
بسم الله الرحمن الرحيم

kingdom of Saudi Arabia
Ministry of Higher Education
king Saud University
College of Science
Biochemistry Department

Practical Note

Nutritional Biochemistry (BCH 445)

Table of Contents

** تعني أن هذه التجارب أضافية ممكن الاستفادة منها لإكمال 12 تجربه في حالة نقص الاحتياجات للتجارب الاساسيه

INTRODUCTION

Preparation of Laboratory Samples

The food material within the sample selected from the population is usually heterogeneous, *i.e.*, its properties vary from one location to another. Sample heterogeneity may either be caused by variations in the properties of different units within the sample (*inter-unit* variation) and/or it may be caused by variations within the individual units in the sample (*intra-unit* variation). The units in the sample could be apples, potatoes, bottles of ketchup, containers of milk etc. An example of inter-unit variation would be a box of oranges, some of good quality and some of bad quality. An example of intra-unit variation would be an individual orange, whose skin has different properties than its flesh. For this reason it is usually necessary to make samples *homogeneous* before they are analyzed, otherwise it would be difficult to select a representative *laboratory sample* from the *sample*. A number of mechanical devices have been developed for homogenizing foods, and the type used depends on the properties of the food being analyzed (*e.g.*, solid, semi-solid, liquid). Homogenization can be achieved using mechanical devices (*e.g.*, grinders, mixers, slicers, blenders), enzymatic methods (*e.g.*, proteases, cellulases, lipases) or chemical methods (*e.g.*, strong acids, strong bases, detergents).

Once the sample has been made homogeneous, a small more manageable portion is selected for analysis. This is usually referred to as a *laboratory sample*, and ideally it will have properties which are representative of the population from which it was originally selected. Sampling plans often define the method for reducing the size of a sample in order to obtain reliable and repeatable results.

Preventing Changes in Sample

Once we have selected our sample we have to ensure that it does not undergo any significant changes in its properties from the moment of sampling to the time when the actual analysis is carried out, *e.g.*, enzymatic, chemical, microbial or physical changes. There are a number of ways these changes can be prevented.

Enzymatic Inactivation

Many foods contain active enzymes they can cause changes in the properties of the food prior to analysis, *e.g.*, proteases, cellulases, lipases, etc. If the action of one of these enzymes alters the characteristics of the compound being analyzed then it will lead to erroneous data and it should therefore be inactivated or eliminated. Freezing,

drying, heat treatment and chemical preservatives (or a combination) are often used to control enzyme activity, with the method used depending on the type of food being analyzed and the purpose of the analysis.

Lipid Protection

Unsaturated lipids may be altered by various oxidation reactions. Exposure to light, elevated temperatures, oxygen or pro-oxidants can increase the rate at which these reactions proceed. Consequently, it is usually necessary to store samples that have high unsaturated lipid contents under nitrogen or some other inert gas, in dark rooms or covered bottles and in refrigerated temperatures. Providing that they do not interfere with the analysis antioxidants may be added to retard oxidation.

Microbial Growth and Contamination

Microorganisms are present naturally in many foods and if they are not controlled they can alter the composition of the sample to be analyzed. Freezing, drying, heat treatment and chemical preservatives (or a combination) are often used to control the growth of microbes in foods.

Physical Changes

A number of physical changes may occur in a sample, *e.g.*, water may be lost due to evaporation or gained due to condensation; fat or ice may melt or crystallize; structural properties may be disturbed. Physical changes can be minimized by controlling the temperature of the sample, and the forces that it experiences.

Sample Identification

Laboratory samples should always be labeled carefully so that if any problem develops its origin can easily be identified. The information used to identify a sample includes: a) Sample description, b) Time sample was taken, c) Location sample was taken from, d) Person who took the sample, and, e) Method used to select the sample. The analyst should always keep a detailed notebook clearly documenting the sample selection and preparation procedures performed and recording the results of any analytical procedures carried out on each sample. Each sample should be marked with a *code* on its label that can be correlated to the notebook. Thus if any problem arises, it can easily be identified.

Sources of Error

There are three common sources of error in any analytical technique:

Personal Errors (Blunders)

These occur when the analytical test is not carried out correctly: the wrong chemical reagent or equipment might have been used; some of the sample may have been spilt; a volume or mass may have been recorded incorrectly; etc. It is partly for this reason that analytical measurements should be repeated a number of times using freshly prepared laboratory samples. Blunders are usually easy to identify and can be eliminated by carrying out the analytical method again more carefully.

Random Errors

These produce data that vary in a non-reproducible fashion from one measurement to the next *e.g.,* instrumental noise. This type of error determines the standard deviation of a measurement. There may be a number of different sources of random error and these are accumulative (see "Propagation of Errors").

Systematic Errors

A systematic error produces results that consistently deviate from the true answer in some systematic way, *e.g.,* measurements may always be 10% too high. This type of error would occur if the volume of a pipette was different from the stipulated value. For example, a nominally 100 cm^3 pipette may always deliver 101 cm^3 instead of the correct value.

To make accurate and precise measurements it is important when designing and setting up an analytical procedure to identify the various sources of error and to minimize their effects. Often, one particular step will be the largest source of error, and the best improvement in accuracy or precision can be achieved by minimizing the error in this step.

EXPERIMENT 1

1. Determination of food total acidity

There are two fundamentally different methods of expressing acidity: (a) titratableacidity and (b) hydrogen ion concentration or pH.

Titratable acidity (also called total acidity) provides a simple estimate of the total acid content of a food and it is not a good predictor of pH. The pH indicates the strength of the acid condition

Food acids are usually organic acids, with citric, malic, lactic, tartaric, and acetic acids being the most common. However, inorganic acids such as phosphoric and carbonic (arising from carbon dioxide in solution) acids often play an important and even predominant role in food acidulation.
The organic acids present in foods influence the flavor (i.e.,tartness), color (though their impact on anthocyanin and other pH-influenced pigments), microbial stability (via inherent pH-sensitive characteristics of organisms),and keeping quality (arising from varying chemical sensitivities of food components to pH).

-Determination of acidity are important in :

1- determine the degree of maturity of fruits and vegetables such as: If the determination of organic acids contained in the grape malic acid, the description is not yet ripe grapes, ripe grapes contains a lot of tartaric acid.

The titratable acidity of fruits is used, along with sugar content, as an indicator of maturity,

generally the higher the maturity, the lower the acid content.

e.g.in the ripening process, such as tomatoes from green to mature stage , there is an increase in sugar content.

2- To determine the freshness of foods such as: fresh milk, the lactic acid levels in the high, that milk is rotten.

3. Acidity indicators reflect the quality of food; the amount of organic acids in food directly affects the food flavor, color, stability, and the level of quality.

4-Determination of acid on the microbial fermentation process has a certain significance. Such as: fermentation products in the beer and soy sauce, vinegar and other acids is an important indicator of quality.

Titratable acidity is determined by neutralizing the acid present in a known quantity (weight or volume) of food sample using a standard base. The endpoint for titration is

usually either a target pH or the color change of a pH-sensitive dye, typically phenolphthalein.

The volume of titrant used, along with the normality of the base and the volume (or weight) of sample, is used to calculate the titratable acidity, expressed in terms of the predominant organic acid

13-3 table Concentrations of H_3O^+ and OH^- in Various Foods at 25 °C

Food	$[H_3O^+]^a$	$[OH^-]^a$	K_w
Cola	2.24×10^{-3}	4.66×10^{-12}	1×10^{-14}
Grape juice	5.62×10^{-4}	1.78×10^{-11}	1×10^{-14}
SevenUp	3.55×10^{-4}	2.82×10^{-11}	1×10^{-14}
Schlitz beer	7.95×10^{-5}	1.26×10^{-10}	1×10^{-14}
Pure water	1.00×10^{-7}	1.00×10^{-7}	1×10^{-14}
Tap water	4.78×10^{-9}	2.09×10^{-6}	1×10^{-14}
Milk of magnesia	7.94×10^{-11}	1.26×10^{-4}	1×10^{-14}

table Molecular and Equivalent Weights of Common Food Acids

Acid	*Chemical Formula*	*Molecular Weight*	*Equivalents per Mole*	*Equivalent Weight*
Citric (anhydrous)	$H_3C_6H_5O_7$	192.12	3	64.04
Citric (hydrous)	$H_3C_6H_5O_7 \cdot H_2O$	210.14	3	70.05
Acetic	$HC_2H_3O_2$	60.06	1	60.05
Lactic	$HC_3H_5O_3$	90.08	1	90.08
Malic	$H_2C_4H_4O_5$	134.09	2	67.05
Oxalic	$H_2C_2O_4$	90.04	2	45.02
Tartaric	$H_2C_4H_4O_6$	150.09	2	75.05
Ascorbic	$H_2C_6H_6O_6$	176.12	2	88.06
Hydrochloric	HCl	36.47	1	36.47
Sulfuric	H_2SO_4	98.08	2	49.04
Phosphoric	H_3PO_4	98.00	3	32.67
Potassium acid phthalate	$KHC_8H_4O_4$	204.22	1	204.22

13-6 table Acid Composition and °Brix of Some Commercially Important Fruits

Fruit	*Principal Acid*	*Typical Percent Acid*	*Typical °Brix*
Apples	Malic	0.27–1.02	9.12–13.5
Bananas	Malic/citric (3:1)	0.25	16.5–19.5
Cherries	Malic	0.47–1.86	13.4–18.0
Cranberries	Citric	0.9–1.36	
	Malic	0.70–0.98	12.9–14.2
Grapefruit	Citric	0.64–2.10	7–10
Grapes	Tartaric/malic (3:2)	0.84–1.16	13.3–14.4
Lemons	Citric	4.2–8.33	7.1–11.9
Limes	Citric	4.9–8.3	8.3–14.1
Oranges	Citric	0.68–1.20	9–14
Peaches	Citric	1–2	11.8–12.3
Pears	Malic/citric	0.34–0.45	11–12.3
Pineapples	Citric	0.78–0.84	12.3–16.8
Raspberries	Citric	1.57–2.23	9–11.1
Strawberries	Citric	0.95–1.18	8–10.1
Tomatoes	Citric	0.2–0.6	4

1.A-Determination of Milk Acidity (Titratable Acidity)

1.1.A. Introduction

-The acidity of fresh milk is due to phosphates, casein and whey proteins, citrates and carbon dioxide dissolved during the process of milking.

Developed acidity which is due to lactic acid produced by the action of bacteria on lactose in milk.

Generally the acidity of milk means the total acidity (Natural + developed) or titrable acidity. It is determine by titrating a know volume of milk with standard alkali to the point of an indicator line phenolphthalein

The acidity of cow milk ranges from 0.10 to 0.26 %.

Acidity is expressed as percent lactic acid. Since 1 ml of 0.1 N lactic acid contains 0.009 grams of lactic acid, the number of ml. of 0.1 N NaOH required to neutralize the lactic acid in the sample, multiplied by 0.009 will give the amount of lactic acid (grams) in the sample, when the result is divided by weight of milk sample and multiplied by 100 the percent lactic acid will be obtained

1.2.A.Material

Fresh and sour milk) (Milk sample)
N/10 NaOH (Use fresh solution
Phenolphthalein indicator
100 ml conical flask , 50 ml capacity burette with stand

1.3.A. Method

Fill the burette with N/10 NaOH solution .

Mix the milk sample thoroughly by avoiding incorporation of air .

