M.Sc. FOOD SCIENCE & NUTRITION LAB MANUAL

1st Semester

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PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for PG Food Science & Nutrition first Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers , and non-teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

Laboratory Practice Safety Rules

1. Use safety glass when dealing with fire and chemical.
2. Should use front cover clothes during biochemistry practical.
3. Always use hand wash after dissection and any type of chemical use.
4. Carefully handle needles , forceps, microscope and any other dissecting instrument.

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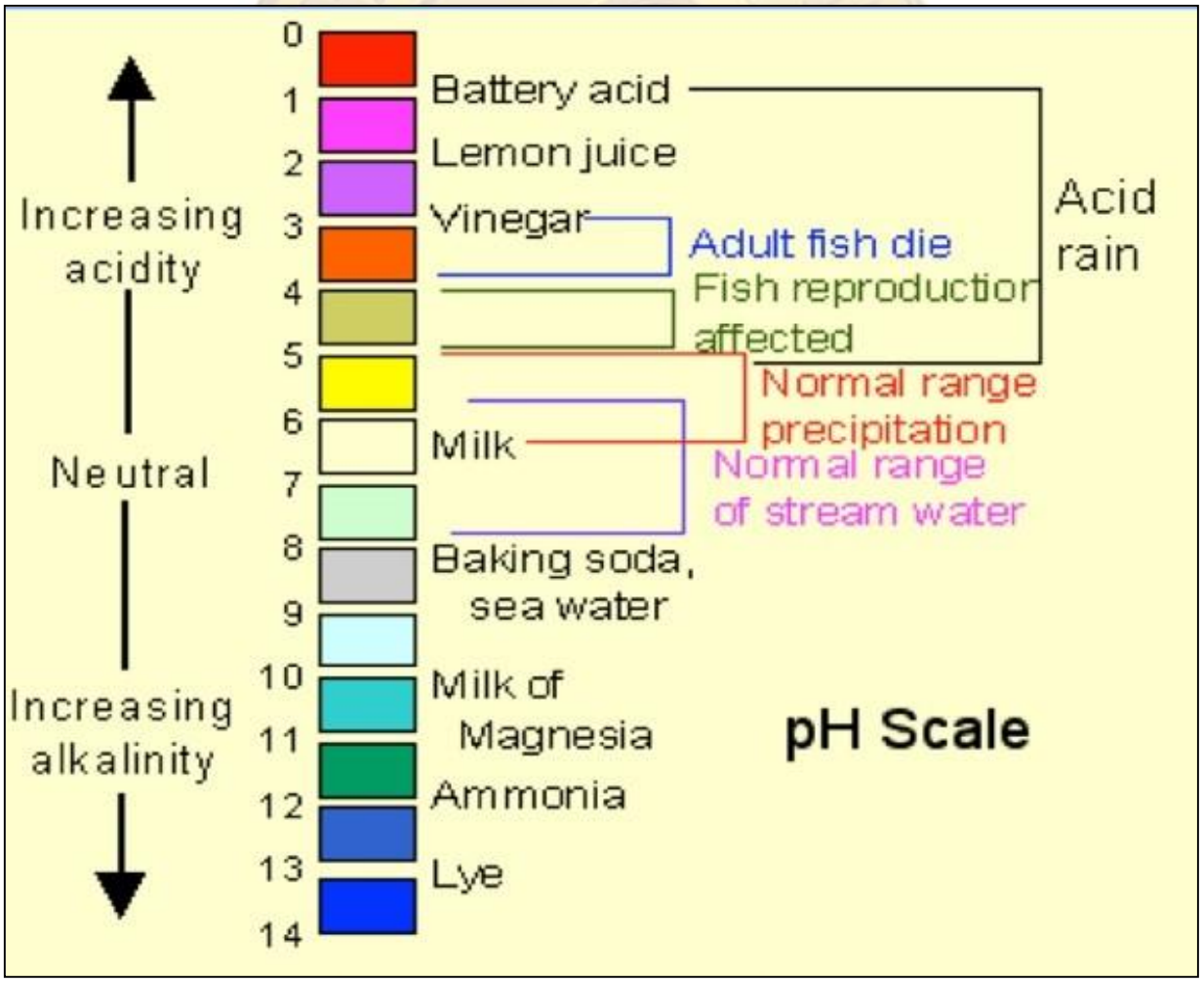
FSN – 195 NUTRITIONAL BIOCHEMISTRY AND ANTHROPOMETRY LAB

UNIT-I

1. Determination of pH: in acids, alkalis and buffers using pH meter and indicators.

Principle:

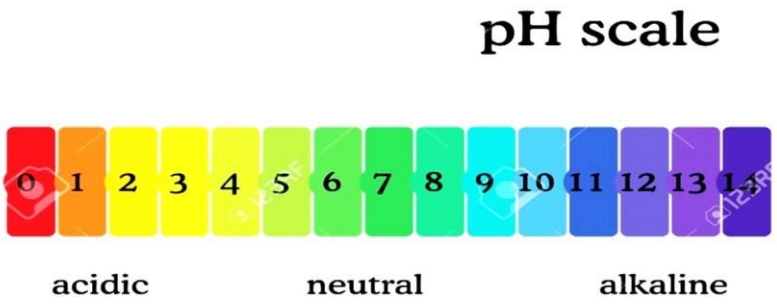
Acids and bases are substances capable of donating protons (H^+) and hydroxide ions (OH^-), respectively. They are two extremes that describe chemicals. Mixing acids and bases can cancel out or neutralize their extreme effects. A substance that is neither acidic nor basic is neutral. The values of proton concentration ($[H^+]$) for most solutions are inconveniently small and difficult to compare so that a more practical quantity, pH, has been introduced. pH was originally defined as the decimal logarithm of the reciprocal of the molar concentration of protons, but was updated to the decimal logarithm of the reciprocal of the hydrogen ion activity. The pH scale typically ranges from 0 to 14. Pure water is neutral with pH=7. A pH less than 7 is acidic, and a pH greater than 7 is basic.



Different methods available for measuring pH:

a. pH paper:

pH paper is a strip of special paper that is prepared by dipping the strip in different chemical solution and then drying it. It can be used to find the approximate pH of any solution. These paper changes colour when immersed in acidic or basic solutions. To determine the pH, the pH paper is dipped in a given sample solution and the colour developed in the paper is compared with the colour chart and the approximate pH of the solution can be identified. It is commercially available as test papers.



b. Universal indicator:

Universal indicator is a pH indicator composed of different chemical compounds. When the indicator is poured in the sample solution, the mixture obtained exhibits a smooth colour change over a pH value ranging from 1-14 that indicates the acidic or basic property of the solution. It is commercially available as test solutions.

c. pH meter:

pH meter is an electronic instrument consisting of a special bulb that is sensitive to hydrogen ions that are present in the test solution. The signal produced by the bulb is amplified and sent to an electronic meter connected to the bulb.

For very precise measurement the pH meter should be calibrated before each measurement. The calibration should be performed with at least two buffer solutions with known pH. For general purpose buffer solution with pH 4 and pH 10 are used.

After each single measurement the bulb is rinsed with distilled water or deionized water to remove any traces of solution being measured. Then the bulb is bottled by swab the tissue paper to remove remaining water that could dilute the sample and after the reading. When not in use, the bulb must be immersed in distilled water or pH 7 buffer all the times to avoid dehydration of the pH sensing membrane.

Fig: pH Meter

Requirement:

i. Beaker
ii. pH meter
iii. Tissue paper
iv. Distilled water
v. Sample solution (May be some fruit juices used to measure the pH)

Result:

Sample A- pH 12.05

Sample B- pH 1.98

Interpretation:

The pH of the sample A was found to be 12.05 which indicates that the sample is basic

The pH of the sample B was found to be 1.98 which indicates the sample is strongly acidic.

2. Colorimeters: Use of colorimeter in UV and visual range:

Principle:

A colorimeter is a light sensitive device used for measuring the transmittance and absorbance of light passing through a liquid sample. It measures the intensity or concentration of the colour that develops upon introducing a specific reagent into a solution. It is most commonly used to determine the concentration of a known solute in a given solution by the application of Beer's and Lambert's law.

Beers law:

According to Beer's law, when monochromatic light passes through the coloured solution, the amount of light transmitted decreases exponentially with increase in concentration of the coloured substance.

Lambert's law:

According to this law, the amount of light transmitted decreases exponentially with increase in the thickness of coloured solution.

Biological application of colorimeter:

- The colorimeter is commonly used for determination of the concentration of a coloured compound by measuring the optical density or its absrbance.
- It is used widely to monitor the growth of a bacterial or yeast culture.
- Colorimeter is used to measure and monitor the colour in various foods and beverages including vegetable products.
- It is used in estimation of biochemical compounds like glucose, urea, creatinine, uric acids, enzymes etc in blood or plasma sample.
- It is used to test the quality of water by screening for chemical such as chlorine, chloride Iron, zinc etc.

Components of colorimeter and their uses:

- In a colorimeter a beam light with a specific wave length is cast through a solution via a series of lenses which navigate the coloured light in the measuring device. This analyzes the colour compared to an existing standard.
- If the concentration of the solution is greater more light will be absorbed.
- The colorimeter determines the intensity and concentration of compound in a coloured solution. This is done by passing light of specific a wave length of visible spectrum through the solution.
- Bsed on the nature of coloured compound specific light filters are used.
- Three types of filters are available- blue, green and red with corresponding light wave length, transmission rays from 470-490nm, 500-530nm, 620-680 nm respectively.

- For most of the experiments, the common wave length range is between 400-7—nm but when some analytes absorb in the ultra violet range(<400nm) then modification of the colorimeter is required.

Fig: Colorimeter

3. Separation techniques: Thin layer Chromatography

Thin layer chromatographic analysis for qualitative assessment of antioxidant:

Thin layer chromatography (TLC) is a chromatographic technique used to separate non volatile mixtures. Thin layer chromatography id performed on a sheet of glass, plastic or aluminum oxide or cellulose. Thin layer of absorbent is known as stationary phase. After the sample has been applied on the plate a solvent or solvent mixture (known as mobile phase) is drawn up the plate via capillary action. The mobile phase has different properties from the stationary phase. For example, with silica gel, a very polar substance, non polar mobile phases such as methanol: chloroform: hexame (7:2:1 v/v/v) may be used.

Principle:

Thin layer chromatography is based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and the mobile phase.

The compounds under the influence of the mobile phase (driven by capillary action) travel over the surface of the stationary phase. During this movement the compounds with higher affinity travel slower while the other travel faster. Thus separation of compounds in the mixture is achieved.

TLC system components:

I. The plates:
The plates available readymade with a stationary phase. These are stable and chemically insert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particle size.

II. TLC chamber:
This is used for the development of Chromatography. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents and keeps the process dust free.

III. Mobile phase:
This comprises of solvent or solvent mixture. The mobile phase used should be particulate free and of the highest purity for proper development of TLC spots.

IV. A Filter paper:
This is moistened in the mobile phase to be placed inside the chamber. This helps develop a uniform rise in mobile phase over the length of the stationary phase.

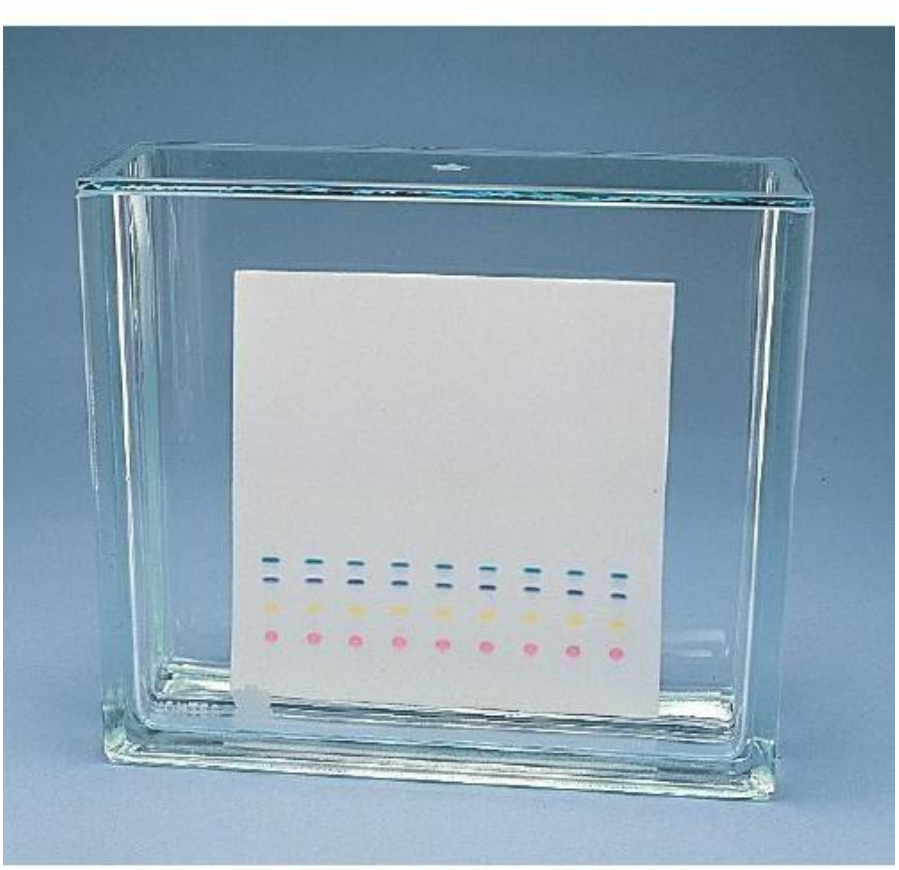


Fig: TLC Chamber with TLC plate

Requirements:

i. Methanol: Chloroform: Hexane (1:1:1 v/v/v)
ii. Standard ascorbic acid solution
iii. Lemon juice
iv. TLC jar and TLC plate
v. 0.05% DPPH in ethanol/methanol
vi. Spray bottle

The thin layer chromatography is performed on 20X 20 cm plate precoated with silica gel. The plates were run in the mobile phase developed by Methanol: Chloroform: Hexane (1:1:1 v/v/v).

