B.Sc. NUTRITION LAB MANUAL

1st Semester

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Nutrition

MIDNAPORE CITY COLLEGE

C1P1 Basic Nutrition (Practical)

Contents

1. Use and care of kitchen equipments

Introduction: Quality kitchen equipment is essential for any food service business. Because it is used frequently an increased emphasis needs to be placed on cleaning and maintenance. Neglect can put customers and staff at risk due to germs on rice. In addition costly food wasre or equipments failure can occur if proper care is not taken. This article covers cleaning and maintenance tips for our kitchen equipments.

However busy schedule and rushed can cause cleaning tasks to be done poorly altogether. This is a big problem because of the sanitation risks. Bacteria and mold growth on kitchen surface can spread to plate, utensils, or directly in food and cause people to get sick. This risk can easily avoided by following of strict cleaning schedule grills, ranges, fryers, meat scissors, most cooking and pre equipments should be cleaned and disinfected daily. Others appliances like refrigerators and others weekly cleaning. While cleaning freezers and wmptying grease traps can be done monthly.

Kitchen food safety and sanitation standards:

1. Do not come to work when worker are sick with a contagious illness, such as influenza (chest cold fever), have a bad cold or symptom of a stomach alignment (diarrhoea or vomiting), or if you had diarrhoea in the past 72 hours, please call the event kitchen manager, if you are ill and can not attend or if you are unsure whether you should stay home.
2. Dress in layers so you can comfortable during your work shift.
3. Remember to bring any medication or reading glasses that yoy may need.

4. Bring some kind of hair net, cap to wear in the kitchen. That will keep hair in place and out of the food.
5. Of worker have long polished hair nails. Consider trimming your nails and remaining nail polish. If you came to work with polished nail, you will be required to wear gloves at all times.
6. Wear comfortable close shoe for safety and sanitation reasons, sandal and other open load shoe should not allow in kitchen.

Food safety standards: Kitchen food safety standards are based on these in the USA kitchen companion booklet given to each. We ask all kitchen worker to remember that safety and sanitation should always come first. When making work decision in the preparation dish wash the dining room areas. This will keep food safe and avoid accidents.

Follow the guideline in the kitchen companion to:

- Keep food and work areas clean.
- Don't cross-contaminate. Keep raw meat, poultry, fish, and their juices away from other food.
- Always refrigerate perishable food within 2 hours—1 hour when the temperature is above 90 °F (32.2 ºC).
- Check the temperature of your refrigerator and freezer with an appliance thermometer.
- Always wash hands with warm water and soap for 20 seconds before and after handling food.
- Cover hand with a glove at all time if you have a cut or infection on your hand.
- Cutting boards, utensils, and countertops can be sanitized by using a solution of 1 tablespoon of unscented, liquid chlorine bleach in 1 gallon of water.
- After cutting raw meats, wash cutting board, utensils, and countertops with hot, soapy water.
- Use different cutting board for different food items.

- Hot food should be held at 140 °F (60 °C) or warmer.
- Cold food should be held at 40 °F (4.4 ºC) or colder.
- Perishable food should not be left out more than 2 hours at room temperature—1 hour when the temperature is above 90 °F (32.2 ºC).

Kitchen and equipments use, maintenance and cleaning

All Kitchen/Food Production facility and equipment will be maintained, cleaned and sanitized on a regular basis to ensure the safety of the food prepared and served to customers.

The Kitchen Staff/Chef's who use such equipment will be responsible for washing and sanitizing removable parts after each use. Equipment that handles potentially hazardous or easily contaminated foods is cleaned at least every eight hours.

i. Sanitizing spray:

- Put cold water into spray bottle

- Using the measuring spoon attached to the bleach bottle, measure 1 tbsp bleach and mix with the water.
- Rinse the spoon in clean water and re-attached to bleach bottle.
- Effectiveness of spray does not last during long storage, throw away leftover spray before your group leaves.

ii. Sanitizing counters and tables:

- Spray kitchen counter and work surfaces before you begin cooking and again before your group leaves.
- Spray table before sitting, and again before your group leaves.

iii. Dishwashers:

Follow the dishwashers instruction:

- Wash all the dishes except the following- wash the coffee maker and coffee ground buskets.
- Wash the food thermometer and replace them in their cases.
- Rinse call the item before sending them through the dishwashers.
- Replace dishes, cook ware and utensils in their drawers and cupboards after air drying. Towel drying of dishes.

iv. Cookware and utensils:

- Use only the metal and glass containers in the kitchen for cooking. Plastic cannot be sanitized well enough to be included as a cooking utensils.
- Never put plastic in the microwave oven

v. Towels and dish cloth:

- Soak them in a bucket of warm water and vinegar for about 15 minutes. ...
- If your dish cloths/towels are white, add bleach into the load when you wash them.
- Wash your towels with hot water. ...
- Never leave your towels in the washing machine overnight or for an extended period of time.

vi. Tableclothes:

- Wash all the table clothes that have been placed on tables even if the tables were not occupied or tablecloths appear clen.
- Dry your tablecloth on the Delicate setting until it is *mostly* dry.
- Iron your tablecloth on the highest setting the fabric can tolerate.
- wrapping a tablecloth around an old inner tube from a roll of carpet.

vii. Cutting board:

- Do not use glass cutting board.
- Do not use worn boards with hard to clean grooves.
- Wash all the cutting board in dish washers.

- Cutting boards, utensils, and countertops can be sanitized by using a solution of 1 tablespoon of unscented, liquid chlorine bleach in 1 gallon of water.
- After cutting raw meats, wash cutting board, utensils, and countertops with hot, soapy water.
- Use different cutting board for different food items- Green: Fruit and vegetables.
  Red: Raw meat, poultry, fish
  White or clear: cheese, bread, cooked food.

viii. Refrigerator:

- Claen any spills and each shelf of the refrigerator that you used with baking soda water before leaving.
- Remove all the leftover food from the freezers when you are finished with your event.

ix. Oven and cooking surface:

- After cooking the surface should be cleaned also for oven. Remove only spills.
- Do not use steel wool on the grill surfaces this will causes the surface to rust.

x. Microwave oven:

- clean all sides of the inside of oven.

xi. Garbage disposal and sinks:

- Drain water from sinks.
- Remove any non-food item from sink before running the disposal, and keep utensils, dish clothes, and hands, out of drains.
- Turn cold water on.
- Do not put hands in the drains.

2. Weights and measures standards, household measures of raw and cooked foods

Purpose and use of different kitchen equipments: **1. Measuring tools:**

Fig: Liquid measuring cup

Liquid measuring cup: Made up of clear glass or plastic measures liquid ingradient.

Fig: Dry measuring equipments

Dry measuring equipments: Made up of metal or plastic ingradient.

Fig: Measuring spoon

Measuring spoon: Made up of metal measures small quantities of dry or liquid ingradients 1 tbsp.

Fig: Instant read thermometer

Instant read thermometer: Accurate reading in second measures the temperature of food at end of cooking time.

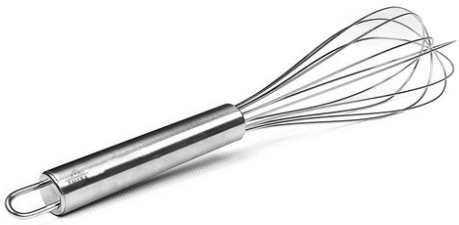
Fig: Straight edge spatula

Straight edge spatula: A straight edged spatula is used to level off ingredients.

2. **Mixing tools:**

Wooden spoon: Used for stirring and mixing.

Whisk: Incorporate air into loads whisk eggs and soufflés. Prevents lumps in sauces.



Mixing bowl: A large bowl in which ingradient are mixed for the purpose of preparing food.

Rubber Spatula: Used to scrape bowls and saucepans used to fold one ingradient into another.

3. **Baking Tools:**
   a. **Bench tools (scraper):**
      - Portion or cut dough
      - Clean surface after making dough
      - Diced foods from cutting board to pan.

   b. **Sifter:**
      - Blends dry ingradients
      - Removes lumps from flour and sugar

   c. **Pastry Blender:**
      - Blends shorting into flour
      - Blends butter and cheese mixtures.

   d. **Pastry brush:**
      - Brushes butter or sauces on foods
      - Removes crumbs from cakes before froasting
   e. **Rolling pin:**

- Rolls dough and pastries flat.

4. **Cutting tools:**

a. kitchen shears:

- Snips herbs
- Trims vegetables
- Cuts meat, dough, cooking and pizza.

b. Peeler:

- Removes the outer surface of fruit and vegetables.

c. Shredder greater:

- Used to shread and grate foodssuch as: cheese and cabbage
- Different sized opening for small and large pieces.

d. Cutting Board:

- Made up of wood or plastic
- Protects tables and counters tops when cutting or chopping foods.

e. Chef's knife:

- Most used knife
- To out chopped dice fruits and vegetables.

Introduction: To standardized some commonly consumed cooked food in terms of household measures and raw foods into equivalent weight and to ensure the accuracy in diet planning the evaluation requirement content of diet or intake of different food group, it is essential to have an idea of one serving portion of the cooked food for the various age group. The amount of these food in terms of common household measures used for eating or serving conversion of the amounts in household measures to the constituent weight of the raw foods.

Commonly used household measures inclue large, medium or small katorie, bowl or cups, teaspoon, tablespoon and serving spoon.

For ensuring accuracy of the standardization of size of the container in terms of diameter and height or the voume must be known. Incase of food item like chapaties, parathas, dosa, idli the size and the thickness needs to be noted. For pulse preparation like sambar the consistency nd the texture should be known. Ythese details facilitate better assessment of the raw weight of ingradients. An idea of the weight of food stuff which may e eaten raw as related to their size is also beneficial. For example it is desirable to know the weight of a small medium and large apple or any other fruit nay other vegetables, a spicy bread or a bun or a cup of milk.

Weighment of raw food: This method has been practiced by majority of the workers in India. The survey team visits the household and weighs all the food that is going to be cooked and eaten. Also the amount which is left over or discarded is weighed. A survey needs to make at least two visits prior to the main meals being cooked.

Weighment of cooked food: Cooked food is weighed instead of the raw materials. In Indian homes, this is not a very acceptable thing. Hence, this is more appropriate for dietary assessment of institutions, hostels, etc. This method consists of determine the quantity of each food taken for cooking the kitchen and also the weighment of cooked food. The cooked food leftover after consumption also weight. From this data actual quantity of food consumption by the entire family can be calculated.

