# FN 502 ADVANCED NUTRITION (2+1)

## INDEX

## EXPERIMENT NO.1

# DETERMINATION OF ACIDITY OF FRUIT JUICE

**AIM:**

To determine the acidity of fruit juice (lime).

**PRINCIPLE:**

A known weight of fruit juice is treated with standard alkali using phenolphthalein as indicator. The acidity of the fruit juice is expressed in terms of the most predominant organic acid in the fruit.

**REAGENTS:**

1. Standard alkali – 0.1 N Na OH
2. Phenolphthalein

**PROCEDURE:**

- ❖ Pipette 10 ml of the filtered fruit juice and make up to 100 ml with distilled water.
- ❖ Take 10 ml of the sample add 2-3 drops of phenolphthalein indicator.
- ❖ Titrate against 0.1N sodium hydroxide till a faint pink colour is obtained.
- ❖ Express the acidity as percent or anhydrous citric acid or other words.

**OBSERVATION: For Example:**

| Sl. No. | VOLUME OF SAMPLE TAKEN (ml) | BURETTE READING (ml) | |
|---|---|---|---|
| | | Initial | Final |
| 1. | 10 | 2.1 | 10 |
| 2. | 10 | 10 | 18.2 |
| 3. | 10 | 18.2 | 26.2 |

**CALCULATION: For Example:**

$$\text{Acidity of fruit} = \frac{\textbf{Titre Value X Normality of alkali X Vol.made up X Eq.Weight of acid X100}}{\textbf{Vol.of sample taken for estimationX Vol.of sample taken X1000}}$$

$$= \frac{8.03X0.1X100X64X100}{10X10X1000}$$

**Acidity =5.139%**

**RESULT: For Example:**

The acidity of given lemon juice in terms of citric acid is **5.139 %**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of acidity present in various fruit juice (other samples)
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and major points of discussion
- Write about the beneficial usage of this test and its importance in food industry

## EXPERIMENT NO.2

# DETERMINATION OF MOISTURE CONTENT OF FOOD

**Aim:**

To determine the moisture content of the given sample and calculate the percentage of moisture.

**PRINCIPLE:**

Estimation of moisture is one of the most often performed determinations in food analysis. Moisture is lost when food is heated not much higher than the temperature of boiling water or by allowing standing overnight over dehydrating agent or by heating over a vacuum.

**PROCEDURE:**

Moisture content of a sample is estimated by heating the sample in an oven to a constant weight at $100^0$C for overnight or $130^0$C for 2 hours, in an oven.

**EQUIPMENT:**

Balance, Hot air oven to maintains temperature, desiccator.

**PROCEDURE:**

- Dry the moisture in an oven at $100^0$C cool it in a desiccator and record the weight.
- Take 10 g of the sample in the moisture cup and weigh.
- Dry the sample in a hot air oven at $100^0$C for longer period (0 to 4 hours) at $135^0$ C for a shorter period (about 2 hours) or less than $100^0$ C with air under vacuum.
- Remove the moisture cup from the oven and cool it in a dessicator.
- Repeat the process of heating and cooling till a constant weight of moisture cup is achieved, with sample.

**CALCULATION: For Example:**

$$\text{Moisture (\%)} = \frac{\text{Initial Weight of moisture cup and sample)} - \text{(Final weight after drying)}}{\text{Weight of the sample}} \times 100$$

$$= \frac{58.9 - 52.08}{10} \times 100 = \frac{6.64}{10} \times 100 = 66.4\ \%$$

**RESULT: For Example:**

The **moisture** percentage of **drumstick leaves = 66.4 %**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of moisture content present in (various) other samples.
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and important points of discussion
- Write about the beneficial usage of this tests and its importance in food industry

## EXPERIMENT NO. 3

# ESTIMATION OF FAT

**AIM:** To determine the fat content of the given sample.

**Introduction**: (For Example)

- Fats are fatty acids esters of glycerol.
- Fat as liquid is called oil.
- Seeds like gingerly, groundnut, sun flower etc contain oil as reserve food material.(write more special features with structures)

**PRINCIPLE:**

- Oil from a known quantity of the seed is extracted with petroleum ether.
- It is then distilled.
- Completely dried.
- The oil is weighed and the percent oil is extracted.