.Transfer 10 gm milk to conical flask .

.Add equal quantity of glass distilled water .

Add 3-4 drops of phenolphthalein indicator and stir .

Rapidly titrate the contents with N/10 NaOHsolution ,continue to add alkali drop by . the drop and stirring the content till first definite change to pink colour which remains .constant for 10 to 15 seconds

.Complete the titration within 20 seconds .

.Note down the final burette reading .

1.4.A. Result and Calculation

No of ml. of 0.1 NNaOHRequired for neutralization x 0.009

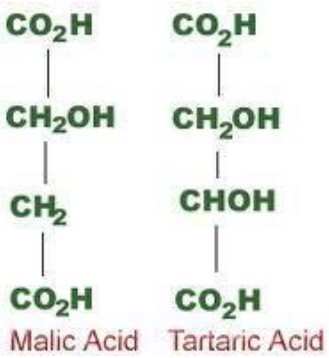
$$\text{Lactic acid\%} = \frac{(\text{No of ml. of 0.1 N NaOH Required for neutralization x 0.009}) \times 100}{\text{Weight of sample}}$$

1.5.A.Discussion

B .Total acidity of fruit juices.

1.1.B. Introduction

The acidity of natural fruit juices is the result mainly of their content of organic acids. For example, most fruits contain the tricarboxylic acid (citric acid) whereas grapes are rich in tartaric acid & peaches, apricots & plums in malic acids. Both tartaric & malic acids are dicaroxylic acids.



The acidity of fruit juice may be determined by simple direct titration with 0.1M sodium hydroxide, using phenolphthalein as an indicator.

1.2.B.Objective

To determine the total acidity in various fruit juice samples.

1.3.B. Materials

0.1 M NaOH
1% phenolphthalein indicator
Fresh fruit juice (orange or lemon)

1.4.B. Method:

1. Weight 10g of sample in conical flask & add 25ml distilled water
2. Titrate using 0.1M NaOH& phenolphthalein as an indicator
3. *Calculate* the total acidity of your fruit juice

Calculate the total acidity:

eq . OH (moles) $= 0.1 \times \text{vol.ofNaOH (ml)} \times 10^{-3}$
g. citric acid $= 0.1 \times \text{vol.ofNaOH (ml)} \times 10^{-3} \times 192.43/3$
g. tartaric acid $= 0.1 \times \text{vol.ofNaOH (ml)} \times 10^{-3} \times 150.09/2$
g. malic acid $= 0.1 \times \text{vol.ofNaOH (ml)} \times 10^{-3} \times 134.09/2$

% acidity = Wt of acid / wt. Of sample ∗100

1.5.B. Results and Calculation:

1.6.B. Discussion:

C. Determination of vinegar total acidity.

1.1C.Introduction:

The total acidity of vinegars is derived both from the original fermentation process &from acidic salts present in the original used for fermentation .It may be determined titrimetrically using phenolphthalein as an indicator .

The natural acidity of vinegar is mainly due to the presence of acetic acid , CH_3COOH , which is volatile .

1.2.C.Objective:

To determine the total acidity in samples of vinegar by titration with alkali

1.3.C. Material:

0.1 M of NaOH
Phenolphthalein indicator
Vinegar

1.4.C. Method:

1- Weight 25 gm vinegar
2- Add 50 ml of distilled water
3- Titrate with 0.1M NaOH , using 2 drops of phenolphthalein as an indicator

1.5.C. Result and Calculations:

Calculate percent acidity as acetic acid (MW=60.05)
Wt. of acetic acid= (0.1M NaOH X ml titre X MW)/1000
%of total acidity= (wt. of acid / wt. of sample) X 100

1.6.C. Discussion:

D.Determination of oil acid value

1.1 .D.Introduction:

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid.

1.2 .D.Objectives:

To determine the concentration of fatty acids that are removed from a triacylglycerols (lipid) due to hydrolysis

1.3 .D.Principle:

The value is a measure of the amount of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and/or lypolytic enzyme lipase.

The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution.

1.4 .D.Materials:

95 % ethyl alcohol or ethanol.

Phenolphthalein indicator solution :- Dissolve one gram of phenolphthalein in 100 ml of 95 % ethyl alcohol.

Standard aqueous potassium hydroxide or sodium hydroxide solution (0.1 N). The solution should be colorless and stored in a brown glass bottle.

1.5.D.Methods

1. Mix the oil or melted fat thoroughly before weighing.
2. Weigh accurately about 5 g of cooled oil sample in a 250 ml conical flask and add 50 ml of freshly neutralised hot ethyl alcohol and about one ml of phenolphthalein indicator solution.
3. Boil the mixture(in water bath) for about five minutes and titrate while hot against standard alkali solution shaking vigorously during the titration.
   The weight of the oil/fat taken for the estimation and the strength of the alkali used for titration shall be such that the volume of alkali required for the titration does not exceed 10 ml.

1.6.D.Results and Calculations:

Acid value = 56.1(V x N) / W

Where V = Volume in ml of standard potassium hydroxide or sodium hydroxide used

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution; and W = Weight in g of the sample

The acidity is frequently expressed as free fatty acid for which calculation shall be

Free fatty acids as oleic acid per cent by weight W = 28.2 (V x N) / W

Acid value = Percent fatty acid (as oleic) x 1.99

1.7.D.Discussion

- Comment on the values obtained for FFA determination for oil sample. What can you say about the samples based on these values?

1.References

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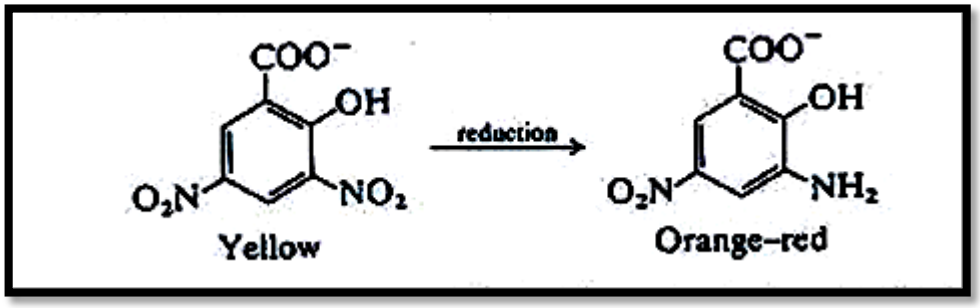
EXPERIMENT-2

Estimation of reducing sugars by dinitrosalicylic acid method:

2.1 Introduction and Principle:

-When alkaline solution of 3,5-dinitrosalicylic acid reacts with reducing sugars it is converted into 3-amino-5-nitrosalicylic acid that impart colour to the solution. Intensity of the colour is an index of reducing sugar.



2.2 Material:

- Dinitrosalicylic acid reagent (DNS) (1 g of dinitrosalicylic acid + 200 mg of crystalline phenol + 50 mg of sodium sulphite in 100 ml of 1% NaOH. (Store at 4□C).
  Since the reagent deteriorates due to sodium sulphite , if long storage is required sodium sulphite, may be added at the time of use .
- 40% sodium potassium tartrate.
- Glucose standard solution 1%
- Working glucose standard 100 mg/ 100 ml (by dilute 10 ml of stock solution to 100 ml)
- Sample preparation :

1. -Homogenize 1g of sample (honey , skimmed milk) in 50 ml of 80% hot ethanol (twice).
2. Centrifuge the content and and collect the supernatant .
3. Evaporate alcohol by heating the contents over water bath. (Don't heat directly, alcohol is highly inflammable).
4. Dissolve the contents left over in 100 ml of distilled water.

2.3Method:

-Pipette out the chemicals in the test tubes as given in the table

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

- 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.

2.4Results and Calculations

Tubes No.	Absorbance	CHO Conc.
B		
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
S 1		
S 2		

Amount of CHO present in 1g sample = $\frac{\text{mg of glucose}}{\text{Volume of test}}$ X 100

2.5.Discussion:

2.6.Reference:

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EXPERIMENT-3

3.Quantitativeof Food Carbohydrate Levels

3.1 Introduction and principle

In food science and in many informal contexts, the term carbohydrate often means any food that is particularly rich in the complex carbohydrate starch (such as cereals, bread, and pasta) or simple carbohydrates, such as sugar (found in candy, jams, and desserts)

In hot acid polysaccharides hydrolyzed into monosaccharide and react with anthrone reagent to form a green colored complex.

3.2 Material:

- Anthrone reagent(0 .2% in ice cold H2SO4)
- Glucose standard solution 1%
- Working glucose standard 100 mg/ 100 ml (by dilute 10 ml of stock solution to 100 ml)
- Sample: skimmed milk, honey, ground nut, guava, grape, banana, carrot, bean ,dates and corn .

(Homogenize 1g of sample in 100 ml of distilled water)

3.3 Method:

1. pipette out various reagents as given in the table below:

Experimental Protocol													
Reagent	B	1	2	3	4	5	6	7	8	9	10	S1	S2
1.Standard Glucose	-	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	0.4	0.6
2.Distilled water	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0	0.6	0.4
Cool all tubes on ice													
3.Anthrone reagent	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

2. Mix the content and keep in boiling water bath for 10 minutes.
3. Cool and read the absorbance at 630 nm
4. Find the value of S1 and S2 from the standard curve and calculate the amount by multiplying with dilution factor, then per gram of sample.

3.4Results and Calculations:

Tubes No.	Absorbance	CHO Conc.
B		
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
S 1		
S 2		

Amount of starch present in 1g sample = $\frac{\text{mg of glucose}}{\text{Volume of test}}$ X 100

3.5 Discussion :

3.6 Reference:

Prasad.M. S; Madhu1 CH ;Venkateshwalu .G and Sabath M. Quantitative evaluation of carbohydrate levels in different natural foodstuffs by uv-visible spectrophometer . Asian J. Pharm. Ana. 2012; Vol. 2: Issue 1, Pg 10-11.

EXPERIMENT-4

4.Estimation of starch byanthrone method

4.1 Introduction:

Starch is an important storage carbohydrate found abundantly in roots, fruits , tubers, stems and cereals. It is a polymer of glucose monomers which exist as amylose and amylopectin units. It is easily hydrolyzed by diluted acids into monomeric units which can be calorimetrically measured.

4.2 Principle:

The sample treated with 80% ethanol to remove reducing sugars. The starch extracted thereafter with perchloric acid. In hot acid starch hydrolyzed into glucose,which get dehydrated to hydroxymethylfurfural that react with anthrone reagent to form a green colored complex.

4.3Materials:

- Anthrone reagent(0 .2% in ice cold H2SO4 –fresh prepared)
- 52% Perchloric acid
- 80% Hot Ethanol
- Glucose standard solution 1%
- Working glucose standard 100 mg/ 100 ml (by dilute 10 ml of stock solution to 100 ml)
- Sample preparation :

  -Homogenize 1g of sample (flour, banana and corn) in 50 ml of 80% hot Ethanol.
  -Centrifuge and collect the ppt.
  - Make 3 washing with 80% hot Ethanol
  -Dry the residue in water bath (Don’t heat directly, alcohol is highly inflammable)..
  -Add 10 ml of water and 13.0ml of 52% Perchloric acid
  -Extracted at 0 °C for 20 minutes.
  -Centrifuge and collect the supernatant.
  -Repeat the extraction step two or three times and make the volume to 100 ml

4.4 Method:

5. Pipette out various reagents as given in the table below:

	Experimental Protocol												
S.No.Reagent	B	1	2	3	4	5	6	7	8	9	10	S1	S2
1.Standard Glucose	-	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	0.4	0.6
2.Distilled water	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0	0.6	0.4
	Cool all tubes on ice												
3.Anthrone reagent	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

6. Mix the content and keep in boiling water bath for 10 minutes.
7. Cool and read the absorbance at 630 nm
8. Find the value of S1 and S2 from the standard curve and multiply the value by factor 0.9 as 0.9 g of starch yield 1 g of glucose on hydrolysis
9. Calculate the amount by multiplying with dilution factor , then per gram of sample.