Household Measure	Volume (ml)
1. Katori medium	125ml
2. Glass large	240ml
3. Glass medium	200ml
4. Cup large	250ml
5. Serving spoon	50ml
6. Cup medium	200ml
7. Serving spoon medium	25ml
8. 1 tsp	15ml
9. 1tbsp	5ml
	15ml

The portion size and balanced diets for an adult and for different age groups. The following table give the portion size for menuplanning and the number of portion to be given to each age group to have a balanced doet such as suggested by ICMR.

Table: Portion size for menu plan

Food groups	Energy	Carbohydrate	Protein	Fat
1. Cereals and millets	100 100	3 6	20 15	0.08 07
2. Pulses	85	7	-	7
3. Egg	100	9	-	7
4. Meat chicken or fish	80	1.3	19	-
5. Milk	45	3.6	-	0.4
6. Roots & tubers	30	1.7	-	0.2
7. Green leafy vegetables	40	-	10	-
8. Other vegetables	20	-	5	-
9. Fruits	45	-	-	5
10. Sugar	-	-	-	-
11. Fats and oil				

Source: Dietary guidelines for Indians_ A manual 1999, National Institute of Nutritional, ICMR Hyderabad.

Uses:

- It is easy to understand the amount of food to be taken.
- Bu choosing healthy foods and appropriate portion size that a person can eat regularly and still maintain a healthy body weight.

Portion Size

Portion size is an important component of menu planning in food exchange list. A key part of healthful eating means choosing appropriate amounts of different foods. ... Portion size is the amount of a food you choose to eat — which may be more or less than a serving. For example, the Nutrition Facts label may indicate ½ cup cereal for one serving but if you eat ¾ cup, that is your portion size.

The food are choosen from all the food groups.

Beverages: Chocolate Milk Shake

Ingradient

4 scoops	vanilla or chocolate ice cream (about 2 cups)
1/2 cup	milk cold
1/4 cup	HERSHEY'S Syrup
4pcs	Ice cubes
	Cashew nut

Procedure

1. **Place ice cream, milk and syrup in blender container.**
2. **Poured the whole mixture in to a glass.**
3. **Now garnish with cashew nut.**
4. **Now it is ready to serve.**

Nutritional importance

Chocolate milk provides important nutrients — such as calcium, protein, and vitamin D — which may benefit health. However, it's high in calories and added sugar, which can contribute to weight gain and may increase your risk of certain chronic diseases.

Cereals and Flour Mixture
Chapathi

Ingradient

- 1 Cup Whole Wheat Flour, Atta
- ½ Cup Water, at room temperature
- 1 teaspoon salt

INSTRUCTIONS

1. Put one cup of flour in a bowl. Add half a cup of water and mix with a spoon. When there is no more water visible, stop mixing. Cover the bowl and let it rest for 30 minutes.
2. After 30 minutes, add the salt, gently mix the dough for 2 minutes, just enough to gather the dough into one piece. Cover the bowl and set aside for 30 minutes.
3. Divide the dough into lime size balls. Set an iron pan on medium high heat. Let it become hot. Take a dough ball and generously dust it with flour. Roll the dough into a 4 inch round on a flat smooth surface using a rolling pin. Place the chapati on the hot griddle. Let it cook for 10-15 seconds until bubbles starts to form on top. Flip the chapati and cook for 30 seconds more. Gently press on top of the chapati to make the chapati fluff up. Flip one more time and press on top. Chapati should beautifully fluff up. If making phulkas, just flip on direct flame and it will fluff up.

4. Line a bowl with cotton towel. Place the cooked chapati on the bowl and cover with a towel. Chapati will stay beautifully soft for a long time.

Nutritive Importance:

Roti or **chapati** is an inalienable part of Indian diet. ... A plain roti is an excellent source of soluble fibre, which helps lower blood cholesterol levels, prevents constipation and helps keep our digestive system healthy.

Nutrition Facts

Amount Per Serving

Calories 82

	% Daily Value*
Total Fat 0.6g	**1%**
Saturated Fat 0.1g	**1%**
Cholesterol 0g	**0%**
Sodium 83.4mg	**3%**
Total Carbohydrate 17.3g	**6%**
Dietary Fiber 2.6g	**10%**
Sugars 0.1g	
Protein 3.2g	6%

Vitamin A 0% • Vitamin C 0%

Calcium 1% • Iron 5%

Rice Polao

Ingradient: Basmati rice-1 cup
Onion-2 Big Piece
Carrot- 1 pice
Capsicum-1 piece
Beans- 50 g
Peas- 30 g
Clove- 2g
Cumin-2g
Cinnamon- 1g
Cardamom- 1g
Salt-3g
Green chilies- 7 g
Bay leave- 1g

Procedure:

1. Begin by rinsing 1.5 cups basmati rice in water until it runs clear of starch. Next, soak the rice in enough water for 30 minutes.
2. After 30 minutes strain the rice of all the water and keep aside.
3. While the rice is soaking, prep the veggies. Rinse and chop them. Make sure to chop the vegetables in small cubes. For cauliflower, chop them in medium-sized florets. Peel, rinse and slice 1 large onion thinly.
4. Keep all the whole spices aside. From the below list of spices, you can skip black pepper, black cardamom.
5. Add chopped 1 to 2 green chilies (green peppers) in a mortar-pestle.
6. Heat 3 tablespoons ghee or oil in a deep thick bottomed pot or pan.
7. Once the ghee has melted and become hot, add all whole spices and fry for a few seconds until they become fragrant. The spices will also splutter while frying. The ghee should not smoke or become too hot.
8. Add 1 cup of thinly sliced onions.
9. Mix the onions with the ghee and begin to sauté them.
10. Now add all the chopped veggies. You will need about 1 to 1.5 cups of mixed chopped vegetables.
11. Mix and sauté again for 2 to 3 minutes on a low flame.
12. Add the rice.
13. Mix rice gently with the rest of the ingredients.
14. Sauté rice gently for 1 to 2 minutes on a low to medium-low flame, so that the rice gets well coated with the oil or ghee.
15. Pour 2.5 to 3 cups water into the pan. I added 3 cups of water. Depending on the quality of rice you have used, you can add less or more water. You can replace water with vegetable stock.
16. Season with salt. Mix very well. To figure out if you have added the right amount of salt, check the taste of the water.
17. Once the rice grains are cooked, fluff them and let the rice stand for 5 minutes covered with the lid.
18. Serve pulao garnished with coriander leaves (cilantro), mint leaves or fried onions and fried cashews or fried raisins.

Nutritive Importance:

It is composed of many vegetables which is good sourse of vitamin and minerals which play an important role to maintain a healthy lifestyle.

Sandwich

Ingradient:

- 10 slices white or brown bread
- 2 small to medium potatoes, boiled and sliced into rounds
- 1 cucumber, thinly sliced
- 1 small to medium onion,
- 1 medium tomato, thinly sliced
- butter softened and at room temperature, salted or unsalted, as required, cheese
- chaat masala as required
- black salt to taste
- roasted cumin powder as required- optional
- freshly ground black pepper , optional
- tomato ketchup or sauce to be served with the sandwiches

Procedure:

1. Trim the edges of the bread slices if you want.
2. Butter the bread slices evenly and very well and also add the cheese slice.
3. Place all the veggie slices one by one.
4. Sprinkle the black salt and chaat masala on each one of them or alternately.
5. Cover the sandwich with the other bread slices.
6. Slice into triangles and rectangles.
7. Then placed into asandwich maker.
8. Serve bombay veg sandwich immediately with tomato ketchup and the coriander chutney.

Nutritive value:

1. As sandwich is composed of green vegetables, so that it provides essential vitamin and minerals that nourish our body.
2. It is made up of non irritating without spice so it is best for all individuals and patients.
3. It is consist of butter and cheese so, it provides energy anf helps to transport fat soluble vitamin.

Cookies

1. Drop Cookies: These are made from relatively soft dough that is dropped by spoonful on to the baking sheet. During bak-ing, the mounds of dough spread and flatten. Chocolate chip cookies, oatmeal cookies and rock cookies are popu-lar examples of drop cookies.

Quantity Ingredients

Maida : 2½ cups
Butter : 1 cup
Sugar : 1 cup
Egg : 1
Milk : 2 table spoons
Vanilla essence : 1 teaspoon
Salt : a pinch

▲ Plate 5.6 Drop Cookies

Method

· Preheat oven to 190°C.
· Lightly coat 2 cookie sheets with veg-etable oil.
· Sift flour, baking powder and salt together.
· Beat egg yolks in a mixer bowl until pale and thick.
· In a clean mixer bowl, with clean beat-ers, beat egg whites to soft peaks.
· Beat in sugar 1 table spoon at a time, until stiff and glossy.
· Gently fold egg yolks into egg whites.
· Fold in dry ingredients and milk until just blended.
· Drop by level tablespoonfuls 2 inches apart onto prepared cookie sheets.
· Bake for 10 minutes or until golden.
· Carefully transfer to wire racks to cool

Nutritive value: The following nutrition information is provided by the USDA for one serving (three cookies or 34g) of Oreo cookies.

- **Calories:** 160
- **Fat:** 7g
- **Sodium:** 135mg
- **Carbohydrates:** 25g
- **Fiber:** 1.2g
- **Sugars:** 14g
- **Protein:** 1g

Vegetables and fruits
Fruit juice

Ingradient:

- 1 cup pomegranate seeds
- 1 cup grapefruit
- 2 tablespoon lemon juice
- 1 tablespoon honey
- 1/2 cup water
- 1 orange
- 1 mango
- 1/2 cup ice cubes
- 2 sprigs mint leaves

How to make Healthy Fruit Juice

- Step 1

  To prepare this healthy juice, wash all the fruits properly and peel and chop them, if required.
- Step 2

  Add them to a food processor with water, lemon juice, ice cubes and honey and blend them together.
- Step 3

  Then, pour the juice into a chilled glass using a strainer to remove the pulp. Garnish with mint leaves and serve.

Nutritional Importance:

Fruit juices contain a range of minerals, vitamins and bioactive compounds, such as phytochemicals, that are important for good health. Guidelines for a healthy, balanced diet typically recommend plenty of fruits and vegetables are required to supply our vitamin and mineral needs.