**MATERIALS:**

- **Petroleum ether ($40^0$ -$160^0$C)**
- **Waterman No.2 Filter paper**
- **Absorbent Cotton**
- **Soxhlet apparatus**

**PROCEDURE**

- Fold a piece of filter paper in such a way to hold the milk peda, wrap around a second filter paper which is left at top like thimble.
- A piece of cotton wool is placed at the top, to evenly distribute the solvent,as it drops on the sample during extraction.
- Place the sample packet in the soxhlet tube of the soxhlet extraction apparatus
- Extracted with petroleum ether for 6 hours without interruption by gentle heating
- Allow to cool and dismantle the extraction flow to evaporate, the ether, once, steam or water bath until no odour of ether remain. Cool at room temperature

- Carefully remove the dirt or moisture outside the flask and weigh the flask
- Repeat heat until constant weight is recorded

**CALCULATION: For Example:**

$$\text{Oil in ground sample (\%)} = \frac{\textbf{Weight of Oil (g)X100}}{\textbf{Weight of Sample}}$$

Weight of oil = 1.87 g

Weight of sample = 5 g

$$\text{The given sample of milk peda contain} = \frac{1.87\ X100}{5} = \textbf{37.4\%}$$

**RESULT:** The given milk **peda** sample contain **37.4% of fat.**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of fat present in (various) other samples.
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and major points of discussion
- Write about the beneficial usage of this tests and its importance in food industry

## EXPERIMENT 4

## ESTIMATION OF FREE FATTY ACIDS

**AIM:** To determine the free fatty acids in the given sample.

**PRINCIPLE:**

The free fatty acids in an oil is estimated by titrating it against potassium hydroxide in the presence of phenolphthalein indicator. The acid number is defined as mg KOH required to neutralize the free fatty acids present in 1g of sample. However, the free fatty acids content is expressed as oleic acid equivalents.

**MATERIALS:**

- 1% Phenolphthalein in 95% ethanol
- O.1N Potassium hydroxide
- Neutral solvent: Mix 25ml ether,25ml 95% alcohol

**PROCEDURE:**

- Dissolve 1-10g of oil or melted fat in 50ml of neutral solvent in a 250ml conical flask
- Add few drops of phenolphthalein
- Titrate the contents against 0.1N potassium hydroxide
- Shake constantly until a pink colour which persists for fifteen seconds is obtained.

**CALCULATION: For Example:**

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titre Value X Normality of KOH X 56.1}}{\text{Weight of the sample (g)}}$$

**Polythene:**

$$\text{Acid value} = \frac{0.3 \text{ X } 1.402 \text{ X } 56.1}{10} = 2.3595 \text{ mg KOH /g}$$

**Bottle:**

$$\text{Acid value} = \frac{0.6 \text{ X} 1.402 \text{ X } 56.1}{10} = 4.7191 \text{ mg KOH /g}$$

**Sack:**

$$\text{Acid value} = \frac{0.2\text{X}1.402\text{X}56.1}{10} = 1.5730 \text{ mg KOH /g}$$

**RESULT: For Example:**

Free fatty acids of Cocoa butter was estimated for different packing conditions. It was found that acid value of bottle was high compared to polythene and sack. (Add on)

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of free fatty acids present in (various) other samples.
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and major points of discussion
- Write about the beneficial usage of this tests and its importance in food industry

# EXPERIMENT No.5

# ESTIMATION OF PEROXIDE VALUE

**Aim:**

To determine the peroxide value

**PRINCIPLE:**

- ❖ Peroxide value is a measure of peroxides contained in the oil.
- ❖ The peroxides present are determined by titration against thiosulphate in the presence of KI
- ❖ Starch is used as indicator.

**MATERIALS:**

- **Solvent Mixture:**
- Mix two volumes of glacial acetic acid with one volume of chloroform
- 5% Potassium iodide Solution
- 1% Starch Solution
- $\frac{N}{500}$ Sodium thiosulphate solution: Prepare $\frac{N}{10}$ Solution and dilute to $\frac{N}{500}$ on the day of use

**PROCEDURE:**

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

**CALCULATION: For Example:**

Peroxide value (milli equivalent peroxide/ kg sample)

$$= \frac{\mathbf{S\ X\ N\ X\ 100}}{\mathbf{g\ of\ Sample}}$$

**Where S= ml; N= Normality of $Na_2S_2O_3$**

**RESULT: For Example:**

The **cocoa butter** taken for analysis had **no peroxide value**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of peroxide value present in other (various) samples.
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and major points of discussion
- Write about the beneficial usage of this tests and its importance in food industry

# EXPERIMENT No.6

# ESTIMATION OF BMR

## INTRODUCTION:

- Basal metabolism is the minimum amount of energy needed by the body for maintenance of life when the person is at post absorptive state, physical and emotional rest
- Thus basal metabolism represented as basal metabolic rate (BMR) represents the minimum amount of energy expended in a fasting state to keep a resting awake body live in warm quiet environment
- Thus basal metabolism represented as basal metabolic rate (BMR) represents the minimum amount of energy expended in a fasting state to keep a resting awake body alive in warm quiet environment
- The BMR can be measured by directly from the heat produced or indirectly from $O_2$ intake and $CO_2$ expenditure when the subject is at rest
- BMR calculated by using equations

**Predictive equations:**

- **Estimation using body weight**
- **Harris Benedict equation**
- **FAO/WHO equation**
- **ICMR fore predicting BMR**

