STUDENTS' LABORATORY MANUAL

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY
FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI
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CHAPTER ONE

1.1 PROCESSING OF LEGUMES

SUBJECT: PROCESSING OF COWPEAS

AIM: Preparation of moimoin through baking.

MATERIALS NEEDED:

Ingredients: cowpea, salt, pepper, tomato puree, crayfish, edible oil, water (onions).

Equipment/Apparatus: Basins, spoons, knives, small baking pans, plastic film, oven, grinder, sealer, freezer.

PROCEDURE:

(i) Clean the seeds.
(ii) Soak them in cold water until the skin can be rubbed off (for about 15 minutes).
(iii) Dehull by working the soaked seeds between the palms.
(iv) Separate dehulled seeds, and grind into smooth pasty-shurry.
(v) Prepare the other accompanying condiments, and also oil the pan.
(vi) Mix the slurry and the condiments very intimately, and place portions inside the pans up to a height of 2cm.
(vii) Charge the pans into the oven and bake (or steam) at 105°C at first then finish off at about 150°C.
(viii) Cool, and place inside plastic films and seal, store in deep freezer.

QUESTIONS/EXERCISES

(i) Use flow diagram to depict the process.
(ii) What roles do the condiments play?
(iii) Why do we oil the pans first?
(iv) Why do we bake at lower temperature firstly?
(v) Estimate the moimoin (cake) yield.

1.2 SUBJECT: PROCESSING OF SOYABEANS

AIM: Production of whole, liquid soy milk.

MATERIALS NEEDED:

Ingredients: soybean seeds, water, sugar.

Equipment/Apparatus: Basins, grinder, steam chest, bottles with covers, lacquered cans, perforated metal bowls, cooker, pots, calibrated beakers or cylinders, sifter.

PROCEDURE:

(i) Clean and soak in lake-warm water for a few hours.
(ii) Dehull and separate the beans.
(iii) Mash the dehulled beans in the grinder.
(iv) Add water to the mash to make a 40% suspension of the mash.
(v) Strain or sieve the suspension to separate the filtrate (milk).
(vi) Sweeten (optional).
(vii) Fill into sterilized bottles or cans and heat-treat.
(viii) Cool and store.
1.3 SUBJECT: Processing of Groundnuts

AIM: Production of peanut-butter

MATERIALS NEEDED:
Ingredients: Groundnuts, salt, spices, water.
Chemicals: Antioxidants.
Equipment/Apparatus: Bowls, steam chest, oven, grinding machine, glass and plastic containers with covers, frying pan, cooker, stirring wood.

PROCEDURE:
(i) Clean fresh groundnuts
(ii) Place thinly on trays and dry in the oven till the hull is easily removed.
(iii) Discharge, cool and dehull, and winnow to separate the seeds from the hulls.
(iv) Place the seeds on the pan and roast over the fire with continuous stirring till the colour is golden brown.
(v) Discharge and cool
(vi) Blanch for 5 minutes via steaming.
(vii) Discharge, cool and hull into the grinder. Add condiments and grind to a very smooth slurry.
(viii) Fill hot or cold into sterilized containers and cool.
(ix) Dry and label.
(x) Store.

QUESTIONS/EXERCISES
(i) Illustrate the process using a flow diagram. Also
(ii) Attempt an estimate of the yield of the peanut butter you produced.

1.4 SUBJECT: Processing of Soybeans

AIM: Production of soy flour: Full-fat flour.

MATERIAL NEEDED:
Ingredients: Soybean seeds, water
Equipment/Apparatus: Basin, pulverize, steam chest, plastic film, sealer, perforated metal bowls, spoons, balance, trays, oven

PROCEDURE:
(i) Clean and soak for a few hours.
(ii) Dehull, and separate the beans
(iii) Blanch the dehulled beans using live steam for 15 minutes.
(iv) Discharge from steam chest, and spread thinly on tray and place inside oven and dry at 50°C till breaking point is reached.
(v) Remove from oven, cool and pulverize.
(vi) Pack in film and label, and seal.
(vii) Store.
QUESTIONS/EXERCISES
(i) Use a simplified flow diagram to depict the process of full-fat soyflour products.
(ii) Determine the moisture contents of both the fresh and dry, pulverized soybean.
(iii) Estimate the soy flour yielded or extraction rate.
(iv) What is the importance of blanching process?

1.5 SUBJECT: Processing of Cowpeas (or Soybeans)
AIM: Preparation of flours from cowpeas of soybeans
MATERIALS NEEDED:
Ingredients: Cowpeas, soybeans, water, steam.
Equipment/Apparatus: Blancher, basins, cabinet dryer, plastic film, trays, grinder, sealer.

PROCEDURE
(i) Winnow the shelled seeds, and wash.
(ii) Soak in water for about 15 minutes.
(iii) Dehull and separate the fractions.
(iv) Steam-blanch the peeled seeds at 100°C.
(v) Drip-dry, and spread thinly on the trays and dry in the oven to the breaking point.
(vi) Cool and grind to fine powder.
(vii) Package and store.

QUESTIONS/EXERCISES
(i) Use flow diagram to express the whole process operation.
(ii) What is the function of the steaming or blanching process?
(iii) What are the moisture contents of the raw seeds and the flour, respectively?
(iv) Evaluate the flour yield.

1.6 SUBJECT: Particles Size Distribution in flours
AIM: To determine particle size distribution in a given flour.
MATERIALS NEEDED:
Grain, grinder, a set of sieves, sieves, shaker, containers, weighing balance.

PROCEDURE:
(i) Grind conditioned grain into flour.
(ii) Arrange sieves on a sieve shaker in decreasing pore diameter or increasing mesh size.
(iii) Place a pan collector at the bottom of the sieves.
(iv) Operate the sieve shaker for 10 minutes.
(v) Determine percentage particle retention on each sieve.
(vi) Repeat experiment using flour of different milling passes.
(vii) Repeat experiment varying shaking time.

QUESTIONS/EXERCISES
(i) What effect has number of milling passes on particle size distribution of flour?
(ii) What is the pattern of particle size distribution in a given milling operation?
(iii) What effect if any, has shaking time on particle size distribution?
CHAPTER TWO

PROCESSING OF SELECTED TROPICAL FRUITS INCLUDING OIL PALM FRUIT

2.1 SUBJEC1: Processing of Selected Common Tropical Fruits.

AIM: Conversion of locally available food: Jam

MATERIALS NEEDED:

Ingredients: Oranges, grape, uduara (African star apple), pineapple, pawpaw, guava, lime, water, sugar, carrot, mango (mature, firm and unripe).

Chemicals: NaOH, indicator, buffer solution pH 2-4, paraffin wax.

Equipment/Apparatus: Thermometer (0-100°C), refractometer (0-70%), pH-meter, cooker, top loading balance, basins, beakers, stirring rod, pot (25cmx15cm=diameter x height), moisture-extraction oven, stainless steel knives and large spoon, plastic bowl, aluminum tray, blender, bottles with covers.

PROCEDURE:

(i) Wash the lime, cut, and express to obtain the juice. Sieve and store in a clean, closed container. Sterilize the bottles and keep in oven at 100°C.

(ii) Wash the named fruit(s) to be used.

(iii) Peel and cut.

(iv) Grind or pulp (optional).

(v) Mix or blend (optional) where a mixed jam product is desired.

(vi) Place sugar inside the pot in the ratio of 55:45 (i.e. sugar : prepared fruit(s)). Add water and boil.

(vii) Introduce the fruit when sugar solution has started boiling, and stir continually.

(viii) Boil to concentrate it while checking the temperature intermittently. At temperature of about 105°C, add the lime juice, pulsatively, while stirring continually. When it sets, stop.

(ix) Fill hot into the bottles, cool the jars, and seal off with paraffin wax. Close with the lid, label and store.

QUESTIONS/EXERCISES

(i) Use a simplified flow diagram to express the process.

(ii) What are the functions of the fruits and the lime juice?

(iii) Determine the acidity of the lime juice and the jam.

(iv) Determine the % soluble solids content of the fruit(s) used and the jam.

(v) Determine the jam yield.

2.2 SUBJECT: Processing of Palm Fruit.

AIM: Palm oil production.

MATERIALS NEEDED:

Ingredients: Ripe palm fruits, water.

Equipment/Apparatus: Basins, pots, press, spoons, mortar and pestle, matchet, fine filter.
PROCEDURE:
(i) Keep bunch for 3 days after it is cut from the palm tree.
(ii) Strip or thresh the bunch and pick the fruits.
(iii) Clean and wash the fruits.
(iv) Boil the ripe fruits till the pericarp is soft.
(v) Mash the boiled fruit in a tall, narrow mortar with long pestle.
(vi) Heat the mashed fruit, and introduce the mess into the press.
(vii) Press to extract oil.
(viii) Heat the crude oil to 80-100°C, filter hot and allow the filtrate to cool and separate the oil from sludge (if any).
(ix) Fill into calibrated bottles and store.

QUESTIONS/EXERCISES:
(i) Use a flow diagram to illustrate the process.
(ii) Estimate the extraction rate of the crude palm oil.
(iii) What is the moisture content of the oil?
(iv) To what extent is the cake from the oil extraction useful?
(v) What is the efficiency of your method?

2.3 SUBJECT: Processing of Oil Palm Kernel

AIM: Production of palm kernel oil.

MATERIALS NEEDED:
Ingredients: Oil palm kernels, water.
Equipment/Apparatus: Basins, pots, steam chest, metal bowl, fine filter, bottles with cover or plastic cans press, funnel, grinder.

PROCEDURE:
(i) Clean the kernels and wash.
(ii) Break or grind very coarsely.
(iii) Place the grinder inside metal bowl and charge into the steam chest. Render or hot condition at 80°C.
(iv) Place the rendered grind inside the press and express the oil.
(v) Strain the filtrate (oil).
(vi) Bottle or fill into plastic containers and store after labeling.

QUESTIONS/EXERCISES:
(i) Depict the process by the use of flow diagram.
(ii) Determine the moisture content of both the kernels used and oil extracted.
(iii) What is the extraction rate of the palm kernel oil?
(iv) What is the essence of the rendering?
(v) Compute the efficiency of your method.

2.4 SUBJECT: Fruit Juice Production

AIM: To produce single strength juice.

MATERIALS NEEDED: Sound fruits, juice press, filter or sieve, stainless steel knives, refractometer, large spoon big pot, basin, bottles, sodium benzoate 0.2% crown cork machine.
PROCEDURE:
(i) Sort and wash fruits.
(ii) Wash bottles, sterilize and oven dry.
(iii) Cut up fruits or peel fruit as required.
(iv) Extract fruit juice as appropriate for the fruit.
(vi) Add sodium benzoate 0.1-0.15% to juice.
(vii) Heat juice to 60°C and fill not into bottles.
(viii) Cork bottles and pasteurize in a water bath for 10 minutes.
(ix) Cool and label.

QUESTIONS/EXERCISES
(i) Why should the level of sodium benzoate limited to 0.1-0.15%?
(ii) What advantage has sodium benzoate over other preservative?
(iii) Why is it necessary to fill the juice hot?
(iv) Explain the need to pasteurize juice in water bath.

2.5 SUBJECT: Frozen Fruit Juice
AIM: To produce concentrated fruit juice.
MATERIALS NEEDED: Sound fruits, juice press, stainless steel knives, refractometer, large spoon, plastic bottles, sodium benzoate, freezer.

PROCEDURE:
(i) Sort and wash fruits.
(ii) Peel and cut up fruit as required.
(iii) Wash plastic bottles.
(iv) Extract juice as appropriate for the fruit.
(v) Concentrate fruit juice to 60°C solids.
(vi) Add sodium benzoate 0.1% and 1/3 fresh fruit juice.
(vii) Fill into clean plastic bottles leaving sufficient head space.
(viii) Freeze the product.

QUESTIONS/EXERCISES
(i) Explain the wisdom of adding fresh fruit to the concentrated juices.
(ii) Why is sufficient headspace recommended for bottles?
(iii) In the preservation of fruit juice, glass bottles are recommended, justify.
(iv) Compare the sensory qualities of frozen juice with bottle juice.

2.6 Manual Extraction of Oil from Oil Seeds
AIM: Production of Vegetable Oil
MATERIALS NEEDED:
Oil seed (Groundnut, Melon, Soyabean) and Water
EQUIPMENT/APPARATUS: Milling machine, source of heat, basin, filter, packaging material, weighing balance.
PROCEDURE:
(i) Dehull the seeds
(ii) Sort and clean the seeds
(iii) Mill the seeds.
(iv) Express the oil manually by pounding the seeds with a mortar and hot to aid oil release.
(v) Separate the oil and heat for the water to evaporate.
(vi) Package and label.