1.A. Basic Concept of Carbohydrate

❖ *Quality analysis of carbohydrate compounds:*

- **Introduction:**

A carbohydrate is an organic compound with the general formula Cm (H2O) n, that is, consists only of carbon, hydrogen and oxygen, with the last two in the 2:1 atom ratio. Carbohydrates make up the bulk of organic substances on earth and perform numerous roles in living things.

The carbohydrates (saccharides) are divided into four chemical groups: monosaccharaides, disaccharides, oligosaccharides and polysaccharides. Polysaccharides serve for the storage of energy (e.g., starch in plants and glycogen in animals) and as structural components (e.g., cellulose in plants and chitin in arthropods). Structural polysaccharides are frequently found in combination with proteins (glycoproteins or mucoproteins) or lipids (lipopolysaccharides). The 5-carbon monosaccharide ribose is an important component of coenzymes (e.g., ATP, FAD and NAD) and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA. Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting and development [1]. This experiment aims to introduce you with the identification of unknown carbohydrates. To gain maximum benefit, observations should be related, as far as possible, to the structure of the substances examined.

TESTS ON CARBOHYDRATES:

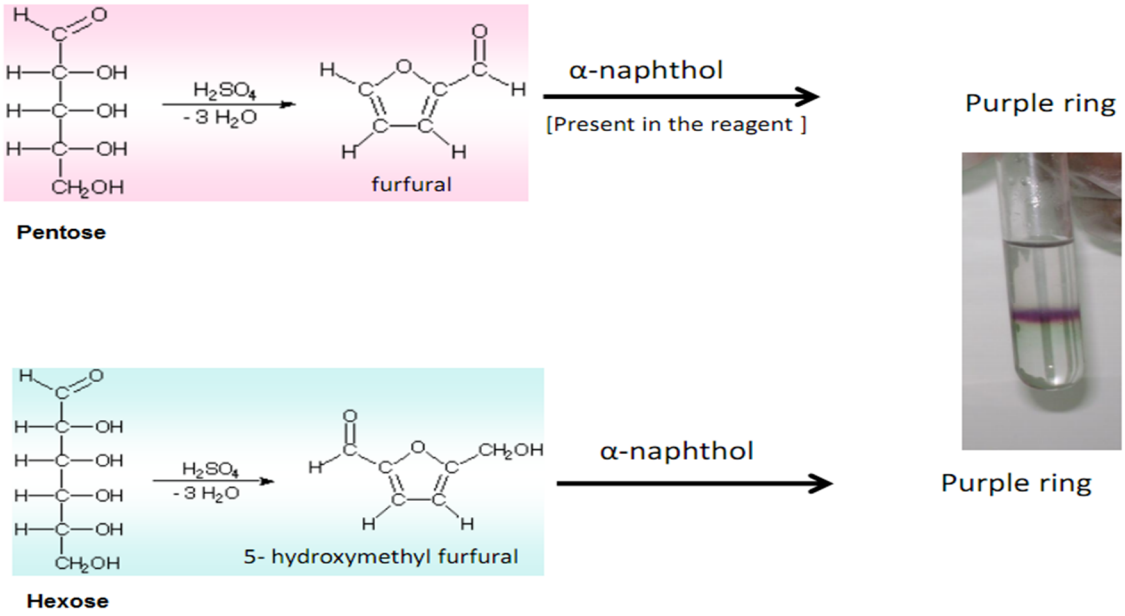
1) Molisch's Test:

Molisch's Test is a sensitive chemical test for all carbohydrates, and some compounds containing carbohydrates in a combined form, based on the dehydration of the carbohydrate by sulphuric acid to produce an aldehyde (either furfural or a derivative), which then condenses with the phenolic structure resulting in a red or purple-colored compound.

- **Procedure: -**

- Apply this test two different carbohydrate solutions of your own choice, preferably to one monosaccharide and one polysaccharide.
- Place 2 mL of a known carbohydrate solution in a test tube, add 1 drop of Molisch's reagent (10% α-naphthol in ethanol).
- Pour 1-2 mL of conc. H2SO4 down the side of the test tube, so that it forms a layer at the bottom of the tube.
- Observe the color at the interface between two layers and compare your result with a control test.

A brown color due to charring must be ignored and the test should be repeated with a more dilute sugar solution.



2) Carbohydrates as Reducing Sugars:

A reducing sugar is any sugar that, in a solution, has an aldehyde or a ketone group. The enolization of sugars under alkaline conditions is an important consideration in reduction tests. The ability of a sugar to reduce alkaline test reagents depends on the availability of an aldehyde or keto group for reduction reactions. A number of sugars especially disaccharides or polysaccharides have glycosidic linkages which involve bonding a carbohydrate (sugar) molecule to another one, and hence there is no reducing group on the sugar; like in the case of sucrose, glycogen, starch and dextrin. In the case of reducing sugars, the presence of alkali causes extensive enolization especially at high pH and temperature. This leads to a higher susceptibility to oxidation reactions than at neutral or acidic pH. These sugars, therefore, become potential agents capable of reducing Cu+2 to Cu+, Ag+ to Ag and so fort. Most commonly used tests for detection of reducing sugars are Fehling's Test, Benedict's test and Barfoed's Test.

a) Fehling's Test: Fehling's Solution (deep blue colour) is used to determine the presence of reducing sugars and aldehydes. Perform this test with fructose, glucose, maltose and sucrose.

- **Procedure:**

- To 1 mL of Fehling's solution A (aqueous solution of CuSO4) add 1 mL of Fehling solution B (solution of potassium tartrate).
- Add 2 mL of the sugar solution, mix well and boil.

- **Try to see the red precipitate of cuprous oxide that forms at the end of the reaction.**

b) Barfoed's Test:

Barfoed's reagent, cupric acetate in acetic acid, is slightly acidic and is balanced so that is can only be reduced by monosaccharaides but not less powerful reducing sugars. Disaccharides may also react with this reagent, but the reaction is much slower when compared to monosaccharaides. Perform this test with glucose, maltose and sucrose.

- **Procedure:**
  - To 1-2 mL of Barfoed's reagent, add an equal volume of sugar solution.
  - Boil for 5 min. in a water bath and allow to stand.

- **You will observe a brick-red cuprous oxide precipitate if reduction has taken place**

c) Seliwanoff's Test:

Seliwanoff's Test distinguishes between aldose and ketoses' sugars. Ketoses are distinguished from aldoses via their ketone/aldehyde functionality. If the sugar contains a ketone group, it is a ketoses' and if it contains an aldehyde group, it is an aldose. This test is based on the fact that, when heated, ketoses are more rapidly dehydrated than aldoses. Perform this test with glucose, fructose, maltose and sucrose.

- **Procedure:**
  - Heat 1 mL of sugar solution with 3 mL Seliwanoff's reagent (0.5 g resorcinol per litter 10% HCl) in boiling water.
    - **In less than 30 seconds, a red color must appear for ketoses.**
    - **Upon prolonged heating, glucose will also give an appreciable color.**

d) Bial's Test:

Bial's Test is to determine the presence of pentose (5C sugars). The components of this reagent are resorcinol, HCl, and ferric chloride. In this test, the pentose is dehydrated to form furfural and the solution turns bluish and a precipitate may form. Perform this test with ribose and glucose.

- **Procedure:**
  - To 5 mL of Bial's reagent, add 2-3 drops of sugar solution and boil.
    - **Upon boiling, note the green-blue color formed. The boiling step is common for each test for the reducing sugars**.

3) Action of Alkali on Sugars:

- **Procedure:**
  - Heat 1 mL glucose solution with 1 mL 40% NaOH for 1 min.
  - Cool and apply test for reducing sugars (e.g.; Fehling's Test).
  - Apply a control test with glucose solution to observe the difference.

4) The Inversion of Sucrose:

Sucrose is a disaccharide, which means that it is a molecule that is derived from two simple sugars (monosaccharaides). In the case of sucrose, these simple sugars are glucose and fructose. Inverted sugar is a mixture of glucose and fructose. It is obtained by splitting sucrose into these two components. The splitting of sucrose is a hydrolysis reaction which can be induced simply by heating an aqueous solution of sucrose. Acid also accelerates the conversion of sucrose to invert.

- **Procedure:**
  - Add 5 mL of sucrose solution to two test tubes.
  - Add 5 drops of conc. HCl to one test tube.
  - Heat both tubes in boiling water bath for 10 min.
  - Col and neutralize with diluted NaOH (use litmus paper).
  - Test both solutions for the presence of reducing sugar with Fehling's Test.

5) Iodine Test: Iodine test is an indicator for the presence of starch. Iodine solution (iodine dissolved in an aqueous solution of potassium iodide) reacts

with starch producing a blue-black color. Apply this test to all the polysaccharides provided.

- **Procedure:**
  - To 2-3 mL of polysaccharide solution, add 1-2 drops of iodine solution.
  - Observe the different colours obtained for each of the polysaccharide solutions.
- **Starch turns into an intense "blue-black" colour.**

❖ Known test for Glucose:

Sl. No.	Experiment	Observation	Inference
1.	**Molisch's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Molisch's reagent is added to it and mix well. After that 1-2 ml of concentrated H_2SO_4 solution is added slowly added down the sides of the sloping test-tube, without mixing, to form a layer.	A distinct purple coloured ring developed within the junction of two liquids of the test tube	This is general test for all Carbohydrate due to the formation of hydroxymithyle furfural. As glucose is a monosaccharide it reacts with the solution.
2.	**Benedict's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then ½ of Benedict reagent is added to it and mix. Then boil the solution using a Bunsen burner on the reducing flame.	At first greenish yellow colour is developed just after heating then it turns to brick red colour after vigorous boiling.	This is the positive test for all reducing sugar. As glucose is a reducing monosaccharide so, it gives red colour due to formation of Cu_2O (Cuprous oxide)
3.	**Barfoed's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube.	Red precipitate is developed at the	This is a positive test for only reducing monosaccharide it

	Then just 2-3 drops of Barfoed's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	bottom of the test tube	reacts with the solution due to the formation of Cu_2O.
4.	**Fehling's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Fehling's reagent is added to it and mix well. Then heat vigorously.	At first yellow colour developed and then red precipitate is formed.	This is the positive test for reducing sugar due to formation of Cu_2O. As glucose is a reducing monosaccharide, it reacts with the solution.
5.	**Saliwanoff's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Saliwanoff's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	No changed	This is the positive sugar for keto sugars only. As glucose is an aldosugar it does not react with the solution.
6.	**Iodine Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Iodine solution is added to it and mix well and weight for 3 minutes.	No changed.	This is the positive test for the polysaccharide. As glucose is a reducing monosaccharide so, it does not react with iodine.