4.5. Results and Calculations:

Tubes No.	**Absorbance**	**CHO Conc.**
B		
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
S 1		
S 2		

Amount of starch present in 1g sample = $\frac{\text{mg of glucose}}{\text{Volume of test}}$ X 0.9 X 100

4.6 Discussion:

4.7 Referance:

1. Sharma R.K.andSangha S.P.S.(2009). Basic techniques in biochemistry and molecular biology.I.K. Internatioal publishing house .New Delhi .p 81.

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EXPERIMENT-5

5. Determination of sample fructose content

5.1 Introduction:

Fructose is a ketohexose. Fruits and honey are rich source of this sugar.

Fructose formshydroxymethyl furfural when treated with acid.

This hydroxymethyl furfural react with resorcinol to form a red color product.

5.2 Objective:

Determine the content of fructose in samples.

5.3Material:

- resorcinol reagent (1g resorcinol and 0.25 gm of thiourea in 100 ml of glacial acetic acid. keep the solution in dark)
- Diluted HCl (5:1 HCl: Water)
- Standard fructose solution (100mg /100ml of water, then take 10 ml of this solution and dilute to 100 ml to preparing working solution)
- Sample : Fruit juice and honey (1g of sample in 100 ml of distilled water)

5.4 Method:

- Add the different solutions as given in table below:

Experimental Protocol													
. Reagent	B	1	2	3	4	5	6	7	8	9	10	S1	S2
.Working Standard Solution	-	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2		
Sample												1.0	2.0
D.H_2O	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2	-	1.0	-
Resorcinol reagent	1 ml												
diluted HCl	7 ml												

- Mix the content and keep at 90^0C in water bath for 10 minutes.
- Cool the tubes under tap water and read the absorbance at 520 nm within 30 minutes.

- Plot the standard curve and calculate the quantity of fructose present in the sample by multiplying its value with dilution factor.

5.5 Results and Calculations:

Tubes No.	Absorbance	fructose Conc.
B		
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
S 1		
S 2		

Amount of starch present in 1g sample = $\frac{\text{mg of fructose}}{\text{Volume of test}}$ X 100

5.6. Discussion:

5.7. Referance

- Sharma R.K.andSangha S.P.S.(2009). Basic techniques in biochemistry and molecular biology. I.K. Internatioal publishing house . New Delhi .p 82

EXPERIMENT-6

6. Crude Fiber Digestion and Analysis

6.1. Introduction and principle

Dietary fiber is defined as plant polysaccharides that are indigestible by humans, plus lignin. The major components of dietary fiber are cellulose, hemicellulose, pectin, hydrocolloids and lignin.

The crude fiber method gives an estimate of indigestible fiber in foods. It is determined by sequential extraction of a defatted sample with 1.25% H_2SO_4 and 1.25% NaOH. The insoluble residue is collected by filtration, dried, weighed and ashed to correct for mineral contamination of the fiber residue. Its analysis is also important for detecting adulteration in quality and quantity of foods.

6.2.Objective

To determine the amount of crude fibrein whole flour

6.3. Material

6.3.1.Material for Procedure 1:

- petroleum ether
- Sulphuric acid (0.225 N) (1.25 ml of conc. H_2SO_4 diluted to 100 ml)
- Sodium hydroxide (0.313N) (1.25g NaOH diluted to 100 1 H2O).

6.3.2. Material forProcedure 2:

1. Dilute Sulphuric acid – 1.25 % (w / v), accurately prepared
2. Sodium hydroxide Solution – 1.25 % (w / v), accurately prepared
3. Ethanol – 95 % (v / v)
4. Petroleum ether.
5. soxhlet extractor, and thimbles

CAUTION*: Always add acid to water in making solutions of acid. Do not add water to acid. Wear goggles when working with these solutions.*

6.4. Method:

6.4. 1. Method for Procedure 1:

1. Take 2g of powdered material and pour it in 100 ml petroleum ether to removefat
2. Boil at 52^0C in water bath if necessary.
3. Boil 2g of dried sample after extraction with 200 ml of H_2SO_4 for minutes using bumping chips.
4. Filter through muslin and wash the contents with the hot water to remove if any acid left.
5. Boil the residue in 200ml of NaOH for 20 minutes.
6. Filter like step 3 and wash with 25 ml of 1.25% H2SO4.
7. Remove the residue and transfer in a pre-weighed dish (W_1).
8. Dry the residue at 130^0C for 2 hours cool and weigh (W_2).
9. Ignite at 600^0C for 30 minutes. Cool and weigh (W_3).

6.4.1.Methodsfor Procedure 2:

1. Weigh accurately about 2 gm ground sample into a thimble (e.g whole flour)
2. Extract for about 1 hour with petroleum ether in a soxhlet extractor.
3. Transfer the material in the thimble to a 1 litre flask.
4. Take 200 ml of dilute sulphuric acid in a beaker and bring it to boil.
5. Transfer the whole of the boiling acid to the flask containing fat free material and immediately connect the flask to a water cooled reflux condenser and heat so that the contents of the flask begin to boil within 1 minute.
6. Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid. Continue boiling for exactly 30 minutes.
7. Remove the flask and filter through fine linen (about 18 threads to a cm) or through a coarse acid washed, hardened filter paper held in a funnel and wash with boiling water until the washings are no longer acid to litmus paper.

8. Bring some quantity of sodium hydroxide solution to boil under a reflux condenser. Transfer the residue on the filter into the flask with 200 ml of boiling sodium hydroxide solution.
9. Immediately connect the flask with the reflux condenser and boil for exactly 30 minutes.
10. Remove the flask and immediately filter through the linen or filter paper.
11. Thoroughly wash the residue with hot water and transfer to a gooch crucible prepared with a thin but compact layer of asbestos.
12. Wash the residue thoroughly first with hot water and then with about 15 ml of ethanol and with 3 successive washings of petroleum ether.
13. Dry the gooch crucible and contents in an air oven at 105°C for 3 hours. Cool and weigh.
14. Repeat the process of drying for 30 minutes, cooling and weighing until the difference between two consecutive weighings is less than 1 mg.
15. Incinerate the contents of the gooch in a muffle furnace at 550°C until all carbonaceous matter is burnt.
16. Cool the gooch crucible in a dessicator and weigh

6.5. Results and Calculations:

6.5.1. By Procedure 1

% crude fiber=

$$\frac{\text{Loss of weight on ignition } (W_2 - W_1)-(W_3 - W_1)}{\text{Weight of the sample}} X100$$

6.5.1. By Procedure 2

Crude fibre (on dry basis) = 100 (W1 – W2) x 100

Percent by weight W 100 – M

Where W1 = wt of gooch crucible + contents + asbestos before ashing

W2 = wt of gooch crucible + ash and asbestos after ashing

W = wt of sample taken for test

M = Percent moisture content.

6.6. Discussion:

Comment on the values obtained for the crude fibre content in the flour sample. What is the importance of crude fibres for the human digestive system?

6.7. References

1. Roehrig, K. Carbohydrate Biochemistry and Metabolism. Westport, CT: The AVI Publishing Co. 1984.
2. Sharma R.K.andSangha S.P.S.(2009). Basic techniques in biochemistry and molecular biology. I.K. Internatioal publishing house .New Delhi .p 90.

EXPERIMENT-7

7. Estimation oftotal proteins in milk and eggwhite.

7.1. Introduction and principle

The major constituents of milk are lactose , fats and proteins . The proteins include casein (aphosphoprotein and 80% of total protein) , lactoalbumin and a little lactoglobulin. There are important amounts of calcium ,phosphorus and vitamin A and B_2 with rather small quantities of vitamin B_1 , C and D_3 or iron . The white colour of milk is due to emulsified lipid and to the calcium salt of casein . That fat content of human milk is similar to that of cow`s milk but there is more lactose and less protein ,calcium and phosphorus. Colostrums is more yellowish and contains about twice as much protein including immunoglobulin, The protein content falls with time reaching average levels after about one month. Fat and lactose change little.

Total protein can be estimated by the Lowry method and Turbidimetric method .

Lowry Procedure can detect protein levels as low as 5ug.Two reactions account for the intense blue color that develops;

(1)that coordination of peptide bonds with alkaline copper (biuret reaction)and

(2)the reduction of the Folin-Ciocalteu reagent (phosphomolybdatephosphotungstate) by tyrosine and tryptophan residues in the protein .

Determination of total protein by measurement of protein turbidity produce by mixed with an anionic organic acid such as sulfosalicylic acid , TCA , or benzethonium chloride. These methods are sensitive , but the reagent does not react equally with each protein fraction. This is particularly true of sulfosalicylic acid , which produces 4 times more turbidity with albumin than with α-glubulin .

7.2. Objectives

To estimate the total protein in milk and egg by :

1- the Lowry method

2-Turbidimetricmethods(bysulfosalicylic acid).

7.3. Materials

A. Lowry Procedure

Albumin standard (100 µg/ml): Dissolve 0.01g albumin in water and make up to 100ml with water

.Reagent A: Dissolve; 2 g Na_2CO_3 (2%), 0.4 g NaOH (0.4%), 0.16 g sodium, potassium tartarate (0.16%), 1g SDS (1%) in water and make up to 100 ml.Store at room temperature

Reagent B: 4% $CuSO_4.5H_2O$, Dissolve 0.4g $CuSO_4.5H_2O$ in a little volume of water and make up to 10 ml. Store at room temperature.

.Reagent C: 100 parts of reagent A + 1 part reagent B .

Take 100 ml reagent A and add 1ml reagent B.

Folin-Ciocalteu reagent: Dilute commercial reagent by 1: with water.
Prepare fresh

B.Turbidimetric method

Bovine albumin standard. 50mg/dl-

sulphosalicylic acid 3%

Spectrophotometer , test tubes .Glass cuvettes

Preparation of sample:

A.. Lowry Method

fat-free milk

Centrifuge cold milk at 0 - 4ºC for 30 min. at 4,000 rpm. Quickly remove the lipid layer from the top. Or use skimmed cow`s milk

Dilute fat-free milk 1: 1000

Pipette 0.1ml milk into a 100ml volumetric flask and make up to the mark with distilled water

Egg white

*(Note : weigh the egg , then weigh the egg white)

1g of egg white is mixed with 100 ml of distilled water(slowly mix egg white before used)

B. Turbidimetric method

Dilute fat-free milk 1: 100

1g of egg white is mixed with 30 ml of distilled water

7.4. Method

A. Lowry Procedure

Pipette out the chemicals in the test tubes as given in the table

Tube	Water (ml)	Standard(ml)	Sample (ml)
A(blank)	1.0	-	-
B	0.8	0.2	-
C	0.6	0.4	-
D	0.4	0.6	-
E	0.2	0.8	-
F	-	1.0	-
G	-	-	milk 1.0
H	-	-	milk 1.0
I			Egg 1.0
L			Egg 1.0

- Add 3ml reagent C to all tubes. Mix and let stand at room temp. for 15 min.
- Add 0.3 ml of Folin-Ciocalteu reagent. (Add this reagent to one tube at a time and immediately after adding it mix well.
- Let the tubes stand at room temperature for 45 min.
- Read absorbance at 660 nm against the blank

.