## 1. ESTIMATION USING BODY WEIGHT:

**Body weight for females= Weight in Kg × 0.9 kcal ×24hrs**

**For Example:**

| | |
|---|---|
| Subject 1 | Body weight = 51× 0.9 kcal ×24 = 1101.6 kcal |
| Subject 2 | Body weight = 46× 0.9 kcal ×24 = 993.6kcal |
| Subject 3 | Body weight = 45× 0.9 kcal ×24 = 972 kcal |
| Subject 4 | Body weight = 35× 0.9 kcal ×24 = 756 kcal |

## 2. HARRIS BENEDICT EQUATION

**BMR= 655.5 + (9.56 X W) + (1.85 X H) – (4.68 X A)**

**$A = W^{0.425} X H^{0.725} X .007184$**

**For Example:**

| |
|---|
| Subject 1: BMR = 655.5 + 487.56 + 290.45 – 6.9877 = 1426.52 Kcal |
| Subject 2: BMR = 655.5 + 439.76 + 284.9 – 6.5950 = 1373.565 Kcal |
| Subject 3: BMR = 655.5 + 430.2+ 284.9 – 6.53 = 1301.07 Kcal |
| Subject 4: BMR = 655.5 + 430.2+ 271.95 – 5.6768 = 1351.9732 Kcal |

## 3. FAO / WHO EQUATION

**BMR = 8.7 X W +829**

**For Example:**

| |
|---|
| Subject 1: 8.7 X 51 +829 = 1272 Kcal |
| Subject 2: 8.7 X 46 +829 = 1229.2 Kcal |
| Subject 3: 8.7 X 45 +829 = 1220.5 Kcal |
| Subject 4: 8.7 X 35 +829 = 1133.5 Kcal |

## 4. ICMR EQUATION: BMR = 14.0 X BW (kg) + 471

**For Example:**

| |
|---|
| Subject 1: 14 X 51+ 471= 1185 Kcal |
| Subject 2: 14 X 46+ 471= 1115 Kcal |
| Subject 3: 14 X 45+ 471= 1101 Kcal |
| Subject 4: 14 X 35+ 471= 961Kcal |
| |

| EQUATIONS FOR CALCULATING BMR :For Example: | | | | |
|---|---|---|---|---|
| **EQUATIONS** | **SUBJECT 1 (Kcal)** | **SUBJECT 2 (Kcal)** | **SUBJECT 3 (Kcal)** | **SUBJECT 4 (Kcal)** |
| Using Body weight | 1101.6 | 993.6 | 972 | 756 |
| Harris Benedict | 1426.52 | 1373.565 | 1301.07 | 1351.97 |
| ICMR | 1185 | 1115 | 1101 | 961 |
| FAO/ WHO | 1272 | 1229.2 | 1220.5 | 1133.5 |

**RESULT: For Example:**

BMR was calculated using body weight, height, surface area with different equations. BMR estimated using body weight was found to be high in subject 1 and lowest in subject 4, Out of 4 subjects taken for estimating BMR, Harris benedict equation estimation of BMR was high for subject 1, followed by subject 2, subject 4 and subject 3. For ICMR, FAO / WHO, BMR was high in subject 1 and lowest for subject 4

**Exercise:**

- **Conduct the experiment using other subjects**
- Calculate the BMR of various subjects using different Equations.
- Take the action pictures
- Pictures of the Subjects (Optional)
- Collect reviews
- References
- Write inference with supportive studies and major point of discussions
- Write about the beneficial usage of these equations and its importance in maintaining health of each individuals

# EXPERIMENT No. 7

# ESTIMATION OF CAROTENE

**AIM:**

To estimate the amount of total carotene present in the given sample.

**PRINCIPLE:**

- Carotene is estimated by extraction of total pigment with alcoholic KOH and portioning with petroleum ether after saponification.
- The other pigments are removed by treatment with calcium carbonate and yellow colour is measured as carotene.

**PROCEDURE:**

- Method of Arnone (1949) is a classical method for the estimation of total carotene.80% acetone need to be prepared ,250 mg of fresh (Sample) need to be ground into fine paste and a green supernatant is been obtained,by using small quantities of acetone
- The extract has to be centrifuged repeatedly till the sediments become colourless.
- The supernatant is taken together and volume is made upto 25 ml with 80% acetone
- The extract is kept away from direct sunlight.
- The optical density is read in a spectrophotmetric or calorimeter at $480^0$ and 510 nm wave length.
- **Samples need to be analyzed in duplicate.**
- From the optical density, the carotenoid content need to be calculated using the formula.

$$\text{Total Carotene (mg/g)} = 7.6\ (\mathbf{D\ 480\text{-}1.49\ X\ D\ 510})\ X\frac{\mathbf{v}}{\mathbf{1000}}\ \mathbf{X\ W}$$