Procedure:

I. 1 drop of standard ascorbic acid and 1 drop of lemon juice, 1 drop of spinach extract were spotted on the TLC plate.
II. The plate is placed in a TLC chamber after adding the solvent for mobile phase.
III. The chamber is covered by its lead and wait for the sample and the standard to be run.
IV. The plate is run upto just below the upper edge of the plate.
V. Then the plate was removed from the chamber and dried.
VI. The plate is sprayed with 0.05% DPPH reagent.
VII. Dried the plate and purple colour of DPPH will be bleached by the yellow spots.

Interpretation:

Yellow spot was the indication of positive antioxidant activity. Lemon juice, spinach extract and ascorbic acid have a similar spot on the TLC plate. It indicates the presence of strong antioxidant activity in lemon juice and spinach extract.

4. Enzyme Assays: GOT, GPT by semiautoanalyser by kit method:

a. Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT):

Principle:

In this test L aspartate and alpha keto glutarate react in the presence of GOT in the sample to yield oxaloacetate and L glutamate.

The Oxaloacetate is reduced by malate dehydrogenase (MDH) to yield L malate with the oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340nm.

The rate of reduction in absorbance is proportional to GOT activity in the sample.

Requirements:

i. Eppendorf
ii. 100 and 1000µl Micropipette and tips.
iii. SGOT Kit

Procedure:

Reagent/Sample	**Amount (**µl)
Working reagent	**500**
Sample	**50**

Mixed the sample well and incubate 37^0C for 60 seconds and measure the absorbance at interval of 30 seconds for 2 times at 340 nm.

Result:

Serum SGOT (IU/l) = 24.44

Standard value = 0-55 IU//lit.

Interpretation: SGOT is an important parameter to analyse the liver function. A high SGOT level indicates the toxiaemia of liver. Here the serum sample have a normal SGOT level.

b. Estimation of Serum Glutamate Pyruvate Transaminase (SGPT):

Principle:

In this test, L- alanine and alpha keto glutarate react in the presence of GPT in the sample to yield pyruvate and L glutamate. Pyruvate is reduced by lactate dehydrogenase to yield lactate with the oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340nm.

The rate of reduction in absorbance is proportional to SGPT actively in sample.

Procedure:

Reagent/Sample	**Amount (**µl)
Working reagent	**500**
Sample	**50**

Mixed the sample well and incubate 37^0C for 60 seconds and measure the absorbance at interval of 30 seconds for 2 times at 340 nm.

Result:

Serum SGPT (IU/l)= 19.2

Standard value = 5-55 IU//lit.

Interpretation: SGPT is an important parameter to analyse the liver function. A high SGPT level indicates the toxiaemia of liver. Here the serum sample have a normal SGPT level.

5. Estimation of Creatinine in blood by kit method:

a. Principle:

Creatinin react with picric acid in an alkaline medium to form an orange coloured complex. The rate of formation of this complex is measured by reading the change in absorbance at 505 nm in a selected interval of time and is proportional to the concentration of creatinin. The reaction time and the concentration of picric acid and sodium hydroxide have been optimized to avoid interference from ketoacidosis.

Creatinin + Picric acid $\xrightarrow{\text{Alkaline medium}}$ Orange colour complex

b. Requirement:

i. Semiautoanalyser/ Colorimeter
ii. Eppendorf
iii. Plasma or serum sample
iv. 1000ml and 100ml micropipette with tips
v. Distilled water
vi. Creatinine kit

c. Procedure:

Reagents/sample	Standard	Test
Working creatinine reagent	500µl	500µl
Standard reagent	50µl	-
Plasma /serum	-	50µl

d. Result:

Absorbance can be measured in colorimeter or by using semiautoanalyser. If the absorbance is measured in colorimeter the total volume of standard and sample should be at least 3 ml. A blank as distilled water or working creatinine reagent will be used in both the cases.

Here the result of colorimetric analysis is given below-

$$\text{Creatinine} \quad \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X concentration of standard}$$

$$= \frac{0.276}{0.356} \text{X } 2$$

$$= 1.55 \text{ mg/dl}$$

e. Interpretation:

Reverence value of creatinine level of adult female- 0.6- 1.1 mg/dl
Reverence value of creatinine level of adult male- 0.9- 1.3 mg/dl
The creatinine level of plasma sample is 1.55 mg/dl. So the sample have a high creatinine level.

6. Estimation of uric acid in blood by kit method:

a. Principle:

Uricase is a very specific enzymeacting on uric acid and the end products are allantoin and peroxide. Peroxidase is used to utilize hydrogen peroxide (proportional to uric acid concentration) to convert chromogen to coloured complex. The intensity of colour produced is proportional to uric acid concentration and is measured photometrically at 500- 550 nm.

Uric acid+O2+ H2O $\xrightarrow{\text{Uricas}}$ Allantoin +CO2+H2O2

H2O2 +4-Aminoantipyrine + TBHS $\xrightarrow{\text{Peroxidas}}$ Coloured complex

TBHS- 2,4,6 – Tribromo -3-hydroxybenzoic acid

b. Requirement:

i. Semiautoanalyser/ Colorimeter
ii. Eppendorf
iii. Plasma or serum sample
iv. 1000ml and 100ml micropipette with tips
v. Distilled water
vi. Uric acid kit

c. Procedure:

i.

Reagents/sample	Blank	Standard	Test
Working reagent	200µl	200µl	200µl
Standard reagent	-	10µl	-
Plasma /serum	-	-	10µl

ii. Mix the above contents well and incubate at 37^0c for 5 minutes

iii. Then add 300µl of distilled water to the blank, standard and test.

d. Result:

Absorbance can be measured in colorimeter or by using semiautoanalyser. If the absorbance is measured in colorimeter the total volume of standard and sample should be at least 3 ml.

Absorbance of standard- 0.143

Absorbance of test- 0.105

$$\text{Uric acid concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X concentration of standard}$$

$$= \frac{0.105}{0.143} \text{X } 6$$

$$= 4.40 \text{mg/dl}$$

e. Interpretation:

Reference value of uric acid in male is- 3.2- 7 mg/dl
Reference value of uric acid in adult female is- 2.6- 6mg/dl
Uric acid is the end product of purine metabolism and that are catalyzed by uricase enzyme to form allantoin that are excreted through urine. The sample have uric acid value of 4.40mg/dl of uric acid. A serum uric test is done to detect arthritis, gout and kidney disorder patients.

7. Estimation of Serum cholesterol by kit method

a. Principle:

Cholesterol ester are hydrolysed by cholesterolesterase (CE) to give free cholesterol and fatty acid. In subsequent reaction, cholesterol oxidase (CHOD) oxidises the 3-OH group of free cholesterol to liberate cholest 4 en 3 one and hydrogen peroxide. In presence of peroxidase(POD), hydrogen peroxidase couples with 4 amino antipyrine (4AAP) and phenol to produce red quinineimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of total cholesterol concentration in the sample.

$$\text{Cholesterol ester} \xrightarrow{CE} \text{Cholesterol + fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{Cholest 4 en 3 one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4- AAP} \xrightarrow{POD} \text{Quinoneimine dye} + H_2O$$

b. Requirements:

i. Semiautoanalyser/ Colorimeter
ii. Eppendorf
iii. Plasma or serum sample
iv. 1000ml and 100ml micropipette with tips
v. Distilled water
vi. Cholesterol kit

c. Procedure:

i.

Reagents/sample	Blank	Standard	Test
Reagent 1	500µl	500µl	500µl
Standard/ reagent 2	-	5µl	-
Plasma /serum	-	-	5µl

ii. Mix the above contents well and incubate at 37^0c for 10 minutes
iii. Blank the analyser with reagent blank.
iv. Measure absorbance of standard followed by the test.
v. Calculate results as per given calculated formula.

d. Result:

Absorbance of standard- 0.318
Absorbance of test- 0.142
Standard concentration- 200mg/dl

Cholesterol concentration (mg/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$ X concentration of standard

$= \frac{0.142}{0.318} X\ 200$

= 89.308mg/dl

e. Interpretation:

Desirable standard cholesterol level < 200mg/dl
Borderline high risk- 200-239 mg/dl
High risk- >239mg/dl

Serum cholesterol is an important diagnostic criteria that are used to detect the cardiovascular disease risk. The supplied serum sample have 89.30 mg/dl serum cholesterol level which is under the normal range.

8. Estimation of serum triglyceride by kit method:

a. Principle:

Triglyceride are hydrolysed by lipoprotein lipase (LPL) to produce glycerol and free fatty acids (FFA). In the presence of glycerol kinase (GK) and Adenosine tri phosphate (ATP), glycerol converts to glycerol 3 phosphate and adenosine di phosphate (ADP). Glycerol 3 phosphate is further oxidized by glycerol 3 phosphate oxidase (GPO) to produce Dihydroxy acetone phosphate (DHAP) and H2O2. In presence of peroxidase (POD), hydrogen produces red quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to triglyceride concentration in the sample.

Triglyceride $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol + Free fatty acids

Glycerol +ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3-phosphate+ ADP

Glycerol-3-phosphate +O2 $\xrightarrow{\text{Glycerol-3-phosphate oxidase (GPO)}}$ DHAP +H2O2

2 H2O2 + 4-AAP+4 Chlorophenol $\xrightarrow{\text{Peroxidase (POD)}}$ Quinoneimine dye +4H2O

b. Requirements:

i. Semiautoanalyser/ Colorimeter
ii. Eppendorf
iii. Plasma or serum sample
iv. 1000ml and 100ml micropipette with tips
v. Distilled water
vi. Triglyceride kit

c. Procedure:

i.

Reagents/sample	Blank	Standard	Test
Reagent 1	1000µl	1000µl	1000µl
Standard/ reagent 2	-	10µl	-
Plasma /serum	-	-	10µl

ii. Mix the above contents well and incubate at 37^0c for 10 minutes

iii. Blank the analyser with reagent blank.

iv. Measure absorbance of standard followed by the test.

v. Calculate results as per given calculated formula

d. Result:

Absorbance of standard- 0.227
Absorbance of test- 0.104
Standard concentration- 200mg/dl

Cholesterol concentration (mg/dl) = $\frac{\text{Absorbance of}}{\text{Absorbance of standard}}$ X concentration of standard

$$= \frac{0.104}{0.227} X\ 200$$

$$= 91.62 \text{ mg/dl}$$

e. Interpretation:

Desirable standard Triglyceridel level < 150mg/dl
Borderline- 150-199 mg/dl
High risk- 200-499 mg/dl
Very high- >500 mg/dl

Serum triglyceride is an important diagnostic criteria that are used to detect the cardiovascular disease risk. The supplied serum sample have 91.62mg/dl serum cholesterol level which is under the normal range.

9. Estimation of Blood glucose by Glucose oxidase peroxidase (GOD POD) method

a. Principle:

Colorimetric or spectrophotometric estimation of serum glucose using glucose oxidase peroxidase (GODPOD) is a standard method. Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconate. The formed hydrogen peroxide (H2O2) is detected by a chromogenic oxygen acceptor, phenol, 4- Aminophenazone (4-AP) in the presence of peroxidase (POD). Peroxidase enzyme acts on hydrogen peroxide to liberate nascent oxygen (O). Nascent oxygen couples with 4 amino antipyrine and phenol to form red quinone dye. The intensity of colour is directly proportional to concentratin of glucose in plasma. The intensity of colour is measured colorimetrically at 530nm.