QUESTIONS/EXERCISES:
(i) With the use of a flow diagram show the steps involved for production of vegetable oil.
(ii) Determine the oil yield.
(iii) Determine the free fatty acid content of the oil.
(iv) What is the importance of adding hot water?

2.7 SUBJECT: Solvent Extraction Of Oil From Oil Seeds
(Cold Extraction)
AIM: Use of Solvent Extraction Method for Vegetable Oil Production
MATERIALS NEEDED
Ingredients: Oil seeds, Solvent (hexane)
Equipment/Apparatus: Air tight container, Filter paper, grinder or blender, Source of heat, oil extractor, hot air oven, packaging materials.

PROCEDURE
(i) Dehull the seeds
(ii) Sort, clean and dry the seeds
(iii) Grind into fine particle size
(iv) Wrap the grind material with filter put into an air-tight container
(v) Pour in the hexane and cork
(vi) Allow to stand for 12h
(vii) Remove the filter paper with the sample.
(viii) Recover the solvent using solvent extraction apparatus
(ix) Further dry the oil in the flask using hot air oven.
(x) Package the oil.

Questions
(i) Use flow diagram to illustrate the process
(ii) Determine the moisture content of the product
(iii) What is the essence of adding hexane?
(iv) Evaluate the percentage yield of the oil
(v) Determine the percentage loss of the solvent.

2.8 Production of Ade Drink (limeade/lemonade)
AIM: to produce Ade Drink (limeade/lemonade)
Ingredients: Lime juice / lemon juice 100-200ml; Sugar, 250g; lime or lemon essence, 2-5ml; Sodium benzoate, 1.0g; Sorbic acid, 1.0g; and Ascorbic or citric acid 7.0g/1000ml water.

Methods
(i) Mix all the ingredients with warm water and continue as in single strength juice.

Question
(i) Explain the role of the various ingredients used in the production of limeade and lemonade.
(ii) Which of the two products do you prefer and give reason for your choice.
CHAPTER THREE
PROCESSING OF VEGETABLES INCLUDING FRUITS

3.1 SUBJECT: Processing of Fruity vegetables

AIM: Production of Drum dried whole tomato products:
(i) Tomato flakes
(ii) Tomato powder

Materials Needed:
Ingredients: Wholesome, ripe tomatoes, tap water.
Chemicals: NaOH, washing solution, buffer solutions.
Equipment/Apparatus: Knives, basins, stirring rod, beakers, grinder, spoons, drums, dryer,
moisture-extraction oven, pH-meter, plastic films, sealing machines.

Procedure:
(i) Clean the batch tomatoes and inspect
(ii) Cut.
(iii) Place the cuts in the grinder and effect grinding.
(iv) Switch on the drum dryer after setting at pre-determined temperature and rpm. Gradually
and pulsatively pour the tomato pulp onto the hot, revolving surface of the dryer, collect
the flakes.
(v) Cool at ambient condition
(vi) Package as is and store as tomato flakes, after
(vii) Pulverize the flakes, and package as tomato powder.

Question/Exercises
(ii) Also depict the entire process with the aid of a simplified flow diagram.
(iii) Determine the acidity of the fresh tomato, and the reconstituted tomato,
titrimetrically.
(iii) Determine also the moisture contents of both fresh tomato and the dry product.
(iv) Estimate the % yield of the product.

3.2 SUBJECT: Processing of Fruity vegetables

AIM: Production of low-pressure oven-dried tomato.

Materials Needed:
Ingredients: Wholesome, ripe tomatoes, tap water.
Chemicals: NaOH, washing solution, buffer solution, phenolphthalein.
Equipment/Apparatus: Knives, basins, stirring rod, beakers, grinder, spoons, moisture
extraction oven, aluminium pump, pH-meter, plastic film, sealing machine.

Procedure:
(i) Clean the tomatoes, and drip-dry.
(ii) Slice thinly, axially or laterally (about 2mm thick).
(iii) Spread the slices on the tray to 5cm thickness and place it inside a sub-atmospheric
pressure oven, with temperature set at < 450°C.
(iv) When dried, discharge them and cool at ambient package some quantities as dry tomato
shles.
(v) Place the dry slices in the grinder and pulverize them, discharge, and package as tomato
powder.
Questions/Exercises:
(i) Use flow diagram to show the whole process.
(ii) Repeat all the exercises in the processing of leafy vegetable (3.1).
(iii) What is the implication of the low-pressure drying process?

3.3 Processing of Leafy Vegetables
Ingredients: Fresh bitter leaves, tap water, edible oil.
Equipment/Apparatus: Kitchen knives, basins, plates, sieve, plastic films, oven freezer, trays, sealing machine.

PROCEDURE:
(i) Pick the leaves from the stalks.
(ii) Wash them.
(iii) Reduce the size, noting the technique you adopt.
(iv) Wash the cut bitter leaves while intermittently testing for bitterness. When bitterness reaches acceptable level, stop washing, settle the product stream. Decant and collect the solid fraction for further handling.
(v) Drip-dry one portion of the edible fraction, package in plastic film-eliminating air as much as possible and place the packs inside the freezer.
(vi) Thin-spread the 2nd portion on a tray, place it inside the oven set at 60°C, withdraw when it has dried to a safe level. Cool at ambient condition. Package in films, seal and store.

Questions/Exercises
(i) In addition to your report in longhand, use a flow-diagram to illustrate the entire process operations.
(ii) State the essence of the size reduction if it foams so much, a few drops of oil will help to check the foaming leaves.
(iii) State the basic principle underlying the bitter processing.
(iv) Evaluate the edible fraction yield.

3.4 SUBJECT: Canned tomato paste
AIM: To process and preserve fresh into paste.
Materials Needed
Ingredients: Fresh ripe tomatoes, red ripe pepper (paprika), salt.
Chemical: Sodium bicarbonate
Equipment/Apparatus: Thermometer (0-200°C), refractometer, jacketed kettle or large spoon, blender, sieve, 300x208 enamel cans.

Procedure:
(i) Wash the tomato and blanch for 3 minutes.
(ii) Peel tomatoes and separate the seeds from the pulp.
(iii) Grind tomato pulp and pepper into sherry; the pepper should not exceed 10% of tomato.
(iv) Concentrated pulp to 25% solids, checking with a refractometer.
(v) Then add sodium bicarbonate and salt at the rate of 3g and 30g per litre of paste, respectively.
(vi) Fill hot into washed cans.
(viii) Seal and process cans for 25 minutes in boiling water.
(iii) What attraction has pear-shaped variety of tomato?
3.5 SUBJECT: Tomato ketchup

AIM: To produce tomato ketchup

Materials Needed:

Ingredients: Ripe tomato preferably pear-shaped variety; dome-shaped red pepper (paprika), onions, garlic, salt, sugar, vinegar.

Equipment/Apparatus: Blender, jacketed kettle or large pot, large spoon, thermometer (0-200°C), refract meter, sieve, stainless knives, metal cap bottle, oven.

Procedure:

(i) Wash bottles and caps and sterilize for 20 minutes in boiling water. Oven dry.
(ii) Weigh out ingredients as required and wash vegetables.
(iii) Wash and blanch tomato for 3 minutes.
(iv) Peel tomatoes and separate seeds from pulp.
(v) Grind tomato into a slurry and sieve.
(vi) Grind pepper and onions carefully removing the seeds.
(vii) Dissolve sugar and salt in vinegar.
(viii) Concentrate tomato to 20% solids.
(ix) Add all other ingredients and concentrate to 25% solids.
(x) Fill into bottles, cap and sterilize in boiling water.
(xi) Cool and dry bottles and label before storage.

Questions/Exercises

(i) Calculate the percent tomato solids in the product on salt-free basis.
(ii) Calculate product yield based on:
(a) Raw material
(b) Partially concentrated juice.
(iii) Compare the colour of tomato pulp with that of final product and account for change, if any.
(iv) Explain why ketchup is packaged in glass bottles and not in cans?
(v) Why process tomato ketchup in water-bath?

SUBJECT: Processing of Plantain

AIM: Production of Flour from Plantain

Materials Needed:

Ingredients: Unripe Plantain, Water.


Procedure:

(i) Wash the unripe plantain
(ii) Peel the plantain with knife
(iii) Slice the plantain
CHAPTER FOUR

PROCESSING OF TROPICAL ROOTS AND TUBERS

4.1 SUBJECT: Processing of Stem Tubers.

Aim: Extraction of starch from potatoes

Materials Needed:

Ingredients: Potatoes, water.

Chemicals: so.

Equipment/Apparatus: Basins. Rasp, screen, metallic trays, dryer, plastic film, pulverizer.

Procedure:

(i) Clean and wash the potatoes.
(ii) Rasp the clean, washed potatoes into slurry.
(iii) Make suspension by adding water to the slurry. Add SO₂ (0.01-0.0% w/w) to the suspension.
(iv) Screen the diluted slurry to obtain white starch suspension.
(v) Stand the suspension to settle, and decant the water and recover the wet starch or centrifuge.
(vi) Spread thinly on trays and dry at 60°C.
(vii) Cool and de-clump or pulverize.
(viii) Pack, label and store.

Questions/Exercises

(i) Illustrate the entire process by use of a flow-diagram.
(ii) What is the essence of making a suspension before separation?
(iii) Estimate the moisture content of both the fresh potato and the extracted starch.
(iv) Evaluate the potato starch extraction rate.
(v) What is the efficiency of your method?

4.2 SUBJECT: Processing of Root Tubers

AIM: Extraction of starch from cassava tubers

Materials Needed:

Ingredients: Fresh, wholesome cassava roots, water.

Equipment/Apparatus: Basins, trays, knives, grinding machine, sifter/screen, moisture-extraction oven, top-loading balances, plastic film, bottle, sealer.

Procedure:

(i) Pre-wash the cassava roots (if need be), and drip-dry.
(ii) Peel, and wash the resultant roots.
(iii) Grind them.
(iv) Suspend the mash in water and then screen.
(v) Stand the starch solution to settle.
(vi) Decant the water, and discharge the wet starch.
(vii) Spread thinly on the trays and place in the oven. Dry at 60°C.
(viii) Discharge dry starch when Me is 13%, cool, package (*) and store.
Questions/Exercises
(i) By the aid of a simplified flow-diagram, show also the whole process.
(ii) Determine the starch yield with respect to both the fresh roots and peeled root; (before grinding, and after packaging;)
(iii) Determine the starch yield with respect to both the fresh roots and peeled roots; (before grinding, and after packaging;)
(iv) State five uses of (cassava) starch both in the food industry and other industries.
(v) How efficient is your extraction operation?
(*) It will be in chunks or cakes after drying and requiring pulverization before packaging.

4.3 SUBJECT: Processing of Root Tubers

AIM: Production of Gari from cassava

Materials Needed:
Ingredients: Fresh cassava roots, water, palm oil (optional).

Equipment/Apparatus: stainless steel knives, drums, or coal-fired tripod stand, wooden scoop, flattened wooden stirrer, screen, plastic film or bags, jute bags.

Procedure:
(i) Clean and wash the tubers;
(ii) Weigh and peel, weigh the peeled cassava, after washing.
(iii) Rasp or grate the clean cassava tuber.
(iv) Introduce the slurry into jute bags and allow fermentation to take place while being pressed to de-water afterwards.
(v) Delump the solid cake and sieve to separate the fibre.
(vi) Fry or garify at 250°C for 20 minutes and dry to the storage moisture content.
(vii) Cool at ambient condition.
(viii) Package and store.

Questions/Exercises
(i) Illustrate the process by the aid of a flow diagram.
(ii) Estimate the moisture contents of both the fresh cassava roots and the gari.
(iii) Evaluate the extraction rate of gari, with reference to the whole tuber as well as the peeled tuber.
(iv) What are the functions of the grating?
(v) How many stages are there in cassava fermentation?
(vi) State the significance of each of the stages, highlighting the micro-organisms involved.

4.4 SUBJECT: Production of Milled Cassava Product (garisava).  

AIM: To produce garisava from tubers using upgraded local technique.

Materials Needed: Cassava tubers, big basins or pots, drying trays, stainless steel knives, mill or grinder, bags, sieves.