**Where D = Optical Density**

**V= Volume of Acetone** (25ml)

**W= Weight of Sample** (0.25mg)

**CALCULATION: FOR EXAMPLE:**

$$\text{Total Carotene (mg/g)} = 7.6\,(D\,480 - 1.49 \times D\,510) \times \frac{V}{1000} \times W$$

$$= 7.6\,(1.499 - 1.49 \times 0.583) \times \frac{25}{1000} \times 0.25$$

$$= 7.6 \times 0.63033 \times \frac{25}{1000} \times 0.25$$

$$= \frac{29.9406}{1000}$$

Total Carotene (mg/g) =0.0299

Total Carotene (mg/100g) =0.029 X 100 =2.99

**RESULT: For Example:**

**Total carotene of red amaranthus = 2.99/100g**

**Exercise:**

- **Conduct the experiment using various other samples**
- Calculate the Carotene Content of various other samples
- Take the action pictures
- Pictures of the samples and ongoing changes as per the procedure
- Collect reviews
- References
- Write inference with supportive studies and major points of discussion
- Write about the beneficial usage of this test and its importance in food Industry and carotene content in daily Balanced diet.

# EXPERIMENT NO.8

# ESTIMATION OF IRON

## AIM:

To estimate the iron content of the given sample

## PRINCIPLE:

- Iron is determined calorimetrically with ferric iron which gives a blood red colour with potassium thiocyanate.
- The intensity of the colour, which is measure of the concentration of the iron present is determined calorimetrically using standard iron solution.

## REAGENTS:

1. 30% of $H_2SO_4$.
2. 7% potassium per sulphate solution
3. 30% potassium thiocyanate solution: Dissolve 40 g **of KCNS** in 90 ml glass distilled water and add 4 ml of acetone and make up to 100ml.
4. **Standard iron solution**
   - Dissolve 0.702 g of ferrous ammonium sulphate in 100ml of glass distilled water and add 5 ml 1:1 Hcl and make upto 1 L and mix thoroughly.
   - Prepare fresh standard solution once in 6 months.
   - Prepare the working standard solution of different concentration.

## PROCEDURE:

- To an aliquot (2ml) of the mineral solution add enough water. if necessary to make up to a volume of 6.5 ml followed by 10 ml of 30% $H_2SO_4$, 1ml of potassium per sulphate solution and 1.5ml of 30% KCNS solution.
- Prepare a blank by **adding all** reagents **except the mineral solution.**
- Measure the red colour with 20 minutes, at 540 nm.

- Table 1, 2, 3. 4 and 5 ml of working standard in a series of test tubes and add enough water to make up to 6.5 ml, followed by 1 ml of saturated potassium per sulphate
- 1 ml of 30% $H_2SO_4$ and 1.5 ml of 30% KCNS solution
- Read the red colour at 540 nm with 20 minutes

**OBSERVATION: FOR EXAMPLE:**

| VOLUME OF SOLUTION (ml) | READING |
|---|---|
| 1ml | 0.301 |
| 2ml | 0.425 |
| 3 ml | 0.647 |
| 4 ml | 0.876 |
| 5ml | 1.007 |
| Sample | 0.4 |

**CALCULATION: FOR EXAMPLE:**

Concentration of unknown from graph = 2 r

2 ml of mineral solution contains = 2 r

100 ml of mineral solution contains $= \frac{2}{2} \times 100 = 100$ r

100 ml of mineral solution was made from 5 g of sample

There for 5g of sample contains 100 r

There for 100 g of sample will contain $= \frac{100}{5} \times 100 \times \frac{1}{1000} = 2$mg

**RESULT; FOR EXAMPLE:**

Amount of Iron in 100 g of red amaranthus is 2 mg (Add more points)

**Exercise:**

- **Conduct the experiment using various other samples**
- Calculate the amount of iron present in various (other) samples.
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and major points of discussion
- Write about the beneficial usage of this tests and its importance in food industry

## EXPERIMENT NO.9 (1)

# ESTIMATION OF SUGARS

**AIM:**

To estimate the amount of reducing sugar, non-reducing sugar and total sugar in the given sample.

## 1. REDUCING SUGAR.

- Reducing sugar present in the fruit juice reduces the alkaline solution of the cupric salt (Fehlings Solution) is coloured cuprous oxide.
- Methylene blue redox indicator is employed to detect the end point of filtration.
- The red colour and cuprous oxide is turned during the reaction will be marked by the intense blue colour of the indicator.
- But when all the cupric ions of the Fehlings solutions are reduced to cuprous by sugar solution (Fruit juice).
- Then the next few drops of sugar solution added will dissolve the indicator, so that the red colour of the cuprous oxide become visible, which is taken as the end point of the titration.
- The reducing sugar will be taken often in terms of glucose since glucose is the most predominant reducing sugar present in fruit.

