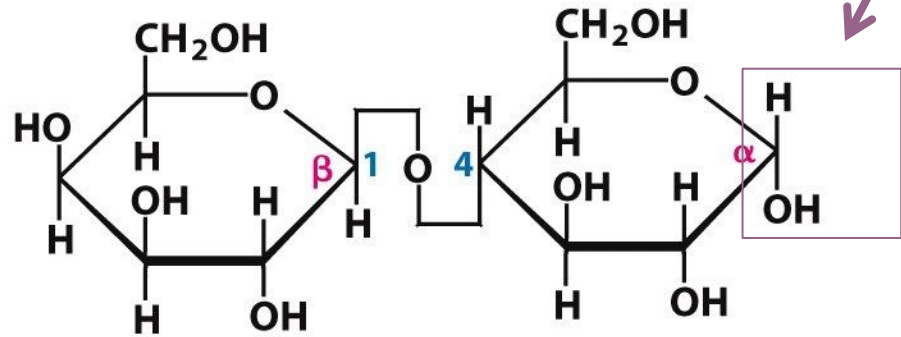
carbonyl group part of glyosidic linkage

Free carbonyl group

Sucrose
(α-D-Glucopyranosyl-(1→ 2)-β-D-fructofuranose
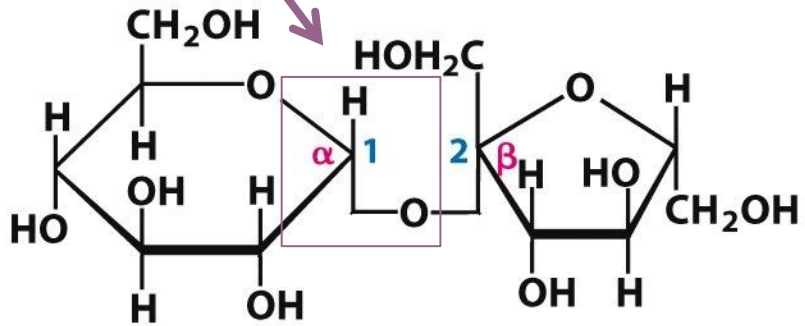
Lactose
(β-D-Galactopyranosyl-(1→ 4)-α-D-glucopyranose

Non-reducing sugar

Reducing sugar

Principle

- When alkaline solution of 3,5-dinitrosalicylic acid reacts with reducing sugars (e.g. Glucose, lactose, etc.) it is converted into 3-amino-5-nitrosalicylic acid with orange color (which absorbs at 540 nm).

3,5-dinitrosalicylic acid(DNS) + reducing sugar $\xrightarrow{100\ ^\circ C}$ 3-amino-5-nitro salicylic acid

(Orange complex)
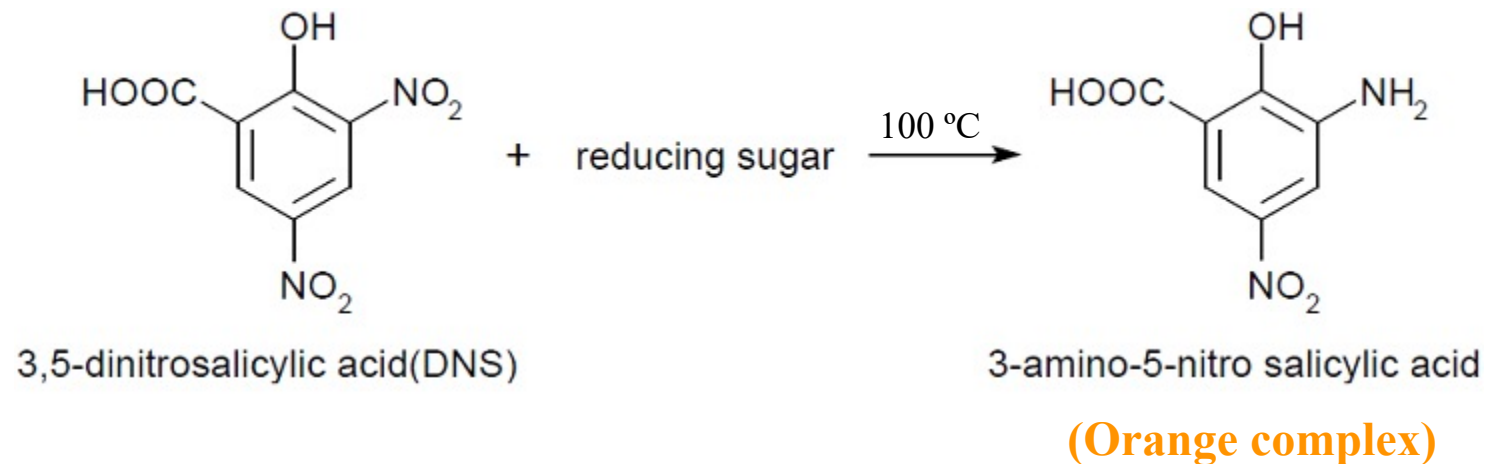
- Intensity of the color is an index of reducing sugar.