Procedure:
(i) Cut the cassava tuber into about 3cm lengths and peel.
(ii) Wash the tubers and boil for about 15 minutes.
(iii) Drain and cool.
(iv) Slice cooked tuber into 1.0mm thickness.
(v) Soak in water overnight.
(vi) Wash cassava slices until sour taste disappears.
(vii) Sundry slices to at least 10% moisture.
(viii) Mill the dry slices and sieve.
(ix) Bag the flour and analyze for cyanide.

Questions/Exercises
(i) Monitor cyanide level at each step of operation.
(ii) What is the role of cooking in this experiment?
(iii) Why is thin slices recommended?
(iv) Justify the soaking operation.
(v) Reconstitute the product and evaluate sensory qualities.

4.5 SUBJECT: Improved Fufu Production
AIM: To produce fufu with reduced odour.
Materials Needed: Cassava tubers, basins or pots, trays, sieves, citric acid.

Procedure:
(i) Cut cassava tuber into 3cm length and peel.
(ii) Wash peeled tubers and soak in water overnight.
(iii) Drain cassava, wash and pulverize with a greater.
(iv) Bag the pulp and allow to partially ferment for 24 hours but not more than two days.
(v) Wet sieve fermented pulp.
(vi) Add citric acid 0.1% and allow the starch to sediment.
(vii) Decant and bag sherry.
(viii) Dewater by pressing.
(ix) Sundry to at least 12% moisture.
(x) Pulverize and store.

Questions/Exercises
(i) Illustrate the process with the aid of a flow-chart.
(ii) What role does citric acid play in the process? Estimate cyanide level after soaking, fermentation, dewatering, and sun-dry.
(iii) Compare odour of fufu with market samples.

4.6 SUBJECT: Production of High Quality Cassava Flour
AIM: to produce High quality cassava flour
Materials Needed
Freshly harvested Cassava roots, Grating Machine (manual), calico bag, sifter, Water

Procedure:
(i) Peel and wash fresh cassava roots
(ii) Grate the roots into a mash
(iii) Dewater the mash by pressing inside a clean calico bag
(iv) Break the pressed mash (cake) into fine granules
(v) Spread thinly on clean trays (or black polythene sheets) on a raised platform to prevent contamination by dust, stones etc. and allow to dry.
(vi) Mill the dry sample and sift if necessary.
(vii) Package in polythene bags or any other airtight containers.

Note: it is important for the drying to be completed in one day.

Questions/Exercises
(i) Why is it necessary to dry the mash on raised platform
(ii) Why is it important to complete the processing within 24h.
(iii) Why is it necessary to sift the dry flour.
(iv) Describe the process with a flow diagram.

4.7 SUBJECT: Production of Cassava Flour Doughnuts

AIM: the production of Doughnuts from Cassava Flour

Ingredients:
Cassava flour 200g; Sugar, 50g; Margarine, 20g; Egg, one; Baking powder, 5.0g; Grated nutmeg, 1.5g; Water, as necessary.

Method:
(i) Put 50g of cassava flour into ½ cup of boiling water, removed from the fire and stir 4-5 times.
(ii) Mix the remaining flour with sugar, margarine, baking powder, and nutmeg.
(iii) Add the cooked cassava flour and mix together until mixture resembles bread crumbs.
(iv) Whisk the egg until light, and add to the mixture. Mix into a stiff dough which leaves the sides of the bowl clean.
(v) Mold into doughnut shapes and fry in oil (127°C) until golden colour.
(vi) Serve the doughnut warm.

Note: the doughnut may also be baked and in doing so beating egg is brushed the molded doughnut to give a good crust.

Questions/Exercises
(i) Why is it necessary to boil a portion of the cassava flour.
(ii) What is the importance of prolong mixing of the dough.
(iii) What role does the egg play in the mix.

4.8 SUBJECT: Production of Cassava Biscuits

AIM: To Produce Biscuits from Cassava Flour

Ingredients: Cassava flour, 200g; Margarine, 100g; Sugar, 65g; Baking Powder, 5.0g; Salt, 0.5g; Grated nutmeg, 1.5g and water as appropriate.

Methods
(i) Sift flour and baking powder into a dry bowl, add sugar, salt, and grated nutmeg.
(ii) Mix thoroughly.
(iii) Mix the margarine into the flour
(iv) Add enough water to make a stiff dough (the dough should not stick to the sides of the bowl)
(v) Roll out the dough on a board covered with flour and cut into shapes with biscuit cutters and also make holes in them.
(vi) Bake in a hot oven until brown (about 15 min at 175°C).

Questions/Exercises
(i) What important role does margarine play.
(ii) Why is it necessary to make holes in shaped biscuits.
CHAPTER FIVE

PROCESSING OF CEREAL GRAINS AND SUGAR CANE

5.1 SUBJECT: Processing of Maize (Sorghum or Millet)
AIM: Extraction of starch from maize (sorghum or millet).

Materials Needed:
Ingredients: maize grains, water.
Chemicals: Sulphur dioxide.
Equipment/Apparatus: Basins, trays, grinding machine, sifter/screen, moisture-extraction oven, top-loading balance, plastic film, sealer, pulverizer.

Procedure:
(i) Clean the maize grains, and soak in water.
(ii) Add to a concentration of 0.02-0.03% $\{\text{w/w}\}$, and allow the steeping for up to 48 hours.
(iii) Discharge the steeped grains, wash and grind.
(iv) Suspend the wet grind in water, and the screen to extract the starch fraction.
(v) Stand the starch solution to settle the starch.
(vi) Decant the water (top layer), and drip dry the wet starch.
(vii) Spread thinly on a tray, place inside the oven and dry at 60°C.
(viii) Discharge, cool, pulverize, package, seal and store.

Questions/Exercises
(i) Describe the process with the aid of simplified flow-diagram, also.
(ii) What is the importance of the steeping operation?
(iii) What are the functions of the S0.2?
(iv) What is the essence of drying at the temperature indicated in the procedures?
(v) Evaluate the dry starch yield.
(vi) Estimate the efficiency of your method.

5.2 SUBJECT: Processing of Maize (Sorghum or Millet)
AIM: Preparation of flour (meal) from maize (sorghum or millet).

Materials Needed: Dried maize (sorghum or millet) grains, mill or grinder, sieves, bags/containers, water and commercial semolina sample (for reference).

Procedure:
(i) Determine the fineness of the commercial semolina sample by sieving (sieve analysis).
(ii) Grind dried (or conditioned) grains into flour.
(iii) Sieve the ground sample.
(iv) Repeat the grinding and sieving operations until the bulk of the sample has attained the fineness of the commercial semolina sample.
(v) Repeat the experiment using dried or conditioned grains, as appropriate.

Questions/Exercises
(i) What is the fineness of the commercial semolina sample?
(ii) What is the yield of the flour (meal)?
(iii) What is the moisture content of the flour (meal)?
(iv) How are flour (meal) samples from maize, sorghum or millet utilized.
5.3 SUBJECT: Baking of White Bread

AIM: To produce bread from wheat flour.

Materials/Needed: Flour, fat, baker's yeast, salt, scale, sugar, dough mixer, baking pans, oven, thermometer, smooth board, humidity chamber (1kg flour, 20g salt, 20g yeast, 100g sugar, 400g fat, 500ml water).

Procedure:
(i) Propagate yeast by dissolving it in a little water at 40°C for 3 minutes.
(ii) Place all the measured dry ingredients in dough mixer and mix thoroughly.
(iii) Add yeast and mix.
(iv) Add remaining water and mix for 10 minutes at low speed initially and increase speed later.
(v) Allow dough to rest for 45 minutes.
(vi) Knock dough and work it on a flat board.
(vii) Grease baking pans and place weighed dough into pans.
(viii) Proof dough in a humidity chamber for 1 hour by placing boiled water and in same chamber.
(ix) Bake at oven temperature of 250°C for 25 minutes or until desire crust is reached.

Questions/Exercises
(i) Explain the phenomenon of sticky dough.
(ii) What is an "over worked dough"? What effect, if any, does over working the dough have on the quality of bread?
(iii) What effect does the hot water have on the proofing operation?
(iv) Why is it desirable to minimize dough cracking.

5.4 SUBJECT: Baking of Cake

AIM: To produce semi-rich cake by creaming (sugar batter) or blending (flour batter) method.

Materials needed: Wheat flour, baking powder, caster sugar, flavours, (vanilla, etc.), fat (margarine), baking oven cake pans, sieve, cake (dough) mixer, plastic bowl and weighing scale.

Procedure:
Recipe: 60z flour, 4 oz margarine (cold), 4 oz caster sugar, 2 level teaspoon baking powder, 2 drops of flavour, 2-4 tablespoon milk (mixed).

A. Creaming (sugar batter) method:
(i) Sieve flour, baking powder and salt.
(ii) Cream the margarine and sugar in a bowl until soft and fluffy.
(iii) Slowly add the egg, mixing continuously.
(iv) Lightly mix the sieved flour and baking powder and salt.
(v) Add 2-4 tablespoon milk and fold well.
(vi) Pour into a greased tin and bake for 30 minutes.

B. Blending (flour batter) method:
(i) Sieve the flour, baking powder and salt.
(ii) Rub in margarine to sandy texture.
(iii) Add the sugar.
(i) Make a well in the centre of the flour.
(ii) Gradually add the well-beaten eggs and mix as lightly as possible until combined.
(iii) Add 2-4 tablespoon milk and fold well.
(iv) Pour into a greased tin and bake for 30 minutes.

Questions/Exercises
(i) Illustrate the procedure for baking cake with a flow chart.
(ii) What is the role of margarine (fat)?
(iii) What is the difference in texture of cake and bread?
(iv) Explain the phenomenon of dipping in cakes and it is prevented

5.5 SUBJECT: Baking with Composite Flour
AIM: To evaluate baking potential of composite flour.

Materials needed: Wheat flour, any other flour (maize, soybean, sorghum, cassava, cocoyam, etc.) salt, sugar, baker's yeast, fat, scale, baking pans, dough mixer, smooth board, thermometer, baking oven, bread volumeter, humidity chamber.

Procedure:
(i) Use formula for whole wheat bread but gradually replace wheat flours.
(ii) Follow the steps outlined earlier for white bread

Questions/Exercises
(i) Measure the volume of the various bread samples.
(ii) Explain the variation in volume in the composite bread.
(iii) Slice the loaves and compare crumb texture.
(iv) Chemically explain the low volume in bread with relatively high replacement of wheat flour.
(v) What is the function for fat in the bread formulas?
(vi) Explain the cracking phenomenon observed in some samples.

5.6 SUBJECT: Baking With Non-Wheat Composite Flours
AIM: To investigate potential of non-wheat flour composite.

Materials needed: Dough mixer, baking pans, baking oven, humidity chamber, maize flour, cowpea flour, soybean flour, cassava starch, sugar, salt, baker's yeast, fat, thermometer, bread volumeter.

Recipe: A (600gm maize flour, 300gm cassava starch, 100gm soybean flour sugar 40gm, salt 15gm and 980ml of water); B (800gm maize flour, 100gm cassava starch, 100gm cowpea flour, sugar 800gm, fat 60gm, yeast 20gm, salt 15gm and 980ml of water).

Procedure:
(i) Dissolve yeast in warm water (38°C) 1:5 ratio.
(ii) Mix all dry ingredients in a dough mixer at slow speed for 5 minutes.
(iii) Add yeast suspension and remaining water and mix for 10 minutes at medium speed.
(iv) Allow resulting batter to bulk ferment for 30 min.
(v) Mix at medium speed for 5 minutes.
(vi) Weigh batter and place in already greased pan.
(vii) Proof in a humidity chamber for at least 30 minutes.
(viii) Bake at 22°C until crust is brown.
Questions/Exercises

(i) Measure the volume of baked product.
(ii) Evaluate product in terms of appearance, crust colour, crumb, grain, texture and flavour.
(iii) Why use batter instead of conversational dough method?
(iv) Explain the cracks evident in the samples.

5.7 SUBJECT: Plain Biscuit

AIM: To produce biscuit

Materials Needed: Wheat flour, fat, sugar, milk, baking powder, sieve, dough mixer, plastic bowl, improvised biscuit cutter (flour - 800g, fat - 375g, sugar - 450g, milk - 75g, baking powder - 8g, water - 75ml, vanilla - 5ml)

Procedure:

(i) Sieve flour and mix with baking powder in the mixer
(ii) Add fat and blend.
(iii) Mix sugar, milk, water, vanilla to make a cream.
(iv) Add cream to the flour mix and blend for 15 seconds.
(v) Transfer dough kneading board and roll with a rolling pin.
(vi) Cut dough into desired shape with improvised biscuit cutter and transfer to baking trays.
(vii) Bake at 200°c for 20 minutes or until brown colour is developed.
(viii) Cool, product and package.