❖ Known test for Fructose:

Sl. No.	Experiment	Observation	Inference
1.	**Molisch's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Molisch's reagent is added to it and mix well. After that 1-2 ml of concentrated H_2SO_4 solution is added slowly added down the sides of the sloping test-tube, without mixing, to form a layer.	A distinct purple coloured ring developed within the junction of two liquids of the test tube	This is general test for all Carbohydrate due to the formation of hydroxymithyle furfural. As fructose is a monosaccharide it reacts with the solution.
2.	**Benedict's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then ½ of Benedict reagent is added to it and mix. Then boil the solution using a Bunsen burner on the reducing flame.	At first greenish yellow colour is developed just after heating then it turns to brick red colour after vigorous boiling.	This is the positive test for all reducing sugar. As fructose is a reducing monosaccharide so, it gives red colour due to formation of Cu_2O (Cuprous oxide).
3.	**Barfoed's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Barfoed's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	Red precipitate is developed at the bottom of the test tube	This is a positive test for only reducing monosaccharide. As fructose is a reducing monosaccharide so it gives positive result. It reacts with the solution due to the formation of Cu_2O.
4.	**Fehling's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube.	At first yellow colour developed and then red precipitate is formed.	This is the positive test for reducing sugar due to formation of Cu_2O. As fructose is a

	Then just 2-3 drops of Fehling's reagent is added to it and mix well. Then heat vigorously.		reducing monosaccharide, it reacts with the solution.
5.	**Saliwanoff's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Saliwanoff's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	Red colour developed.	This is the positive sugar for keto sugars only. As fructose is a ketosugar, it react with the solution and give red colour.
6.	**Iodine Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Iodine solution is added to it and mix well and weight for 3 minutes.	No changed.	This is the positive test for the polysaccharide. As fructose is a reducing monosaccharide so, it does not react with iodine.

❖ Known test for Lactose:

Sl. No.	Experiment	Observation	Inference
1.	**Molisch's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Molish's reagent is added to it and mix well. After that 1-	A distinct purple coloured ring developed within the junction of two liquids of the test tube.	This is general test for all Carbohydrate so, lactose (reducing disaccharide) react with molisch's test due to the formation of

	2 ml of concentrated H_2SO_4 solution is added slowly added down the sides of the sloping test-tube, without mixing, to form a layer.		hydroximethayl furfural.
2.	**Benedict's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then half of benedict reagent added to it and mix. Then boil the solution using a Bunsen burner on the reducing flame.	At first greenish yellow colour is developed just after heating then it turns to brick red colour after vigorous boiling.	This is the positive test for all reducing sugar. As lactose is a reducing monosaccharide so, it gives red colour due to formation of Cu_2O (Cuprous oxide).
3.	**Barfoed's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Barfoed's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	Red precipitate is developed at the bottom of the test tube	This is a positive test for only reducing sugar. As lactose is reducing sugar. It react in benedict test due to the formation of cuprous oxide (cu_2o).
4.	**Fehling's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Fehling's reagent is added to it and mix well. Then heat vigorously.	At first yellow colour developed and then red precipitate is formed.	This is the positive test for reducing sugar due to formation of Cu_2O. As lactose is a reducing monosaccharide, it reacts with the solution.
5.	**Saliwanoff's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Saliwanoff's reagent is added to it and mix well. Then heat vigorously and	Red colour developed.	This is the positive sugar for keto sugars only. As lactose is an aldosugar, it does not react with the solution.

	finally cooled at room temperature.		
6.	**Iodine Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Iodine solution is added to it and mix well and weight for 3 minutes.	No changed.	This is the positive test for the polysaccharide. It does not react with iodine reagent.

❖ Known test for Maltose:

Sl. No.	Experiment	Observation	Inference
1.	**Molisch's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Molish's reagent is added to it and mix well. After that 1-2 ml of concentrated H_2SO_4 solution is added slowly added down the sides of the sloping test-tube, without mixing, to form a layer.	A distinct purple coloured ring developed within the junction of two liquids of the test tube.	This is general test for all Carbohydrate so, maltose (reducing disaccharide) react with molisch's test due to the formation of hydroximethayl furfural.
2.	**Benedict's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then half of benedict reagent added to it and mix. Then boil the solution using a Bunsen burner on the reducing flame.	At first greenish yellow colour is developed just after heating then it turns to brick red colour after vigorous boiling.	This is the positive test for all reducing sugar. As maltose is a reducing monosaccharide so, it gives red colour due to formation of Cu_2O (Cuprous oxide).

3.	**Barfoed's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Barfoed's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	Red precipitate is developed at the bottom of the test tube	This is a positive test for only reducing sugar. As maltose is reducing sugar. It react in benedict test due to the formation of cuprous oxide (cu_2o).
4.	**Fehling's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Fehling's reagent is added to it and mix well. Then heat vigorously.	At first yellow colour developed and then red precipitate is formed.	This is the positive test for reducing sugar due to formation of Cu_2O. As maltose is a reducing monosaccharide, it reacts with the solution.
5.	**Saliwanoff's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Saliwanoff's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	Red colour developed.	This is the positive sugar for keto sugars only. As Maltose is an aldosugar so, it does not react with the solution.
6.	**Iodine Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Iodine solution is added to it and mix well and weight for 3 minutes.	No changed.	This is the positive test for the polysaccharide.as maltose is a disaccharide so, it does not react with iodine reagent.

❖ Known test for Sucrose:

Sl. No.	Experiment	Observation	Inference
1.	**Molisch's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Molish's reagent is added to it and mix well. After that 1-2 ml of concentrated H_2SO_4 solution is added slowly added down the sides of the sloping test-tube, without mixing, to form a layer.	A distinct purple coloured ring developed within the junction of two liquids of the test tube.	This is general test for all Carbohydrates. As sucrose is carbohydrate so, it reacts with molisch's test due to the formation of hydroximethayl furfural.
2.	**Benedict's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then half of benedict reagent added to it and mix. Then boil the solution using a Bunsen burner on the reducing flame.	No change.	This is the positive test for all reducing sugar. As sucrose is a non-reducing sugar so, it does not react with this solution.
3.	**Barfoed's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Barfoed's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	No change.	This is the positive test for all reducing sugar. As sucrose is a non-reducing sugar so, it does not react with this solution.
4.	**Fehling's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube.	No change.	This is the positive test for all reducing sugar. As sucrose is a non-reducing sugar so, it

	Then just 2-3 drops of Fehling's reagent is added to it and mix well. Then heat vigorously.		does not react with this solution.
5.	**Saliwanoff's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Saliwanoff's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	Red colour developed.	This is the positive sugar for keto sugars only. As Sucrose is an aldosugar so, it does not react with the solution.
6.	**Iodine Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Iodine solution is added to it and mix well and weight for 3 minutes.	No changed.	This is the positive test for the polysaccharide.as sucrose is a disaccharide so, it does not react with iodine reagent.
7.	**Hydrolysis Test:** About 2-3ml of supplied carbohydrate solution is taken in a dry test tube. Then few drops of conc. H_2SO_4 con. HCl is added to it and then boiled. After boiling cooled the solution under tap water and then neutralize the solution by adding Na_2CO_3 until no further bubbles occur. Then perform Benedict's Test.	At first greenish yellow colour is developed just after heating then it turns to brick red colour after vigorous boiling.	This is the positive test for all carbohydrate. As sucrose is a non-reducing sugar and converted to glucose after acid hydrolysis. And the reducing sugar test is perform by using Benedict's Test and it gives the brick red colour due to formation of Cu_2O.

❖ Known test for Starch:

Sl. No.	Experiment	Observation	Inference
1.	**Molisch's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Molish's reagent is added to it and mix well. After that 1-2 ml of concentrated H_2SO_4 solution is added slowly added down the sides of the sloping test-tube, without mixing, to form a layer.	A distinct purple coloured ring developed within the junction of two liquids of the test tube.	This is general test for all Carbohydrates. As starch is carbohydrate so, it reacts with molisch's test due to the formation of hydroximethayl furfural.
2.	**Benedict's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then half of benedict reagent added to it and mix. Then boil the solution using a Bunsen burner on the reducing flame.	No change.	This is the positive test for all reducing sugar. As starch is a non-reducing sugar so, it does not react with this solution.
3.	**Barfoed's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Barfoed's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	No change.	This is the positive test for all reducing sugar. As starch is a non-reducing sugar so, it does not react with this solution.
4.	**Fehling's Test:** About 2-3 ml of supplied carbohydrate solution is	No change.	This is the positive test for all reducing sugar. As starch is a non-

	taken in a dry test tube. Then just 2-3 drops of Fehling's reagent is added to it and mix well. Then heat vigorously.		reducing sugar so, it does not react with this solution.
5.	**Saliwanoff's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Saliwanoff's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	Red colour developed.	This is the positive sugar for keto sugars only. As Starch is an aldosugar so, it does not react with the solution.
6.	**Iodine Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Iodine solution is added to it and mix well and weight for 3 minutes.	Blue colour developed.	This is the positive test for the polysaccharide.as starch is a polysaccharide so, it reacts with iodine reagent.
7.	**Hydrolysis Test:** About 2-3ml of supplied carbohydrate solution is taken in a dry test tube. Then few drops of conc. H_2SO_4 con. HCl is added to it and then boiled. After boiling cooled the solution under tap water and then neutralize the solution by adding Na_2CO_3 until no further bubbles occur. Then perform Benedict's Test.	At first greenish yellow colour is developed just after heating then it turns to brick red colour after vigorous boiling.	This is the positive test for all carbohydrate. As starch is a non-reducing sugar and converted to glucose after acid hydrolysis. And the reducing sugar test is perform by using Benedict's Test and it gives the brick red colour due to formation of Cu_2O.