B. Turbidimetric method

Set up a series of test tubes as follows:

Tube No.	**Protein Soln. (ml)**	**H_2O (ml)**	**sulphosalicylic acid**
Blank(0)	**0**	**2**	**8ml**
1	2	0	
2	1.8	0.2	
3	1.5	0.5	
4	1.2	0.8	
5	1.0	1.0	
6	.8	1.2	
7	0.5	1.5	
Milk sample	2	0	
Milk sample	1	1	
Egg white	2	0	
Egg white	1	1	

-Mix the contents of each tube well and allow standing for 5 minutes.

-Using the spectrophotometer for the blank solution at 500 nm, set the transmittance at 100%.

Record the tubes transmittance.

-Read the protein concentration of the “unknown sample” from the standard curve.

7.5. Results and calculation:

A. Lowry Procedure

Tube	protein concentration(µg/ml)	A_{660}
A	0	
B	20	
C	40	
D	60	
E	80	
F	100	
G		
H		
I		
L		

Plot a standard curve for absorbance at 660 nm againstprotein concentration (µg/ml).
From the standard curve obtain the concentration of protein in the diluted sample.

Averageprotein concentration in diluted sample= (µg/ml).

Calculate the concentration of protein in the original sample (g/ 100 ml)

Concentration in original milk(g/L) = $\frac{\text{Average conc. in diluted milk} \times 10^3 \times 10^3}{10^6}$

B. Turbidimetric method

Solutions	**Transmittance at 500nm**	**Protein conc. (mg./dl)**
1		
2		
3		
4		
5		
6		
7		
Milk sample		
Milk sample		
Egg white		
Egg white		

Plot transmittance against protein concentration on semi-logarithm paper (standard curve).

-Read the protein concentration of the “unknown sample” from the standard curve.

Calculate the concentration of protein in the original sample (g/ 100 ml)

.

7.6. Discussion

7.7 References:

- Neide K.K. et al.2003, Determination of total proteins in cow milk powder samples: a comparative study between the Kjeldahl method and spectrophotometric methods. J. Food Anal., 16: 507-516.

- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent.Journal of Biological Chemistry, 193,265–275.

EXPERIMENT-8

8. Determinationof HoneyProline

8.1. Introduction:

The analysis of proline in natural honey should point out the determination of ripeness and genuiness. Most of the proline in the honeys originates from the secretion of honeybees.

Determination theprolineconcentration in honey contributes to the knowledge of one of the parameters that can be used as a quality parameter for the samples.

8.2. Objective:

Determine the proline concentration in honey.

8.3. Material:

Formic acid (98-100%)
Ninhydrin solution (3% in ethylene glycol monomethylether)

2-propanol-water solution (1:1)

proline standard:
Range 0- 200 mg /dl

Honey sample(1)
2.5 g. of honey dissolved in about 25 ml distilled water. Then the solution was transferred quantitatively to a 100 ml. volumetric flask, diluted to volume with distilled water and was very well shaken.

8.4. Method:

Follow the procedure given in table

ml	blank	1	2	3	4	5	6	7	8	S1	S2
standard	-	0.1	0.2	0.3	0.4	0.5	0.6	0.8	1		
Sample	-	-								1	1
H_2O	1	0.9	0.8	0.7	0.6	0.5	0.4	0.2	0	-	-
formic acid	0.5 ml										
ninhydrin	2 ml										

-The tubes were capped carefully and then they were shaken vigorously.

-Placed tubes in a boiling water bath for 15 minutes and then transferred to a water bath of 22ºC for 10 minutes.
-10 ml. of the 2-propanol-water solution (1:1) were added to each tube in regular intervals of time.
-Tubes cooled at 22ºC were removed before 35 minutes and the absorbances at 520 nm determined. Strict control of time in each step is critical.

The proline content was expressed as milligrams of proline per kilogram of honey.

8.5. Results and Calculations:

Normal proline concentrations range from 250 to 2712 mg/kg depends on type of honey.

8.6. Discussion:

8.7. References

- Gerónimo .J. Di and Fritz .R. Proline in argentine honeys.*Proc. 37th Int. Apic.Congr., 28 Oct – 1 Nov 2001, Durban, South Africa*

- Khalil.I ,Moniruzzaman. M , Boukraâ .L , Benhanifia .M, Islam A, Islam .N, Sulaiman .S.A and Gan .S.H. Physicochemical and Antioxidant Properties of Algerian Honey . *Molecules* 2012, *17*, 11199-11215; doi: 10.3390/molecules170911199

. www.mdpi.com/journal/molecules

EXPERIMENT -9

9. Estimationofprolinein plant tissue

9.1. Introduction:

Proline is found in high percentage in basic protein . Free proline is said to play a role in plants under stress conditions. Though the molecular mechanism has not yet been established for the increased level of proline , one of the hypotheses refers to breakdown of proteins into amino acids and conversion to proline for storage .

9.2. Principle:

During selective extraction with aqueous sulphosalicylic acid, proteins are precipitated as a complex. Other interfering materials are also presumably removed by absorption to the protein –sulphosalicylic acid complex.

The extracted proline is made to react with ninhydrin in acidic conditions (PH 1.0) to form the chromophore(redcolour) and read at 520nm

9.3. Materials

- Acid Ninhydrin

Warm 1.25g ninhydrin in 30ml glacial acetic acid and 20ml 6MPhosphoric acid, with agitation until dissolved. Store at 4^0C and use within24h.

- 3% Aqueous Sulphosalicyclic Acid
- Glacial Acetic Acid
- Toluene
- Proline 0.1 M (115 mg/ 100 ml)
- ice bath

Sample preparation:

1. Extract 0.5g of plant material by homogenizing in 10ml of 3% aqueous sulphosalicylic acid.
2.Filter the homogenate through Whatman No. 2 fiter paper ,or centrifuge and take the supernatant .

9.4. Method:

- The sample extract was S1, S2, and S3

Reagent	B	1	2	3	4	5	6	7	8	9	10	S1	S2	S3
Std.	-	.2	.4	.6	.8	1.0	1.2	1.4	1.6	1.8	2	1	1.5	2
H2O	2	1.8	1.6	1.4	1.2	1.0	.8	.6	.4	.2	0	1	.5	0
G.acetic acid	2													
Ninhydrin	2													
Heat it in the boiling water bath for 1hour. Terminate the reaction by placing the tube in ice bath.														
Toluene	4													

- Stir well for 20-30sec.
-Separate the toluene layer and warm to room temperature.
-Measure the red colour intensity at 520nm.
-Find out the amount of proline in the test sample from the standard curve

9.5. Results and Calculation:

Express the proline content on freshweightbasis as follows:

$$\mu \text{ moles per g tissue} = \frac{\mu \text{ g proline /ml} \times \text{ml toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

Where115.5 is the molecular weight of proline.

Notes

1. Thecolour intensity is stable for at least 1h.
2. The relationship between the amino acid concentration and absorbance is linear in the range 0.02 to 0.1µ M per ml of proline.

9.6. Discussion:

9.7. References

Sadasivam .S and. Manickam . A. 1996 .Biochemical Methods.New age international (p) ltd; publisher. New Delhi

Sharma R.K.andSangha S.P.S.(2009). Basic techniques in biochemistry and molecular biology.I.K. Internatioal publishing house .New Delhi .p121.

EXPERIMENT-10

10. **Estimation of cholesterol in egg yolk**

10.1 Introduction:

Cholesterol is essential for the structure and function of every cell in the body. The body makesthe cholesterol daily, soany extra cholesterol eaten increases the body's total fats. The rise of cholesterol in the body can give a condition in which excessive cholesterol is deposited in artery walls called atherosclerosis .

Cholesterol content of various foods

High cholesterol foods	Cholesterol mg per 100 grams
Egg yolks (about 6)	**1458**
Whole milk	**11**
Skimmed milk	**4**
Egg whites	**0.3**
Fruits	**0**

10.2 Objective:

Estimate the concentration of cholesterol in egg yolk

10.3Material

- Methanol
- Chloroforom
- 2 % $CaCl_2$
- Cholesterol standard (1 mg/ml): weigh 100mg of cholesterol and dissolve in chloroform : methanol mixture (2:1) and make the volume to 100ml
- Glacial acetic acid
- Ferric chloride (2.5 % w/v phosphoric acid)
- Phosphoric acid
- Sulphuric acid

Test tube, quick fit conical flasks, glass centrifuge tubes

Pasteur pipette, pipettes, measuring cylinders

Volumetric flasks, glass cuvettes

Preparation of sample

*(Note: weigh the egg , then weigh the egg yolk)

1. Weigh approximately 1 g of egg yolk in a test tube.

2. Add 10 ml of chloroform:methanol (2:1 v/v) and strongly mix with vortex for 10 min.

3. Filtrate the extract by filter paper (or centrifuge).

4. Measure the volume of filtrate and add 2 times that volume of a 2% $CaCl_2$ solution.

5. Mix with the vortex (at high speed) for 5 min, then centrifuge at 3000 rpm for 10 min.

6. Remove the upper phase carefully with a Pasteur pipette.

7. Double the total volume of the lower phase by the addition of Chloroform:methanol: water (2:50:50)

8. Homogenize vigorously in the vortex for 5 min .then centrifuge at 3000 rpm for 10 min .

9. Carefully remove the upper phase with a Pasteur pipette and record the volume of the lower phase and use the lower layer for cholesterol estimation.

10.4. Method:

-Set up 8 test tubes as following

Tube	**Chloroform: Methanol Mixture (2:1) (ml)**	**Cholesterol Standard (ml)**	**Egg yolk extract (ml)**	**Glacial Acetic acid (ml)**	**Ferric Chloride (ml)**	**Conc. H_2S0_4 (ml)**
Blank	1.0	-	-	3.0	0.3	3.0
1	0.9	0.1	-			
2	0.8	0.2	-			
3	0.6	0.4	-			
4	0.4	0.6	-			
5	0.3	0.7	-			
6	0.2	0.8				
7	0.1	0.9				
Sample 1	-	-	1.0			
S 1			1.0			
Sample.2	0.5	-	0.5			
S2	0.5		0.5			

11. Mix the tubes slowly and allow the tubes to stand at room temp for 10 min

12. Read the absorbance at 560 nm. using glass cuvettes.

13. Plot a standard curve for absorbance against cholesterol concentration.

14. Read the conc. of cholesterol in egg yolk extract from the standard curve.

10.5. Result and calculation

Total extract volume =

Tubes	A	C
1		
2		
3		
4		
5		
6		
7		
S1		
S1		
S2		
S2		

Conc.of diluted sample = mg/ml

Cholesterol Conc .mg/1g egg yolk= mg/ml X Total extract volume

10.6. Discussion:

10.7. References:

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- Tietz, N. W. (1987) .Fundamentals of Clinical Chemistry .