β-D-Glucose + O2 + H2O ---(GK) (Glucose oxidase) ----------Gluconic acid +H2O2

H2O2+ Phenol+ 4-AP ---------- (Peroxidase) ---------- Red Quinone dye + H2O

b. Requirements:

i. GOD POD kit
ii. Test tube
iii. 1000µl and 20µl micropipette with tips
iv. Colorimeter or spectrophotometer
v. Distilled water

c. Procedure:

i. Mixed R1 vowel with 100 ml of R2 and stored at 2-80C in a dark coloured bottle provided with the kit. It will prevent the reagent from light source.

Reagents/sample	Blank	Standard	Test
Reagent 1	1500µl	1500µl	1500µl
Standard/ reagent 2	-	20µl	-
Plasma /serum	-	-	20µl

ii. Mix the above contents well and incubate at 37^0c for 10 minutes.

iii. 1500µl of distilled water was added to all the test tubes.

iv. Set the colorimeter with reagent blank at 490-500nm.

v. Measure absorbance of standard followed by the test.

d. Result:

Absorbance of standard- 0.13

Absorbance of test- 0.92

Serum glucose concentration (mg/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X } 100$

$= \frac{0.92}{0.13} \text{X } 100$

= 707.69 mg/dl

e. Interpretation:

Normal fasting plasma glucose level= 65-110 mg/dl
Post prandial= <120mg/dl
Hyperglycemia is an abnormal condition with excess blood glucose level often associated with diabetes mellitus. The sample have a blood glucose level of 707 mg/dl which is very considered to the normal level. So the patient should follow a strict diet along with follow medical guidelines to reduce the complications of diabetes mellitus.

10. Estimation of Serum proteins by Biuret method

a. Principle:

Protein contain COO and NH2+ groups joined together by a covalent bond directly to form peptide bond (O=C-N-H). Two or more peptide bond give purple colour when mixed with blue colour alkaline copper solution. Biuret reagent is composed of Sodium potassium turtarate, copper sulphate and NaOH/KOH. When this reagent is mixed with protein solution biuret substance bind with cupric ion and give the purple colour. The number of peptide bond present in protein is dit=rectly proportional to the intensity of the purple colour. Colorimeter or spectrophotometer is used to measure the intensity of the colour in the form of optical density (OD). Thereby OD of unknown serum sample was compared with OD of standard.

b. Requirement:

i. Working biuret reagent
ii. BSA standard (Concentration- 2mg/dl)
iii. Pipette- 5 ml
iv. 1ml micropipette with tips
v. Test tubes
vi. Serum sample

c. Procedure:

i. Test tubes was marked with S as standard, T as unknown test and B as blank.

Reagents/sample	Blank	Standard	Test
Working biuret reagent	3ml	3ml	3ml
Standard/ reagent 2	-	3ml	-
Plasma /serum	-	-	0.1 ml
Distilled water	3 ml	-	2.9 ml

ii. Mix the above contents well and incubate at 37^0c for 10 minutes.

iii. 1500µl of distilled water was added to all the test tubes.

iv. Set the colorimeter with reagent blank at 440-540nm.

v. Measure absorbance of standard followed by the test.

d. Result:

Absorbance of standard- 0.13

Absorbance of test- 0.11

Serum glucose concentration (mg/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 2$

$= \frac{.\text{ଭ}\text{ଭ}}{.\text{ଭ}\text{ଷ}}$

= 1.69 mg/dl

e. Interpretation:

The important function of serum is to transport minerals, vitamins and other nutrients from intestine to tissue or organs. Low serum protein causes muscle wasting called marasmus in children. Normal level of serum protein is 6-8 g/dl. So the samples have very low serum protein level of about 1.69 g/dl.

11. Extraction method of foods by various solvents

Extraction is a separation technique employed in several food industries. The extraction can be from solid to liquid, liquid to liquid. It is essentially the process of moving one or more compounds of interest from one phase or from the original location to another phase or the physically separated location where further processing and analysis is done. The use of a convenient type of extraction not only influences the accuracy of result but also determines total analysis time and in this way affects the sample and analysis.

Solvent extraction method for lipid from foods:

The total lipid content of a food is commonly determined by its organic solvent extraction. The choice of solvents is highly critical in fat analysis. The most commonly used solvents are ethyl ether and petroleum ether, but pentane and hexane are used for extracting oil from sea fish. Lipid may be conjugated with proteins and polysaccharide, thus a successful extraction requires that the lipid can be made free by alkaline or acid hydrolysis and then extracted into the organic solvent. Solvent from a boiling continuously flows over the sample. Fat content is measured by the weight of fat loss of the sample. Soxhlet method is a semi continuous solvent extraction approach. In this procedure the sample is soaked completely for 5-10 minutes in a solvent and then transferred to a chiffon based boiling flask. A commercial soxhlet apparatus is used for the extraction of fat from sea fish.

Requirements:

i. Food powder
ii. Soxhlet apparatus
iii. Solvents (Hexane and isopropanol 6:4 v/v)
iv. Filter paper
v. Petridish
vi. Measuring cylinder

Procedure for sample preparation:

- Seafish like Bhola is collected from Digha mohona.
- First dry the sample before extraction. Drying is necessary because many organic solvent can not penetrate into the foods containing water and therefore extraction would be inefficient.
- Dried samples were usually finely grinded in a mixer grinder to produce a homogenous mixture and to increase the surface area of lipid in foods to be exposed with the solvent during extraction
- The ideal solvent for lipid extraction would completely extract all the lipid components from food, while leaving all the other components behind. The efficiency of solvent extraction depends on the polarity of the lipid present compared to the polarity of the solvent. Polar lipids like glycolipids, phospholipids are more soluble in polar solvents such as alcohol, then in non polar solvent such as hexane. On the other hand non polar lipids such as triglycerols are more soluble in non polar solvents than in polar ones. Here we used Hexane and isopropanol (6:4 v/v) for fat extraction from bhola fish.

Procedure for extraction of fat from sea fish:

- 10 g of fish powder is taken in a 250ml conical flask.
- 60 ml of hexane and 40 ml of isopropanol is poured into the conical flask and mixed well.
- Transfer the whole mixture to the shaker for overnight.
- After shaking whole mixture is transferred to the soxhlet for heating for 6-6 hours.
- After heating the mixture is cool down.
- Centrifuge the mixture for 20 minute at 5000 RPM.
- Oil part from the sample is comes at the upper part of the test tubes.
- Oil is collected and kept in an opened Petridis in an appropriate position for evaporation. It takes about 30-40 minute to evaporate the solvents.
- After evaporation of solvents the oil is collected**.**

Fig: Soxhlet apparatus

FSN – 195 NUTRITIONAL BIOCHEMISTRY AND ANTHROPOMETRY LAB

UNIT-II

1. Introduction of Anthropometry

The word anthropometry comes from the Greek word "anthropos" means human and "Methron" means measures refers to the measurement of the human individual of an early tool of physical anthropometry, it has been used for identification, for the purpose of understanding human physical variation.

Anthropometric measurement also have used in epidemiology and medical anthropology foe example in helping to determine the relationship between various body measurement (height, weight, percentage of body fat etc).

Importance of Anthropometric measurement:

The anthropometric measurement is used in nutritional assessment. Those that are used to assess growth and development in infant, children and adolescent include length and head circumference, height, weight, weight for length, MUAC.

Individual measurements are usually compared to reference, standard on a growth chart.

Anthropometric measurements used for adult usually include height, weight, BMI, waist hip ratio and percentage of body fat. This measure is compared to reference standard to assess weight status and the risk of various diseases.

Anthropometric measurement requires precise measuring techniques to be valid.

Length is used for infant those who are unable to stand.

Nutritional growth and metabolic disorder assessment by anthropometric measurement:

Anthropometric data of an individual, group or community, is a major determinant of health like malnutrition, over nutrition, over weight and underweight. Obese and also control obesity which provide useful information to study the relationship among diet nutritional status health. The assessment of nutritional status requires following parameters.

Weight for age: It reflects growth of children. Their weight changes significantly with age, unlike in adults. This index uses as an indicator underweight for child.

Height for age: When a child has under nourished for a long time, the bone growth is affected**.** Low height for the age indicate stunted child.

Weight for height: When a child has been malnourished for a long time, will be short and underweight. A child whose weight for height is low indicates recent weight loss and said wasted child.

Mid Upper Arm Circumference (MUAC): It is used for rapid screening of malnutrition from the 6-59 months.

Body Mass Index (BMI): It is measured by body weight (kg) and body height(m^2) indicate the weight status as underweight, normal weight, overweight, obesity of adult person.

Waist-Hip Ratio: High waist-hip ratio indicate excess abdominal fat or central obesity risk for metabolic disorder.

Anthropometric indicators, site of measurement and Inferences:

Indicator	Site of measurement	Inference
Birth weight	The weight at which the baby is born	It is actually an indicator of maternal nutrition and health status but has implications for the baby's health.
Weight	Measured as weight in kg (to the nearest 100g)	Mainly affected by acute infection and acute food storage. If after the infection the child is an adequate diet weight demonstrates a period of rapid growth (catch-up-growth).
Head Circumference	Measured around the head	Useful in the first 2 years mainly as a measure of brain development
Mid Upper Arm Circumference (MUAC)	It is the **circumference** of the left **upper arm**, **measured** at the **mid**-point between the tip of the shoulder and the tip of the elbow (olecranon process and the acromium).	MUAC is a measure of adequacy of nutrition. A useful measure for screening acute malnutrition in the community. Also used for patient whose weight/height can't be taken e.g. those who are bed ridden.
Weight for age	Measure of weight compared to the weight of children of the same age and sex from a reference population.	It is indicator of both acute and chronic malnutrition used to identify underweight child.
Height for age	Measure of height compared to the height of children of the same age and sex from a reference population.	It is indicator of chronic malnutrition and is used to identify stunted children.
Weight for height	Weight is below -2SD of expected height of children of same age from a reference population.	It is indicator of malnutrition and is used to identify wasted children.

Stunting	Whose height for age is below minus -2SD (moderate and severe *stunting*) and -3SD (severe *stunting*) from the median of the WHO Child Growth Standards.	It is an indicator of chronic malnutrition
Wasting	Weight is below -2SD of expected Weight of children of same age from a reference population.	It is an indicator of chronic malnutrition
Body Mass Index (BMI)	Weight (kg) divided by height (meter)^2 means $\frac{9AEC\hbar P(GC)}{*AEC\hbar P(I\ 2)}$	An indicator of nutritional status
Body Surface Area	$\frac{\text{Height(cm)} \times \text{Weight(kg)}\ 300}{}$	Used mainly for drug prescription for children

2. Measurement of body fat percentage by skinfold thickness:

Principle:

Skinfold thickness describes the amount of subcutaneous fat when the fold is lifted and its thickness measured by specialized calipers. Skinfold thickness values can also be introduced into predictive mathematical formulae to derive body fat indices like percentage body fat and hence derive fat mass and fat-free mass. Measurements from various sites can also be used in a prediction equation to estimate body density before conversion to percent body fat.

Sites of measurement:

Biceps: Measure midline of the anterior aspect of the arm over the biceps muscle, midpoint on the arm as above.

Triceps: Mark is made in the mid upper arm, mid line of the posterior aspect of the arm over the triceps muscle. Measures with the elbow bend at 90^0 angle for identifying the biceps and triceps. During the measurement the arm should be hanging freely.

Sub scapular: Found just below and lateral to the bottom tip of the scapula, measured in 45^0 angle. Subjects stand with their arm raised by them. The scapula was hold by the finger tips to find the bottom of the bone. Skinfold thickness is measured in the natural process. Subject should be in relaxed condition**.**

Supra iliac: Found 1 cm above the anterior superior iliac spine (top of the hip bone) in the mid auxillary line (waist line), measured horizontally with the subject breathing normally.

Requirements:

- Human subject
- Skinfold caliper

Procedure:

i. Skin fold caliper was used for measuring the skin fold thickness of the four different position of the body I e. biceps, triceps, subscapular and supra iliac.
ii. Measurement was taken from a healthy, undamaged and uninfected dry skin. Moist skin is harder to assess and can influence the measurement.
iii. First mark the skin fold site using a pen.
iv. The skinfold should be firmly grasped by the thumb and index finger using the pads at tip of the thumb and finger. Gently pull the skinfold away from the body.
v. The caliper should be placed perpendicularly to the fold on the site which is marked at approximately 1 cm below the finger and thumb while maintaining the grasp of the skinfold, allow the caliper to be released so that full tension is placed on the skinfold.

vi. Record the measurement in the skin fold caliper.

vii. There are 4 site for the measurement of skin fold for male and female which are biceps, triceps, subscapular and supra iliac.