❖ Known test for Dextrin:

Sl. No.	Experiment	Observation	Inference
1.	**Molisch's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Molish's reagent is added to it and mix well. After that 1-2 ml of concentrated H_2SO_4 solution is added slowly added down the sides of the sloping test-tube, without mixing, to form a layer.	A distinct purple coloured ring developed within the junction of two liquids of the test tube.	This is general test for all Carbohydrates. As dextrin is carbohydrate so, it reacts with molisch's test due to the formation of hydroximethayl furfural.
2.	**Benedict's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then half of benedict reagent added to it and mix. Then boil the solution using a Bunsen burner on the reducing flame.	No change.	This is the positive test for all reducing sugar. As Dextrin is a non-reducing sugar so, it does not react with this solution.
3.	**Barfoed's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Barfoed's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	No change.	This is the positive test for all reducing sugar. As dextrin is a non-reducing sugar so, it does not react with this solution.
4.	**Fehling's Test:** About 2-3 ml of supplied carbohydrate solution is	No change.	This is the positive test for all reducing sugar. As starch is a non-

	taken in a dry test tube. Then just 2-3 drops of Fehling's reagent is added to it and mix well. Then heat vigorously.		reducing sugar so, it does not react with this solution.
5.	**Saliwanoff's Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Saliwanoff's reagent is added to it and mix well. Then heat vigorously and finally cooled at room temperature.	Red colour developed.	This is the positive sugar for keto sugars only. Dextrin is an aldosugar so, it does not react with the solution.
6.	**Iodine Test:** About 2-3 ml of supplied carbohydrate solution is taken in a dry test tube. Then just 2-3 drops of Iodine solution is added to it and mix well and weight for 3 minutes.	Blue colour developed.	This is the positive test for the polysaccharide.as dextrin is a polysaccharide so, it reacts with iodine reagent.
7.	**Hydrolysis Test:** About 2-3ml of supplied carbohydrate solution is taken in a dry test tube. Then few drops of conc. H_2SO_4 con. HCl is added to it and then boiled. After boiling cooled the solution under tap water and then neutralize the solution by adding Na_2CO_3 until no further bubbles occur. Then perform Benedict's Test.	At first greenish yellow colour is developed just after heating then it turns to brick red colour after vigorous boiling.	This is the positive test for all carbohydrate. As dextrin is a non-reducing sugar and converted to glucose after acid hydrolysis. And the reducing sugar test is perform by using Benedict's Test and it gives the brick red colour due to formation of Cu_2O.

B. Estimation of Percentage (%) of reducing sugar (glucose) by Benedict's Method using Benedict Quantitative Reagent:

- **Principle:**
  a) Copper sulphate present in Benedict Quantitative Reagent (BQR) then copper is cupric state (Cu^{2+}).
  b) Glucose can able to reduced cupric ion and produce cuprous ion that react with potassium thiocyanate present in BQR and the compound which is produced cuprous thiocyanate which is produced cuprous thiocyanate, which form white precipitate.
  c) Potassium ferrocyanate is also present in BQR that keeps the cuprous oxide in soluble form which is responsible for red colour.
  d) Na_2CO_3 is another component of BQR and it provides two major functions-
     1. In alkaline medium glucose is transformed to enediol form which is highly reducing nature that reduced Cu^{2+}
     2. This alkaline media also protect the glucose from denaturation.

- **Requirement:**
  1. Glucose solution(supplied)
  2. Benedict Quantitative Reagent.
  3. Distilled water.
  4. 100ml and 50ml conical flask.
  5. 10ml and 5ml pipette.
  6. 100ml volumetric flask.
  7. 25 ml burette with burette stand.
  8. Bunsen burner
  9. Filter paper.

- **Procedure:**
  1. At first 10ml of supplied solution is taken in a volumetric flask and make the volume up to the marks with distilled water.
  2. Transfer this solution in to a burette.
  3. Noted the initial burette reading.
  4. 10ml of benedict Quantitative Reagent was taken by 10ml of volumetric pipette and transferred it into a 100ml conical flask.
  5. 1-2 pinch of anhydrous sodium carbonate (Na_2CO_3) into conical flask and placed it over a tripod stand.
  6. Heat the reagent gently on a low flame.
  7. While it started boiling, add the diluted glucose solution drop by drop by burette (drop the solution only during boiling)

8. Disappearance of blue colour and appearance of pale white precipitate indicates end point of titration.
9. Final burette reading is noted down.

- **Observation:**

No. of observation	Burette Reading(ml)		Volume of dilute glucose solution required(Final burette reading – Initial burette reading)ml	Mean volume of glucose solution required(ml)
	Initial(ml)	Final(ml)		
1.	0	5.3	5.3	5.3

- **Calculation:**

10ml of BQR reduced by 0.02g glucose
So, 5.3 ml glucose solution reduce 10ml of BQR.
So we can told that,

10ml = 0.02g

5.3ml of glucose solution contains 0.02ml
1ml of glucose solution contains 0.02/5.3 ml
100ml of glucose solution contains (0.02*100)/5.3 = 3.37g

Hence, the supplied sample glucose solution contain 3.37g glucose.

Estimation of Total reducing sugar in foods (rice power) by Benedict's method using Benedict Quantitative Reagent:

- **Introduction:** Starch enriched cereals are rice, wheat etc. In refined rice, starch level is 75.2g% and in non-refined it is 7.4g%. In non-refine wheat power starch level is 75g5 and in refined wheat power its level is 74g%. Starch is non reducing polysaccharide of carbohydrate. For starch quantification, it is subjected to hydrolysis by the treatment of 1(N) HCL that produced free glucose. Reduction capacity of glucose is used and amount of starch in food stuff is express in terms of glucose.

- **Principle:** Starch is hydrolysis by treatment of 1(N) HCL and produced glucose molecule and are used for titration after separation of protein by titrating the solution with Ba $(OH)_2$ and $ZnSO_4$. Starch is quantified in the foods in terms of glucose.

- **Biochemical reaction in this experiment:**
  1. Copper sulphate present in BQR when copper is present in cupric state(Cu^{2+})
  2. Glucose can able to reduced cupric ion and produced cuprous ion that react with potassium thiocyanated present in BQR and the compound which is produced cuprous thiosyanate which form white precipitate.
  3. Potassium ferrocyanate is also present in BQR that keep the cuprous oxide in soluble form which prevents the appearance of the red colour.
  4. Na2Co3 is another component of BQR and it provides two major functions-
     a. In alkaline media glucose is transfer into enediol form which is high reducing in nature that reduce Cu^{2+} to Cu^{+} in very short time.
     b. This alkaline media protect the glucose from denaturation.

- **Requirements:**
  1. Food powder
  2. Benedict Quantitative Reagent
  3. Distilled water
  4. 1(N) HCl
  5. 10% $Ba(OH)_2$ Solution
  6. 100ml and 50 ml conical flask
  7. 10ml and 5ml pipette
  8. 100ml volumetric flask
  9. 25ml burette with burette stand
  10. Bunsen burner
  11. Filter paper
  12. Anhydrous Na_2CO_3
  13. Water bath.

- **Procedure:**

a) Hydroxylation of food starch by acid:

1. 1g of supplied food powder was transfer to 100/250ml conical flask.
2. 50ml 1(N) HCL was added and the mouth of this flask plugged by cotton.
3. This conical was placed in a tripod stand under Bunsen burner for boiling (10-15min).
4. After boiling the total set allow to cool at room temperature and neutralize by Na_2CO_3 unless the frothing is abolished.

b) Titration against BQR:

1. Neutralize solution was transfer in to 100ml volumetric flask where 10ml 10% $ZnSO_4$, 10% Ba $(OH)_2$ solution was added and mixed.

2. The volume of flask was made up to the mark by distilled water.
3. The supernatant is filtered and filtrate was collected and transfer to the burette.

c) Titration against BQR:

1. 10ml of BQR was transfer to 50ml conical flask and 1 pinch of Na_2CO_3 was added.
2. The conical flask was place over the tripod stand of under the Bunsen burner.
3. Heat the reagent gently on low flame.
4. While it started boiling, the diluted glucose solution was add drop by drop from the burette (drop the solution only during boiling)
5. Disappearance of blue colour and appearance of pale white precipitate indicate the end point of titration.
6. Final burette reading is noted down.

- **Observation table:**

No. of observation	Burette Reading(ml)		Volume of dilute glucose solution required(Final burette reading – Initial burette reading)ml	Mean volume of glucose solution required(ml)
	Initial(ml)	Final(ml)		
1.	0	3.7	3.7	3.6
2.	3.7	7.2	3.5	
3.	7.2	10.8	3.6	

- **Computation and result:**

Given data 10ml BQR = 20mg glucose

3.6ml diluted sample contain 20mg of glucose
1ml diluted sample contain 20 / 3.6 mg of glucose
100ml diluted sample contain (20*100)/ (3.6) = 555.5mg of glucose

1g food powder contain 555.55mg of glucose
100g food powder contain (555.55*100)/ (1000) g% starch in terms of glucose
=55.5g%
Hence, the supplied food powder contain 55.5g5 of glucose.

- **Interference:** According to Gopalan, 100g of rice powder contain 79g% glucose, but here the supplied food power contain 55.5g% glucose, s, it is very low in reducing sugar.

C. Estimation of Percentage of Lactose in Milk by Benedict Quantitative Method

Principle:

The protein of milk consist of casinogen, lactoalbumin and lacto globulin. The protein are precipitated by tungstic acid. Lactose is the only reducing sugar present in milk, filtrate of milk is estimated by the reduction of BQR.

Requirement:

A. Chemical Reagent:-

1. 10ml 10% sodium tungstate- 1g sodium tungstate added in 10ml distilled water.
2. 10ml 2/3 normal H_2SO_4
3. Anhydrous Na_2Co_3
4. Benedict Quantitative Reagent (BQR)

B. Glass Goods:-

1. 50ml conical flask
2. 100ml conical flask
3. 100ml volumetric flask

c. Miscellaneous:-

1. Supplied milk
2. Bunsen Burner
3. Filter paper
4. Distilled Water
5. Glass Beads

Procedure:

A. Preparation & Dilution of Milk:

250ml of mother dairy milk is supplied in the lab.

B. Preparation of Protein free titrate:

1. Pipetting of 10ml milk and transfer it to the 100ml volumetric flask.
2. Added it to 10% 10ml Sodium tungstate and 10ml 2/3 normal H_2SO_4.
3. Make the volume upto 100ml marked with distilled water.
4. Then mixed thoroughly and allow the solution to stand for 5 min.
5. Filter the solution and taken the filtrate in a 25ml burette.

C. Preparation of Filtration against BQR:

1. Taken 10ml BQR in a 50ml conical flask add about 1 pinch of anhydrous Na_2Co_3 and 2-3 pieces of glass beads.
2. The conical flask was placed over the tripod stand and heat the solution gently over a control flame and while boiling added lactose solution from the burette drop by drop.
3. The disappearance of blue colour and appearance of white gelatinous PPT indicates the end point of titration.
4. The same procedure was repeated 2 times.