Questions/Exercises

(i) Why is wheat flour used for bread unsuitable for biscuit making?
(ii) Illustrate the procedure for margarine of fat?
(iii) What will be the effect of over-working the dough on quality of biscuit?

5.8 SUBJECT: Processing of Sugar Cane

AIM: Extraction of raw cane sugar (brown sugar)

Materials Needed:
Ingredients: mature canes of sugar grass, water, chemical Ca(OH)₂, NaOH
Equipment/Apparatus: Knives, basins, cooker, pots/kettles, press, vacuum concentrator, centrifuge stirring rod, pH-meter.

Procedure:

(i) Clean the canes, de-back, and shred them.
(ii) Express the sugar cane shreds to obtain the juice.
(iii) Strain the juice.
(iv) Neutralize the clear juice by adding either Ca(OH)₂ directly or lime and water.
(v) Heat the neutralized solution to precipitate impurities.
(vi) Stand to cool and settle out the lime.
(vii) Decant the clear juice.
(viii) Heat the juice under vacuum to concentrate the sugar.
(ix) Centrifuge the concentrated solution, and segregate the brown sugar from the molasses.

Questions/Exercises

(i) Illustrate the process by a flow diagram also.
(ii) What is the pH of the juice in step (iii)?
(iii) What is the soluble solid content of the juice in (iii)?
(iv) What is the mass density of the juice?
(v) Evaluate the yield of the raw sugar.
(vi) Evaluate the efficiency of your extraction.
CHAPTER SIX
PRODUCTION OF SPECIAL FOOD PRODUCTS

6.1 SUBJECT: Canned Snail Meat
AIM: To preserve snail meat by canning.
Materials Needed: Snail, tomato sauce or 10% brim, 300x208 enamel cans, can sealer, retort, lime juice, long pin, stainless steel knives.

Procedure:
(i) Wash snail
(ii) Cut the pointed end of the snail shell.
(iii) Heal in boiling water for 10 minutes
(iv) Remove the snail meat by spinning the long pin from the pointed end of the shell and pull from the large end
(v) Shrub and wash the snail with water and lime juice.
(vi) Rinse with water and drain
(vii) Pack into washed 300x208 enamel cans and hot fill with either tomato juice or 10% brime.
(viii) Seal cans with can sealer.
(ix) Retort cans for 30 mins at 115.6°c.
(x) Cool cans and label.

Questions/Exercises
(i) Explain the role of the initial hot water treatment.
(ii) Why is it necessary to use lime juice, alum or similar material in washing snail?
(iii) Illustrate the canning of snail meat with a flow chart and identify the critical check points in the operation.

6.2 SUBJECT: Sausage Manufacture
AIM: To produce specific sausage
Materials Needed: Beef, pork, spices (onions, paprika), ice blocks, meat grinder.

Procedure:
(i) Grind chilled beef and pork separately through a 3.4mm plate.
(ii) Add ice blocks during the grinding operation.
(iii) Grind the other ingredients (5g onions, 3g pepper) separately.
(iv) Add 70g beef, 30g pork, 2g salt and the other ingredients and grind for 10 minutes.
(v) Stuff the meat blend into pre-heated animal casing and water cook at 105°c for 2 hours.
(vi) Cool and package.

Questions/Exercises
(i) What role, if any, does ice block play in the production of sausage
(ii) Why is it desirable to pre-heat the animal casing prior to stuffing?
(iii) Calculate the shrinkage in your sausage.
6.3 SUBJECT: Imitation of Cooked Sausage

Aim: To utilize local ingredients in sausage production.

Materials Needed: Chilled beef, chilled pork, oilseed meal, salt, local spice ('ehuru', 'Uda', or 'uziza'), onions, pepper, ice blocks, animal casing, stainless steel knives, meat grinder, cooking pot.

Procedure:

(i) Wash the chilled meat.
(ii) Thoroughly wash the animal intestine and boil it for 5 minutes.
(iii) Cut up the chilled meat and grind with a meat grinder separately and add ice block.
(iv) Grind the other ingredients separately.
(v) Add 56g beef, 2g pork, 20g oilseed meal, 2g salt, 0.5g 'uziza' or 'uda', or 'ehuru', 5g onions, 3g pepper and chop for 10 minutes.
(vi) Stuff the blend into animal casing and put in water; cook at 105°C for 2 h.
(vii) Cool and package.

Questions/Exercises

(i) Evaluate the sensory properties of the imitation sausage and compare with the exercise.
(ii) What effect, if any, does the local flavouring ingredients have on the quality of the imitation sausage?
(iii) Calculate the cooking yield of product.

6.4 SUBJECT: Production of Wine.

Aim: To demonstrate principles of the alcoholic fermentation and also to illustrate some quality tests applied to finished wine.

Many beverages are produced with the aid of the alcoholic fermentation brought about by yeasts. Examples are beer, ale, wine, brandy and whisky. This experiment demonstrates the principles of the alcoholic fermentation and also illustrates some quality tests which are applied to finished wine.

(i) Inoculation and incubation of grape juice:
A. Inoculate 100ml samples of pasteurized grape juice in 6 oz bottles as follows:
   1. With a 10% inoculum of the burgundy strain of wine yeast.
   2. With a 10% inoculum of an apiculate yeast.
   3. With a 10% inoculum from crushed grapes previously fermented.
B. To 100ml of pasteurized grape juice in a 6 oz bottle add 4g of sucrose and inoculate with a 10% inoculum of burgundy strain of wine yeast. After 2 days of incubation add an additional 4g of sugar and make a similar additional 4-6 days later.
C. Incubate all bottles of inoculated grape juice at room temperature, taking care to leave the caps loose.

(ii) Examination of wine:
A. After 4-5 weeks, note the aroma, taste, and appearance of the wines. Pool each of 4 types of wine (1-A-1, 1-A-2, 1-A-3, and 1-B) as directed by the instructor. Perform the following tests on the pooled wines.

1. Total acidity (expressed as per cent tartaric acid). Compare with that of undermented controls. (see Appendix 1.B.3, all for details on titration).
2. Volatile acidity (expressed as volume percent) can be determined by means of an ebulliometer and interpreted results.

Questions/Exercises
(i) Why does the commercial maker of wine add potassium metabisulfite to the must? How much is added? Why was it not used in this experiment?
(ii) What are the differences between sparkling and still wines? Between fortified and regular wines?
(iii) Why is large yeast inoculum used in wine making?
(iv) What is the effect of too high or too low a temperature of incubation during wine making?
(v) What are apiculate yeasts? What is their source and their importance in wine making?
(vi) What is the significance of volatile acids in wine?
(vii) What defects of microbial origin can occur in wine? How can each be prevented?

6.5 Subject: Production of Vinegar

Production of vinegar from a raw material such as apple juice or grain employs a two-step fermentation. Yeasts ferment the carbohydrate in the raw material to ethanol. The ethanol is then oxidized to acetic acid by an acetobacter sp. The generator or submerged processes may start with ethanol.

(i) THE SLOW (ORLEANS) PROCESS (Used now only for small scale production; it demonstrates the principles of the fermentation).
A. Sterilize or pasteurize a bottle containing 200 ml of pure apple juice and inoculate heavily (2-3 loopfuls from a slant culture) with a high alcohol yielding yeast such as saccharomyces cerevisiae var. ellipsoides. Include at room temperature until activity dies down (a week or more). Leave the cap loose on the bottle during incubation.
B. Inoculate the bottle with vinegar film (mother of vinegar). Place a small piece of Acetobacter film inside the bottle near the neck, so that it will be exactly at the level of the liquid when the bottle is inclined by means of bottle supports furnished. Incubate at room temperature. Allow aceticification to proceed until a strong vinegar is produced. This should take about 7 weeks.
C. Examine the finished vinegar for color, odor, and microscopic appearance. Pool all good vinegar as directed by the instructor. Filter the vinegar through filter paper. Titrate the filtered vinegar as instructed. Calculate the percent of acetic acid in the vinegar according to the formula given in Appendix 1.B.3.a.
D. The students will be assigned to clarify the vinegar for the class. Filter by means of an infusoria-earth filter aid. Distribute filtered vinegar among a series of 6-8 oz bottles, filling them about an inch from the top. Place the filled bottles into a water bath at 145°F for 30 min. This serves to pasteurize the vinegar and improves its keeping quality. (Note: In commercial practice, aging—usually in wooden tanks—generally precedes filtration and pasteurization).
E. Examine the "mother of vinegar." After a gross observation, prepare a smear and stain it with the gram procedure.
A. Vinegar cells (nematodes, *Anguilla aceti*) often infect vinegar plants, especially if vinegar is made from fruit juices. Vinegar infected with the cells will be provided. Examine it microscopically and record results.

(i) Production of vinegar by submerged fermentation: (New vinegar plants generally employ fermentors equipped to support a submerged fermentation).

A. Add 5ml ethanol (not denatured) and 1ml glacial acetic acid to each of 3-500 ml Erlenmeyer flasks containing 94ml of the acetobacter broth (medium 1). Titrate the resulting mixture and express results as percent acetic acid. A suitable closure for these flasks consists of 3 thicknesses of cotton milk filter discs placed flat over the opening of the flask, with the edges folded down around the neck of the flask, and the discs secured with a rubber band placed over them around the neck of the flask.

B. Inoculate the flasks as follows:

1. To one flask add 3ml of an active culture of *acetobacter acetici*.
2. To a second flask add 3ml of an active culture of *acetobacter suboxydans*.
3. To a third flask add a piece of vinegar film the size of a dime. Macerate the film well before adding it.

C. Incubate the flasks on a rotating or reciprocal shaker at room temperature. Operate the shaker at a speed sufficient to provide good agitation without excessive splashing.

D. Titrate at 2 days intervals and record the amount of acetic acid present. When the titratable acid is approximately 3%, and 3ml of ethanol to each flask and continue incubation the shaker. Titrate regularly and note when the maximal concentration of acetic acid is attained.

E. Continue to incubate the flasks for 5 days after the maximal acid concentration has been attained. Titrate at regular intervals and note any changes in the acid content of the culture in each flask.

Questions/Exercises

1. What are the principal chemical steps in the vinegar making process? Give equation.
2. What is meant by “fining” of vinegar? How may it be done?
3. What is the relationship between “grain” of vinegar and the percent acid?
4. How much 45-grain vinegar would you expect to get from 100 gallons of 95% ethanol, assuming maximal efficiency in the fermentation?
5. Is the acetic acid fermentation carried out under aseptic conditions? Why?
6. Devise a scheme to produce vinegar from corn cobs. From where?
CHAPTER SEVEN
PROCESSING OF MISCELLANEOUS FOOD PRODUCTS

7.1 SUBJECT: Production of Ugha (Fermented African oil bean seeds)
Aim: Production of ugha from Raw African oil bean seeds
Materials Needed:
Ingredients: Raw mature African oil bean seeds, clean water.
Equipment/Apparatus: Pressure pot/aluminium pot, cooking spoon, local vegetable shredder (Nkwoor) or knife, sieve, basin, tray, wrapping material (akwukwo ugha (Alchornea laxiflora benth) leaves or polyethylene) and twin or rope.

Procedure:
1. Sorting, cleaning and washing
2. Put clean seeds in pot, covered with water
3. Boil for 45min with stirring at intervals
4. Dehull seeds while hot in batches
5. Shred with nkwoor or knife
6. Put the shreds in boiling water in a pot and boil for 30 minutes with stirring at intervals
7. Pour shreds into clean sieve to drain out hot liquor
8. Spray water on the shreds to completely remove hot liquor
9. Wash the shreds 3times
10. Drain wash water
11. Cover shreds with clean water and steep for 8-10hrs
12. Drain off steep liquor and wash the shred
13. Put shreds in clean sieve lined with the leaves and completely drain out the wash water
14. Cover the shred with clean akwukwo ugha
15. Incubate samples at 34°C (warm environment) for 5h
16. Weight samples in small portion and wrap in clean leaves or polyethylene bags
17. Heap wrapped samples together and cover them
18. Ferment for 3 days or 5 days depending on end use
19. Stop fermentation by steaming product for 20 min.