a   0.2   0.5   1   2   5   10 (mg/ml)

DNS
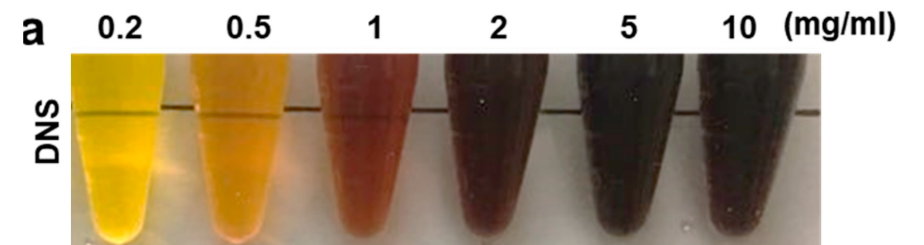
Method

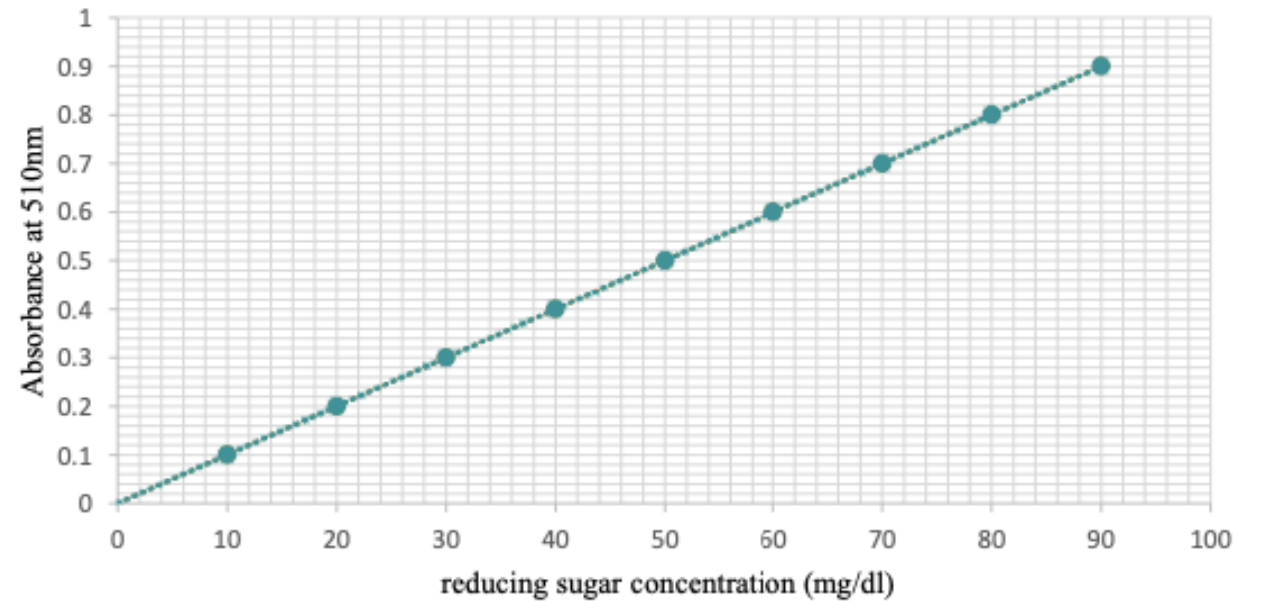| Tubes	Glucose solution (100mg/dl) (ml)	Sample (ml)	Water (ml)	DNS reagent (ml)		Sodium potassium tartrate (ml)
B	--	--	1	3		1
1	0.1	--	0.9	3		1
2	0.2	--	0.8	3		1
3	0.3	--	0.7	3		1
4	0.4	--	0.6	3	Cover the tubes (with aluminum foil) And heat for 5 min. in a boiling water bath	1
5	0.5	--	0.5	3		1
6	0.6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

Method cont.

1. Mix the contents.

2. Place test tubes in boiling water bath for 5 min.

3. Cool by immersing in cold water and read at 540 nm.

4. Plot the standard curve and calculate the amount in the sample from standard curve and calculate the contents.

Results

Tube	Absorbance at 540nm	CHO content (mg/dl)
B	--	--
1		C1xV1=C2xV2 100X0.1=C2x1 C2= 10
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

Homework

1. DNS is one method used to determine reducing sugar content, name other methods. "*list 3*"

2. Explain how lactose can be an indicator of milk spoilage.