Result:

Sl No.	Burette Reading		Difference	Average
	Initial (ml)	Final (ml)		
1	**0.00**	**5.2**	**5.2**	
2	**5.2**	**10.4**	**5.2**	**5.2**
3	**10.4**	**15.6**	**5.2**	

Calculation:

10ml BQR= 0.0268g lactose

5.2ml supplied milk solution reduced lactose- 0.0268g

1ml supplied milk solution reduced lactose- $\frac{0.0268}{5.2}$

100ml supplied milk solution reduced lactose- $\frac{0.0268}{5.2} \times 100$

$= 0.515$ g

Here the supplied milk solution is diluted by 10 times, so, the percentage of lactose is supplied milk solution = 0.515×10g% lactose= 5.15g% lactose.

2. GENERAL QUALITATIVE ANALYSIS OF LIPIDS

- **Introduction:** Lipids are biological molecules characterized by limited solubility in water and solubility in non-polar organic solvents. Their intermolecular interactions are dominated by the hydrophobic effect and van der Waals interactions. Many lipids are, however, amphipathic molecules, which interact with other molecules and with aqueous solvents via hydrogen bonding and electrostatic interactions.

  Importance:

  Lipids are major components of cell membranes, and are responsible for most of the permeability filter functions of membranes. Membranes act as barriers to separate compartments within eukaryotic cells, and to separate all cells from their surroundings. Lipid are store in the adipose tissues for energy production when necessary. Lipid are also deposited under the skin, tissues, and other vital organ in the form of adipose tissue. Phospholipid, glycolipid, lipoprotein and steroids from structural compound like plasma membrane, mitochondria, endoplasmic reticulum and myelin sheath. Phospholipid helps in intestinal absorption for the nonpolar nutrient like fat, fatty acid, function as regulatory and information molecules. Phospholipid helps in the excretion of cholesterol in the bile.

- **Classification of Lipid:**

  Lipids are classified broadly into three groups-

  1. Simple Lipid
  2. Compound lipid
  3. Derived lipid.

1. Simple Lipid: These are ester of fatty acid with different alcohol and carry other substances e. g., acylglycerol. It also classified into monoacylglycerol, diacylglycerol and triacylglycerol. Triacylglycerol are against classified in to

a) Simple triacylglycerol and b) mixed triacylglycerol

2. Compound lipid: These are the ester of fatty acid with alcohol but carry in addition other substances, phosphate, nitrogen etc. These are divided into-

a) Phospholipid

b) Glycolipid

c) Lipoprotein

3. Derived lipid: These are derived from simple or compound lipid by their hydrolysis.

- **General Quantitative Test for Lipids:**

Sl. No.	Experiment	Observation	Inference
1.	**Smell test:** Smell taken from the supplied sample.	Smell of chloroform occur	May be presence of cholesterol.
		No smell of chloroform	There may be presence of glycerol.
2.	**Solubility Test:** 1-2ml of supplied sample taken in a dry test tube and add water in the test tube. In a test tube few ml of supplied sample is taken and add chloroform.	Soluble in water.	Glycerol may present.
		Soluble in chloroform	Cholesterol may be present.
3.	**Salkowski's Test:** About 2-3ml of supplied fat is taken in a test tube. Then equal volume of concentrated H_2SO_4 is added to it.	Upper layer turns in to red to purple colour but lower layer exhibited green fluorescence colour.	Presence of cholesterol
		No change	Absence of cholesterol.
4.	**Libermann-burchared Test:** About 2-3ml of supplied fat sample is taken in a dry test tube. Then 10-15 drops of acidic anhydrous and 3 drops of conc. H_2SO_4 added to it.	At first cherry red colour appear and then it turns to blue.	Presence of cholesterol.
		No change	Absence of cholesterol.
5.	**Acrolin Test:** About 2-3 ml of supplied sample is taken in attest tube and then add potassium bisulphate($KHSO_4$)	A pungent odour is developed	May be presence of glycerol
		No odour occur	Absence of glycerol
6.	**Dunston's Test:** About 4-5ml of 0.5% borax s solution is taken in a dry test tube. 1-2 drops of 1% alcoholic phenoptheline is added to it drop wise and 1-2ml of supplied sample is added.	At first red colour of Borax and phenoptheline mixture discharge by lipid solution.	May be presence of glycerol.
		No change.	Absence of glycerol.

- **General Quantitative Test for Cholesterol:**

Sl. No.	Experiment	Observation	Inference
1.	**Smell test:** Smell taken from the supplied sample.	Smell of chloroform occur	Presence of cholesterol.
2.	**Solubility Test:** 1-2ml of supplied sample taken in a dry test tube and add water in the test tube. In a test tube few ml of supplied sample is taken and add chloroform.	Insoluble in water. Soluble in chloroform	Cholesterol may be present.
3.	**Salkowski's Test:** About 2-3ml of supplied fat is taken in a test tube. Then equal volume of concentrated H_2SO_4 is added to it.	Upper layer turns in to red to purple colour but lower layer exhibited green fluorescence colour.	Presence of cholesterol
4.	**Libermann-burchared Test:** About 2-3ml of supplied fat sample is taken in a dry test tube. Then 10-15 drops of acidic anhydrous and 3 drops of conc. H_2SO_4 added to it.	At first cherry red colour appear and then it turns to blue.	Presence of cholesterol.

- **General Quantitative Test for Glycerol:**

Sl. No.	Experiment	Observation	Inference
1.	**Smell test:** Smell taken from the supplied sample.	No smell of chloroform occur	Presence of glycerol.
2.	**Solubility Test:** 1-2ml of supplied sample taken in a dry test tube and add water in the test tube. In a test tube few ml of supplied sample is taken and add chloroform.	Soluble in water. Insoluble in chloroform	Water soluble lipid like glycerol present.
3.	**Acrolin Test:** About 2-3 ml of supplied sample is taken in attest tube and then add potassium bisulphate($KHSO_4$)	A pungent odour is developed	May be presence of glycerol
4.	**Dunston's Test:** About 4-5ml of 0.5% borax s solution is taken in a dry test tube. 1-2 drops of 1% alcoholic phenoptheline is added to it drop wise and 1-2ml of supplied sample is added.	At first red colour of Borax and phenoptheline mixture discharge by lipid solution.	May be presence of glycerol.

Determination of Acid Value of Fats & Oils

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 enymetic hydrolysis of fats into free fatty acids that can be determined volumetrically by titrating the sample with 'KOH'. The acidity of fats and oil is expressed as its acid value or number which is defined as an mg of KOH required to neutralize the free fatty acid present in 1mg of fats and oils. The amount of free fatty acid present or acid value of fat is a useful parameter which give an indicator about the age and extend of its deterioration.

Requirements:

Chemicals:

1% phenopthelin solution in 95% alcohol

0.01 normal KOH-0.56gm of KOH dissolved in distilled water and make the final volume of 1 liter.

Fat solvent in 95% alcohol.

Glass Goods:

Burette with burette stand

50ml conical flask

Pipette

Miscellaneous:

Olive oil (fresh sample that have been stored at room temperature for several days may be used for comparison).

Procedure:

Sample Preparation:

1. A clean and dry 50ml conical flask is weight by rough balance then than 1ml graduated pipette is taken.
2. 1ml olive oil is taken by this graduated pipette and poured in the conical flask slowly.
3. Conical flask with olive oil is also weight.
4. Then olive oil (g) = weight of conical flask with oil weight of the conical flask.
5. 25ml of solvent added in the set conical flask and shake well.
6. A few drops of phenopthelin is added and whole content is mixed thoroughly.
7. The above solution is filtrated against 0.01normal KOH until a faint pink colour persist.
8. The volume of KOH is noted.
9. Then the above procedure is repeated for 2 times.

Observation of Result:

Weight of dry conical flask- 36.398g

Weight of dry conical flask with olive oil- 37.420g

Net weight of olive oil- (37.420-36.398) g= 1.022g.

Result of Conical flask with olive oil:

Sl No.	Burette Reading		Difference	Average
	Initial (ml)	Final (ml)		
1	0.00	2.2	2.2	
2	2.2	4.5	2.3	2.23
3	4.5	6.7	2.2	

Calculation:

Titrate value- 2.23
Weight of sample- 1.022
Acid value (mg KOH/g Fat) - 56.1

Result:

$$\frac{\text{Titrate value} \times 0.01(\text{N})\ \text{KOH} \times \text{molecular weight of KOH (56.1)}}{\text{Weight of Sample (g)}}$$

$$= \frac{2.23 \times 0.01 \times 56.1}{1.022}$$

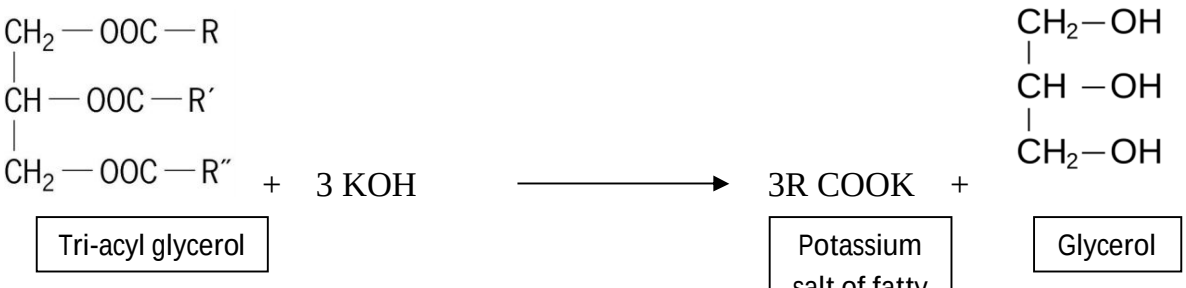
$$= (1.25103) / (1.022)$$

= 1.22 mg KOH/g of Fat

Determination of Saponification Value of Fats and Butter

- **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 utilized 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 1g of the given fat.



From the above reaction, it may be noted that 3 molecules of KOH are consumed for saponification of each molecule of tri-acylglycerol irrespective of chain length of fatty acids. Evidently each gram of tri-acylglycerol with shorter chain of fatty acid will contain longer number of molecule of the tri-acylglycerol and will thus require much more KOH. The saponification value is thus an indication of severest molecular weight of the fatty acid in an aceyl glyceride. The procedure involves refluxing of known amount of fat or oil with a fixed but an 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 utilized for saponification can thus be calculated.