**REAGENTS:**

1. Fehlings Solution A and B
2. Methylene blue indicator
3. Standard [illegible] Solution 0.5% solution —

   Dissolve 0.5g [illegible] in 100ml distilled water in a standard flask.
4. Hydrochloric Acid 25% solution —

   Dissolve 25g Hydrochloric Acid in 100 ml distilled water in a standard flask.
5. A Sodium hydroxide

Dissolve 22 gm Sodium Hydroxide in 100 ml distilled water in a standard flask

**PROCEDURE:**

- Pulp the fruit in a blender and filter it through what man No.40 filter paper.
- Transfer 25 ml of fruit pulp to 250 ml conical flask and 100 ml water is added.
- Neutralize it by 1N Sodium Hydroxide using phenolphthalein as indicator (Then end point is to appearance of pink colour).
- Add 2ml of the lead acetate to clarify the solution and kept still for 10 minutes.
- Then add 2 ml of potassium oxalate solution and allowed to settle as precipitate.
- Wait for 5 minutes and filter the solution for 50 ml standard flask and make up the volume.
- Pipette out 5 ml of Fehling Solution A and B into a 250 ml conical flask.
- Add about 50 ml of distilled water and 2 to 3 glass heads.
- Boil the contents vigorously and while boiling added the clarified juice taken in burette till the blue colour just appear.
- Then add 0.5 ml of methylene blue indicator and allow it to boil for one minute.
- While boiling, complete the titration as quickly as possible by adding 2 to 3 drops of sugar solution at 5 to 6 seconds interval until the indicator is completely discoloured and the brick red colour of cuprous oxide becomes dominant.(not interrupt boiling), for more than a few seconds as the indicator undergo into the flask.
- Calculate the content of reducing sugar of glucose/100 ml of the juice.
- **CALCULATION : For Example:**

10 ml of Fehling Solution (A+B) = 0.05 gm of glucose

Weight of fruit juice taken for analysis = 25 ml

Volume made up =250 ml

Volume of clarified juice recanted =12.8 ml

With 10 ml of Fehling solution A+B.

% of reducing sugar in the juice

**Glucose / 100 g of juice** $= \frac{\text{Fehlings solution x dilution}}{\text{Titre Value X Weight of Sample}} \text{ X } 100$

$= \frac{0.05 \text{ x } 250 \text{ x } 100}{12.8 \text{ x } 25}$

$= \frac{1250}{320}$

**= 3.9% (B)**

**Reducing Sugar in guava** = 3.9 %

**Result: FOR EXAMPLE:**

Percentage of reducing sugar in guava juice is **3.9%.**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate. amount of reducing sugar in the given sample
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and important points of discussion
- Write about the beneficial usage of these tests and its importance in food industry

## EXPRIMENT NO.9 (2) (Contd...)

# TOTAL SUGARS AND NON REDUCING SUGAR

**PRINCIPLE:**

- The non reducing sugar of the fruit (Sucrose) is hydrolysed to reducing non Saccharides by treatment with dilute acid.
- The total reducing sugars are then estimated by titration with Fehlings solution.

**REAGENTS**

1. Fehlings Solution A and B
2. Methylene blue indicator
3. Phenolphthalein.
4. Neutral lead acetate 45% solution. Dissolve 45g lead acetate in 100ml distilled water in a standard flask.
5. Potassium oxalate 22% solution. Dissolve 22 g Potassium Oxalate in 100 ml distilled water in a standard flask.
6. 1 N Sodium Hydroxide. Dissolve 22 g potassium Oxalate in 100 ml distilled water into standard flask.
7. Citric acid- Soluble.

**PROCEDURE:**

- Pipette out 50 ml of clarified juice solution (Prepared for the estimation of reducing sugar) into a 250 ml conical flask.
- Add 50 g of citric acid and 50 ml of water.
- Boil gently for 10 minutes to complete the inversion of sucrose and then cool.
- Transfer the contents to a 250 ml volumetric flask and neutralize with 1 N sodium hydroxide using phenolphthalein as the indicator.
- Make up the volume.

- Titrate the makeup solution with 10 ml of Fehlings Solution as detailed in the total sugar, as g of glucose per 100g of original juice.

The difference between the total sugars and reducing sugar will represent non reducing sugar will represent non reducing sugar will be expressed as g of sucrose per 100 g of the juice.

**CALCULATION- For Example:**

**Total Sugar:**

Weight of the fruit juice for analysis = 50g

Volume made up = 250 ml

Volume of the clarified juice used for inversion =50 ml

Volume made up after the inversion = 250 ml

Volume of the made up solution reacted with

10 ml of Fehlings Solution => Titre Value = 15.0ml

Percentage of total sugar in the original juice

$$= \frac{\text{Fehlings Solution X Dilution Factor X 250 x100}}{\text{Titre Valuue X 50 X Weight of the Sample}}$$

$$= \frac{0.05X250X250\ X100}{15X50\ X50} = \frac{312500}{37500} = 8.33\ \%$$

Let the percentage of reducing in the same juice

Expressed as glucose = 3.9 % (B)