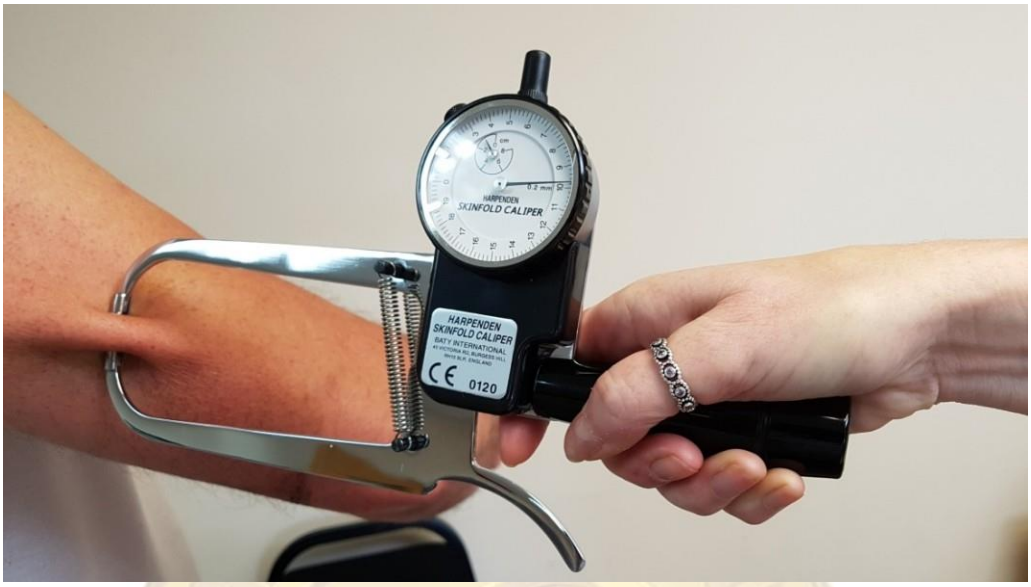


Fig: Measurement of skin fold thickness by skin fold caliper

Result:

Age- 17 years

Sex: Female

Total skin fold thickness for four sites of measurement (biceps + triceps + sub scapular + supra iliac) = 8.6+19.2+18.4+12.7 =58.9 cm

THE SUM OF 4 SKINFOLDS (BICEPS, TRICEPS, SUBSCAPULAR, SUPRAILLIAC), BY DURNIN & WOMERSLEY TABLE FOR PERCENTAGE OF BODY FAT:

Skinfold	MEN (age in years)				WOMEN (age in years)			
	17-29	30-39	40-49	50+	17-29	30-39	40-49	50+
15	4.8				10.5			
20	8.1	12.2	12.2	12.6	14.1	17.0	19.8	21.4
25	10.5	14.2	15.0	15.6	16.8	19.4	22.2	24.0
30	12.9	16.2	17.7	18.6	19.5	21.8	24.5	26.6
35	14.7	17.7	19.6	20.8	21.5	23.7	26.4	28.5
40	16.4	19.2	21.4	22.9	23.4	25.5	28.2	30.3
45	17.7	20.4	23.0	24.7	25.0	26.9	29.6	31.9
50	19.0	21.5	24.6	26.5	26.5	28.2	31.0	33.4
55	20.1	22.5	25.9	27.9	27.8	29.4	32.1	34.6
60	21.2	23.5	27.1	29.2	29.1	30.6	33.2	35.7

65	22.2	24.3	28.2	30.4	30.2	31.6	34.1	36.7
70	23.1	25.1	29.3	31.6	31.2	32.5	35.0	37.7
75	24.0	25.9	30.3	32.7	32.2	33.4	35.9	38.7
80	24.8	26.6	31.2	33.8	33.1	34.3	36.7	39.6
85	25.5	27.2	32.1	34.8	34.0	35.1	37.5	40.4
90	26.2	27.8	33.0	35.8	34.8	35.8	38.3	41.2
95	26.9	28.4	33.7	36.6	35.6	36.5	39.0	41.9
100	27.6	29.0	34.4	37.4	36.4	37.2	39.7	42.6
105	28.2	29.6	35.1	38.2	37.1	37.9	40.4	43.3
110	28.8	30.1	35.8	39.0	37.8	38.6	41.0	43.9
115	29.4	30.6	36.4	39.7	38.4	39.1	41.5	44.5
120	30.0	31.1	37.0	40.4	39.0	39.6	42.0	45.1
125	30.5	31.5	37.6	41.1	39.6	40.1	42.5	45.7
130	31.0	31.9	38.2	41.8	40.2	40.6	43.0	46.2
135	31.5	32.3	38.7	42.4	40.8	41.1	43.5	46.7
140	32.0	32.7	39.2	43.0	41.3	41.6	44.0	47.2
145	32.5	33.1	39.7	43.6	41.8	42.1	44.5	47.7
150	32.9	33.5	40.2	44.1	42.3	42.6	45.0	48.2
155	33.3	33.9	40.7	44.6	42.8	43.1	45.4	48.7
160	33.7	34.3	41.2	45.1	43.3	43.6	45.8	49.2
165	34.1	34.6	41.6	45.6	43.7	44.0	46.2	49.6
170	34.5	34.8	42.0	46.1	44.1	44.4	46.6	50.0
175	34.9	-	-	-	-	44.8	47.0	50.4
180	35.3	-	-	-	-	45.2	47.4	50.8
185	35.6	-	-	-	-	45.6	47.8	51.2
190	35.9	-	-	-	-	45.9	48.2	51.6
195	-	-	-	-	-	46.2	48.5	52.0
200	-	-	-	-	-	46.5	48.8	52.4
205	-	-	-	-	-	-	49.1	52.7
210	-	-	-	-	-	-	49.4	53.0

Body fat percentage:

Body Fat	**Male%**	**Female%**
Essential body fat	2-5%	10-15%
Storage	10-15%	10-15%
Total	12-20%	20-30%
Border line	21-25%	31-35%
Obesity	>25%	>33%

Interpretation:

The subjects total skin fold thickness is 58.9cm. As the subject is female and within the age group of 17-29 years old the body fat percentage will be 27.8, which is below the borderline of obesity. So the person have a normal body fat percentage.

3. Nutrition status of Pre-school children using anthropometric parameters.

Weight Measurement: Weight of individual was taken by portable body weight machine. The machine was placed on the plane surface and subject was asked to stand to erect the head and reading was taken in kg (accuracy -0.5 kg). Subject should wear simple clothes.

Height Measurement: At first subject is asked to stand to erect and his/her hand oriented in the eye-ear plane. The anthropometric rod is placed in front of subject and sliding caliper is placed on the vertex of head and read out the measurement in cm (accuracy-0.1cm).

Mid-upper Arm Circumference (MUAC): Subject is standing erect hanging freely with forearm extended loosely. The circumference is taken in plane and right angle to the long axis of the humorous through a point midway between the axilla and skin flexion line at the cuboidal fossa by placing the tape.

Alternatively MUAC is measured at the mid-point between the tip of shoulder (acromion) and the tip of the elbow by placing the tape (accuracy-0.1cm).

Head Circumference: Wrap the tape around the widest possible circumference from the most prominent part of the forehead often (one to two fingers above the eyebrow). Around to the widest part of back of head.

Measured three times and take the longest number.

46cm is the standard head circumference of preschool children according to ICMR 2010.

Chest Circumference: The tape is placed interscapular level posteriorly and anteriorly over the edge of the chest. Measure at the fullest part of your bust, wrap it around (under your armpits, around your shoulder blades, and back to the front) to get the measurement.

32cm is the standard chest circumference of preschool children according to ICMR, 2010.

Result:

Age- 3 years

Sex- Female

Sl No.	Parameters	No. of Observation	Observation Result	Average
1	Body Weight	1	18.0	18.16 kg
		2	18.5	
		3	18.0	
2	Body height	1	90.0	90.16 cm
		2	90.5	
		3	90.0	
3	Mid Upper Arm Circumference	1	15.0	15.1 cm
		2	15.5	
		3	15.0	
4	Head Circumference	1	49.0	49.33 cm
		2	49.5	
		3	49.5	
5	Chest Circumference	1	48.0	48.16 cm
		2	48.0	
		3	48.5	

Interpretation:

Interpretation of Body Weight: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, they observed that the normal weight of preschool children (3yrs) 12.9 kg.

So, my subjects body weight is 18.16 kg so, my subject's body weight is overweight.

Interpretation of Body Height: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, they observed that the normal height of preschool children (3yrs) 99.1 cm.

So, my subjects body height is 90.16 cm so, according to ICMR 2010, my subject's body height is low.

Interpretation of MUAC: These are the cut off value of MUAC-

Age Group (yr)	Cut off value of MUAC (cm)	Category
0-5	>13.5 12.5-13.5 <12.5	Normal Moderate malnourished (PEM) Severe Malnourished
Adult (Female)	27-30 <27	Normal Under nutrition
Adult (Male)	30-33 <30	Normal Under nutrition

My subject MUAC is 15.1 cm. So, according to this chart my subject MUAC is normal.

Interpretation of Head Circumference: The head circumference standard level is 46 cm for preschool children according to ICMR 2010. My subject's head circumference is 49.33 cm which is higher than normal.

Interpretation of Chest Circumference: The chest circumference standard level is 32 cm for preschool children according to ICMR 2010. My subject's chest circumference is 48.16 cm which is higher than normal.

4. Nutrition status of school going children using anthropometric parameters:

Weight Measurement: Weight of individual was taken by portable body weight machine. The machine was placed on the plane surface and subject was asked to stand to erect the head and reading was taken in kg (accuracy -0.5 kg). Subject should wear simple clothes.

Height Measurement: At first subject is asked to stand to erect and his/her hand oriented in the eye-ear plane. The anthropometric rod is placed in front of subject and sliding caliper is placed on the vertex of head and read out the measurement in cm (accuracy-0.1cm).

Mid-upper Arm Circumference (MUAC): Subject is standing erect hanging freely with forearm extended loosely. The circumference is taken in plane and right angle to the long axis of the humorous through a point midway between the axilla and skin flexion line at the cubital fossa by placing the tape.

Alternatively MUAC is measured at the mid-point between the tip of shoulder (acromion) and the tip of the elbow by placing the tape (accuracy-0.1cm).

Result:

Age- 6 years

Sex- Male

Sl No.	Parameters	No. of Observation	Observation Result	Average
1	Body Weight	1	17.0	17.16 kg
		2	17.5	
		3	17.0	
2	Body height	1	110.0	110.33 cm
		2	110.5	
		3	110.0	
3	Mid Upper Arm Circumference	1	14.0	14.16 cm
		2	14.5	
		3	14.0	

Interpretation:

Interpretation of Body Weight: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, they observed that the normal weight of school going children is (6yrs) 25.97 kg.

So, my subjects body weight is 17.16 kg so, my subject's body weight is low.

Interpretation of Body Height: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, the normal height of school going children is (8yrs) 127.22 cm.

So, my subjects body height is 110.33 cm so, according to ICMR 2010, my subject's body height is low.

Interpretation of MUAC: These are the cut off value of MUAC-

Age Group (yr)	Cut off value of MUAC (cm)	Category
0-5	>13.5 12.5-13.5 <12.5	Normal Moderate malnourished (PEM) Severe Malnourished
Adult (Female)	27-30 <27	Normal Under nutrition
Adult (Male)	30-33 <30	Normal Under nutrition

My subject MUAC is 14.16 cm. So, according to this chart my subject MUAC is normal.

5. Nutritional status of adolescence using anthropometric parameters:

a. Weight Measurement: Weight of individual was taken by portable body weight machine. The machine was placed on the plane surface and subject was asked to stand to erect the head and reading was taken in kg (accuracy -0.5 kg). Subject should wear simple clothes.

b. Height Measurement: At first subject is asked to stand to erect and his/her hand oriented in the eye-ear plane. The anthropometric rod is placed in front of subject and sliding caliper is placed on the vertex of head and read out the measurement in cm (accuracy-0.1cm).

c. Mid-upper Arm Circumference (MUAC): Subject is standing erect hanging freely with forearm extended loosely. The circumference is taken in plane and right angle to the long axis of the humorous through a point midway between the axilla and skin flexion line at the cubital fossa by placing the tape.

Alternatively MUAC is measured at the mid-point between the tip of shoulder (acromion) and the tip of the elbow by placing the tape (accuracy-0.1cm).

d. Waist Circumference Measurement: At first take the waist circumference measurement of the parameter indicators the status of the abdominal fat deposition. It is helpful for assessment of different type of metabolic disorder. The measurement is taken by the tape horizontally and finally over the midpoint between the terminal end iliac crest particularly the last rib above the iliac crest.

e. Hip Circumference Measurement: It is also good index of body fat distribution. The study associated with waist circumference. The measurement is taken by placing the tape over the area maximum bulge of hip. The end of the tape should meet at the lateral surface of the hip.

f. Waist-Hip Ratio: The waist hip ratio obtained by dividing the waist circumference and hip circumference. So, waist-hip ratio of the subject- $\frac{\text{L gqr Ggpaskdcpclac}}{\text{Lgn Ggpaskdcpclac}}$

g. Measurement of skin fold thickness for body fat percentage:

Measurement of skin fold thickness was taken from the biceps, triceps, subscapular and supra iliac by using a skin fold caliper.