- ## Materials and reagents:

1. **Fat solvents-** A mixture of 95% ethanol and ether (1.1 v/v).
2. **0.5(N) alcoholic KOH-** 0.5 (N) solution of KOH is prepared by dissolving 28.5 g of KOH pellets in 20 ml water and making the volume to 1 lit with 95% alcohol.
3. **1% Phenlopthelin solution in 95% alcohol.**
4. **0.5 (N) HCL.**

Glass goods:

1. 25 ml burettes with burets stands
2. 100 ml conical flusks
3. Pipette
4. Reflex condenser.

Instrument:

1. Boiling water bath.

- ## Procedure:

1. Blank 50 ml conical flask is measured by rough balance.
2. Taken 1 g of fats sample (butter) measured by weight machine and furthered weight.
3. Added 3 ml of fat solvent and add 25 ml of 0.5(N) alcoholic KOH on boiling water bath for 30 minutes.
4. Then cool it room temperature and add 2-3 drops of phenlopthelin into the conical flask.
5. Titrated the above solution with 0.5 (N) HCL till the pink colour disappear.
6. Noted the volume of HCl from burette.

- **Observation Table:**

No. of observation	Burette reading		Difference volume of 0.01(N)KOH required	Mean volume of 0.01(N) KOH required
1	**Initial**	**Final**	1.2	1.2
	0	1.2		

- **Composition and result:**

Weight of blank conical: 35 g

Conical with water: 36 g.

Weight of sample: (36-35) = 1 g

Saponification value = $\frac{(28.05 * \text{Titrate value})}{\text{Weight of sample}}$

= $\frac{28.05 * 1.2}{1}$

= 33.66 mg

The multiplication factor of 28.05 in the above equation is included since 1 ml of 0.5 (N) KOH contains 28.05 mg of KOH.

- **Interpretation:**

Here calculated saponification value is 33.66 mg/g of fat.

It provides information of the average chain length of the fatty acid in fat. In varies inversely with chain length of fatty acid.

Some fats are saponification number gives below-

Name of the fats and oil	Saponification no.
1. Butter fat	210 -230
2. Castor Oil	175 - 180
3. Cotton seed oil	190 - 198
4. Safflower oil	188 - 198

GENERAL QUALITATIVE ANALYSIS OF PROTEIN

- **Introduction:** Proteins are macromolecular polymers of many amino acids. Practically, proteins are polypeptides of higher molecular weight. The poly-peptide molecule having molecular weight more than 10000 (or 10KD) is treated as protein.

- **Classification of protein:** Proteins are classified into three group depending upon mainly on the solubility. These are the following-

A. Simple protein: This protein contains only amino acids. The following are different types of simple proteins.

- **Albumins:** These are soluble in water and dilute salt solution, coagulated by heat and precipitated by the full separation of the aqueous solutions with solid ammonium sulphate. This indicates that they are profusely hydrated. Serum albumin, lactalbumine and ovalbumin are example.
- **Globulin:** These are insoluble in water water but soluble in dilute neutral salt solutions. These are coagulated by heat, and precipitated by half saturation with ammonium sulphate solution. These are less hydrated then albumin. Examples are oval globulin and serum globulins.
- **Prolamines:** Soluble in about 70 per cent alcohol, insoluble in water, absolute alcohol and other neutral solvents. They are rich in praline. Zain of corn and gliadin of wheat are good example.
- **Glutelins:** These are soluble in dilute acids and alkalis, insoluble in water and neutral solvent and are coagulated by heat, e.g., gluten from wheat.
- **Histones:** These are soluble in water and vary dilute in acids, insoluble in dilute NH_4OH and not coagulated by heat. They are strongly basic and occur in nucleoproteins.
- **Globins:** They are referred to as examples of histones but are classified separately since they are not basic like histones and are not precipitated by ammonium hydroxide, e.g., globins of haemoglobin.

B. **Compound protein:** These are composed of simple proteins and are associated by some non-protein substances as prosthetic factor.
   - **Nucleoproteins:** These are combinations of simple basic proteins, i. e., protamines or histones with nucleic acids (DNA or RNA). These are soluble in dilute NaCl. The chromatein of cell nuclei and the virus are nucleoproteins.
   - **Glycoprotein and mucoprotein:** These are simple protein combined with carbohydrates (glycosaminoglycan) and on hydrolysis give amino acids, ammo sugars and uronic acids. The glycosaminoglycan may be hyaluronic acid, chondroitin sulphates and heparin.
   - **Lipoproteins:** This are proteins conjugated to lipid like lecithin, cephalin, neutral fat, fatty acid or cholesterol. Lipoproteins are different from proteolipids in that the latter are soluble in organic solvents and insoluble in water. Lipoproteins occur in blood and cell membranes.

C. **Derived proteins:** These protein are derived from simple or compound protein. Example- Denaturated protein, Peptides

• Different qualitative tests for Proteins:

Sl. No.	Experiment	observation	Inference
1.	**Biuret test:** Just 1 drop of 1% copper sulphate solution taken in a dry test tube. Then about 3ml of 10% NaOH solution added to it. Then equal volume of supplied protein solution is taken and mixed well.	A violet colour developed.	Presence of higher protein like albumin and globulin.
		A bluish violet colour developed.	This is the positive test for giletin due to the formation of coordination complex between COO and NH group of the peptide linkage.
		A rose pink colour developed.	Presence of lower protein like peptone.
2.	**Million's Test:** About 2-3 ml of supplied protein solution taken in a dry test tube. Then 3-4 drops of Million's reagent	Initially precipitated and gradually white.	This is the positive test for albumin and globulin.
		No change	This is the positive test for gelatin.

	added to it after that slightly heated with mixing.	Very little bit of precipitate developed and changed the colour after heating.	This is the positive test for peptones.
3.	**Xanthoprotein Test:** About 2-3ml of supplied protein solution is taken in a dry test tube then HNO_3 is added to it. Then heated and boiled and cooled	A white precipitate is formed and turns yellow after boiling.	This is the positive test for albumin and globulin
		No precipitate developed	This is the positive test for gelatin.
		Yellow colour developed without ppt.	This is the positive test for peptones.
4.	**Adamkiewicz Test:** About 2-3 ml of supplied protein solution is taken in a dry test tube. Then 2ml of glacial CH_3COOH added to it and mixed well. After that con. H_2SO_4 poured into the test tube.	Purple colour developed in the junction of two liquids.	This is the positive test for albumin and globulin.
		No change	This is the negative test for gelatin.
5.	**Heat coagulation:** Few drops of supplied protein solution is taken in a dry test tube. Then few drops of glacial CH_3COOH is added to it after heating	A feculent coagulation is developed.	This is the positive test for globulin.
		No coagulation is occur	This is the negative test for peptone and gelatin.
6.	**Esbach test:** Few ml of supplied protein sample is taken in a dry test tube. Then few ml of Esbach solution is added to it.	A yellow precipitate is appeared which is disappeared on heating.	This is the positive test for albumin and globulin.
		Precipitate disappeared during heating but reappeared after cooling.	This is the positive test for gelatin.
		No change	This is the positive test for peptone.

- **Known tests for Albumin:**

Sl. No.	Experiment	observation	Inference
1.	**Biuret test:** Just 1 drop of 1% copper sulphate solution taken in a dry test tube. Then about 3ml of 10% NaOH solution added to it. Then equal volume of supplied protein solution is taken and mixed well.	A violet colour developed.	Presence of higher protein like albumin and globulin.
2.	**Million's Test:** About 2-3 ml of supplied protein solution taken in a dry test tube. Then 3-4 drops of Million's reagent added to it after that slightly heated with mixing.	A white precipitate is formed and it is turn into red.	This is the positive test for albumin or globulin.
3.	**Xanthoprotein Test:** About 2-3ml of supplied protein solution is taken in a dry test tube then HNO_3 is added to it. Then heated and boiled and cooled	Yellow precipitate is developed.	This is the positive test for albumin.
4.	**Adamkiewicz Test:** About 2-3 ml of supplied protein solution is taken in a dry test tube. Then 2ml of glacial CH_3COOH added to it and mixed well. After that con. H_2SO_4 poured into the test tube.	Purple colour developed in the junction of two liquids.	This is the positive test for albumin.
5.	**Heat coagulation:** Few drops of supplied protein solution is taken in a dry test tube. Then few drops of glacial CH_3COOH is added to it after heating	A feculent coagulation is developed	This is the positive test for albumin.

6.	**Esbach test:** Few ml of supplied protein sample is taken in a dry test tube. Then few ml of Esbach solution is added to it.	A yellow precipitate is appeared which is disappeared on heating but reappear after cooling.	This is the positive test for albumin

- **Known tests for Gelatin:**

Sl. No.	Experiment	observation	Inference
1.	**Biuret test:** Just 1 drop of 1% copper sulphate solution taken in a dry test tube. Then about 3ml of 10% NaOH solution added to it. Then equal volume of supplied protein solution is taken and mixed well.	A bluish violet colour developed.	This is the positive test for gelatin.
2.	**Million's Test:** About 2-3 ml of supplied protein solution taken in a dry test tube. Then 3-4 drops of Million's reagent added to it after that slightly heated with mixing.	No precipitate is formed.	This is the positive test for gelatin.
3.	**Xanthoprotein Test:** About 2-3ml of supplied protein solution is taken in a dry test tube then HNO_3 is added to it. Then heated and boiled and cooled	No precipitate is formed.	This is the positive test for gelatin.

4.	**Adamkiewicz Test:** About 2-3 ml of supplied protein solution is taken in a dry test tube. Then 2ml of glacial CH_3COOH added to it and mixed well. After that con. H_2SO_4 poured into the test tube.	No changed	This is the negative test for gelatin.
5.	**Heat coagulation:** Few drops of supplied protein solution is taken in a dry test tube. Then few drops of glacial CH_3COOH is added to it after heating	No coagulation occurred.	This is the negative test for gelatin.
6.	**Esbach test:** Few ml of supplied protein sample is taken in a dry test tube. Then few ml of Esbach solution is added to it.	A yellow precipitate is appeared which is disappeared after cooling.	Presence of gelatin.