Exercise/Questions
1. State the importance of Blanching the seed and dehulling hot?
2. What are the critical control points in the production of ugha?
3. Make a flow diagram for the traditional production of ugha
4. Explain the role of steeping and fermentation in the production of ugha.

7.2 SUBJECT: Production of Dawadawa or (iru) from locust beans
AIM: To produce dawadawa from raw locust beans
Materials Needed:
Ingredients: Mature dry locust beans, water, aluminium, pot, mortar and pestle, sieve, potash (kanwa), trays, blanched banana leaves, salt.
Procedure:
1. Sorting, cleaning and washing
2. Soak overnight or 12h
3. Boil seeds for 12h or more
4. Cool and dehull by pressing between palms or use mortar and pestle i.e use abrasive method
5. Wash the cotyledons and discard seed coats and undehulled beans
6. Boil cotyledons for 2h (add potash for softening)
7. Drain off liquor through sieve
8. Spread dehulled beans while hot in trays lined with blanched banana leaves (10cm deep)
9. Stack trays together and wrap with jute bags
10. Ferment for 36h or more
11. Salt and mold (to desired shapes)
12. Air and/or sundry

Questions/Exercise
1. Make a flow diagram for the production of dawadawa
2. Point out the critical control points in the production of dawadawa
3. How can this product be preserved?

7.2.1 Subject: Production of dawadawa from Soybeans
AIM: To produce Dawadawa from soybeans
Materials: Mature dry soybeans, pot, sieve, trays/basket, blanched banana leaves.

Procedure:
1. Sort and Clean
2. Fry or toast to a brown colour
3. Dehull to remove the seed coats
4. Cook or boil for 3h
5. Drain liquor through a sieve
6. Spread in a leaf lined baskets/trays
7. Mixed with previously fermented soybean dawadawa if available
8. Cover trays with the same leaves used for lining trays/baskets
9. Cover the trays and place in warm place
10. Ferment for 3 days
11. Sundry the product and grind to a fine powder

Questions/Exercise
1. Make a flow diagram for production of soybean dawadawa
2. What is the effect of mixing the cooked dehulled beans with a previously fermented soybean dawadawa?
3. Why must fermentation be carried out in a warm environment?
7.3 **Subject: Production of Butter Scotch**

**Method 1:**

**Ingredients**

- Brown sugar: 400 gms
- White sugar: 100 gms
- Glucose: 400 gms
- Water: 130 ml
- Butter: 80 gms
- Lemon oil: 3 ml

Mix the sugar (brown and white), glucose and water in a sauce pan. Boil to temperature of 280°F or 285°F if a more brittle product is desired. Add 80 gms of butter (in small pieces). Stir well to homogenize, heat a little if not well mixed. Cool. Add 3 ml of lemon oil and pour between bars on greased metal slab. Aim at 1/4 inch thickness. Wrap and store as soon as it gets cool.

**Production Butter Scotch**

**Method 2:**

**Ingredients**

- Granulated sugar: 450 g
- Butter (diced): 75 g
- Cream of tartar: 1-5 ml
- Water: 275 ml

Few drops of vanilla extract

Grease a 28 x 18 cm flat square cake pan with oil. Dissolve 450 g sugar in the water with gentle heat. Add cream of tartar and stir into the solution. Bring the solution to boil without stirring and continue to heat until a temperature of 116°C is reached. Remove from heat, stir in the butter, return the solution to heat to a temperature of 136°C without stirring. Add the vanilla and boil to a temperature of 168°C (the product thickens). Pour mixture into the prepared pan and use an oiled knife to mark off into oblong shapes. As soon as the product get cool, break out the shapes and wrap.

7.4 **Subject: Production of Chocolate Fudge**

**Sugar** 60 g

**Cocoa powder** 50 g

**Honey** 150 g

**Butter** 150 ml

**Milk** 150 ml

**Vanilla extract** 2 ml

Grease a 28 x 23 cm flat tin with oil. The sugar, honey, cocoa powder, butter and liquid milk are put into a sauce pan & placed over a gentle heat. Stir mixture until sugar and cocoa dissolve. Heat mixture to a temperature of 116°C. Take off pan from heat and add vanilla flavor to the mixture. Beat fudge until it thickens and becomes creamy. Pour into the grease pans and mark off in squares, wrap and store in tight containers.
CHAPTER EIGHT
TOTAL QUALITY MANAGEMENT

8.1 ATTRIBUTE CHARTS
X-chart and R-range (mean and range charts) and critical control points

Procedure:
(i) Ten batches of cowpeas are provided and each batch comprised of five (5) grains.
(ii) Use the venier caliper and measure the length of each grain in each batch.
(iii) Record your observations and use the values to calculate the control limits.
(iv) Plot (a) The mean chart (X-chart) and (b) The range chart (R-chart)

Questions/Exercises:
(i) Discuss the charts in details, stating whether the parameters are under control or out of control.
(ii) Explain the implications of the obtained results.

8.2 FRACTIONAL DEFECTIVE CHART

Procedure:
(i) Five batches of grains is provided each containing 50 grains.
(ii) Sort each batch by removing any grain with any form of defect such as broken grains, weevil-infested, shriveled and coloured grains etc.
(iii) Count the number of defective grains in each batch and record same.
(iv) Calculate the fractional defective values and the control limits.
(v) Plot the p-chart

Questions:
(i) Discuss the chart, explaining the results that fall within or out of the limits and the applications of each deviation or conformity.
CHAPTER NINE

9.1. MEASUREMENT OF RHEOLOGICAL PARAMETERS

The Newtonian viscosity of fluids can be calculated from the measured ratio of shear stress and shear rate. One of various types of viscometers such as: capillary, falling ball, rotational, and cone-and-plate are generally used. For non-Newtonian materials there are several methods of measurements. To obtain the power law parameters to describe their behavior involves the measurement of shear stress and shear rate relationship using a rotational or a cone-and-plate viscometer.

9.2. Capillary Viscometer

The experimental setup used in capillary flow measurements are of different types. One type of capillary viscometer is shown in Fig. 3.2. The ratio of the reservoir bulb radius to that of the capillary should be greater than 10 so that the pressure drop due to the flow in the reservoir can be neglected.

For steady laminar flow of Newtonian fluids in a circular capillary, the classical Hagen-Poiseuille equation (3.6), provides the basis for the measurement of viscosity.

\[ \dot{V} = \frac{\Delta P \cdot \pi \cdot R^4}{8 \mu \cdot L} \]

where \( \dot{V} \) is the volumetric flow rate, \( \Delta P \) is the pressure drop across the capillary, \( R \) is the capillary radius, \( \mu \) is the fluid viscosity, and \( L \) is the capillary length.

However, for a mean static fluid height \( h \), the pressure drop is given as

\[ \Delta P = \rho g h \]

where \( \rho \) is the fluid density and \( g \) is the acceleration due to gravity. Substitution of equation 3.7 into equation 3.6 and rearrangement gives

\[ \frac{\mu}{\rho} = \frac{\pi \cdot R^4 \cdot g \cdot h}{8 \cdot L \cdot \dot{V}} \]

where \( \mu/\rho \) is the kinematic viscosity of the fluid if volume \( V \) of the fluid flows through the capillary in time \( t \), then

\[ \dot{V} = \frac{V}{t} \]

Now equation 3.8 can be rewritten as

\[ \mu = \left( \frac{\pi \cdot R^4 \cdot g \cdot h}{8 \cdot L \cdot \dot{V}} \right) \]

When the viscometer shown in Fig. 3.2 is allowed each time to drain between etched marks \( \xi \) and \( \xi \), the volume \( V \) and mean height \( h \) become fixed, and the terms in parenthesis become constant. Equation 3.10 may be simply written as

\[ \dot{V} = \frac{h \cdot \dot{V}}{b} \]

where \( b = \left[ R^2 \cdot g \cdot h / (8 \cdot L \cdot \dot{V}) \right] \), a viscometer constant.

The viscometer constant can be either determined by obtaining values of the parameters needed or, more conveniently, by measuring the efflux time for a fluid of known kinematic viscosity, as shown follows.

\[ b = \frac{1}{t_{\text{minimum}}} \]
Once the value of the viscometer constant is known, the kinematic viscosity of a test fluid (unknown) can be determined by measuring its efflux time and using equation 3.11. From this knowledge of the density, the viscosity, \( \eta \), is then readily calculated.

For efflux times less than 200 s, the capillary entrance and exit effects (called the end effects) become important and a modified version of equation 3.11 is used. (See Van Wazer, Lyons, Kim, and Colwell, 1963)

### 9.3. Rotational Viscometer

Rotational viscometers allow continuous measurements of the shear stress vs. shear rate relationship. The concentric-cylinder or coaxial-cylinder viscometer is one type of rotational viscometer commonly used in rheological studies (Fig. 3.3). In principle, the torque \( M \) required to rotate the inside cylinder at a given number of revolutions per minute is measured to obtain the flow curve for the material filled in the gap between the concentric cylinders. The equation of motion for a coaxial-cylinder viscometer gives

\[
\frac{1}{R^2} \frac{d}{dR} (R^2 T_{R\theta}) = 0
\]

or \( R^2 T_{R\theta} \)

where \( T_{R\theta} \) is the shear stress exerted in the angular direction \( \theta \) by a fluid surface at radius \( R \) on the fluid in the region \( R < R < R_c \). \( R_c \) is the radius of the inner cylinder and \( R \) is the radius of the outer cylinder. Now the torque \( M \) exerted at the radial position \( R \) is given by

\[
M = \int F R dR
\]

where \( F \) is the force exerted. From the definition of shear stress, \( T_{R\theta} \), and substituting for the force from equation 3.15,

\[
T_{R\theta} = \frac{F}{A} = \left( \frac{M}{R} \right) \left( \frac{1}{2 \pi R L} \right)
\]

where \( A \) is the surface area at radius \( R \), and \( L \) is the length. Therefore,

\[
R^2 T_{R\theta} = \frac{M}{2 \pi L}
\]

For a power-law fluid without yield stress, the shear-stress vs. shear-rate relationship in cylindrical coordinates is

\[
T_{R\theta} = -m \left( \frac{d\omega}{dR} \right)^n
\]

where \( \omega \) is the angular velocity (radians per unit time) of the inner cylinder. Substituting for \( \omega \), from equation 3.17 into equation 3.18, separating variables, and integrating between the radius of the inner cylinder and the radius of the outer cylinder gives the following expression:

\[
- \int_0^{R_c} \omega d\omega = \left( \frac{M}{2 \pi m L} \right) \int_{R_i}^{R_c} R^{-\frac{2+n}{n}} dR
\]

Upon integration, equation 3.19 becomes

\[
\omega = \left( \frac{M}{2 \pi m L} \right)^{\frac{1}{n}} \left( \frac{n+2}{2} \right) \left( \frac{R_c^{\frac{2+n}{n}} - R_i^{\frac{2+n}{n}}}{} \right)
\]

Taking the natural logarithm of equation 3.20 and rearranging it gives
\[ \ln(M) = n \ln(o) + \ln(2m - 1) - n \ln(n/2) - n \ln \left( \frac{R_i^n - R_o^n}{R_i^n - R_0^n} \right) \]

Thus, rheological parameters \( m \) and \( n \) can be determined by plotting the experimental data in the form \( \ln(M) \) versus \( \ln(o) \). The slope of the line of best fit gives the flow behavior index \( n \). The consistency coefficient \( m \) is obtained from the intercept.

In case of Newtonian fluids, \( n = 1 \) and \( m = 1 \). On substitution and rearrangement, equation 3.20 can be written as

\[ \frac{1}{(M/(4L))} = \left( \frac{1}{R_i^n} - \frac{1}{R_o^n} \right) \]

The foregoing expression is the Margules equation for Newtonian viscosity. Thus, a knowledge of torque \( M \) and angular velocity \( \omega \) is all that is needed to determine Newtonian viscosity.

In a few viscometers the outer cylinder is a large container. In such systems it is able to assume that \( R_i \gg R_o \). On ignoring \( R_i \), equations 3.20 and 3.22 for non-Newtonian fluids become

\[ \frac{1}{(M/(4L))} = \frac{1}{(M/(4R_0^2L))} \]

Where \( \eta \) is the apparent viscosity. Combining equations 3.23 and 3.24 yields

\[ (2)^{(m-1)}(1/n)^n \]

Taking the natural log of equation 3.25 gives:

\[ \ln(o) = (n - 1) \ln(2) + \ln(m) - n \ln(n) \]

Therefore, on plotting the measured values in the form \( \ln(o) \) versus \( \ln(2) \), a straight line is obtained. From the slope and intercept of the line of best fit, the flow behavior index \( n \) and the consistency coefficient \( m \) are determined.