Result:
Age- 17 years
Sex- Female

Sl No.	Parameters	No. of Observation	Observation Result	Average
1	Body Weight	1	56.0	56.16 kg
		2	56.5	
		3	56.0	
2	Body height	1	146.5	146.16 cm
		2	146.0	
		3	146.5	
3	Mid Upper Arm Circumference	1	28.0	28.23 cm
		2	28.0	
		3	28.5	
4	Waist Circumference	1	72.5	72.33 cm
		2	72.5	
		3	72.0	
5	Hip Circumference	1	98.0	98.16 cm
		2	98.0	
		3	98.5	
6.	Skin fold thickness (Biceps +Triceps + Subscapullar + Supra iliac)	1	8.6+19.2+18.4+12.7) =58.9	58.9
		2	8.6+19.2+18.4+12.7) =58.9	

Interpretation:

Interpretation of Body Weight: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, the normal body weight of adolescence is 55.0 kg.

So, my subjects body weight is 56.16 kg so, her body weight is over than normal.

Interpretation of Body Height: A report of the expert group of the Indian council of Medical Research 2010, nutrient requirement and recommended dietary allowances for Indians, the normal height of adolescence is 150 cm.

So, my subjects body height is 146.16 cm which is low.

Interpretation of MUAC: These are the cut off value of MUAC-

Age Group (yr)	Cut off value of MUAC (cm)	Category
0-5	>13.5 12.5-13.5 <12.5	Normal Moderate malnourished (PEM) Severe Malnourished
5-9 years	<13.5 ≥13.5-14.5 ≥ 14.5	Severe acute malnutrition Moderate acute malnutrition Normal
10-14 years	<16 ≥16.0-18.5 ≥18.5	Severe acute malnutrition Moderate acute malnutrition Normal
Adult (Female)	27-30 <27	Normal Under nutrition
Adult (Male)	30-33 <30	Normal Under nutrition

My subject MUAC is 28.23 cm. So, according to this chart my subject MUAC is normal.

Interpretation of Waist circumference:

Sex	Cut off value
Male	85-90 cm- Normal >90cm- Obese
Female	<80 cm – Normal >80 cm- Obesity

My subjects Waist circumference is 70 cm which is lower the normal value.

Interpretation of Waist-hip Ratio:

This is the cut off value of waist-Hip Ratio

Sex	Cut off value
Male	< 0.89- Normal >0.90- Abdominal Obesity
Female	0.81-0.85- Normal >0.85- Abdominal obesity

My subject's waist hip ratio- $\frac{\text{WaistCircumference}}{\text{Hip Circumference}}$

$$= \frac{72.33}{98.16} \text{ cm}$$

$$= 0.73\text{cm}$$

So, my subject waist hip ratio is 0.73 cm, which is low.

Interpretation of BMI:

BMI or body mass index provides a reasonable indication of nutritional status of adults. The BMI has good correlation with adiposity. The BMI classification according to WHO-

BMI (Kg/Mt^2)	**Presumptive diagnosis**
<16.0	Chronic energy deficiency (CED) Grade III underweight
16.0-16.99	CED Grade II underweight
17.0-18.49	CED grade I underweight
18.5-24.99	Normal
25.0-29.99	Over weight
30-34.99	Obesity Grade 1
35-39.99	Obesity Grade II
>40	Obesity grade III (Morbid obesity)

My subjects BMI= Weight in Kg/ Height in Mt^2 = 56.16/1.46 = 26.36 kg/mt^2

The subjects BMI is under overweight category according to the classification of BMI by WHO.

Interpretation of Skin fold thickness:

The subjects total skin fold thickness is 58.9cm. As the subject is female and within the age group of 17-29 years old the body fat percentage will be 27.8, which is below the borderline of obesity. So the person has a normal body fat percentage.

6. Assessment of Nutrition status of geriatric person by anthropometric measurement:

Measurement of Body weight, height, Waist circumference, Waist hip ratio and skin fold thickness was taken to assess the nutritional status.

Result:

Age-70 years

Sex- Male

Body weight- 48.16 (Average for three times)

Body height- 158.16 cm

BMI-30.45kg/mt^2

Waist circumference- 74.33

Hip circumference- 52.16

Waist hip ratio-1.42

Skin fold thickness (total of biceps, Triceps, subscapular and supra iliac) = (6.2+16.1+15.1+11.1)= 48.5cm

Interpretation:

The adult male have BMI of 30.45kg/mt^2 which is grade I obesity. Waist circumference is 74.33 cm which is normal, but Waist hip ratio is 1.42 which is higher than the normal value that indicated obesity, and the person's body fat percentage is 24.7 which is also at the borderline of obesity.

7. Determination of Blood pressure:

Measurement of blood pressure by sphygmomanometer using mercurial BP monitor or dial type BP apparatus on human subject at different posture like sitting, standing and supine.

Principle:

During flow of blood through the blood vessels a pressure exerted on the wall of the vessels perpendicularly called blood pressure.

The cuff of the sphygmomanometer is wrapped around the arm of the subject. The bag is then inflated until the air pressure in the cuff overcomes the arterial pressure and obliterates the arterial lumen. This is confirmed by palpating the radial pulse that disappears when the cuff pressure is raised above the arterial pressure.

When the pressure in the cuff reaches just below the arterial pressure, blood escapes beyond the occlusion into the peripheral part of the artery and the pulse starts disappearing. This is detected by the appearance of sounds in the stethoscope and is taken as the systolic pressure. Subsequently, the quality of the sound changes and finally disappears. The point where the sound disappears is taken as the diastolic pressure. The sound disappears because the flow in the blood vessels becomes laminar.

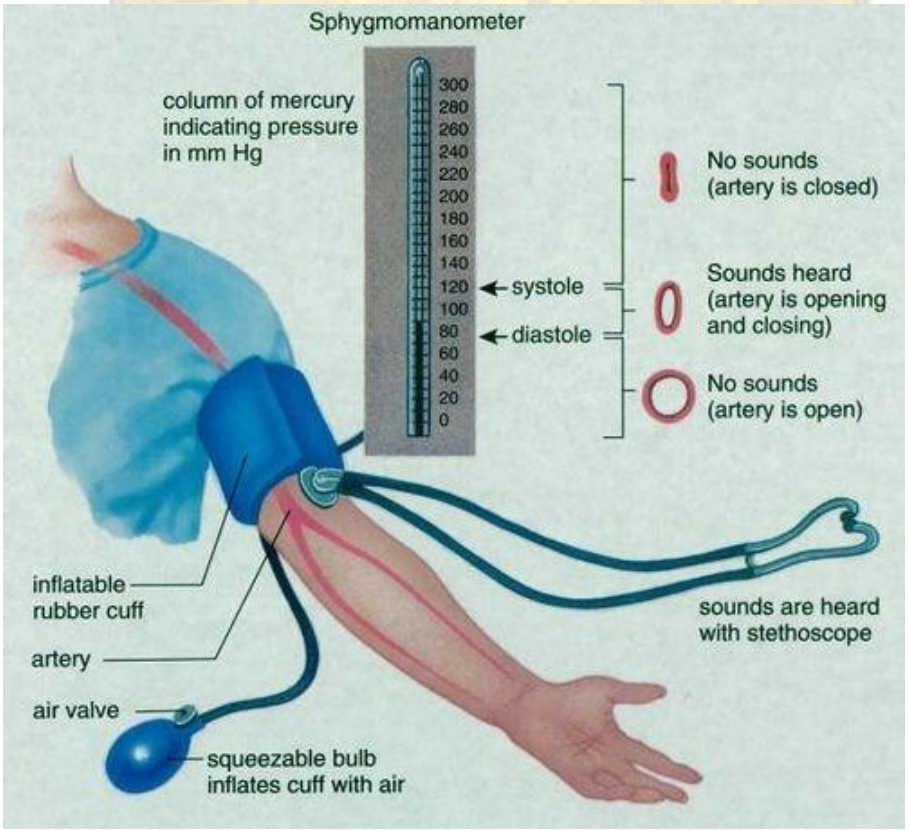


Fig: Measurement of blood pressure by sphygmomanometer

Requirements:

- Sphygmomanometer
- Stethoscope
- A sitting chair
- Human subject

Procedure:

- Subject was asked to sit down on the chair and allow to take rest for 5 minutes.
- Exposed the left arm up to shoulder.
- Wrapped the riva-rocci cuff(which consists of inflatable rubber bag attaching to two tubes. 1 tube attached to mercury manometer and other tube is connected to air pump.
- Raised blood pressure of the manometer by compressing the rubber bulb to disappear the radial point.
- Reduce the pressure gradually and noted the level of indications, where pulse reappears. This is systolic pressure.
- Gradually fall the pressure and again pulse disappears and the level of disappearance is noted as the diastolic pressure.
- Blood pressure is measured in this way at standing and supine posture also for 3 times.

Observation and Result:

- Age of the subject- 21 years
- Sex- Female
- Weight- 45 kg

Different posture	No of observation	Systolic BB (mmhg)	Average Systolic BB (mmhg)	Diastolic BP (mmhg)	Average Diastolic BP (mmhg)	Pulse BP (mmhg) (SBP-DBP)	Mean pressure (DBP + 1/3 x PP)
Sitting	1	112	116	66	68	116-68= 48	68+ 1/3 X 48=83
	2	116		68			
	3	120		70			
Standing	1	112	112	62	62	112-62= 50	62+ 1/3 X 50= 78.5
	2	112		62			
	3	112		64			
supine	1	108	110	64	64	110-64= 46	64+ 1/3 X 46= 79
	2	110		62			
	3	112		66			

Interpretation:

Reference normal value of systolic blood pressure is- 110-140mmhg

Reference normal value of diastolic blood pressure is- 60-90 mmhg

Blood pressure changes due to the pressure exert on the wall of the blood vessels mainly depends on the force of contraction of ventricle on ejection of blood. In standing posture the systolic blood pressure falls slightly in respect to the sitting posture. It is due to immediate standing, blood is pooling towards the lower limb due to gravitational force. So venous return is decreased which reduces the systolic blood pressure. After 3-5 minute of standing the blood pressure will return to the normal level by compensatory mechanism of baro receptor. The subjects blood pressure on sitting, standing and supine position belongs to the normal level.

8. Determination of Respiratory Rate:

Principle:

The respiratory system's major functions are to provide an adequate oxygen (O_2) supply to meet the energy production requirements of the body and maintain a suitable acid-base status by removing carbon dioxide (CO_2) from the body. The respiratory rate in humans is measured when a person is at rest and involves counting the number of breaths for one minute by counting how many times the chest rises. Respiratory rate is a vital sign used to monitor the progression of illness and an abnormal respiratory rate is an important marker of serious illness.

Requirements:

- Human subject
- Watch

Procedure:

- At first the subject is asked to take rest for at least five minutes and then lying down
- It is measured by counting the movements of chest and abdominal movements occur during respiration process.
- One complete breath comprises one inhalation, when the chest rises, followed by one exhalation, when the chest falls.
- Count the number of total respiration for a whole minute.

Result:

Age of the subject: 24 years

Sex: Female

Respiratory rate: 17 breath/ minute

Interpretation:

Average resting respiratory rates by age are:

- birth to 6 weeks: 30–40 breaths per minute
- 6 months: 25–40 breaths per minute
- 3 years: 20–30 breaths per minute
- 6 years: 18–25 breaths per minute
- 10 years: 17–23 breaths per minute
- Adults: 12-18 breaths per minute
- Elderly ≥ 65 years old: 12-28 breaths per minute.
- Elderly ≥ 80 years old: 10-30 breaths per minute.

Here the subject's respiratory rate is 17 breaths/ minute which is under the normal value. So the subject may not have may complications related to respiratory system.