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animal tissues. J Biol Chem 1957;467:226– 509

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EXPERIMENT-11

11. Determination of Iodine Number

11.1. Introduction:

The amount of unsaturation in fat samples is often determined industrially and is termed the iodine number of the fat. The iodine value (*IV*) gives a measure of the average *degree of unsaturation* of a lipid: the higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value is expressed as the grams of iodine absorbed per 100g of lipid. One of the most commonly used methods for determining the iodine value of lipids is "Wijs method".

11.2.Principle:

The lipid to be analyzed is weighed and dissolved in a suitable organic solvent, to which a known excess of iodine chloride is added. Some of the ICl reacts with the double bonds in the unsaturated lipids, while the rest remains: The amount of ICl that has reacted is determined by measuring the amount of ICl remaining after the reaction has gone to completion. The amount of ICl remaining is determined by adding excess potassium iodide to the solution to liberate iodine, and then titrating with a sodium thiosulfate ($Na_2S_2O_3$) solution in the presence of starch to determine the concentration of iodine released:

R-CH=CH-R + ICl (excess)→ R-CHI-CHCl-R + ICl (remaining)

ICl (reacted) =ICl (excess)–ICl (remaining).

ICl (remaining) + 2KI →KCl + KI + I_2.

I_2 + starch + $2Na_2S_2O_3$ (blue) → 2NaI + starch + $Na_2S_4O_6$ (colorless)

Iodine itself has a reddish brown color, but this is often not intense enough to be used as a good indication of the end-point of the reaction. For this reason, starch is usually used as an indicator because it forms a molecular complex with the iodine that has a deep blue color. Initially, starch is added to the solution that contains the iodine and the solution goes a dark blue. Then, the solution is titrated with a sodium thiosulfate solution of known molarity. While there is any I_2 remaining in the solution it stays blue, but once all of the I_2 has been converted to I^- it turns colorless. Thus, a change in solution appearance from blue to colorless can be used as the end-point of the titration. The concentration of C=C in the original sample can therefore be calculated by measuring the amount of

sodium thiosulfate needed to complete the titration. The higher the degree of unsaturation, the more iodine absorbed, and the higher the iodine value. The iodine value is used to obtain a measure of the average degree of unsaturation of oils, and to follow processes such as hydrogenation and oxidation that involve changes in the degree of unsaturation. The sample titration (T) gives a measure of the amount of (iodine) remaining in solution after halogenation.

11.3.Objectives:

To determine the iodine number for fats and oils.

11.4.Materials:

.Lipid solutions: 2.5% w/v in chloroform

Hanus Iodine solutions.dissolve 19.9g of Iodine in 1.5 liters of glacial acetic acid add 12.45 g of Bromine (3.96ml)

Sodium thiosulfate(Na2 S2 O3)0.2M .

.Starch indicator 1%

Potassium iodide (kl),15% .

.Chloroform .

11.5 Method: .

Label two 250 ml flasks “B”(blank)and “T”(test) and set up as follows:(caution: use a fume hood when handling iodine or bromine

	”test “T	”blank “B
Chloroform	l	10ml
Lipid solution	10 ml	
Hanus iodine	25 ml	25 ml

Cover the flasks with aluminium foil and allow to stand for 20 minutes with occasional shaking, then add

	T	B
Distilled water	25	25
Potassium iodide	10	10

Titrate the contents of each flask with 0.2 M thiosulfate until a pale yellow color develops. Add 1 ml of starch indicator to each

Flask and continue the titration until the colour disappears

Result and Calculation :

Amount of $Na_2S_2O_4$(A)=volume taken by blank- volume taken by test

No of moles of Na2S2O4(B) $= \frac{A\ mL \times 0.2M}{1000}$

Since 2 moles of $Na_2S_2O_4$ reacts with one mole of 1_2

So , B moles of $Na_2S_2O_4$ reacts with ?mole of 1_2

No of moles of 1_2(C) $= \frac{B}{2}$

Weigh in gram of 1_2(D) = C moles x M.W

= C X 254

D grams 1_2 saturates 0.25 gm of oiL

? gram 1_2 saturates 100 gm

IN$= \frac{100 \times D}{0.25}$.

11.5.Discussion:

Comment on the values obtained for iodine value determination for your sample. Why it is important to know the iodine number of fats and oils?

11.6.References :

- Aynon. (1964), Official and Tentative Methods of the American Oil Chemists' Society, 2nd. edn., American Oil Chemists' Society, Chicago.
- Aynon. (1954), International Union of Pure and Applied Chemistry, "Standard Methods for the Analysis of Oils and Fats", Paris, France.

EXPERIMENT-12

12. Determination of Peroxide values in Fats and oils

12.1. Introduction:

Foods which contain high concentrations of unsaturated lipids are particularly susceptible to lipid oxidation. Lipid oxidation is one of the major forms of spoilage in foods, because it leads to the formation of off-flavors and potentially toxic compounds. Lipid oxidation is an extremely complex process involving numerous reactions that give rise to a variety of chemical and physical changes in lipids. Two types of lipid oxidation cause the most concern. These are oxidative rancidity and hydrolytic rancidity.

Hydrolytic Rancidity: Hydrolytic rancidity results in the formation of free fatty acids and soaps (salts of free fatty acids) and is caused by either the reaction of lipid and water in the presence of a catalyst or by the action of lipase enzymes.

Oxidative Rancidity: Oxidative rancidity results from more complex lipid oxidation processes. The processes are generally considered to occur in three phases: an initiation or induction phase, a propagation phase, and a termination phase. In complex systems, the products of each of these phases will increase and decrease over time, making it difficult to quantitatively measure lipid oxidation. During the initiation phase, molecular oxygen combines with unsaturated fatty acids to produce hydroperoxides and free radicals, both of which are very reactive. For this phase to occur at any meaningful rate, some type of oxidative initiators must also be present, such as chemical oxidizers, transition metals (i.e., iron or copper), or enzymes (i.e., lipoxygenases). Heat and light also increase the rate of this and other phases of lipid oxidation. The reactive products of this initiation phase will, in turn, react with additional lipid molecules to, form other reactive chemical species. The propagation of further oxidation by lipid oxidation products gives rise to the term “auto-oxidation” that is often used to refer to this process. In the final, termination phase of lipid oxidation, relatively uncreative compounds are formed including hydrocarbons, aldehydes, and ketones.

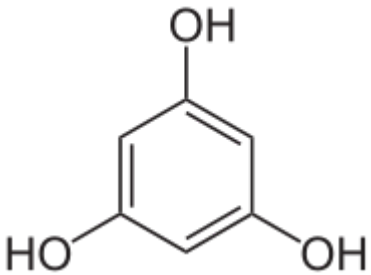
12.2.Principle :

Auto oxidation at fatty acid double bonds occur by reaction with molecular oxygen present in the atmosphere, causing the formation of labile peroxides. These are capable of oxidizing the added potassium iodide, KI, with the liberation of molecular iodine,I_2 The lodine can be determined by titration with standard sodium thiosulphate solution using starch as an indicator. While iodine is present in excess, a purple starch-iodine complex is formed. At the end point the solution becomes colorless.

$$I_2 + 2Na_2S_4O_3 \longrightarrow Na_2S_4O_6 + 2NaI$$

It is generally accepted that the first compounds formed by oxidation of oil are hydroperoxides. The usual method of hydroperoxide assessment is by determination of the Peroxide value, which is reported in units of millimole of hydroperoxide per kilogram of oil (or expressed as milliequivalents of iodine per kilo of oil and fat).

The Lea value is defined as the number of millimoles of peroxide oxygen per kilogram of fat or oil and is therefore numerically half of the peroxide value. The peroxides formed during autooxidation are unstable and decompose into free radicals. These initiates chain reaction which leads eventually to decomposition of the fatty acid into various low molecular weight aldehyde and ketones. One carbonyl compound which is formed early in the process is epihydrinaldehyde. The latter reacts with phloroglucinol (1,3,5-trihydroxybenzene) to give a red colour in acid solution. This reaction is the basis of the qualitative Kreis-kerr test.



Phloroglucinol

12.3.Materials:

A-Determination of peroxide value

- Solvent Mixture : Mix two volumes of glacial acetic acid with one volume of chloroform.

5% Potassium iodide Solution –

- 1% Starch Solution

- N/500 Sodium ThiosulphateSolution . Prepare N/10 solution and dilute to N/500 on the day of use .

- Sample : Edible and rancid oil .

B- Kris- Kerr test

1. 1% phloroglucinol in ether solution
2. concentrated hydrochloric acid

12.4. Method

A. Determination of peroxide value

1- Weigh 1g of oil or fat into a clean dry boiling tube and add 1g of powdered potassium iodide and 20ml of solvent mixture.
2 - Place the tube in boiling water so that the liquid boils within 30 seconds and allow to boil vigorously for not more that 30 seconds.
3 - Transfer the contents quickly to a conical flask containing 20ml of 5% potassium iodide solution.
4- Wash the tube twice with 25ml water each time and collect into the conical flask.
5 - Titrateagsinst N/500 sodium thiosulphate solution until yellow color is almost disappeared.
6 - Add 0.5ml of starch, shake vigorously and titrate carefully till the blue color just disappears.
7- A blank should also be run at the same time.

12.5. Results and Calculation

Peroxide value (milliequivalent peroxide/kg sample)= $\frac{S \times N \times 1000}{\text{g sample}}$

where S = ml $Na_2S_2O_3$(Test –Blank)

and

N = normality of$Na_2S_2O_3$

B. Kreis- Kerr test

1. To 1g of melted sample add a similar amount of concentrated HCl.
2. Add about 1ml of 1% phloroglucinol in either.
3. A slowly- developing red color indicates that the sample of fat or oil is in the early stages of rancidity.

12.6. Discussion

1. Make a table for various peroxide values for different samples of fat and oil, and discuss your result
2. How reliable is the above peroxide value test and describe various other methods used for determining Rancidity of fat and oil.
3. Draw your own conclusion about the nutritional and health hazards involved due to the rancidity of fats and oil.

12.7. Referances:

- Sadasivam .S and. Manickam . A. 1996 .Biochemical Methods.New age international (p) ltd; publisher.New Delhi.
- Aynon. (1964), Official and Tentative Methods of the American Oil Chemists' Society, 2nd.edn., American Oil Chemists' Society, Chicago.
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EXPERIMENT-13

13.Estimation of oil in oil seeds

13.1. Introduction :

Simple lipid are esters of fatty acids with glycerol (triglycerides) .Fat as liquid is called oil. Seeds like groundnut, castor, sunflower etc. contain oil as reserve food material for the embryo.

13.2. Principle:

Oil from a known quantity of the seed is extracted with petroleum ether by Soxhlet extractor. It is then distilled off completely, dried, the oil weighed and %oil is calculated .

13.3. Materials

-Petroleum Ether (40-160^{0}C
-Whatman No.2 filter Paper
-Absorbant Cotton
-Soxhlet Apparatus

13.4. Method:

1- Fold a piece of filter paper in such a way to hold the seed meal. Wrap around a second filter paper which is left open at the top like a thimble . A piece of cotton wool is placed at the top to evenly distribute the solvent as it drops on the sample during extraction.
2- Place the sample in the butt tubes of the Soxhlet extraction apparatus .
3- Extract with petroleum ether (150 droes/min) for 6h without interruption (For castor beans use hexane) by gentle heating.
4- Allow to cool and dismantle the extraction flask . Evaporate the ether on a steam or water bath until no odour of ether remains. Cool at room temperature.
5- Carefully remove the dirt or moisture outside the flask and weigh the flask. Repeat heating until constant weight is recorded.