- **Known tests for Peptone:**

Sl. No.	Experiment	observation	Inference
1.	**Biuret test:** Just 1 drop of 1% copper sulphate solution taken in a dry test tube. Then about 3ml of 10% NaOH solution added to it. Then equal volume of supplied protein solution is taken and mixed well.	A rose pink (Violet) colour developed.	This is the positive test for peptone.

2.	**Million's Test:** About 2-3 ml of supplied protein solution taken in a dry test tube. Then 3-4 drops of Million's reagent added to it after that slightly heated with mixing.	Very little precipitate is formed and dissolved after heating.	This is the positive test for peptone.
3.	**Xanthoprotein Test:** About 2-3ml of supplied protein solution is taken in a dry test tube then HNO_3 is added to it. Then heated and boiled and cooled	Yellow colour is developed without precipitate.	This is the positive test for peptone.
4.	**Adamkiewicz Test:** About 2-3 ml of supplied protein solution is taken in a dry test tube. Then 2ml of glacial CH_3COOH added to it and mixed well. After that con. H_2SO_4 poured into the test tube.	A purple colour is developed at the junction of two liquid.	This is the positive test for peptone.
5.	**Heat coagulation:** Few drops of supplied protein solution is taken in a dry test tube. Then few drops of glacial CH_3COOH is added to it after heating	No coagulation occurred.	This is the negative test for peptone.
6.	**Esbach test:** Few ml of supplied protein sample is taken in a dry test tube. Then few ml of Esbach solution is added to it.	No precipitate is formed.	Presence of peptone.

- **General known tests for aromatic amino acids:**

Sl. No.	Experiment	observation	Inference
1.	**Xanthoprotein Test:** About 2-3ml of supplied protein solution is taken in a dry test tube then HNO_3 is added to it. Then heated and boiled and cooled	Yellow precipitate is developed.	This is the positive test for tyrosine, phenylalanine and tryptophan.
		No change	It is the negative test for arginine and systein
2.	**Million's Test:** About 2-3 ml of supplied protein solution taken in a dry test tube. Then 3-4 drops of Million's reagent added to it after that slightly heated with mixing.	White precipitate is developed then it turns in to red coagulation.	This is the positive test for tyrosine.
		No change	This is the negative test for tryptophan and alanine.
4.	**Adamkiewicz Test:** About 2-3 ml of supplied protein solution is taken in a dry test tube. Then 2ml of glacial CH_3COOH added to it and mixed well. After that con. H_2SO_4 poured into the test tube.	A purple colour is developed at the junction of two liquid.	This is the positive test for tryptophan.
			This is not positive test for alanine.
5.	**Sakaguchi Reaction:** About 3ml of supplied solution is taken in a test tube. Then 5 drops of 20% NaOH , 3 drops of alpha-naphthol and 2 drops of water is added and mix well	A intense red coloured developed	This is the positive test for arginine.
		No change	This is negative test for cysteine.
6.	**Na Nitropruside test:** About 3ml of supplied solution taken is taken in a dry test tube. Then 2ml Na-Nitropruside reagent is added to it and mixed well.	Yellow colour is formed.	This is positive test for cysteine.
		No changed	May be absent of cysteine.

Kjeldahl Method for Determination of Nitrogen

- **INTRODUCTION:**

Nitrogen is one of the five major elements found in organic materials such as protein. The Kjeldahl method of nitrogen analysis is the worldwide standard for calculating the protein content in a wide variety of materials ranging from human and animal food, fertilizer, waste water and fossil fuels. The Kjeldahl method consists of three steps:

A. Digestion
B. Distillation
C. Titration

A. Digestion of the sample:

Digestion is the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This is accomplished by boiling a homogeneous sample in concentrated sulphuric acid. The end result is an ammonium sulphate solution. The general equation for the digestion of an organic sample is shown below:

$$\text{Protein} + H_2SO_4 \rightarrow (NH_4)_2SO_4\,(aq) + CO_2\,(g) + SO_2\,(g) + H_2O\,(g) \quad (1)$$

Sulphuric acid has been used alone for the digestion of organic samples. The amount of acid required is influenced by sample size and relative amount of carbon and hydrogen in the sample, as well as amount of nitrogen. A very fatty sample consumes more acid. Also, heat input and digestion length influences the amount of acid loss due to vaporization during the digestion process. Initially an organic sample usually chars and blackens. The reaction may at first be very vigorous depending on the matrix and the heat input. With organic decomposition the digestion mixture gradually clears as CO_2 evolves.

The problem with using sulphuric acid alone for digestion is very long digestion time's result with many samples due to the slow rate of organic decomposition. The addition of an inorganic salt to the digest elevates the boiling point of the H_2SO_4. The solution temperature of concentrated sulphuric acid alone is about 330° C. Addition of a salt such as K_2SO_4 can elevate the solution temperature of the digestion mixture to 390° C or more, depending on the ratio of salt to acid.

This significantly increases the rate of organic decomposition in the digestion mixture, shortening the length of time required for digestion. There are several precautions to keep in mind concerning salt addition. First, it is possible to raise the solution temperature of the digestion mixture too much. If the temperature goes much above 400° C during any phase of the digestion, volatile nitrogen compounds may be lost to the atmosphere.

Remember that as acid is gradually consumed during the digestion process, for the various reasons mentioned above, the salt acid ratio of the digest gradually rises. This means that the hottest solution temperatures are attained at the end of the digestion.

Heat input, consumption of acid by organic material and vaporization, salt/acid ratio, digestion length, and physical design of the Kjeldahl flask, are all interrelated. Each has an effect on the final solution temperature. A second precaution is that if the salt/acid ratio is too high, a considerable amount of material will "salt out" upon cooling of the digest. Concentrated acid pockets can be contained within the cake. These can react violently when concentrated base is added in the distillation process. A certain amount of salting out can be managed by diluting the digest with water while it is still somewhat warm, but not too hot.

Several catalysts have been employed by Kjeldahl chemists over the years to increase the rate of organic breakdown during the acid digestion. Mercuric oxide has been the most effective and widely used. However, mercury forms a complex with ammonium ions during digestion. The addition of sodium thiosulfate or sodium sulphide after digestion and before distillation will break the complex and precipitate mercuric sulphide. This is also important from a safety point of view, as mercury vapour might escape to the atmosphere during the distillation process. Because of environmental concerns over the handling and disposal of mercury, other catalysts are coming more into favour. Many methods employ copper sulphate. Titanium oxide and copper sulphate in combination have been found to be more effective than copper sulphate alone. Selenium is frequently used. Commercially prepared mixtures of potassium sulphate and a catalyst are available from laboratory chemical suppliers.

B. Distillation:

Distillation is adding excess base to the acid digestion mixture to convert NH4 + to NH3, followed by boiling and condensation of the NH3 gas in a receiving solution. This is accomplished by;

1) Raising the pH of the mixture using sodium hydroxide (NaOH solution). This has the effect of changing the ammonium (NH4 +) ions (which are dissolved in the liquid) to ammonia (NH3), which is a gas.

$$(NH4)2SO4\ (aq) + 2NaOH \rightarrow Na2SO4\ (aq) + 2H2O\ (l) + 2NH3\ (g)\ (2)$$

2) Separating the nitrogen away from the digestion mixture by distilling the ammonia (converting it to a volatile gas, by raising the temperature to boiling point) and then trapping the distilled vapours in a special trapping solution of boric acid (H3BO3). The ammonia is bound to the boric acid in the form of ammonium borate complex.

$$H3BO3 + NH3 \rightarrow NH4 + + H2BO3 -\ (3)$$

The majority of the NH3 is distilled and trapped in the receiving acid solution within the first 5 or 10 minutes of boiling. But depending on the volume of the digestion mixture and the method being followed, 15 to 150 ml of condensate should be collected in the receiving flask to ensure complete recovery of nitrogen. Further extension of the distillation times and volumes collected simply results in more water being carried over to the receiving solution. Excess water does not change the titration results. Distillation times and distillate volumes collected should be standardized for all samples of a given

methodology. The rate of distillation is affected by condenser cooling capacity and cooling water temperature, but primarily by heat input. Typically the heating elements used for distillation have variable temperature controllers. A distillation rate of about 7.5 ml/minute is most commonly cited in accepted methods. Connecting bulbs or expansion chambers between the digestion flask and the condenser is an important consideration to prevent carryover of the alkaline digestion mixture into the receiving flask. The slightest bit of contamination of the receiving solution can cause significant error in the titration step. When very low levels of nitrogen are being determined, it is advisable to "precondition" the distillation apparatus prior to distillation. This can be done by distilling a 1:1 mixture of ammonia free water and 50% NaOH for 5 minutes just before sample distillation to reduce contamination from atmospheric ammonia.

C. Titration:

There are two types of titration: back titration, and direct titration. Both methods indicate the ammonia present in the distillate with a color change and allow for calculation of unknown concentrations.

D. REAGENTS AND APPARATUS:

- Potassium sulphate, K_2SO_4+Se tablets (2 for each digestion tube)
- Concentrated H_2SO_4 (in the hood)
- 35 % (w/v) NaOH for each digestion tube (already prepared).
- 4.0 % (w/v) Boric acid, H_3BO_3 (already prepared)
- 0.1 M HCl (already prepared, exact concentration will be given)
- Methyl orange (in droppers)
- Erlenmeyer flasks
- Burette
- Digestion tubes
- **Milk: Do not forget to bring any brand of milk.**

E. PROCEDURE:

1) **Sample:** 5.0 mL fresh cow's milk in a digestion tube.

2) **Reagents for digestion:** to each milk sample and also to an empty digestion tube (blank) add the followings: - 2 tablets of K_2SO_4 + Se catalyst - 10.0 mL of concentrated H_2SO_4 (98%)

3) Digestion: heat for ca. 30 minutes at 420ºC.
ATTENTION: Do not inhale the gases evolve in reaction 1.

4) Cooling and diluting: let the digestion tubes to cool to 50-60 ºC and add to each add 50 mL of distilled water.
ATTENTION: Let the tubes stand in air to cool, cold water may break the tubes.

5) Distillation:
I. Place the digested samples in digestion tubes to the distilling unit and add 50.0 mL of 35% (w/v) NaOH.
ii. The sample is distilled until 100 mL of distillate are collected in 25.0 mL of 4.0 % (w/v) boric acid.

6) Titration: add 2-3 drops indicator to the Erlenmeyer flask and titrate it with 0.1 M HCl.

7) Calculate the amount of protein (% protein) and compare the result with the value give on the milk.