### 9.3.1. Power Law Fluid with a Yield Value or Stress

When a yield stress \( \sigma_0 \) exists for a fluid in a coaxial viscometer, the relationship between torque \( M \) and the rotor angular speed \( \omega \) is given by (Charm, 1978):

\[ \omega = \left( \frac{1}{m} \right)^{1/n} \int_{R_o}^{R_i} \left( 1 - \frac{M}{2 \pi \sigma_0 R_0^2 L} \right)^{1/n} \frac{dR}{R} \]

Where

- \( R_o \) - distance from the centre at which the velocity of the streamline is zero. This will occur at the point in the gap where the yield stress is equal to the shear stress.
- \( R_i \) - radius of the outer cylinder in wide gap viscometer or equal to \( [M/(2.1)]^{1/2} \) for single-cylinder viscosity.
- \( R \) - radius of the cylinder spindle.
Since the rate of shear (τ) is proportional to the rotational speed of the viscometer, the τ₀ may be determined by plotting (M/L)¹/₃ versus

\[ \tau_0 = \left( \frac{M}{L} \right)^{1/3} \frac{1}{2\pi R_i^2} \]

By plotting \( \log (\tau) \) versus \( \log \left( \frac{M}{L} \right)^{1/3} \frac{1}{2\pi R_i^2} \), the slope of the resulting line will be 1/n. Then, using equation 3.27, m can be calculated.

### 9.4 Bingham Plastic Behavior Fluids

The relationship for Bingham plastics is given by Heldman and Singh (1981). When using coaxial rotational viscometer it becomes

\[ \omega = \frac{M}{m \cdot 4\pi L} \left( \frac{1}{R_i^2} - \frac{1}{R_0^2} \right) - \frac{\tau_0}{m} \ln \left( \frac{R_0}{R_i} \right) \]

**Note:** There are two major points worth noting:

1. The foregoing development does not account for the drag on the top and bottom of the rotating inner cylinder (called the end effects). To overcome this, the actual length of the inner cylinder is replaced by an effective length \( L' > L \). Methods to determine \( L' \) are available in the literature (Van Wazer et al., 1963). Manufacturer's of these viscometers also provide such data for their system.

2. As with capillary viscometers, the assumptions made in the derivation of equations for the calculation of rheological parameters include: steady-state and laminar flow, time independent properties, no-slip condition at the wall, incompressible fluid, and isothermal conditions at measurement. These conditions must be strictly adhered to for the results to be meaningful.

### 9.5 OBJECTIVES

The specific objectives of this laboratory are to:

1. Determine the Newtonian viscosity of a test fluid using a capillary viscometer.
3. Determine the effect of temperature on Newtonian viscosity.

### 9.5.1 APPARATUS

- Capillary viscometer (Cannon —Ferske, size no. 100 (kinematic viscosity range 2 to 30 centistokes cSt) or size no. 150 (kinematic viscosity range 6 to 30 cSt) Constant temperature glass water bath (10° 0 10°C) (preferably Cannon MF Series) Suction type rubber bulb, two 100 ml cylinders, two 100 mL volumetric flasks, stopwatch balance Distilled water, acetone, and trichloroethylene Viscosity standards; silicone oils, water test fluids clarified apple juice, Gatorade, evaporated milk, or sucrose-water solutions with viscosity within the viscometer range
8. Brookfield Synchro-Lelectric, Model LV or RV, or other similar coaxial-cylinder viscometer constant-temperature bath, thermometer, and beakers (600 mL/L)
Suggested test fluids:
Newtonian: corn syrup, corn oil, STP oil additive
Pseudoplastic: mustard paste, French salad dressing, Gerber bananas
Dilatant: corn starch in water, 55% by weight
Brookfield Synchro-Lelectric, RV or LV model, or other similar viscometer
Two constant temperature water baths (30°C to 80°C), thermometers, beakers (600 mL, one per bath)
Test fluid: corn syrup or corn oil

9.5.2 Procedure
9.5.2.1 Determination of the Newtonian Viscosity Using a Capillary Viscometer
The Newtonian viscosity data for this part of the experiment will be obtained with the Cannon—Fenske Capillary Viscometer, shown in Fig. 3.2, size no. 100. Larger size may be used for higher-viscosity fluids.

1. Fill up a 10-mL graduated cylinder with the test fluid.
2. Attach a suction bulb to arm G of the viscometer. Invert the viscometer, dip arm Arm the test fluid, and apply suction until the fluid level reaches etched mark E. Return the viscometer to an upright position.
3. Place the viscometer in a controlled temperature glass water bath and allow the temperatures to equilibrate. Record the temperature.
4. Record the efflux time t for the test fluid to drain between marks C and E.
5. Repeat the measurement by applying suction to arm A to bring the test fluid level above mark C.
6. Rinse the viscometer thoroughly, first with distilled water and then with acetone. Dry the viscometer completely.
7. Follow steps 1 to 5 for other test fluid or the viscosity standards.
8. Clean the viscometer after each standard first with trichloroethylene and then with acetone. Aspirate to dryness.
9. Determine the densities of the test fluid and the standard by filling them in tared 100 mL volumetric flasks and weighing. Record the data in Table 3.1.

9.5.2.2 Determination of Rheological Parameters Using a Coaxial-Cylinder Viscometer
The rheological parameters are determined from a knowledge of the shear-stress vs. shear-rate relationship generated with a coaxial-cylinder viscometer, such as the Brookfield Synchro-Lelectric model RVT or Haake Rotovisco RV series. In such types of viscometers, a spindle rotates in the fluid at a selected angular velocity and the torque required to overcome the viscous resistance of the fluid is measured. The rheological parameters are computed from these measurements at various angular velocities, discussed earlier.

The following procedure is for obtaining the required data with a Brookfield Synchro-Lelectric model RVT, recommended by the manufacturer.

1. Record the product (test fluid) information on the data sheet B of Table 3.1 along with the instrument data.
1. Pour about 500 mL of the test fluid into a 600-mL beaker and place the beaker in a temperature-controlled water bath (30°C). Record temperature of the fluid after equilibration.
2. Attach spindle to viscometer shaft, taking care to avoid exerting side thrust on the shaft.
3. Insert spindle in the test fluid up to immersion groove cut in the spindle shaft.
4. Level the viscometer by adjusting screws on mounting stand and the bubble level on the dial casing.
5. Depress the clutch and start the motor. Release the clutch and allow the dial to rotate until the pointer stabilizes at a position on the dial.
6. Depress the clutch to freeze the pointer. Stop the motor and record the rotational speed and the dial reading (torque M). If the dial reading is less than 50, change to a larger spindle (lower number). For off-scale dial reading, a smaller spindle (higher number) should be used.
7. Stop motor and record the dial reading and the rotational speed.
8. Reset the rotational speed (angular velocity) and repeat steps 6 through 8. For good results, measure torque (dial reading) at four or more angular velocities.
9. Obtain data on at least one Newtonian, one pseudoplastic, and one dilatant and record them in Table 3.1.(B).

**9.5.3. Determination of Effect of Temperature on Newtonian Viscosity**

In addition to the data on the Newtonian viscosity of the test fluid obtained in the previous experiment using a coaxial-cylinder viscometer, two more data sets can be obtained by conducting measurements at two more temperatures (50°C and 70°C). The procedure to be followed is essentially as described for the previous experiment. Although it is possible to determine the Newtonian viscosity from a single measurement of shear rate (angular velocity) and shear stress (torque), measurements at several velocities should be made to show that the flow behavior index n remains unity at all temperatures of measurement.

**Notes**

1. To convert revolutions per minute (rpm) to radians per second, use the following formula:

\[
\frac{\text{revolutions}}{\text{min}} \times \frac{\text{min}}{60 \text{secs}} \times 2 \pi \frac{\text{radian}}{\text{revolution}} = \omega
\]

To convert dial reading to torque \( M (\text{dyne.cm}) \), use

\[
\frac{\text{dial reading}}{100} \times \text{factor} = \text{torque } (M), \text{dyne.cm}
\]

The factor is supplied by the viscometer manufacturer. For example, for the Brookfield Synchroelectric Model RVT, the factor is 673.7.

2. The following data are supplied by Brookfield Engineering Laboratories for their cylindrical spindles.
<table>
<thead>
<tr>
<th>Spindle No</th>
<th>R (cm)</th>
<th>L' (cm)</th>
<th>L (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV-1</td>
<td>0.9421</td>
<td>7.493</td>
<td>6.510</td>
</tr>
<tr>
<td>LV-2</td>
<td>0.5128</td>
<td>6.123</td>
<td>5.395</td>
</tr>
<tr>
<td>LV-3</td>
<td>0.2941</td>
<td>4.846</td>
<td>4.287</td>
</tr>
<tr>
<td>LV-4</td>
<td>0.1588</td>
<td>3.396</td>
<td>3.101</td>
</tr>
<tr>
<td>LV-5</td>
<td>0.1588</td>
<td>1.684</td>
<td>1.514</td>
</tr>
</tbody>
</table>

9.6. RESULTS AND DATA INTERPRETATION

1. Report the capillary viscometer constant \( h \) and the viscosity of one Newtonian test fluid.
2. Plot the flow curves (torque versus angular velocity) for one Newtonian, one pseudoplastic, and one dilatant test fluid using the data obtained with the coaxial-cylinder viscometer.
3. Determine \( m \) and \( n \) for the non-Newtonian test fluids using plots of \( \ln(M) \) against \( \ln(J) \). Write the specific power law for each of test fluid.
4. Determine the viscosity of the Newtonian test fluid at three temperatures and compute Arrhenius parameters by plotting \( \ln(M) \) versus \( 1/T \).
5. A Cannon–Fenske capillary viscometer is to be calibrated. Two standards with known viscosity and density are tested. The following data were obtained:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Viscosity, Pa.s</th>
<th>Density, kg/m³</th>
<th>Efflux Time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1</td>
<td>1.2(10⁻²)</td>
<td>1.01(10³)</td>
<td>59.4</td>
</tr>
<tr>
<td>Standard 2</td>
<td>1.3(10⁻³)</td>
<td>1.3(10³)</td>
<td>63.1</td>
</tr>
</tbody>
</table>

The viscometer is calibrated by finding \( h \), the viscometer constant. From the foregoing data, find \( h \) for this particular viscometer.

6. Flow behavior index \( n \) and consistency coefficient \( m \) of banana puree at various temperatures are listed as follows.

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>V. Pa.sⁿ</th>
<th>M. Pa.sⁿ</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>7.0</td>
<td></td>
<td>0.458</td>
</tr>
<tr>
<td>24</td>
<td>6.5</td>
<td></td>
<td>0.458</td>
</tr>
<tr>
<td>53</td>
<td>3.5</td>
<td></td>
<td>0.458</td>
</tr>
<tr>
<td>74</td>
<td>2.9</td>
<td></td>
<td>0.458</td>
</tr>
</tbody>
</table>

Discuss the effect of temperature on \( m \) and \( n \).

Note: the computer program "Rheology" can be used for most of these calculations.