13.5. Results and Calculation

$$\text{Oil in ground sample \%} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$

$$\text{Oil to dry weight basis} = \frac{\text{\% oil in ground sample}}{\text{100 \% moisture in whole seed}}$$

Note

Sample Preparation:

Sample preparation varies depending upon the materials.

Peanut

Place about 50g of kernels in a drying dish and dry at 130°C for not more than 20min in a forced-draft oven. Cool to room temperature and then pass through the nut slicer. Care is to taken to prevent expressing of any oil while slicing. Mix the sliced sample well. Weight accurately 2g into the filter paperfold.

Sunflower

Wight about 50g of sunflower seeds into a large beaker. Add an equal weight of Hyflowsupercel or equivalent (dried at 130°C overnight before use). Mix well. Grind the mixture immediately.

$$\text{For sunflower seeds oil in ground sample, \%} = \frac{\text{weight of oil (g)} \times 200}{\text{Weight of sample (g)}}$$

Since equal quantity of Hyflowsupercel is added.

Soybean

Dry the sample well either by keeping overnight in a warm room or in an oven so that the moister is within 6-8%. Grind in a micro0sample mill with minimum exposure to air. Mix thoroughly. Weight exactly 2g of the sample and place in the filter paper fold.

Castor Bean

Dry the sample as for peanut. Cool to room temperature and then grind through the food shopper, feeding the beans slowly to prevent expression of oil. Mix and weight 5g into the filter paper fold.

Cotton Seed

Dry about 60g of sample for 2h at 130°Cin a forced-draft oven. Place the dried seed in the prepared vessel and cover with a clay lid. Place the vessel in an oven for 1h. The temperature can be up to 115°C. Grind the sample by rapid feeding through the mill. Mix well and weight 4-5g into the filter paperfold.

Safflower

Weight accurately 10g whole seed and pulverize in an iron mortar. Transfer into the filter paperfold. Wash mortar and pestle carefully with petroleum ether and pour washings into the filter paperfold.

13.6. Discussion:

13.7. References

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Springer .New York

EXPERIMENT-14

14.Estimation of inorganic phosphate in milk and soft drink

14.1. Introduction and Principle :

Soft drinks are complex mixtures containing a variety of substances such as colouring compounds, flavouring agents, acidifiers, sweeteners, preservatives, and caffeine. The most common acidifier used in soft drinks is phosphoric which gives a tangy taste in the mouth. Phosphoric acid can also acts as a preservative, keeping the contents of the bottle fresh.

Inorganic phosphate reacts with ammonium molybdate in an acid solution to form phosphomolybdicacid . A reducing agent { such as metol ,p- methyl amino phenol(Elon) or stannus chloride) is added which reduces the molybdenum to give a blue colour but does not affect the uncombined molybdic acid .

14.2. Materials:

1- Ammonium molybdate

Add 13.6 mL of con.sulphuric acid to 35 mL of water and allow the solution to cool. In a separate vessel add 2.5 g of ammonium molybdate to 50 mL of water. Add sulphuric acid solution to it & make up the volume to 100 .

2-. Reducing Solution(any one of the following):

*Metol-sodium sulphate solution . Dissolve 2g of metol in a 10% solution of sodium sulphite and make up to 100 ml. stable for 10 days in dark bottle

*Fresh .ascorbic acid 3%

*1%Elon,3%$NaHSO_3$

3- Tricholoracetic acid 10%

4- Working phosphate solution

First prepare a 300 ppm solution by accurately weighing about 0.220 g of solid KH_2PO_4 into a 500 mL volumetric flask, and diluting it to the mark. Pipette 10

mL of the standard phosphate into a 200, 250, 500 mL and a 1 L volumetric flask, and fill it to the mark. This will give you phosphate solutions of 15, 12, 6 and 3 ppm solution respectively. Pipetting 15 mL of your standard solution into a 1 L volumetric flask will give you a 4.5 ppm solution.

5-Preparation of sample:

A. Milk

- 10 ml of milk (whole and skimmed milk) are pipetted into each of two 100 ml. volumetric flasks.
- The flasks are filled to the 100 ml mark with 10 per cent trichloroacetic acid

,the acid being added slowly and the flask rotated constantly.

- The contents are thoroughly mixed and allowed to stand stoppered for 20 minutes with frequent stirring.
- Centrifuge the content and collect the supernatant .
- 1ml of milk diluted to 10 ml .

B. Soft drink

- Dilute1 :10

14.3.Method :

Reagent .ml	B	1	2	3	4	5	M	M	S1	S2
Standard	-	2	2	2	2	2				
Sample	-						2	2	2	2
ammonium molybdate	0.5 ml									
Metol Or ascorbic acid	0.5ml									
Mix throughly after each addition .Allow to stand for10 min and read the extinction at 880 nm. In case of use ascorbic acid. Heat this slowly to boiling (a deep blue/green colour should develop) and then allow it to cool .Measure the absorbance at 650 nm										

14.4. Results and Calculations:

- Plot a graph between absorbance and volume of phosphate in various standard solutions and obtain the calibrated curve.

  From the curve determine the amount of phosphate in the test solution.

14.5. Discussion:

14.6. References

-sandersca .g.p.1931.The determination of the calcium, magnesium,and acid-soluble phosphorus of milk by means of trichloroacetic acid filtrates.

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-Determination of Phosphate Concentration in Soil
http://www.outreach.canterbury.ac.nz/chemistry

-Estimation of Phosphate Content in Soft Drinks

http://amrita.vlab.co.in/?sub=2&brch=193&sim=1038&cnt=2

EXPERIMENT-15

15.Estimation of inorganic phosphate in tissue

15.1. Introduction and Principle

Digestion of animal tissue with nitric acid and perchloric acid mixture converts all phosphorus. This orthophosphate reacts with acidified ammonium molybdate solution and form molybdophosphoricacid , which is reduced to a blue coloured complex is the presence of stannous chloride. This is measured spectrophotometrically at 690 mm.

15.2.Material

1- Nitric acid : prechloric acid is the ratio of 10:1 (mix and store)
2- Concentrated HCI
3- Ammonium molybdate solution (Dissolve 5 g of ammonium molybdate $(NH_4)^6$ $Mo_7O_2$4.4 H_2O) is 35 ml of distilled water . Add 62 ml of conc. H_2SO_4 to 80 ml of distilled water, cool and add the molybdate solution and make the volume to 200 ml by adding more distilled water.)
4- Stannous chloride solution (Dissolve 0.5 g of stannous chloride in 2ml of conc. HCl add 200 ml of distilled water to it. Always use freshly prepared solution).
5- Stock phosphate solution for standard curve. (Dissolve 219.5 mg anhydrous KH2PO4 is 1000 ml distilled water stock solution.)
6- Spectrophotometer

15.3.Method :

1- Take 0.1 g of tissue. Add to it 10 ml of HNO3 and perchloric acid mixture.
2- Heat gently till solution becomes colourless and reduced to 2-2.5 ml.
3- Cool and transfer the digest to 50ml volumetric flask and make the volume.
4- Take 25.0 ml of this solution into a conical flask.
5- Add to it 1.0 ml of ammonium molybdate and 0.15 ml of stannous chloride solution.
6- Blue colour will appear.
7- Wait for minutes.
8- Note the absorbance at 690 mm and calculate the value of phosphate from standard curve prepared by making different concentrations from 0.2 to

1.0 mg L^{-1}.

15.4.Results and Calculations:

15.5.Discussion:

15.6.References:

Sharma R.K.andSangha S.P.S.(2009). Basic techniques in biochemistry and molecular biology. I.K. Internatioal publishing house . New Delhi

EXPERIMENT-16

16. Determination of Calcium in Milk and tap water

16.1. Introduction:

This method for determining Ca^{2+}concentration in the presence of Mg^{2+}relies on the fact that the pH of the solution is sufficiently high to ensure that all magnesium ions precipitate as magnesium hydroxide before the indicator is added. (The pH will be approximately 12.5 due to the addition of concentrated NaOH solution). The Patton-Reeder indicator is a suitable indicator in this case as it produces a clear colour change from pink/red to blue in the pH range of 12 – 14.

This blue dye also forms a complex with the calcium ions changing colour from blue to pink/red in the process, but the dye–metal ion complex is less stable than the EDTA–metal ion complex. As a result, when the calcium ion–PR complex is titrated with EDTA the Ca^{2+}ions react to form a stronger complex with the EDTA.

The reaction is:

$Ca\text{-}PR + EDTA_4^- \rightarrow PR + [Ca\text{-}EDTA]^{2-}$

Note: Ca-PR is pink/red and PR is blue.

16.2.Objective:

The purpose of this experiment is to determine the amount of calcium in different typesof milk and also in tapwater.

16.3. Material :

- Fat Free Milk
- 1% Milk
- Full cream Milk
- tapwater
- 0.03408M EDTA (750mL)
- 4.65g EDTA dissolved in 500mL distilled water combined with 2.3g EDTA dissolved in 250mL distilled water.
- 8M sodium hydroxide

Note that the concentrated (8mol L^{-1}) sodium hydroxide solution is highly corrosive and should be handled with extra care: ideally wear rubber gloves when preparing and handling it.

16.4. Method:

1. Combine 10mL of sample, 40mL distilled water, and 4mL of 8M sodium hydroxide solution into an Erlenmeyer flask and allow solution to stand for about 5 minutes with occasional swirling. A small of magnesium hydroxide may precipitate during this time. Do not add the indicator until you have given this precipitate a chance to form. After precipitate forms, add 0.1g of Patton-Reeder indicator to flask and swirl the solution to dissolve the indicator.
2. Titrate with EDTA solution
3. Repeat titration for three trials
4. Repeat for other samples

For tapwater the method is modified due to the much lower Ca^{2+} concentration.

1. Dilute the ETDA standard solution by a factor of 1/50 by pipetting 10 ml into a 500 ml volumetric flask and making up to the mark with distilled water. This will give a 0.0005 mol L−1 solution.

2. Pipette a 50 ml. aliquot of tapwater into a conical flask. Add 4 ml of 8 mol L−1 sodium hydroxide solution, and allow solution to stand for 5 minutes with occasional swirling.

3. Add 0.1 g of Patton-Reeder indicator and swirl the solution to dissolve the indicator.

4. Titrate the sample with the diluted EDTA standard solution to the blue endpoint. Repeat until concordant results are obtained.

16.5.Result and Calculations:

1. Calculate the average volume of EDTA solution used from titres.

2. Calculate the moles of EDTA required to complex the Ca2+ ions in the sample.

3. Using the method ratio Ca^{2+} : EDTA = 1 : 1, calculate the concentration in mol L−1 of Ca2+ in your sample solution.

4. Calculate the concentration, in mg/L (parts per million or ppm), of Ca^{2+}in your sample solution.

Sample	Trial	mL EDTA added
	1	
	2	
	3	
	4	
	Avg.	