Then the percentage of non reducing sugar expressed as glucose = A-B

(Total Sugar – Reducing Sugar) = 8.33-3.9 = **4.43 %**

**RESULT: For Example:**

The **percentage of total sugar** in the juice **= 8.33%**

The percentage of **non-reducing sugar** in

guava juice **expressed as glucose** **= 4.43%**

(Discussion- Sucrose is a non-reducing sugar because it lacks the ability to form either aldehyde or a ketone in a basic solution. Sucrose's anomeric carbon is not free since this carbon is used to link fructose and glucose together. Therefore it cannot open up the ring structure and react with the reagent.The anomeric carbon is a stereo centre. An important feature is the direction of the OH group attached to the anomeric carbon. Depending on the direction of the OH group, the anomeric carbon is either α or β)

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate. amount of total sugars and non reducing sugars in the given samples
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and important points of discussion
- Write about the beneficial usage of these tests and its importance in food industry

## EXPERIMENT NO.10

# ESTIMATION OF CRUDE FIBRE

## Introduction:

- Crude fibre consist of largely of cellulose and lignin (97%) plus some mineral matter.
- It represent only 60%to 80% of the cellulose and 4 % to 6 % of the lignin.
- The crude fibre content is commonly used as a measure of the nutritive value of poultry and livestock, feeds and also in the analysis of various food and food products to detect adulteration quality and quantity.

## PRINCIPLE:

- During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of lignin occurs
- The residue obtained after the final filtration is weighed, incinerated, cooked and weighed again.
- The loss of weight gives the crude fibre content.

## MATERIALS:

1. Sulphuric acid solution (0.255 to 0.05N):1.25g. Concentrated Sulphuric acid diluted to 100 ml (Concentration must be checked by titration).
2. Sodium Hydroxide Solution: 1.25g sodium hydroxide in 100 ml distilled water.

## PROCEDURE:

1. Extract 2g of ground materials with ether or petroleum ether to remove fat (initial boiling temperature ($35^0$C to $38^0$C) and final temperature $52^0$C. If fat content is below 1% extraction may be omitted.
2. After fat extraction with either, boil 2 g of dried material with 200 ml of sulphuric acid for 30 minutes with bumping chips.
3. Filter through muslin cloth again and wash with 25 ml of boiling 1.25% of $H_2SO_4$ Then with 50 ml of portions of water and alcohol 25 ml.
4. Remove the residue and transfer to washing dish in a desiccator and weigh ($W_2$).
5. Ignite for 30 minutes at 600 $\pm 15^0$C
6. Cool in a desiccator and re-weight($W_2$)

**CALCULATION: For Example:**

Percentage of crude fibre in ground sample

$$\frac{\textbf{Loss in weight on ignition (W2 – W1) – (W3 – W1)X100}}{\textbf{Weight of sample}}$$

W1 = 22.14

W2 = 22.72

W3 = 22.53

$$= \frac{(W2-W1)-(W3-W1)}{\text{Weight of Sample}} X100$$

$$= \frac{(22.72-22.14)-(22.53-22.14)}{2} X100$$

$$= \frac{0.58-0.39}{2} X\ 100$$

$$= \frac{0.19}{2} X100$$

= 9.5%

**The Percentage of crude fibre in fenugreek seeds = 9.5%**

**RESULT: For Example:**

The **crude fibre** content of **fenugreek seed** was found to be **9.5 % on dry weight basis.**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of crude fibre present in the various (other) samples
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews / References
- Write inference with supportive studies and major points of discussion
- Write about the beneficial usage of these tests and its importance in food industry

## EXPERIMENT NO.11

# DETERMINATION OF TOTAL CARBOHYDRATE BY ANTHRONE REAGENT

**AIM:** To determine carbohydrate in the given sample by anthrone reagent.

**PRINCIPLE:**

- Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid.
- In hot acidic medium, glucose is hydrolysed to hydroxyl methyl furfural.
- This compound forms with anthrone as green coloured product with an absorption maximum at 630 nm.

**MATERIALS:**

1. 2.5 N Hcl
2. Anthrone reagent- Dissolve 200 mg anthrone reagent in 100ml ice cold 25% $H_2SO_4$. Prepare fresh before use.
3. Standard glucose- Stock is prepared by dissolving 100 mg glucose in 100ml of distilled water. For working standard, dilute10 ml of stock standard solution in100ml with distilled water in volumetric flask and store in refrigerator after adding a few drop of toluene

**PROCEDURE:**

- Weigh 100 mg of the sample into a boiling tube.
- Hydrolyze by keeping it in a boiling bath for three hours with 5 ml of 2.5N.Hcl and cool to room temperature.
- Neutralize with solid sodium carbonate until the effervescence ceases
- Keep up the volume to 100 ml and centrifuge.
- Collect the supernatant and take 0.5 and 1 ml aliquots to analyse.
- Prepare the standard by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard, along with blank.
- Make up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water.
- Then add 4 ml of anthrone reagent

- Heat for eight minutes in a boiling water bath
- Cool rapidly and read the green to dark colour at 630 nm.
- Draw a standard graph by plotting concentration of standard on the X axis verses absorbance on Y axis.
- From the graph calculate the amount of carbohydrate present in sample tube.