FSN – 196 **FOOD ITEMS AND ITS CONSTITUENTS LAB**

Study of preparation variables and quality factors of products from the following food commodities

1. Determination of glucose contents from various rice, wheat and millets

a. Principle:

Starch is hydrolysed by treatment of 6(N) HCL and produce glucose molecule are use for titration after separation of protein by titrating the solution with Barium hydroxide (Ba(OH)2) and Zinc sulphate (ZnSO4).

b. Requirements:

i. Benedicts quantitative reagent (BQR)
ii. 6(N) HCL
iii. 10% Ba(OH)2
iv. 10% ZnSO4
v. 100ml and 50 ml conical flask
vi. 10ml and 5 ml pipette
vii. 100ml volumetric flask
viii. 25 ml burette with burette stand
ix. Bunsen burner
x. Filter paper
xi. Anhydrous Na2CO3
xii. Food powder (Wheat)
xiii. Distilled water

c. Procedure:

a. Hydrolyzation of food starch by acid

i. Supplied food powder was transferred to a 100/250 ml conical flask (1g).

ii. 50 ml of 6(N) HCL was added and the mouth of this flask was plugged by cotton

iii. This conical was placed in a tripod stand under burner for boiling (10-15 min).

iv. After boiling the total set was allow to cool at room temperature and neutralized by Na2CO3 unless the frothing is abolished.

b. Titration against BQR

v. Neutralized solution was transferred to 100ml volumetric flask where 10ml of 10% Ba(OH)2 and 10 ml ZnSO4 was added and mixed well.

vi. The volume of the flask was made up to the mark of 100ml and the set was allowed to stand for a few minutes.

vii. The whole solution is filtered and supernatant was collect and transfer to the 25ml burette.

viii. 10ml BQR was transferred to 50ml conical flask & 1 pinch of Na_2CO_3 was added.

ix. The conical was placed over the tripod stand under the burnar.

x. Heat the reagent gently on low flame.

xi. While it started boiling add the sample supernatant from burette drop by drop (drop the solution only during boiling).

xii. Disappearance of blue colour and appearance of pale white precipitate indicate the end point of titration.

d. Observation:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**0**	**2.8**	**2.8**	**2.8**
2	**2.8**	**5.6**	**2.8**	

e. Result:

10ml BRQ = 20mg glucose

2.8 ml dilute sample contain 20mg glucose

1ml dilute sample contain 20/2.8 mg glucose

100ml dilute sample contain 20X100/2.8 mg glucose

= 714.28 mg glucose

1 g food powder contain 714.28 mg glucose.

So, 100g food powder contain 714.28 X 100 mg glucose

So, percentage of glucose is= 71428mg = 71428/1000 = 71.42 g% glucose

f. Interpretation:

According to the Nutritive value of Indian foods by C. Gopalan the carbohydrate content in wheat powder is 71.2 g%. The supplied sample contains 71.42 g% glucose which is similar to the standard value.

2. Determination of Protein from various pulses and legumes

Principle:

Protein contain COO and NH2+ groups joined together by a covalent bond directly to form peptide bond (O=C-N-H). Two or more peptide bond give purple colour when mixed with blue colour alkaline copper solution. Biuret reagent is composed of Sodium potassium turtarate, copper sulphate and NaOH/KOH. When this reagent is mixed with protein solution biuret substance bind with cupric ion and give the purple colour. The number of peptide bond present in protein is directly proportional to the intensity of the purple colour. Colorimeter or spectrophotometer is used to measure the intensity of the colour in the form of optical density(OD). Thereby OD of unknown samples was compared with OD of standard.

Requirement:

i. Working biuret reagent
ii. BSA standard (Concentration- 2g/15ml)
iii. Pipette- 5 ml
iv. 1ml micropipette with tips
v. Test tubes
vi. Food powder (here Bengal gram was taken
vii. Colorimeter or spectrophotometer

Procedure:

Extraction of protein: 1gram food powder was added with 15ml of 0.1M NaOH. Keep 30 minutes in a magnetic stirrer. Then centrifuge at 4000rpm at 10 minutes. Collect the supernatant for estimation of protein.

Test tubes was marked with S as standard, T as unknown test and B as blank.

agents/sample	**nk**	**ndard**	**st**
rking biuret reagent	l	l	l
ndard		ml	
mple			ml
stilled water	hl		ml

ii. Mix the above contents well and incubate at 37^0c for 10 minutes.
iii. 1500µl of distilled water was added to all the test tubes.
iv. Set the colorimeter with reagent blank at 440-540nm.
v. Measure absorbance of standard followed by the test.

Result:

Absorbance of standard- 1.62
Absorbance of test- 0.12

Total protein concentration (g)= $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{X } 2$

$= \frac{0.11}{1.62} X\ 2$

$= 0.13\text{g}$

So the supplied sample of 100g Bengal gram powder contains 0.13X 100= 13g of protein.

Interpretation:

The supplied sample contain about 13gram of protein/ 100 gram. Normally pulses contain high amount of protein approximately 20-25 gram. Here the supplied sample contains low amount of protein.

3. Estimation of calcium in milk by using EDTA by titrimetric method

Principle:

In acid base titration the end point is marked by sudden change in pH and it can be detected by an indication. Here the determination of calcium in milk is based on a complexometric titration of calcium with an aqueous solution of EDTA bind to free calcium ion in the solution and the solution become calcium free. In presence of calcium in a particular pH, the calcon shows pink or purple colour. In absence of calcium calcon shows blue colour.

Requirements:

i. Alcoholic calcon solution
ii. NaOH – 2 Molar (2.05 g of NaOH is added to 250ml of distilled water
iii. EDTA – 0.01 Molar (Dissolve 3.723 g of EDTA in 1 litre of distilled water in a volumetric flask.
iv. Burette with stand
v. Conical flask
vi. 5 ml pipette
vii. 25ml measuring cylinder
viii. Funnel
ix. Milk sample

Procedure:

i. A 50ml conical flask was weighed and 1 ml supplied milk was taken. Then the conical is again weighed to get the weight of the supplied milk.
ii. 25ml of distilled water and 4 ml of 2M NaOH was added to it.
iii. Then 2-3 drops of calcon solution was added and mixed well. The solution will become purple colour.
iv. Titrate the solution against 0.01M EDTA solution in a burette.
v. When purple colour is turned into blue colour that time will be the end point of the titration and the volume of EDTA used was noted in the burette.

Observation:

No of observation	Burette reading (ml)		Difference	Average
	Initial	Final		
1	15.5	19.5	4	4.15
2	19.5	23.8	4.3	

Result:

Volume of EDTA required for titration is- 4.15 ml= 0.0041 litre

Weight of milk- 0.98g

So,

$$\text{Calcium (mg\%)} = \frac{\text{Molarity of EDTA X volume of EDTA (lit) X Molecular weight of calcium X 100}}{\text{Weight of milk (g)}}$$

$$= \frac{0.01 \text{ X } 0.004 \text{ X } 40.078}{0.98} \text{ X } 100$$

$$= 0.0017 \text{ X } 100$$

$$= 0.172 \text{ g}$$

$$= 172 \text{mg}/100 \text{g}$$

Interpretation:

Calcium level of Amul milk is 102mg/100ml. Supplied sample contain 183.5mg% calcium. So, supplied sample contain 172mg% calcium. So this value is higher than the normal value.

4. Estimation of Ascorbic acid in lemon

Vitamin C or ascorbic acid standard in metaphosphoric acid is titrated against 2,6dichlorophenol indophenol salt. This salt is blue in colour in alkaline medium and became pink in acidic medium. Pink colour indicates the complete oxidation of ascorbic acid. The dye in this titration is coloured in the oxidised form and colourless in reduced form. Ascorbic acid is a strong reducing agent because of which it reduces the dye and converted to dehydro ascorbic acid (Oxidised form).

Requirements:

i. 2,6, dichlorophenol indophenol was taken and added with 150 ml of distilled water. Then 42 mg of NaHCO3 was added to it. Then 50 ml of distilled water was added with it or volume upto 200 ml with distilled water.
ii. 6% meta phosphoric acid
iii. 50mg of ascorbic acid was added with 100 ml of 6% meta phosphoric acid and the concentration is 50mg%.
iv. 50ml conical flask
v. 10 ml pipette, burette and beaker
vi. Measuring cylinder
vii. Lemon juice

Procedure:

- 1 ml of lemon juice was taken in a 50ml conical flask. Similarly 1ml of standard Vitamin C was taken in a conical flask
- 9 ml of 6% metaphosphoric acid was added to each conical flask
- Both standard vitamin C and sample was titrated against the 2, 6, dichlorophenol indophenol in a burette
- Volume of the dye was recorded when a light pink colour persists for 30 seconds.
- The total procedure of titration was repeated for 3 times.

Observation of the sample:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**0**	**3.3**	**3.3**	**3.15**
2	**3.3**	**6.3**	**3**	

Observation of the standard vitamin C:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**0**	**2.5**	**1.5**	**1.5**
2	**2.5**	**5**	**1.5**	

Result:

Titration value of standard vitamin C is 2.5 ml.

Titration value of lemon juice sample is 3.1

1 ml standard vitamin C solution contain 0.5mg vitamin C

So, 2.5 ml dye reduces 0.5 mg of vitamin C

1ml dye reduces 0.5/2.5 mg vitamin C

3.15 ml dye reduces 0.5/2.5 X 3.15

=0.63 mg

As 1 ml of lemon juice was taken so 1 ml lemon juice contain 0.63 mg vitamin C,

So, 100ml lemon juice contain 0.63 X 100 =63.0 mg

Interpretation:

The supplied lemon juice contains 63mg% Vitamin C. As per the standard value available at the book of C Gopalan, the vitamin C content in lemon juice is 40mg%. So the supplied lemon juice contain a high amount of vitamin C which may be due to the variety or the change in climate for cultivation of vitamin C.

5. Determination of Acid Number from oils:

i. Principle:

Different fat sample may contain varying amount of fatty acid. In addition the fat often become rancid during storage and this rancidity is caused by chemical or enzymatic hydrolysis of fats into free fatty acids, which can be determined volumetrically by titrating the sample with KOH. The acidity of fats and oils is expressed as its acid value or number which is defined as mg of KOH required to neutralize the free fatty acid present in 1g of fats and oils. The amount of free fatty acids present or acid value of fat is a useful parameter which gives an indicator about the age and extends of its deterioration.

ii. Requirement:

- 1% phenopthalein solution in 95% alcohol
- 0.01 (N) KOH- 0.56 g of KOH dissolved in distilled water and make the final volume at 1 lit in a volumetric flask
- Fat solvent- A mixture of 95% ethanol and diethyl ether (1:1 v/v)
- Burette with stand
- 50ml conical flask
- Pipette
- Fat sample stored at room temperature

iii. Procedure:

- A clean and dry 50 ml conical flask was weighed then 1ml fat sample was taken in that conical flask and again weight is measured.
- 25ml of fat solvent is added to the conical flask and shake well.
- A few drops of phanopthaleinis added and the content is mixed well.
- The solution is titrated against 0.02(N) KOH until a faint pink colour persists.
- The volume of KOH in the burette is noted before and after titration.
- The same procedure is repeated for two times.

iv. Observation:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**8.5**	**14.5**	**6**	**6**
2	**14.5**	**20**	**5.5**	
3	**20**	**26.5**	**6.5**	

v. Result:

Weight of blank conical- 33.01g

Conical with fat sample- 33.88 g

Weight of sample- 0.87 g

vi. Calculation:

$$\textbf{Acid value} = \frac{\text{Titrated value X 0.01(N)KOH X Molecular weight of KOH Weight of sample}}{\text{(g)}}$$

$$= \frac{6X0.01X\ 56.1}{0.87}$$

= 3.86 mgKOH/g of fat

vii. Interpretation:

Hence the supplied sample contain 3.86mg KOH/g of fat

6. Determination of saponification number from oils:

a. Principle:

Hydrolysis of fat with an alkaline measure in the formation of salt of fatty acid (also called soap) and glycerol. This process is called saponification. From the amount of potassium hydroxide utilize during the hydrolysis, the saponification value of a given fat sample can be calculated. The saponification value is defined as mg of KOH required to saponify 1 g of given fat. The procedure involves refluxing of known amount of fat or oil with a fixed amount of KOH, but on excess of alcoholic KOH, the amount of KOH remaining after hydrolysis is determined by back titrating with standard 0.5(N) Hcl and the amount of KOH utilize for saponification can thus be calculated.

b. Requirements:

i) Fat solvent- A mixture of 95% ethanol and diethyl ether (1:1 v/v)
ii) 1 (N) alcoholic KOH- Dissolving 57 g of KOH pellets in 20 ml water and making the volume upto 1 lit in a volumetric flask by using 95% ethanol.
iii) 0.5 (N) Hcl
iv) 1% Phenopthalein solution in 95% alcohol.
v) 25 ml burette with stand
vi) 100 ml conical flask
vii) 5 ml pipette
viii) Boiling water bath
ix) Fat sample

c. Procedure:

i. Weight of Blank 50 ml conical flask is measured
ii. Then 1 g of fat sample was taken in that conical flask and weighed
iii. 3 ml of fat solvent and 25ml of 0.5 (N) HCL was added to that conical flask and kept in a boiling water bath for 30 minute.
iv. Then cool it at room temperature and add 2-3 drops of phenopthalein into the conical flask.
v. Titrate the solution against 0.5 (N) alcoholic KOH till the pink color appears.
vi. Note the volume of alcoholic KOH from the burette.

d. Observation:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**0**	**10.1**	**10.1**	**10.1**
2	**10.1**	**11.2**	**10.1**	

e. Result:

Weight of blank conical- 35 g

Conical with fat sample- 36 g

Weight of sample-1 g

Saponification value= $\frac{28.05 \text{ X Titrate value}}{\text{Weight of sample}}$

$= \frac{28.05 \text{X } 10.1}{1}$

= 283.3 mg

f. Inference:

Here calculated saponification value is 283.30 mg/g of fat. It provides information of the average chain length of the fatty acid in fat. It varies inversely with chain length of fatty acid.