Calculations:

Grams Ca^{2+} =

$L\ E)TA >$

16.6.Discussion:

16.7. Referances:

http://www.outreach.canterbury.ac.nz/chemistry/documents/calcium.pdf

Real World: Determination of Calcium in Milk

students.ycp.edu/~kvautier/.../Real%20World.docx

EXPERIMENT-17

17.Determination of total phenolic content in food

17.1Introduction:

Fruits and vegtables contain a wide variety of free-radical scavenging molecules, including phenolic compounds, carotenoids, and vitamins.

plant phenolic compounds are extremely heterogeneous and may range from simple monomers to very large polymers and belong to either one of two biochemical groups: (1) the flavanoid compounds (including condensed tannins), or (2) the group of compounds where the 6-carbon ring has a 1 or 3 carbon side chain and their derivatives, e.g. caffeic acid, gallic acid, hydrolysable tannins, tyrosine and lignin. Epidemiological studies have shown that consumption of food rich in phenolics can slow the progression of various debilitating diseases Therefore, mostly, the current focus is on the anti-oxidant action of phenolics. The anti-oxidant activity of phenolics is mainly related to redox properties. Oxygen derived free radicals have played a major role in the pathogenesis of a number of degenerative diseases. These free radical molecules are released during the normal metabolic process of oxidation and thus can lead to cancerous changes, accelerate the aging process etc. Recently phytochemicals in fruits and vegetables have attracted a great deal of attention mainly concentrated on their role in preventing diseases caused as a result of oxidative stress. Oxidative stress, which releases free oxygen radicals in the body, has been implicated in a number of disorders including cardiovascular malfunction, cataracts, cancers, rheumatism and many other auto-immune diseases besides ageing and contributes to heart disease and degenerative diseases such as arthritis .

Interestingly, diets rich in plant-based products such as fresh vegetables and fruits and tea, have been found to protect humans from these degenerative diseases.

Tea remains one of the most popular beverages world-wide and contains a variety of phenolic compounds which are potent antioxidants.

17.2.Principles

In this method, we will use a colorimetric method, the Folin-Ciocalteu assay, to quantify the total phenolic content of tea samples. The Folin-Ciocalteu method involves complexation of phenolic compounds in a sample with a molybdate reagent to form a colored complex that absorbs at 650 nm. Catechol is used to produce a standard curve and final phenolic concentrations in the sample express as mg phenols/100 g material

17.3. Materials :

1- Green tea powder
2- Black tea powder
3- FolinCiocalteu phenol reagent
4- Catechol : prepare 100 mg/100ml
5- working standard: 10 mg /100ml
6- 20% Sodium carbonate
7- 80% Ethanol
8-UV/VIS spectrophotometer
9-Hot plate
10- Ginger

Preparation of Samples:

A. Tea samples

1- Brew about 2 g of tea sample in 250 ml boiling distilled water for 5 min.
2- Cool to room temperature and filter using Whatman No. 2 filter paper.

B. Preparation of ginger sample

1. Weigh exactly 2 g of the grind sample ,then add 10-time volume of 80% ethanol.

Or homogenized 2 g sample with 80% ethanol.

2. Centrifuge the homogenate at 10,000 rpm for 15 min. Keep the supernatant and re-extract the residue with 3 times the volume of 80% ethanol, centrifuge and pool the supernatants.

3. Evaporate the supernatant to dryness.

4. Dissolve the residue in a known volume of distilled water (5 ml).

17.4.Method:

1. Pipette out different solution into test tubes.

Tube	**Catechol standard (ml)**	**Sample**	**Dist. Water (ml)**	**Folin-Ciocalteu reagent (ml)**		**Na2CO3 (ml)**
(blank)	-		3	0.5	**Wait 3 min**	2 ml
1	0.5		2.5			
2	0.8		2.2			
3	1.0		2.0			
4	1.5		1.5			
5	2.0		1.0			
6	2.5		0.5			
7	3	0	0			
Black tea1		0.1	2.9			
Black tea2		0.5	2.5			
Black tea3		1	2.0			
green tea1		0.1	2.9			
green tea2		0.5	2.5			
green tea3		1	2.0			
Ginger1		0.1	2.9			
Ginger2		0.5	2.5			
Ginger3		1	2.0			

.

Mix thoroughly and place the tubes in boiling water for exactly 1 min, cool and measure the absorbance at 650 nm against a reagent blank.

. Prepare a standard curve using different concentrations of catechol

.**Notes**

1. If any white precipitate is observed on boiling, the colour may be developed at room temperature for 60 min.

17.5. Results and Calculations:

Tubes .NO.	**Absorbance**	**Conc.**
(blank)		
1		
2		
3		
4		
5		
6		
7		
Black tea1		
Black tea2		
Black tea3		
green tea1		
green tea2		
green tea3		
Ginger1		
Ginger2		
Ginger3		

From the standard curve find out the concentration of phenols in the test sample and express as mg phenols/100 g material .

17.6. Discussion

17.7.References

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EXPERIMENT-18

18. Estimation of total carotenoids in fruits and vegetables

18.1.Introduction:

Carotenoids are organic pigments that are found in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some bacteria, and some fungi. Carotenoids generally cannot be manufactured by species in the animal kingdom so animals obtain carotenoids in their diets, and may employ them in various ways in metabolism.

There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). All carotenoids are tetraterpenoids, meaning that they are produced from 8 isoprene molecules and contain 40 carbon atoms. Carotenoids in general absorb blue light. They serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage. Probably the most well-known carotenoid is the one that gives this second group its name, carotene, found in carrots (also apricots) and are responsible for their bright orange colour. Crude palm oil, however, is the richest source of carotenoids in nature in terms of retinol (provitamin A) equivalent.

In humans, four carotenoids (beta-carotene, alpha-carotene, gamma-carotene, and beta-cryptoxanthin) have vitamin A activity (meaning they can be converted to retinal), and these with other carotenoids can also act as antioxidants. In the eye, certain other carotenoids (lutein, astaxanthin and zeaxanthin) apparently act directly to absorb damaging blue and near-ultraviolet light, in order to protect the macula of the retina, the part of the eye with the sharpest vision.

Carotenoids have many physiological functions. Given their structure, carotenoids are efficient free-radical scavengers, and they enhance the vertebrate immune system. There are several dozen carotenoids in foods people consume, and most carotenoids have antioxidant activity. Epidemiological studies have shown that people with high β-carotene intake and high plasma levels of β-carotene have a significantly reduced risk of lung cancer.

18.2.Principle

Analysis of total carotenoids is based on the extraction of crude pigment in a lipid solvent and measurement of its optical density at 452 nm. The pigment content is expressed as β-carotene. The sample is extracted in acetone which dissolves both the fat and water-soluble pigments. The acetone extract is then taken petroleum ether layer. The fat soluble carotenoids pass from acetone to the petroleum ether leaving all the rest of the pigments in the acetone.

18.3. Material:

1. Separatory funnel
2. Blender
3. Analytical balance
4. Spectrophotometer
5. Conical flasks
1. Acetone
2. Petroleum ether
3. 5% Sodium sulphate
4. β –carotene standard .

18.4.Method:

1 Weight 1-2 g of fresh sample (carrot ,spinach and avocado fruit) and homogenate it with acetone .
2 Centrifuge at 10000g for 15 min .
3 Pour the extract in a 250-ml conical flask.
4 Continue the extraction of the residue (pellet) with acetone until the residue is colourless (3 times).
5 Pool all the extracts in conical flask and transfer it in to a separatory funnel.
6 The yellow pigment is then transferred into the petroleum ether by diluting the acetone with water containing 5% sodium sulphate.
7 Keep on adding petroleum ether until all colour gets transferred into the petroleum ether layer.
8 Make up the volume (to 100 ml)with petroleum ether and measure the intensity of the colour at 452 nm (blank contains petroleum ether).
9 The results are expressed in term of β-carotene as mg/100 g of the material.

18.5.Result and Calculations:

β-carotene, $E_{452} = 13.9 \times 10^4$

β-carotene mg/ 100g = conc.from standard curve X total volume X 100

sample weigh

or

β-carotene

mg/ 100g = (3.86µg/ml) X A 452 X total volume X 100

sample weigh X 1000

18.6.Discussion:

18.7. References:

-Ranganna.s. Handbook of Analysis and Quality Control for Fruit and Vegetable Products.1986
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-Katoch .R .2011. Analytical techniques in biochemistry and molecular biology Springer .New York.

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EXPERIMENT-19

19.Ascorbic Acid Determination

19.1.Introduction

Vitamins are a group of small molecular compounds that are essential nutrients in many multi-cellular organisms, and humans in particular. The name "vitamin" is a contraction of "vital amine", and came about because many of the first vitamins to be discovered were members of this class of organic compounds. And although many of the subsequently discovered vitamins were not amines, the name was retained. In this exercise you will be studying vitamin C, also known as ascorbic acid.

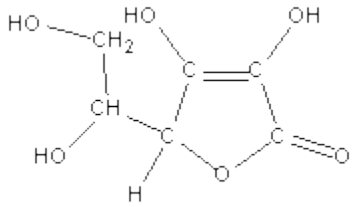

Figure 1. Structure of vitamin C (ascorbic acid).

Ascorbic acid ($C_6H_8O_6$) is a water-soluble vitamin, whose structure is shown in Fig. 1. Vitamin C is easily oxidized, and the majority of its functions *in vivo* rely on this property. It plays a key role in the body's synthesis of collagen and norepinephrine by keeping the enzymes responsible for these processes in their active reduced form. Vitamin C may also play a role in detoxifying by-products of respiration. Occasionally during respiration O_2 is incompletely reduced to superoxide ion (O_2^-) instead of being reduced completely to its -2 oxidation state (as in H_2O). Normally an enzyme called superoxide dismutase converts O_2^- to H_2O_2 and O_2, but in the presence of Fe^{2+} the hydrogen peroxide may be converted into the highly-reactive hydroxyl radical (•OH). The hydroxyl radical can initiate unwanted and deleterious chemistry within a cell when it removes a hydrogen atom (H•) from an organic compound to

form H_2O and a new, potential more reactive free radical. Ascorbic acid can donate a hydrogen atom to a free radical, and thus stop these reactions from occuring.

The human body cannot produce ascorbic acid, and so it must be obtained entirely through one's diet. A vitamin C deficiency in humans results in the disease called scurvy, whose symptoms include hemorrhaging (especially in the gums), joint pain and exhaustion. In its final stages scurvy is characterized by a profound exhaustion, diarrhea, and then pulmonary and kidney failure, which result in death. A very small daily intake of vitamin C (10-15 mg/day for an adult) is required to avoid deficiency and stave off scurvy. However, there has been, and continues to be, vigorous debate on what the optimum daily intake of vitamin C is.

Fruits, vegetables, and organ meats (e.g., liver and kidney) are generally the best sources of ascorbic acid; muscle meats and most seeds do not contain significant amounts of ascorbic acid. The amount of ascorbic acid in plants varies greatly, depending on such factors as the variety, weather, and maturity. But the most significant determinant of vitamin C content in foods is how the food is stored and prepared. Since vitamin C is easily oxidized, storage and the cooking in air leads to the eventual oxidation of vitamin C by oxygen in the atmosphere. In addition, ascorbic acid's water-solubility means that a significant amount of vitamin C present in a food can be lost by boiling it and then discarding the cooking water.

19.2.Objectives:

To estimate the amount of vitamin C in fruit Juices.

19.3.Principle:

Besides being an important vitamin, ascorbic acid is a sugar acid and a powerful reducing agent. The ascorbic acid content in fruits and vegetables can be estimated by macerating the sample with stabilising agents such as 20 % metaphosphoric acid. The most common assay of ascorbic acid is based on its oxidation to dehydroascorbic acid by the redox dye 2,6-dichlorophenol indophenol which is blue in neutral solution and pink in acid. The titration is carried out in acid conditions and at the end point the dye appears rose pink.