**CALCULATION:**

**For example:**

Amount of carbohydrate present in the 100 g of sample (lotus rhizome Flour)

$$= \frac{\text{mg of glucose}}{\text{volume of test sample}} \text{X } 100$$

$$= \frac{0.14}{1} \text{ X100 =14g}$$

**RESULT:**

The amount of carbohydrate present in 100 g of the **lots rhizome Flour** (sample) is **14mg.**

**Exercise:**

- **Conduct the experiment using other samples**
- Draw the graph
- Calculate the amount of carbohydrate present in various (other) samples.
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and write major points of discussion
- Write about the beneficial usage of this tests and its importance in food industry

# EXPERIMENT NO.12

# ESTIMATION OF VITAMIN C

**Aim:**

To estimate the ascorbic acid content in fruit juice (given Sample)

**PRINCIPLE:**

- The reduction of the dye 2, 6 dichloro indo phenol dye by an acid solution of ascorbic acid forms the basis of estimation
- In the absence of interacting agents, the capacity of an extract of sample to reduce a standard solution of the dye is directly proportional to the ascorbic acid content.
- Oxalic acid is used to extract ascorbic acid because it stabilize the vitamin by reducing the pH of the extracting medium and the metallic ions which oxidises the vitamin are removed in complexes with oxalic acid and prevent the catalytic oxidation of ascorbic acid.

**REAGENTS:**

- 4% oxalic acid
- Standard ascorbic acid
- 2,6 dichloro indo phenol dye.

**PROCEDURE:**

**(A) Standardization of dye**

- Take 5 ml of standard ascorbic acid solution and add 5 ml of oxalic acid
- Titrate with the dye solution taken in a burette to a pink colour which should persist at least 15 seconds.
- Determine the dye factor. i.e., mg of ascorbic acid per ml of the dye using the formula.

$$\text{Dye factor} = \frac{0.5}{\text{Titre Vaue}} = \frac{0.5}{3} = 0.166$$

**(B) Estimation of ascorbic acid content in fruit juice.**

- Take 10 ml of fruit and make up to 100 ml with 4 % oxalic acid

- Pipette 10 ml of the made up solution into conical flask and titrate against the dye taken in a burette to a pink end point which should persist for at least 15 seconds.
- Repeat the titration to obtain concordant value of titre value

**CALCULATIONS:**

**A). Dye factor**

1 ml of standard ascorbic acid solution.

5 ml of solution- 0.5 mg of ascorbic acid.

5 ml of made up ascorbic acid solution was reduced by 1 ml of the dye.

$$\text{Dye factor} = \frac{0.5}{3} = \mathbf{0.166}$$

**B). Ascorbic acid content in fruit juice.**

1ml of the dye is reduced by 0.16 mg of ascorbic acid.

7.8 ml of the dye is reduced by – A X B mg of ascorbic acid.

$$= 7.8 \times 0.16 = \mathbf{1.248\ mg}$$

Therefore ascorbic acid present in 100 ml of fruit extract is:

$$1.248 \times 100 = 124.8\ \text{g}$$

Ascorbic acid present in 10 ml of juice is 12.48 mg

Ascorbic acid present in 100 ml of fruit juice = **124.8 mg**

**RESULT:** Ascorbic acid content of 100 ml of orange juice is **124.8 mg**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of ascorbic acid present in the various (other) samples.
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews / References
- Write inference with supportive studies and important points of discussion
- Write about the beneficial usage of this tests and its importance in food industry

## Exercise No. 13

# ESTIMATION OF PHOSPHORUS

**AIM:** To determine phosphorus content in the given sample

**PRINCIPLE:**

- Plant phosphorus is converted to orthophosphates during digestion which react with molybdate and vandate to give yellow coloured complex. The intensity of this yellow colour can be read in spectrophotometer.
- Colour is developed in 30 minutes and is stable for 2-3 weeks.

**REAGENTS:**

- Ammonium molybdate- ammonium vandate in $HNO_3$:22.5g of ammonium molybdate is dissolved in 400ml of distilled water.
- 1.25g of ammonium vandate is dissolved in 300ml of boiling distilled water.
- Vandate solution is added to the molybdate solution and is cooled to room temperature.
- After this, 250ml of $HNO_3$ was added and diluted to 1 litre.
- Phosphate standard solution: 0.2195g of $KH_2PO_4$ is dissolved and distilled to 1 litre.