Some fats and their saponification number is given below-

Butter- 210-230 mg/g of fat

Custard oil- 175- 180 mg/g of fat

Safflower oil- 188-198 mg/g of fat

7. Studying the textural characteristics of curds prepared using different milk (cow, buffalo and dairy milk):

Introduction:

Curd is well known dairy product obtained by lactic acid fermentation of milk. It is generally consumed in its original form as an accompany to the meal or it may be tuned into raita by mixing it with grated cucumber, diced boil potatoes, fried bits of gram flour. Curd or dahi may be consumed as a sweet drink or lassi or as a desert containing sugar and fresh diced bananas, oranges slices, mango and other fruits. In India system of medicine curd has been strongly recommended for curing disorders like dyspepsis, dysentery and other gastrointestinal disorders. The product is also believed to improve appetite and vitality. Some of the beneficial effects of curd are attributed to the antibacterial components formed during the fermentation and the low pH that prevents the growth of many undesirable organisms including pathogenic organisms and possess improved digestive function.

Preparation of curd:

Ingradient

Cow milk, buffalo milk, dairy milk and raw curd

Procedure:

1. First step is to take a thick bottomed pan and rinse it with clean water.
2. Then add ½ litre of whole milk – about 2 cups milk. Keep the pan on the stovetop and begin to heat milk on a low to medium-low heat.
3. When the milk is getting heated, stir once or twice, so that the milk does not get browned or burnt from the bottom.
4. Let the milk come to a boil. Milk will froth and bubble when it comes to boil.
5. Switch off the heat. Remove the pan and keep it aside on the kitchen counter for the milk to cool at room temperature. We just need the temperature of milk to reduce till the milk becomes warm.
6. Now take 1 to 2 teaspoons of the curd starter and add in the warm milk. In winter you can add 2 teaspoons. Whereas in summers 1 teaspoon works fine.
7. With a small wired whisk or a spoon mix very well. A small wired whisk makes the job of mixing the curd starter very well in the milk.
8. Then pour this mixture in a bowl or a pan.
9. Cover the pan or bowl with a lid. Then keep at room temperature for 4 to 5 hours or till the curd is set.
10. The time taken for the milk to get converted to curd largely depends on the temperature. In a warm, hot and humid climate, the time taken for the curd to set will be 4 to 7 hours. Whereas in a cool or cold climate, the time taken can be 8 to 12 hours.
11. Next day you will have a nice well set homemade curd.

Determination of total fat moisture and solid

Principle: Lipids are soluble in organic solvents but sparingly soluble or insoluble in water, solubility in lipids is an important criteria for their extraction from source methanol and depends heavily on the type of lipid present and the proportion of non-polar (principally triglycerol) and polar lipids mainly (phospholipids and lipids) in the sample. A mixture of chloroform methanol allows for extraction of both polar and non-polar lipids from sample matrix. During extraction the lower chloroform layer contains the lipids and the top methanol water layer generally contains non-lipid components.

Apparatus required:

Centrifuge tubes -50ml

Glass beakers- 50ml or 100ml

Whatmann filter paper –no.1
Micro-pipette- 100-1000µl
Pasteur pipette -3ml

Chemical Required:

Choloroform GR
Methanol GR
Mili Q water

Procedure:

- Take approximately 0.5g of sample in duplicate in 50ml centrifuge tube.
- Add 10ml of 2:1 (chloroform : methanol) mixture to each tube, then vortex each tubes for 3 mins.
- Keep in a cold room for overnight.
- Separate the chloroform layer into another screw capped test tube and store it in the cold room.
- Then separate chloroform layer , Now add 2 ml of mili Q water combined chloroform layers and vortex for 1 min and centrifuged at 1500 rpm for 10 min.
- Discard the water layer and evaporate the chloroform layer on a boiling water bath in a pre-weight beaker.
- Keep the beaker in oven at 60^{o} for overnight.
- Take out the beaker from oven and allow it for cooling at room temp, weigh the sample beaker and note down the reading.

Textural characteristic of curd:

Moisture content determination:

The moisture content of the curd products was determined according to the association of official analytical chemists method (AOAC, 1995). Each curd product (near about 7-10g) was placed in an oven at 105^{0}C for 3 hour. Reading was taken at a constant weight. The moisture content was taken expressed as the percentage (%) of dry weight of sample.

Result:

Result of sample A is given below

Total moisture contain = (Weight of sample before put the conical into hot air oven – Weight of sample after remove the conical from hot air oven)
= 54.21 - 54.20
=0.01 g

Total moisture percentage =
0.5g curd contain 0.01g moisture
1g curd ……0.01/0.5 g ……
100g ……0.01/0.5X100g moisture
=2% moisture

Total solid: The weight of residue obtained from moisture content analysis was expressed as percentage of total solids using the formula below

Total solid %= $\frac{\text{Dry weight of curd}}{\text{Weight of sample}}$ x 100

Dry weight = (Curd+ plate after drying) -plate

1.78/7.9 X 100 = 22.53 %

Total fat percentage (g%)
= w2-w1/weight of sampleX100Xmoisture percentage
=54.21-54.20/0.5X100X2
=4%

Physicochemical test:

All the curds were analyzed for colour, flavor, texture and pH.

pH was measured by pH meter at different time interval.

The pH of the sample A is 5.38.

Interpretation:

The samples were prepared from cows' milk and assessed for different physical parameters like moisture content, total solid and pH.

8. Manufacturing of biscuits/ cookies:

A cookie is a baked or cooked food that is typically small and taste sweet. It usually contain flour, sugar and some type of oil or fat. It may include other ingredients such as oats, chocolate nuts etc. In most English speaking countries cookies are called biscuits.

Ingredients required:

i. 1 cup butter, room temperature

ii. 1 cup sugar

iii. 1 egg

iv. Few drops of vanilla essence

v. 2.5 cup flour

vi. 1 teaspoon baking powder

vii. ½ tea spoon salt

Procedure:

i. Preheat the oven at 200^0C

ii. Mix the dry ingredients by mixing the flour, salt and baking powder in a bowl.

iii. Cream the wet ingredients in a separate bowel. Place the butter, sugar, egg and vanilla in a bowel and beat until the ingredients are incorporated and the mixture is light and fluffy.

iv. Mix the dry and wet ingredient. Pour the flour mixture into the bowl at wet ingredient. Use a long handed spoon to stir the batter.

v. Drop the mixture into a baking tray; use a spoon or a small ice cream scoop. Place the baking dough in small quantities on a sheet and placed in a oven

vi. Spread sugar powder on the top of the mixture.

vii. Bake the cookies for 2-3 minutes or until a golden colour occurs at the top of the cookies.

viii. Let the cookies cool down, remove them from the oven and place them on a cooking rack.

Nutrient analysis:

Food stuff	Amount (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (Kcal)
Butter	100	-	81	-	729
Sugar	100	0.1	-	99.4	398
Flour	250	27.5	2.25	173.5	870
Egg	50	6.65	6.65	-	86.5
Baking powder	5	0.005	0.02	2.35	97
Total		34.25	89.92	275.25	2180.5

So, 100g product contains 34.25 g protein, 89.92g fat, 275.25g carbohydrate and 2180.5 kcal energy.

Cost analysis

Food stuff	Amount (g)	Cost(g)
Butter	100	46
Sugar	100	4
Flour	250	16
Baking powder	5	0.93
Egg	50	6
Total cost		74.18

9. Preparation of Kalojam Sweets

Ingredients:

- 1 Cup Powdered Milk
- 2 ½ Tbsp All-Purpose Flour
- 1 Tbsp Semolina
- ¼ Tsp Baking Powder
- 1 Tsp Clarified Butter
- 7-8 Tbsp Milk

For Syrup

- 1 ½ Cup Sugar
- 3 cup of water
- 4 Pc Cardamom

For Coating

- Reduced Dry Milk Powder

Instructions:

1. Mix powdered milk, all-purpose flour, semolina, baking powder, clarified butter, milk until a dough consistency.
2. Make a round-like shape using ghee on the palm.
3. Deep fry in low-medium heat until dark golden brown.
4. In a pot, add sugar, water, cardamom then soak for 1 hour with a lid on with low heat.
5. Remove kalojam from the pot, let it cool then coat with reduced dry milk powder.

Nutritive value:

Ingredients	Amount (g)	Carbohydrate (g)	Protein (g)	Fat (g)	Energy
Wheat flour	100	73.9	11.0	0.9	348
Skimmed milk powder	50	25.5	14	0.05	178.5
Sunflower oil	100			100	900
Ghee	5			5	45
Sugar	500	497	0.5		1990
Baking powder	15	7.04	0.02	0.06	14.55
Total		**603.44**	**25.52**	**106.01**	**3476.05**

Suggestion:

The total energy content of this kalojam dish is 3476.05 kcal, carbohydrate content is 603.44 g, protein content is 25.52 g and fat content is 106.01 g. We make 40-45 sweets and these are divided into 40-45 individuals. This dish is suitable for non-diabetic person and the individuals who don't have heart disease,

obesity etc.

10. PREPARATION OF KACHAGOLLA

Ingredients:

Chana: 100 gm
Powder milk: 22 gm[6 table spoon]
Ghee- 5 gm
Sugar free tablet: 2 gm
Clove: 5 gm

Procedure:

- Take all the ingredients in a bowl knead well until smooth [Remember try to squeeze all the water from channa before making the sweets.
- Now turn on the heat on a low flame. Put the channa on a pan and cook on a low flame for evaporation of excess water.
- Add the powder milk and mix very well with the channa. Transfer it immediately on a plate. Shape in round ball when it cool and for garnishing clove will be added on it.

Nutritive value:

Ingredients	Amount	Energy(kcal)	Carbohydrate(g)	Protein(g)	Fat(g)
Channa	100g	265	1.2	18.3	20.8
Powder milk	22g	109.12	8.36	5.67	5.87
Ghee	5g	44.85	_	_	4.98
Sugar free tablet 1	2g	6.62	1.8	_	_
Cardamom	5g	11.45	2.10	0.51	0.11
Total		437.04	13.46	24.48	31.76

Suggestion: The total energy contain of this Kachagolla dish is 437.19 kcal, carbohydrate contain is 13.46g, protein contain contain is 24.17g, fat contain is 31.76g. 10 piece kachagolla sweets are made from this ingredients and are equally divided into 15-20 individuals. The dish is appropriate for diabetic patients because sugar free tablet are mainly used sweeten this. But there is some condition where provision of sugar free tablet can be dangerous, such as not recommended for phenylketonurias, the children who suffering from seizure, disorders, pregnant and lactating mother.

11. NOLEN GUR ROSOGOLLA

Nolen Gur Rosogolla is one of the most anticipated winter dessert Recipe which spreads happiness and makes any ordinary day special with its comfort, tempting look, unbeatable flavour and irresistible taste. It is an authentic Bengali dessert where Indian Cottage Cheese Balls are cooked in Date Palm Jaggery (Nolen Gur) Syrup.

Nolen Gur Rosogolla is an iconic dessert and pride of Bengal. People urge for it throughout the year and wait for the availability of date palm jaggery (Nolen Gur) in market. Nolen Gurer Rosogolla is a delectable soft, spongy and juicy dessert which drives the person to the taste of divine. Surprisingly, this interesting recipe gets prepared with only three simple ingredients.

Ingredients:

1 litre full fat Milk

1 tbsp vinegar

1 teaspoon fine Rawa (Semolina)

1 cup Date Palm Jaggery

1 cup Sugar

4 cups of Water

For preparing Chenna

1. Take the milk in a heavy bottom pan.
2. Put the flame in medium high and let the milk boil. Tip: While boiling the milk, put the flame in medium and stir occasionally. Otherwise the milk will stick to the bottom of the pan and a burning smell will get into the milk.
3. Put the flame in lowest.
4. Add vinegar slowly into the milk (Step 3).
5. Stir it slowly and let the milk curdle.
6. Stir for 1-2 minutes in lowest flame.
7. On the other hand, place a colander in the sink of kitchen and put a Muslin cloth on the colander.
8. While the water gets separated from the curdled milk, switch off the flame.
9. Pour the curdled milk immediately to the Muslin cloth. You need to be fast in this step otherwise Chenna will get overcooked.
10. With the help of a spatula, stir the Chenna gently for a minute to cool it off.
11. Gather the corners of the cloth.
12. Put the Chenna into the running water.
13. Rinse the Chenna carefully over the cloth, water should not go directly into the Chenna. This helps to remove the Lemon flavour off the Chenna and the Chenna would also become cool.

14. Squeeze the excess water very delicately 2-3 times. Don't over squeeze the Chenna. If the Chenna becomes dry, then the Rasgullas will be hard.
15. Then tie a tight knot of the cloth.
16. Hang the Chenna for 30 minutes.