Table 3.1 DATASHEET FOR RHEOLOGY LAB

<table>
<thead>
<tr>
<th>DATE:</th>
<th>GROUP:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Newtonian Fluids (capillary viscometer)</td>
<td>Instrument data: manufacturer</td>
</tr>
<tr>
<td>Serial No:</td>
<td>Model No:</td>
</tr>
</tbody>
</table>
Product data

Product 1
Product manufacturing and label information

Product 2
Product manufacturing and label information

Product 3
Product manufacturing and label information

Viscometer:

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>DENSITY, g/mL</th>
<th>Viscosity, cp</th>
<th>Efflux Time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.</td>
</tr>
</tbody>
</table>
B. Power Law Fluids (Brookfield or similar viscometer)

Instrument data: manufacturer ____________________________
Serial No: ______________________ Model No: ______________________

**Product data**

Product 1 ____________________________
Product manufacturing and label information ____________________________

Product 2 ____________________________
Product manufacturing and label information ____________________________

Product 3 ____________________________
Product manufacturing and label information ____________________________

Product 4 ____________________________
Product manufacturing and label information ____________________________

**Viscometer**

<table>
<thead>
<tr>
<th>Rotational Speed, rpm</th>
<th>Product 1 ( (T = 0\degree C) )</th>
<th>Product 2 ( (T - 5\degree C) )</th>
<th>Product 3 ( (T - 10\degree C) )</th>
<th>Product 4 ( (T = 15\degree C) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Spindle no. ____________________________
Spindle constant ____________________________
C. Power Law Fluids (Haake or similar viscometer)

Instrument data: manufacturer
Serial No: ___________________________ Model No: ___________________________

Product data
Product 1: ___________________________
Product manufacturing and label information: ___________________________

Viscometer

<table>
<thead>
<tr>
<th>Rotational speed</th>
<th>Torque M (dial reading)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D. Viscosity - Temperature Relationship (Brookfield or similar viscometer)

Instrument data: manufacturer
Serial No: ___________________________ Model No: ___________________________

Product data
Product 1: ___________________________
Product manufacturing and label information: ___________________________

Viscometer

<table>
<thead>
<tr>
<th>Rotational Speed, rpm</th>
<th>Product 1 (T = °C)</th>
<th>Product 2 (T = °C)</th>
<th>Product 3 (T = °C)</th>
<th>Product 4 (T = °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>
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Chapter One

PRODUCTION OF MALT AND MALT EXTRACTS

MALTING

Introduction:
The malting process can be carried out using different cereal grains to obtain malted grains, modified by sprouting. Although barley is the most popular grain for malt, Indigenous cereal grains could be used, such as sorghum, maize and rice. The students are expected to understand the changes that occur during the process, and how these changes affect the overall quality of the final beverage product.

Procedure:
1. Steep 1 kg of cleaned viable/dormant grains in 6-10 litres of H2O for 12 hours. Drain the water. every 3 hours and observe air rest of 30 minutes.
2. Spread steeped grains on a damp jute sack in a dark cupboard another damp jute sack may be laid above, to cover the grains.
3. Allow the grains to sprout for 48h. check every 12h to ensure dampness is maintained. Turn the grains to prevent local overheating.
4. After sprouting, place the grains in an oven at about 55°C to 65°C for about 4 hours and later at 80°C-12°C for another 4h. Observe any changes in colour.
5. Remove the dried rootlets and separate them from the grains.
6. Compare the aroma & colour of the extract, with unmalted grain.
7. Weigh and calculate the total malting loss.

Production of Wort Extract
1. Mill 1 kg of malted grain into malt grists using attrition mill or hammer mill.
2. Mix in 4 litres of H2O, with continuous stirring. Exogeneous enzymes may be added for proteolytic and amylolytic activities. Adjust pH with Ca(OH)2 to alkalinity and with HCl towards acidity, as applicable to the enzymes.
3. Heat up the mash to 45°C and allow for proteolysis for 30 minutes.
4. Slowly heat up to 80°C and ensure starch liquefaction.
5. Carry out iodine test.
6. Add more liquefying amylase and heat up to boiling for 1 hr.
7. Cool the mash to about 65°C and add saccharifying amylase and limit dextrinase.
8. Carry out iodine test.
9. Heat up the mash to 85°C and carry out iodine test.
10. Filter the mash through mash filter or triple-layered calico cloth to obtain the wort.
11. Test for sugar content, pH, colour, starch, aroma, flavor, total solids(% weight).
12. Mash unmalted grain and compare.
Production of Malt Extract

1. Filter 3 litres of cooled wort obtained from the mashing of malted grain, using double layered calico cloth.
2. Concentrate by boiling or using a rotary vacuum evaporator to obtain a syrup of 55%-65% total solids.
3. Test for sugar content, pH, colour density aroma and flavor.
4. Compare these parameters with the unconcentrated extract.
5. Calculate increase in density, as a result of the concentration.
Chapter Two

WINE PRODUCTION

Introduction:
Enology is the science and study of all aspects of wine and winemaking. The purpose of this course is to familiarize the student with winemaking processes and to train students for such production situations. This lab series will include crushing and pressing of fruits, observation and analysis of the fermentation, and analysis of wine to determine the concentrations of various compounds important to wine. Since fermentations must be treated and monitored on a daily basis, you will be expected to visit the regular lab period at least two to four hours per week.

Wine Procedure
1. Extract juice from a suitable indigenous fruit or blend of juices
2. Test for the sugar content, make up to about 16% with sucrose, if necessary.
3. Pasteurize the juice by heating to up to 65°C for 30 min.
4. Add 50 ppm of sodium metabisulphite.
5. Cool to 15°C using ice and add 3% of active yeast slurry from a previous batch.
6. Allow fermentation to proceed for 5-7 days. Test for alcohol, titratable acidity, sugar, pH, every 12 h.
7. Cool the fermented liquor to about 10°C and yeast concentration.
8. Add 3-8% fresh juice to enable secondary fermentation especially if table wine is desired.
9. After fermentation, drain flocculated yeast by releasing from the bottom valve of the fermentor, or rack the liquor from the top by siphon, avoiding contact with air.
10. Allow for yeast flocculation for 2-3 days and drain/rack again.
11. Store in a closed oak wood or glass jar and allow to age for 3-6 months.
12. Test for alcohol content, sugar content, titratable acidity, aroma, taste, and mouth feel and palatefulness.
13. Finished product may be microfiltered, packaged in glass bottles and pasteurized at 65°C for 15°C.
Chapter Three

QUALITY PARAMETERS TESTING FOR NONALCOHOLIC BEVERAGE
Test every 12h for sugar concentration, alcohol concentration, pH, titratable acidity, colour, density, CO₂ production, yeast concentration.

Pasteurization Quality Parameters
1. Pasteurize 20 corked bottles of pineapple juice, 5 per batch for 10, 15, 20, 25 and 30 min at 55°C, 60°C, 65°C and 70°C respectively. Determine microbial concentration/activity by determining colony forming units (CFU). Lipase.
2. Identify the adequately preserved bottles after one month of storage.

Carbonation
1. Dose purified CO₂ (at 2 bar pressure) into pineapple juice at 10°C-20°C in a pressurized contained. Continue CO₂ dosage for separate batches of pineapple juice for 15, 20, 25, and 30 min at 10°C. Repeat this process for higher temperature of 23, 4, 5°C separately.
2. Determine the CO₂ for the different batches and explain any differences.
Chapter Four

PRODUCTION OF NONALCOHLIC BEVERAGE TECHNOLOGY

Preamble: Malt drink is a non-alcoholic beverage obtained from unfermented wort. The manufacturing process of fermentation for malt-based soft drinks is similar to that used in beer production, with the products containing typically malt, sugar, and hops. Fermentation has not only been useful in terms of preservation, but has helped to add flavour and texture. Students are expected to produce a non-alcoholic malt drink, with close attention to the very chemical and biological processes involved. Students will also produce other reconstitutable beverage powders such as cocoa and malted milk. Various laboratory tests will be carried out at the end of the production process to determine the physical, chemical and organoleptic properties of the products as well as the consumer acceptability.

Production of Non-alcoholic malt drink
1. Use 5 litres of wort at 15% sugar content, obtained as described in production of malt extract in BBT 401.
2. Mix with 15% sugar solution.
3. Dilute to 10%.
4. Add caramel colour and hops (0.005%).
5. Boil vigorously to achieve 15%.
6. Cool immediately using ice or heat exchanger to about 100°C and remove sedimented materials after 18-24 hours. While cooling in a freezer to 1°C.
7. Remove more sedimented materials after 18-24h, filter using a microfilter or a suitable filtration equipment.
8. Dose purified CO₂ to achieve 0.60% w.
9. Cork in glass bottles and pasteurize at 68°C for 35 minutes. Test for pH, colour, aroma, flavor, mouthfulness, taste.

Production of Reconstitutable Fruit Juice Beverage Powder
1. Extract and concentrate fresh juice from different fruits to about 30% total solids.
2. Add glucose solids to make up total solids to 50% and spray dry or using a suitable dryer to dehydrate to 5% total solids.
Concentrate wort extract (refer to BBT 401) to 30%, add 10% cocoa powder, 10% sugar and bake at 200°C, until it is hard and brittle.
3. Promptly cool to ambient temperature and promptly grind to coarse powder.
4. Store in an air tight container.
5. Test for solubility, taste (after reconstitution), colour, pH.
**Production of Reconstitutable Cocoa Beverage**

1. Mill 1 kg of malted grain into malt grits using attrition mill or hammer mill.
2. Mix in 4 litres of H₂O, with continuous stirring. Exogeneous enzymes may be added for proteolytic and amylolytic activities. Adjust pH with Ca(OH)₂ to alkalinity and with HCl towards acidity, as applicable to the enzymes.
3. Heat up the mash to 45°C and allow for proteolysis for 30 minutes.
4. Slowly heat up to 80°C and ensure starch liquefaction. Carry out iodine test.
5. Add more liquefying amylase and heat up to boiling for 1 hr.
6. Cool the mash to about 65°C and add saccharifying amylase and limit dextrinase.
7. Carry out iodine test.
8. Heat up the mash to 85°C and carry out iodine test.
9. Filter the mash through mash filter or triple-layered calico cloth to obtain the wort.
10. Add 10-20% Dutched cocoa powder, 1-3% egg powder or Soybean Protein concentrate isolate
11. Add 10-15% skim milk and 10% sugar
12. Concentrate to thick slurry and bake to about 5% moisture content and cool to ambient and promptly and mill
13. Store in an airtight container
14. Repeat steps 1-13 above, but this time, using (a) Sugar syrup, instead of sugar. (b) Soybean flour, instead of egg powder (c) without cocoa powder
15. Mix 40g of each sample in a 40ml cylinder and measure sediment height after 5 min
16. Conduct sensory evaluation on all the samples and note the differences

**Production of Malted Milk**

**Production of Malt Extract**

1. Mill 1 kg of malted grain into malt grits using attrition mill or hammer mill.
2. Mix in 4 litres of H₂O, with continuous stirring. Exogeneous enzymes may be added for proteolytic and amylolytic activities. Adjust pH with Ca(OH)₂ to alkalinity and with HCl towards acidity, as applicable to the enzymes.
3. Heat up the mash to 45°C and allow for proteolysis for 30 minutes.
4. Slowly heat up to 80°C and ensure starch liquefaction.
5. Carry out iodine test.
6. Add more liquefying amylase and heat up to boiling for 1 hr.
7. Cool the mash to about 65°C and add saccharifying amylase and limit dextrinase.
8. Carry out iodine test.
9. Heat up the mash to 85°C and carry out iodine test.
10. Filter the mash through mash filter or triple-layered calico cloth to obtain the wort.
11. Concentrate the wort to about 25% total solids
12. Add 2-4% wheat flour
13. Add about 25% skim milk .1-5% full fat milk
14. Add 10-15% sugar
15. Dry to 5% moisture content, mill and store in an airtight container
16. Conduct Sensory evaluation
Chapter Five

DISTILLERY TECHNOLOGY

1. Prepare pineapple juice and adjust total solids to 5, 10, 15 and 20% respectively.
2. Ferment each for 5 days after adding wine yeast (3% v/v) and distill separately.
3. Determine the yield of alcohol.
4. Store distillate in glass and oak wood cask and test for smoothness and harshness.
Chapter Six

UTILITIES FOR BEVERAGE PROCESSING
Industrial Visit and tours highlighting application of utilities used in water H2O & supply, compressed air supply Boiler operation and steam/Hot H2O supply, electricity management, vacuum, refrigeration. Students will be evaluated based on technical report which will be submitted after the industrial visit.