**PROCEDURE:**

- 0,1,2,3,4,5 ml of $KH_2PO_4$ is pipetted into 50ml of volumetric flask(to get 0,1,…,5ppm P)
- 10ml of Barton's reagent ( ammonium vandate) to each is added
- It is made up to 50ml with distilled water
- It is kept for 30min for developing yellow colour
- "0" ppm is placed in spectro photomter at 470 nm and absorbance is set to zero
- It is repeated for all other standards (1,2,3,4,5 ppm)
- Graph is drawn with
  - Concentration in ppm on X-axis (X-axis – 1cm = 1ppm)
  - spectrophotometer reading on Y-axis (Y-axis – 1cm= 0.02 absorbance)
- **Slope** $= \frac{\text{Absorbance}}{\text{Concentration}} = \frac{\text{Y-axis scale (i.e,0.02)}}{\text{X-axis scale (i.e 1)}}$
- 5ml of plant digest is pippeted out from diacid digest which has been made upto 50ml after digestion ) in 50ml volumetric flask
- 10ml of Barton's reagent is added and made up with distilled water
- It was kept for few minutes till development of yellow colour
- Absorbance is read in spectrophotometer at 470nm
- **Concentration (x – ppm)of unknown plant sample** $= \frac{\text{Absorbance}}{\text{Slope}}$

**OBSERVATION:**

| Concentration (ppm) | Absorbance |
|---|---|
| 1 | 0.018 |
| 2 | 0.039 |
| 3 | 0.051 |
| 4 | 0.078 |
| 5 | 0.068 |
| $R_1$ | 0.021 |
| $R_2$ | 0.023 |
| $R_3$ | 0.021 |

**CALCULATION: For Example:**

$$\text{Phosphorus in ppm} = \frac{\text{xppm} \times 50}{W} \times \frac{50}{5}$$

X ppm= graph value

W = weight of sample - 0.5 g

$$\text{Phosphorus in \%} = \frac{\text{Phosphorus in ppm}}{10000}$$

Graph value: $R_1$ – 1.2 ppm

$R_2$ – 1.3 ppm

$R_3$ – 1.2ppm

$R_1 \longrightarrow$ Phosphorus in ppm $= \frac{1.2 \times 50}{0.5} \times \frac{50}{5} = 1200$

Phosphorus in % $= \frac{1200}{10000} = 0.12\%$

$R_2 \longrightarrow$ Phosphorus in ppm $= \frac{3250}{2.5} = 1300$

$$\text{Phosphorus in \%} = \frac{1300}{10000} = 0.13\%$$

$R_3$ ➡ $$\text{Phosphorus in ppm} = \frac{1.2 \times 50}{0.5} \times \frac{50}{5} = 1200$$

$$\text{phosphorus in \%} = \frac{1200}{10000} = 0.12\%$$

| | P(%) | P(mg/g) | P(mg/100g) |
|---|---|---|---|
| $R_1$ | 0.12 | 1.2 | 120 |
| $R_2$ | 0.13 | 1.3 | 130 |
| $R_3$ | 0.12 | 1.2 | 120 |

**RESULT:**

The **phosphorus** content in **lotus rhizome sample** was found to be **123 mg / 100g**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of **phosphorous** present in **various (other) samples.**
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and important points of discussion
- Write about the beneficial usage of this tests and its importance in food industry

## Exercise No.14

# ESTIMATION OF POTASSIUM

**AIM:** To determine the potassium content in the given sample.

**REAGENTS**

Diacid: Mix 900 ml of Conc. $HNO_3$ and 400 ml of Conc. $HClO_4$in (9:1)

**PROCEDURE**

- 0.2 g sample was taken in digestion tube
- 10 ml of diacid was added along sides
- After that for digestion, it was made up to 50 ml and placed in digester
- Temperature was increased gently at 15 minutes interval from $300^0$ C to $350^0$ C
- Digestion was stopped when solution was colourless (3hrs)

The pre digested sample was used to measure potassium content in flame photometer and was expressed in mg/100g of sample.

**CALCULATION: For Example:**

$$K \text{ in ppm} = \frac{x \text{ ppm}}{W} \times \frac{50 \times 50}{5}$$

$$K \text{ in \%} = \frac{K \text{ in ppm}}{10{,}000}$$

$$LR_1\text{: K in ppm} = \frac{24{,}850}{2.5} = 9940$$

K in % = 0.994 %

$$LR_2\text{: K in ppm} = \frac{23{,}475}{2.5} = 9390$$

K in % = 0.939 %

$$LR_3\text{: K in ppm} = \frac{8.82 X 50 X 50}{0.5 X 5} = \frac{22{,}050}{2.5} = 8820$$

$$K \text{ in \%} = \frac{8820}{10.000} = 0.882\ \%$$

| K (%) | K ( mg/ g) | K (mg / 100g) |
|---|---|---|
| 0.994 | 9.94 | 994 |
| 0.939 | 9.39 | 939 |
| 0.882 | 8.82 | 882 |

**K = 938 mg/ 100 g**

**RESULT**

The potassium content **in lotus rhizome sample** was found to be **938 mg/ 100 g**

**Exercise:**

- **Conduct the experiment using other samples**
- Calculate the amount of potassium present in the various samples.
- Take the action pictures, pictures of the ongoing actual changes in the samples
- Collect reviews
- References
- Write inference with supportive studies and major points of discussion
- Write about the beneficial usage of this tests and its importance in food industry.