19.4.Materials:

50 ml Burette, Pipette, Magnetic stirrer and stirring bars, and 250 ml amber glass bottle.

A. Dye solution (0.5%): Dissolve 0.042 g of NaHCO3 in distilled water and then add 0.050 g of sodium 2,6-dichloroindolphenol, shake vigorously. When dye dissolves, make up to 200 ml. Filter into an amber glass bottle and stored capped in a refrigerator. The solution is good until it fails to give a distinct endpoint.
B. Stabilization Solution- 10% acetic acid. This will keep in the refrigerator for 7 - 10 days.
C. Ascorbic acid standard solution (1 mg/ml): Accurately weigh 0.100 g of ascorbic acid into a 100-ml volumetric flask. Immediately before use, dissolve in 100 ml of acid stabilization solution.

D .Fruit juice samples (2 ml) are to be analysed.

19.5.Method

a. Standardize in triplicate the indophenol solution by rapidly titrating against 2 ml aliquots of standard ascorbic acid solution plus 5 ml of stabilization solution until a distinct rose-pink persists for at least 5 s. Express standardized indophenol solution as mg ascorbic acid equivalent per 1 ml of dye.
b. Titrate in triplicate 2 ml aliquots of the fruit juices (which should contain between 10- 100 mg ascorbic acid/100 m) plus 5 ml extracting solution using the standardized indophenol solution.

19.6.Results and Calculations:

Sample	**Titration volume (1)**	**Titration volume (2)**	**Titration volume (3)**	**Average titration volume**
Blank				
Standard				
Fruit juice				

Take the average of the three titrations, subtract a blank titration (no food sample), and calculate the ascorbic acid as mg / 100 ml by the following formula:

[(mean sample titration volume – blank titration volume)
X (standardized mg AA/ml dye) X 100/2]

Comment on the results obtained for the concentration of ascorbic acid in your fruit juice sample.

19.7. Discussion:

19.8 References:

1. Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 967.21.

EXPERIMENT-20

20.Determination of Caffeine Content in Tea, coffee and soft drink

20.1.Introduction:

Caffeine (1,3,5-trimethylxanthine) and two of itsminor isomeric dimethylxanthines, theobromineandtheophyline, belong to a group of methylxantines.

Caffeine content varies naturally in tea, coffee, and cocoa seeds: tea leaves contains 1.5% to 3.5% caffeine; %), mate tea leaves contains (0.89–1.73%), roasted coffeebeans contain 0.75% to 1.5% caffeine; whereas cocoa bean contains 0.03% to 1.7% caffeine, and the various carbonated beverages contain caffeine in the amount 30 to 60mg per 12 oz. can (or 355 ml)

Caffeine is classified as a central nervous system stimulant
In moderate doses, caffeine can increase alertnessreduce fine motor coordination of the human system, altersleep patterns, and cause headaches, nervousness, and dizziness. In massive doses, caffeine is lethal.
A fatal dose of caffeine has been calculated to be morethan 10 grams (about 170 mg/kg body weight).
Effects of caffeine include an increase in heart rate,constriction of blood vessels, relaxed air passages to improve breathing, and ease of muscle contraction.. It essential to monitor and assess the stipulated concentration of caffeine in their respective products.

20.2. Material :

- chloroform
- soft drink from different brands such as Red Bull, Pepsi , Coca cola ..
- Caffeine Standard Solution containing 10 mg/100ml of chloroform (to get 100µg/ml)
- samples preparation : tea and coffee (0.1 g) were extracted in 10 ml of boiling water (boiled for 5 min) before extracted by chloroform.
- quartz cuvets

20.3.Method

- Ten ml of soft drink samples and hot water extract of tea and coffee samples were taken in separating funnels, and 10 ml of chloroform was added to each sample.
- The separating funnel was shaken vigorously for 5 min. The solutions were then allowed to separate for 10 min at room temperature. Lower chloroform layer was collected for further analysis. One ml of this chloroform layer was diluted with pure chloroform appropriately to read absorbance. Absorbance of these solutions was

measured at 265-277 nm (depend on the λ max of caffeine standard) against pure chloroform as blank .

- A standard curve was prepared for caffeine estimation in the concentration range between 0 and 0.1 mg/ml.
- prepare a calibration curve by graphing absorbance verses concentration.

20.4. Result and calculations:

Caffeine μg/ml in soft drink = $Conc^0$ from. S. curve X10XD.F

20.5. Discussion :

20.6.Referance

http://www.ijplsjournal.com/issues%20PDF%20files/nov2011/6.pdf

http://www.aseanfood.info/articles/11016748.pdf

Komes.D ,Horžić.D , Belščak.A , Kovačevi ć Ganič. K and Baljak.A.2009.Determination of Caffeine Content in Tea and Maté Tea by using Different Methods. Czech J. Food Sci. Vol. 27, Special Issue S213-16 .

EXPERIMENT-21

21. Liquid Chromatographic (HPLC) Determination of Caffeine in Cola Soft Drinks

21.1. Introduction and Principle:

Food additives such as caffeine, saccharin, and benzoate can be quantified in beverages by liquid chromatography on u-Bondapack-C18 column using acetic acid (20%) buffered to pH 3.0 with saturated sodium acetate and modified by adding 0.2% isopropanol. The concentration of the additive in the sample is determined by measuring the peak height monitored at 254 nm using a UV absorbance detector.

21.2.Objectives:

To estimate the concentration of caffeine in beverages by reverse phase high performance liquid chromatography.

21.3. Materials :

Apparatus and Glass ware**:**

a. Liquid Chromatograph equipped with 6000 A solvent delivery system injector,
b. u-Bondapak C18 column,
c. UV. 254 nm detector sensitivity adjustable from 0.02 – 0.05 AUFS.
d. Chart recorder
e. Beaker, pipette, flasks

Reagents**:**

Mobile Phase: 20% acetic acid (v/v) buffered to pH 3.0 with saturated sodium acetate solution modify with 0-2% isopropanol. De-gas prior to use

Standards and Sample Preparation

1. Standard solutions: Prepare individual standard solutions from standard compound to get following concentrations-sodium saccharin: 0.5 mg/ml, caffeine: 0.05 mg/ml and sodium benzoate: 0.5 mg/ml use solutions to determine sensitivity for detector response and retention times of individual standards.

2. Mixed standard solution: Prepare solution containing 0.5 mg/ml sodium, saccharin 0.05 mg/ ml caffeine and 0.5 mg/ml of sodium benzoate. Use this solution to optimise LC conditions for complete resolution and to quantify.

3. Carbonated beverages: Decarbonateby agitation. If free of particulate matter, inject directly.
4. Beverages containing particulate matter: Filter through Millipore filter (0.45 μm) discarding first few ml filtrate. If large amount of particulate matter is present, centrifuge prior to filtration.

21.4. Method:

1. Inject known volume (10 μl) of mixed or individual standard solution in duplicate. Peak heights should agree within ± 2.5%.
2. Inject known volume of prepared unknown sample in duplicate.
3. Measure peak heights of standards and sample components.

21.5. Results and Calculation

Tube	**Conc. (mg/ml)**	**Volume injected**	**Peak Height**
Standard 1			
Standard 2			
Standard 3			
Sample 1			
Sample 2			

Calculate the amount of sample using the following formula:

% compound = $C_1 X (H/H_1) X V_1/V X 0.1$

Where C_1 = concentration of standard in mg/ml

H and H_1 = average peak heights of sample and standard respectively.

V and V_1 = volume injected in μl of sample and standard respectively.

21.6.Discussion:

Comment on the values obtained for the amount of caffeine in beverages sample. Compare your results with amount mentioned on the beverage can.

21.7.Refrences:

Pearson's Composition and Analysis of Foods 9th edn., 1991, p.373

EXPERIMENT-22

22. Benzoate estimation in soft drink

22.1. Introduction:

Sodium benzoate ($C_6H_5COO^-Na^+$, MW=144) is a commonly used preservative which is added to fruit juices to prevent the growth of microorganisms. When added in high concentration, it affects the taste of the juice. Sodium benzoate is usually permitted at a concentration of up to 1.3 grams per litre of juice.

The benzoate anion is not soluble in non-polar solvents because of its negative charge. However, in acid solution, benzoate acid is formed. This is neutral and quite non-polar. Moreover, it is soluble in non-polar solvents, into which it may be extracted at acidic pH, i,e well below the pKa of the carboxyl group, which is 4.20. In this experiment, benzoic acid is extracted into chloroform, which is then removed by evaporation. After dissolving the residue in 50% (v/v) neutralized ethanol, the benzoic acid is titrated with 0.05M sodium hydroxide, using phenolphthalein as an indicator

22.2.Objectives:

To estimate the concentration of benzoate insoft drink

22.3.Principle:

The benzoate anion is insoluble in non-polar solvents. However, in acid solution, benzoic acid is neutral and quite nonpolar. Moreover it is soluble in non-polar solvents, into which it may be extracted at acidic pH. In this experiment, Benzoic acid is separated from a known quantity of the sample by saturating with NaCl and then acidifying with dilute HCl and extracting with chloroform. The chloroform layer is made mineral acid free and the solvent is removed by evaporation. The residue is dissolved in neutral alcohol and the amount of benzoic acid is determined by titration against standard alkali.

22.4.Materials:

1. Electric steam bath
2. Conical flasks.
3. Volumetric flasks
4. Graduated flasks of 250 ml capacity
5. Separating funnels of 250 ml capacity
6. Whatman no. 4 filter paper.
7. Sodium Chloride (AR).
8. Chloroform
9. HCL (dil. 1 : 3)
10. Sodium hydroxide (10%)
11. Standard NaOH solution (0.05N)
12. Saturated Sodium Chloride solution.

Preparation of beverages and liquid products:

Mix the sample thoroughly and transfer 100 ml of the sample into a 250 volumetric flask.

Make alkaline to litmus paper with 10% NaOH solution (add 5 ml)

Make up to volume with saturated NaCl solution. Shake thoroughly and let it stand for 2 hours.

Filter the sample and use the filtrate for determination.

22.5. Method:

1. Pipette 100 ml to 200 ml of the filtrate into a 250 ml separatory funnel.
2. Neutralize to litmus paper using HCI (dil) and add 5 ml excess.
3. Extract carefully with 40, 30, 30 and 20 ml portions of chloroform.
4. Transfer the combined chloroform extract in to a separating funnel and wash it free from mineral acid by shaking gently and rinsing with water.
5. Drain off the water phase.
6. Dry the chloroform layer over anhydrous sodium sulphate and distil off the solvent.
7. Remove the last traces of the solvent under a current of air at room temperature.
8. Dissolve residue in 30-50 ml of alcohol neutralised to phenolphthalein and titrate with 0.05 N NaOH.

22.6.Results and Calculation:

1. Calculate the benzoic acid contents as follows:

$$\text{Benzoic acid (ppm) mg/l} = \frac{122 \times \text{titre} \times \text{dilution} \times 1000 \times \text{NaOH}}{\text{Weight of sample}}$$

2. Determine the concentrations sodium benzoate in your soft drink sample in units of ppm (mg/l).

22.7.Discussion

Compare your results with the total sodium benzoate content in the whole can of the tested soft drink.

22.8. References :

A.O.A.C 17th edn , 2000, Official Method 963.19 Benzoic acid in Foods Titrimeric Method).