For making the Jaggery syrup

1. Add water and sugar into a large pan with wide neck and put the flame on high.
2. Add the Date Palm jaggery into the pan and stir it until the jaggery gets melt.
3. Once it starts boiling check whether it has any dirt inside the syrup.
4. If you observe any dirt, then add 1-2 tablespoons milk.
5. While the syrup starts boiling, the dirt will come on the top like froth.
6. Discard the dirt with the help of a spoon.

For making the Chenna balls

1. After 30 minutes of hanging the Chenna, put it on a large plate.
2. Break the Chenna with the help of fingers.
3. Add 1 teaspoon fine Semolina.
4. Put the pressure of your palm to break the grains of Chenna. Gather them at a place and again knead in the same process for 4-5 minutes.
5. Knead the Chenna until the dough becomes soft and smooth.
6. Divide the dough into 11-12 equal portions.
7. Take one small portion at a time in your hand and fist your hand repetitively by rotating the Chenna portion.
8. This process helps the body of the Chenna portions to become smooth.
9. With the help of your two hands, roll the Chenna portion in between your palm and make a smooth round ball.

For cooking the balls in sugar syrup

1. Dunk the Chenna balls into the boiling sugar syrup one by one immediately.
2. If the flame is too big then put the flame in medium and if the flame is normal, then put the flame on high.
3. Cover the pan and let them cook for 10 minutes.
4. Don't stir the Rasgullas. They are very soft and may break. While they get boiled in the sugar syrup, they will rotate automatically.
5. After 10 minutes, the size of the balls will get doubled.

6. Put the flame in low and cover the pan.
7. Cook them for another 15-20 minutes.
8. Switch off the flame and allow the Rasgullas to cool down for an hour.

Ingredients	Amount	Energy(kcal)	Carbohydrate(g)	Protein(g)	Fat(g)
Milk	1L	670	44	32	41
Semolina	5g	17.4	3.74	0.52	0.04
Jaggery	60g	211.8	51.66	0.9	0.18
Sugar	60g	238.8	59.64	0.06	_
Vinegar	15g	_	_	_	_
Total		1138	159.04	33.48	41.22

Suggestion: The total energy contain of this Nolen Gur Rosogolla dish is 1138kcal, carbohydrate contain is 159.04g, protein contain 33.48g, fat contain 41.22g. We make 11-12 sweets and these are divided into 11-12 individuals. This dish is suitable for non diabetic person and the individuals who do not have heart disease, obesity etc.

12. Imli candy

A unique and tasty candy or toffee recipe made with tamarind or imli with a strong dosage of spice in it. it is one of the popular tangy and sour condiment confectionery which is generally served as a lite snack after a meal or as a mouth refresher. there are different types to these tangy candies, but this is condiment is a combination of spicy and tangy tamarind candies.

Ingredients:

Tamarind (seedless) - 100g
Dates- 80g
Hot water- 2 cup
Jaggery-100g
Chili powder- 10g
Chat masala-10g
Cumin powder-10g
Salt-15g
Ghee- 15g

Procedure:

1. Take 100g tamarind and 80g dates in a bowl then soak it for 30 min in hot water until it goes softens.
2. Make a smooth paste and filter out to remove the fibre.
3. Then pour the paste in a pan add 100g jiggery into it and mix it well, cook in a medium flame.
4. It goes thickens after 15 min and turn to glossy.
5. Add chili powder, chat masala, cumin powder, salt and ghee and mix well.
6. It will be separate from pan and make a thick paste consistency.
7. Cool it completely and make a shape then wrap it tightly.

Nutritive Value:

Ingredients	Amount(g)	Energy (Kcal)	Carbohydrate (g)	Protein (g)	Fat(g)
Tamarind	100	283	67.4	3.1	0.1
Dates	80	253.6	60.64	2	0.32
Jaggery	100	383	95	0.4	0.1
Chili Powder	10	33.33	3.60	1.52	1.40
Chat Masala	10	27.2	5.28	0.58	0.41
Ghee	15	134.55	-	-	14.91
	Total	1114.68	231,92	7.6	17.27

13. SENSORY EVALUATION OF FOODS:

Sensory quality is a combination of different senses of perception coming into play in choosing and eating a food. This practical will focus on the sensory evaluation of foods. We will see how the individual threshold for different sensations varies from individual to individual.

Foods have several characteristics that require evaluation by sensory methods. The various food attributes that are judged on the sensory scale are flavour, texture, aroma and appearance. Every time the food is eaten, the quality is evaluated or the judgment is made. The reaction of the consumer on the basis of the sensory perception serves as an endorsement or proof of the product acceptance.

The sensory evaluation of food is carried out on a scientific basis to ascertain the product formulations or processing techniques that are anticipated to be successful in the market place. In the research and development process of a product, trained sensory panelists evaluate the samples and provide guidance in improvement of the product. This type of testing wherein the scores are determined by individual decisions based on the use of senses and do not rely on the mechanical devices, is known as sensory or subjective evaluation. Thus, sensory evaluation has been defined as a scientific method to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing.

Techniques of sensory evaluation:

Techniques	Test characteristics
Single sample	This type of test is used to test acceptability and to aid in the decision on future development of the product.
Paired comparison	It is the difference test in which a specific characteristic is to be evaluated in two samples, and the sample with the greater level of that characteristic is to be identified
Duo-trio test	Difference test in which two samples are judged against a control to determine which of the two samples is different fro

Triangle test	Difference test in which three samples (of which two are same), and the odd sample is to be identified
Rank order	Preference or difference test in which all samples are ranked in order of intensity of a specific characteristic.
Descriptive test	Use of key or descriptive words in sensory evaluation to characterize food samples.
Profiling	It is a very detailed word description like texture profiling or flavour profiling or characteristics such as chewiness, adhesiveness, hardness.
Descriptive scale	It uses array of words describing a range of intensity of a single characteristic such as surface colour of a cake can be described as pale, slightly brown, pleasing golden brown, dark brown, burned etc. The scales can be designed with an odd number of points usually 5 or 9 point scales. The above example is that of a 5- point rating scale.
Hedonic rating test	Hedonic rating range from unacceptable to very acceptable is relatively easy to construct and is effective when the desirable and undesirable characteristics of a few samples are taken into account. The 9 point hedonic scale can be described as: 1= like extremely, 2= like very much, 3= like moderately, 4= like slightly, 5= neither like or dislike, 6= dislike slightly, 7= dislike moderately, 8= dislike very much, 9= dislike extremely
Smiley scale	This is a sequential series of very happy and continuing through to very unhappy faces used in evaluating food products when respondents are unable to use the language easily.

Requirements for the evaluation process:

Here we will use the hedonic scale test for sensory evaluation of foods. Some of the requirements to be kept in mind for the evaluation of the products are listed herewith:

• People in a sensory test are often placed in individual test booths so that the judgments they give are their own and do not reflect the opinions of those around them.

• Samples are labeled with random numbers so that the people they do not form judgments based on the labels but rather on their sensory experiences.

• Products may be given in different orders to each participant to help measure and counterbalance for the sequential effects of seeing one product after another.

- Standard procedures may be established for sample temperature, volume and spacing in time, as needed to control unwanted variation and improve test precision.
- A well-lighted (white light) room with tables (preferably. white tops) and chairs. Assuming that 4 samples (As for example tomato ketchup as in this test) are evaluated and 10 panellists are going to evaluate the products, the following glassware and other items are also required.

White porcelain saucers : 12 (These are sufficient for serving a set of the four samples to three panellists. The saucers can be cleaned and reused)

Teaspoons : 12

Glass tumblers for water : 10

Bread : 1 loaf

Evaluation cards : 10

- Clear instructions should be provided to the panelists before the evaluation process as described below. These instructions should be pretested by having someone unfamiliar with sensory testing. Instructions regarding the filling of the evaluation sheet should also be provided to the panelists.

Instructions to the panelists:

- Please rinse your mouth with water before starting.
- Different codes have been provided for sensory evaluation.
- Taste each of the coded sample in the sequence presented.
- Rinse your mouth with water and proceed to the next code
- You have to fill the evaluation performa code wise

Hedonic rating test card:

Name.. . . . . Date. . . . . .

Product: Tomato ketchup

Taste the four samples of tomato ketchup and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that describes your feeling about the sample:

Scale	**Ketchup samples**			
	Code No	**Code No**	**Code No**	**Code No**

Like extremely				
Like very much				
Like moderately				
Like slightly				
Neither like nor dislike				
Dislike slightly				
Dislike moderately				
Dislike very much				
Dislike extremely				
Reason for like/ dislike				

(Signature of panellist)

Procedure:

- In this test, four tomato ketchup samples are tested for the preference. One sample Rating Test could be the one prepared in a factory and the others are the three market samples.
- Otherwise, all the four samples could be market samples. The ketchup bottles are first marked with code numbers.
- It is preferable to have three digit random number codes to avoid bias. Single digit numbers like 1,2,3,4 are likely to cause bias.
- For example, some panellists may have a tendency to assume No. 1 is the best and No.4 is the worst and vice versa.
- Random numbers can be taken from random number tables or generated from a calculator. An example of assigning random 11umbers to four samples is shown below.

Ketchup Sample	Code No
A	10
B	20

C	15
D	25

- In the same way, the sets of four saucers are also numbered and smnall quantities of the ketchup samples are taken in them.
- Before starting the evaluation, the panellists are briefed about the test procedure, what to look for in the quality oftomato ketchup, two or more samples may be given the same rating if found so etc. Each product has its own quality attributes.
- For example, in the case of tomato ketchup, good quality attributes are bright characteristic tomato red colour, thick consistency, does not flow easily when the saucer is tilted, does not show separation of serum around the ketchup sample in the saucer etc.
- Ketchup Sample A B C D The set of four saucers containing the samples is given to each panellist along with the evaluation card. Drinking water to rinse mouth in between tasting of two samples to clear the taste ofthe previous sample is provided.
- Simiilarly, cubes of bread are provided for eating for the same purpose. If a panellist requires more samples, the same should be provided.
- On completion of the evaluation, the evaluation cards are collected and the data are analysed as shown below.

Observations and Calculations:

Panelists	Sample A	Sample B	Sample C	Sample D
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Total Score				
Mean score	2.8	3.2	3.1	4.2

Result:

Since the numerical scores are assigned in the reverse order i.e., 1 for the highest quality and 9 for the lowest quality point, lower the score total or score average higher the preference. Therefore, the rating of the quality (preference) of the four tomato ketchup samples will be given accordingly.

From lowest score to highest score. (As for example: Sample A> Sample C> Sample B> Sample D)

Interpretation:

14.

15. Estimation of lactose from milk:

a. Principle:

The protein of milk is consists of casinogen, lactoalbumin and lactoglobulin. The protein are precipitated by tungstic acid. Lactose is the only reducing sugar present in milk. Tiltration of milk is essential by the reduction of BQR.

b. Requirements:

i. 10% sodium tungstate
ii. 2/3 (n) H2SO4
iii. Anhydrous Na2CO3
iv. Benedict quantitative reagent
v. 50 ml and 100ml volumetric flask
vi. 100ml volumetric flask
vii. Filter paper
viii. Burner
ix. Distilled water
x. Supplied milk
xi. Glass beads

c. Procedure:

a. Preparation of protein free titrate

i. 10ml milk was transferred to a 100ml volumetric flask
ii. 10ml of 10% sodium tungstate and 10 ml 2/3 n H2SO4 was added to it.
iii. Make the volume upto 100ml by distilled water.
iv. The mixed thoroughly the whole content and allow to stand for 5 minutes.
v. Then filter the solution and taken in a 25 ml burette.

b. Preparation of filtration against BQR

vi. 10ml of BQR was taken in a 50ml conical flask and add about 1 pinch of anhydrous Na2CO3 and 2-3 pieces of glass beads.
vii. The conical flask was then placed over the tripod stand and heat the solution gently over flame and while boiling added the contents from burette drop by drop.
viii. The disappearance of blue colour and appearance of white gelatinous ppt indicates the end point of filtration.
ix. The titration is repeated for three times.

d. Observation:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**11**	**15**	**4**	**4**
2	**15**	**18.5**	**3.5**	
3	**18.5**	**23**	**4.5**	

e. Result:

10ml BRQ = 0.0268 g of lactose

4 ml dilute sample reduces 0.0268 g lactose

1ml dilute sample reduces 0.0268/4 g lactose

100ml dilute sample reduces 0.0268X100/4 g lactose

= 0.67 g lactose

Here supplied milk is diluted by 10 times. So the percentage of lactose in supplied milk is 0.67X 10 = 6.7g %

f. Interpretation:

According to the standard data available on lactose content in cows milk is about 4.8 %. The supplied sample contains 6.7 g % lactose which is slightly higher than the reference value.