NOTE: ANY OF THESE AREAS COVERED IN THE BBT COURSES CAN BE USED FOR FST 413 (ALCOHOLIC AND NON-ALCOHOLIC BEVERAGES)
Chapter 1  Introduction
   Safety precaution in microbiological laboratory  
   Reporting laboratory practicals  
   Procedures for the use of microscopes  
   Use of colorimeter to estimate microbial growth

Chapter 2  Laboratory media
   Media for separation of mixed cultures  
   Enrichment media  
   Elective media  
   Selective media  
   Differential media  
   Preparation and inoculation of media  
   Choice of media for examination of food and water  
   Canned foods  
   Frozen foods  
   Sugar, Alcohol and other beverages  
   Water

Chapter 3  Method of culturing organism

Chapter 4  Procedure for identification of microorganism
   Simple Stains  
   Gram Stain  
   Principles  
   Ziehl-Neelsen Method for staining Acid-Fast Bacteria  
   Flagella Staining of Bacteria  
   The Capsule Staining  
   Spore Staining  
   Percentage of Positive Reaction of Various Gram Negative Organisms  
   Procedure for identification of Mould Cultures  
   Examination of Yeasts  
   Cultural Characteristics  
   Microscopic Examination of Yeast

Chapter 5  Factors affecting growth of microorganism
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Examination of canned foods  96
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CHAPTER 1
INTRODUCTION

A. SAFETY PRECAUTIONS IN MICROBIOLOGICAL LABORATORY

Some of the micro-organisms with which you will be working with are capable of producing diseases(s). Therefore, great care should be taken in handling cultures, slides, and all materials that may have been in contact with living micro-organisms. The following precautions should always be taken.

i. Only necessary articles should be brought into the laboratory. Coats, cases, etc. should not be brought in.

ii. Laboratory coats must be worn in laboratory and must be left in the lockers provided when not in use. They should never be worn in any other laboratory, and especially not in the food processing laboratory.

iii. Before leaving the laboratory, students must wash their hands. In the event of transfer or possible contamination of hands by cultures, a disinfectant as well as soap and water should be used. If in doubt consult the demonstrator.

iv. Any incident, e.g., spilling of cultures or personal injury, however slight, must be reported to the demonstrator at once. When cultures are spilled on tables, etc., dropped and broken materials must be covered with disinfectant and left for a short time before any attempt is made to clean it up.

v. On no account must any equipment be placed in the mouth except when such use is needed. Items such as pipettes, labels must not be licked.

vi. Smoking and eating in the laboratory is not permitted.

vii. Discarded cultures, empty media tubes and all contaminated materials must be placed in the appropriate container provided for the purpose. Discarded slides and pipettes must be placed in the jars of disinfectant provided.

 REGARD ALL CULTURES AS POTENTIALLY DANGEROUS AND TREAT THEM ACCORDINGLY

viii. At the end of the practical session, remove all apparatus and swab the benches with disinfectant.

ix. Use microscopes carefully and correctly. Report at once any damage which you observe. Do not touch lenses with your fingers; and to clean lenses, use only the special lens tissue at the end of the practical session and return the lens to the technician. Immersion oil, which accidentally contaminates the dry lenses, must be removed immediately.

x. Sterilize inoculating needles and loops before and after use by heating in the Bunsen (or spirit lamp) flame until red hot. Avoid spattering of material by gradually introducing the instrument into the flame.

xi. Return communal apparatus or reagents to their places as soon as you have finished with them.
THESE RULES HAVE BEEN PRODUCED FOR YOUR OWN SAFETY AND CONVENIENCE

B. REPORTING LABORATORY PRACTICAL
   i. Laboratory Report Notebook

   All practicals must be reported directly during the practical class into the laboratory report
   notebook. No student should use loose sheet or loose-leaf folders in the laboratory. During the
   practical examinations, each student must present the laboratory report notebook.

   ii. Reports of experiments and test carried out must be presented in a standard format
       showing:
       a. Title of experiment or test performed with date.
       b. The aim of the experiment or test performed.
       c. An outline of the methods used and if stock cultures were employed, the names
          and brief description of the organisms.
       d. A description of the results obtained. This is often best given as a written report
          with tables or graphs used to clarify results. However, in some cases tables with
          necessary footnotes may be more suitable employed to give the results obtained.
          If class or group experiments are performed, all the results should be recorded.
       e. Discussion and conclusion. This will be drawn from the results obtained.

For a microscope with an oil immersion objective, the following procedures can be followed:

   i. Firstly, ensure that objective, eye-piece, condenser and mirror are clean, removing any
      dust with a soft brush and lens tissue.

   ii. Align the microscope with artificial light source. If an external light source is used, place
       the lamp about 15cm from the mirror.

   iii. Adjust the draw tube to the correct length (usually this will be 160mm when a
        microscope with a rotating nose-piece is used)

   iv. With the x10 objective in position and the eye-piece removed, adjust the plane side of the
       mirror to send the light centrally up the microscope adjustment.

   v. Replace the eye-piece and place the object slide on the microscope stage. Focus the x10
       objective on the object using the coarse adjustment.

   vi. Focus the light source on the object by placing a pencil close against the light source and
       racking condenser up and down to get a sharp image of the pencil in the same field as the
       focused object. (This is termed “critical illumination” the image of the light source being
       in the same plane as the focused specimen).

   vii. Using x10 and/or x40 objectives, select a suitable field for subsequent viewing.

   viii. (a) Rack up the objective (this may be unnecessary if parfocal objective are fitted) and
        rotate the oil-immersion objective into position.
        (b) Place one drop of immersion oil on the microscope slide.
        (c) While viewing the object through the microscope, rack very slowly upwards with the
            coarse adjustment until the specimen comes into view. The restriction in the use of the
            coarse adjustment to upward travel only, while looking down the microscope, lessens the
            danger of slide breakage and consequent damage to the objective lens. Focus sharply
            with the fine adjustment.

   ix. Remove the eye-piece again and adjust the mirror to ensure that the back lens of the
       objective is symmetrically filled with light. To avoid glare, close the iris diaphragm until
       the back lens of the objective is about 1/4 full of light.
x. Replace the eye-piece and refocus with the fine adjustment if necessary. After use, all immersion oil M**UST** be wiped off the objective lens and elsewhere with lens tissue. Failure to remove the immersion oil may allow the oil to penetrate the lens mount and cause a severe loss of definition.

D. **PROCEDURE FOR SETTING UP AND USING COLORIMETER TO ESTIMATE MICROBIAL GROWTH**

i. Switch on the instrument and leave for 10 minutes to warm up.

ii. Using the set matched tubes provided, zero the colorimeter using a blank tube of the medium identical to that in which the cultures have been grown. Place the tube in the colorimeter with the mark on the tube and colorimeter opposite each other and increase the light by moving the light control clockwise until the needle swings over to the zero mark.

iii. Remove the blank tube and replace it with a tube containing the culture whose turbidity is to be measured.

iv. Rinse the tubes out carefully between readings and be careful not to spill the culture.

v. Always check the zero reading of colorimeter with the blank tube between readings to make sure the needle has not drifted off the zero position. Failure to check the zero will result in inaccurate readings.

vi. Do not put your finger on the sides of the colorimeter tubes. Hold the tubes by the rims to prevent finger marks interfering with light passing through them.

The values we obtain for various turbid cultures growing under some experimental conditions will give us comparative values of the growth of a micro-organism under different conditions. The turbidity readings obtained will be proportional to the mass of bacteria or other cells present—the will not be related to the number of cells.
CHAPTER TWO

MEDIA
A culture medium is any nutrient liquid or solid which can be used in the laboratory for the growth of micro-organisms. Such a medium may resemble the natural substrate on which the pathogens, milk for milk micro-organisms, soil extract for soil micro-organisms. Whatever the medium, it must include all the necessary requirements for growth which vary according to the organism it is desired to grow but will include:

1. Water
2. Nitrogen containing compounds e.g. proteins, amino acid, nitrogen containing in organic salts.
3. Energy source, e.g. carbohydrate, protein

SEPARATION OF MIXED CULTURES
A mixed culture can generally be separated into its constituent organisms (provided that the individual strains are present in approximately equal numbers) by using a streak plate method as described in fig.1 isolated colonies can then be picked off and subculture as required.

Enrichment Procedures
When however, the required organism is outnumbered or is accompanied by many unwanted species, as in soil, milk or water, it may be necessary to use enrichment technique to increase the numbers of the required organisms. The methods adopted should be chosen to take advantages of any known physiological characters of the particular organism, e.g. optimum temperature or pH, nutritional requirements, tolerance of added inhibitors.

In some cases, pretreatment of the material itself is appropriate, e.g. heat-treatment of soil suspensions or milk for the isolation of sporng or other heat-resistant organism, followed by plating on soil media. Advantage may also be taken of the differing optimum temperatures of micro-organisms in a mixed population, so that incubation of material at the optimum temperature of the required organism should increase its numbers in relation to other organisms present. Since however, enrichment procedure adopted is unlikely to yield a pure culture, and the final isolation procedure still involves isolation of separate colonies on solid media.

Elective Media
In other cases, particularly in the isolation of soil micro-organisms, a medium which satisfies the minimum nutritional requirements of the organisms concerned known as an elective medium may be very useful, particularly if the required organisms have unusual nutritional characteristics. For example, “wild” yeasts can be isolated using lysine agar, on which organisms cannot grow unless they can use lysine as a sole source of nitrogen.

Selective Media
Another important method of separating mixed cultures is to make use of a selective medium. This basic medium which may support growth of many types but which has been modified to include one or more inhibitory agents, thereby restricting the growth of organisms not required.
The choice of a selective medium must therefore be appropriate for the isolation of the particular organism concerned with reference to the nature of the samples being examined. Inhibitory substances used in preparation of selective media include dyes, antibiotics, bile, salts and various inhibitors affecting the metabolism or enzyme systems of particular species. One form of a selective medium is that in which the pH of the medium has been modified so that it is suitable for the growth of only acid-tolerant or alkali-tolerant species. For example, yeasts, moulds and lactobacilli are acid tolerant organisms and can grow on media at pH 4-5, whereas less acid-tolerant organisms are unable to grow.

(a) **Selective media for Gram-negative bacteria**: Crystal violet used in a medium at a final concentration of 1:500,000 inhibits the growth of many Gram-positive bacteria (though not streptococci) while permitting that of Gram-negative bacteria. Penicillin incorporated at a final concentration of 5-50 units/ml inhibits many Gram-positive organisms, and the medium thus becomes selective medium used for Gram-negative bacteria.

MacConkey agar is a selective medium used for the detection and isolation of coliforms. In this case the bile salts act as the selective agent, intestinal and coliform organisms being inhibited to a lesser extent than other organisms.

(b) **Selective media for Gram-positive bacteria**: Potassium tellurite, thallium acetate and sodium azide added to media to give a final concentration of 1:2000-1:10,000 have been found to inhibit the growth of Gram-negative bacteria, and these substances are therefore frequently used in selective media for Gram-positive bacteria. Glucose azide broth, for example is used in the detection of faecal streptococci in water supplies. Similarly, thallium acetate in a glucose agar has been useful for the isolation of lactic streptococci from sour milk, and potassium tellurite for the isolation of corynebacterium.

**Differential Media**

A differential medium is one in which certain species produce characteristic colonies which can easily be recognized. For example, haemolytic and non-haemolytic species can be distinguished by the examination of colonies formed on blood agar, a non-selective medium. In many cases, however, a medium may be both selective and differential. For example, lactose-fermenting coliforms produce red colonies on MacConkey’s agar (as a result of acid production affecting the neutral red indicator), while non-lactose fermenting intestinal organisms such as Salmonella spp. produce colourless colonies.

**PREPARATION OF MEDIA**

The media are available in either dehydrated or ready-prepared form from Oxoid Ltd., Difco Laboratories and Baltimore Biological Laboratories.

When preparing media, care should be taken that each ingredient or the dehydrated medium is adequately mixed into the water. If mixing is inadequate, stratification of dense, concentrated solutions will occur and on heating there will be deleterious changes caused by caramelisation, hydrolysis, etc. When using dehydrated media it is good practice to allow the powder to soak in the water for 15 minutes with frequent mixing by agitation, before heating.
Agar media being dissolved by the application of heat to the base of the container should be agitated to prevent the agar granules from settling out and being burned. The manuals provided by the manufacturers or dehydrated media give detailed recommendations on the procedures for reconstitution and sterilization and these should be followed closely.

INOCULATION OF THE MEDIUM
After preparation of the agar medium, it is poured in the petri dishes (about 17 ml/plate) aseptically. Allow to solidity.
Once the agar plate is set and ready for use proceed to inoculate culture of desired organisms using inoculating needle or loop. Incubate at the optimum temperature for the organism(s) to grow. Some examples of agar preparations are shown below:

Various Examples of Solid Media
[black = culture]

- Slant
- Stab or Deep
- Streaked Petri Dish

CHOICE OF MEDIA

EXAMINATION OF FOOD AND WATER

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Culture Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>CANNED FOOD</td>
<td>Cooked Meat Medium (Synthetic)</td>
</tr>
<tr>
<td>Anaerobes e.g. Clostridium</td>
<td>Crossley Milk Medium</td>
</tr>
<tr>
<td></td>
<td>Iron Sulphite Agar</td>
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<td></td>
<td>Liver Broth</td>
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<td></td>
<td>Perfringens Agar (OPSP)</td>
</tr>
<tr>
<td></td>
<td>Reinforced Clostridial Medium</td>
</tr>
<tr>
<td>Organisms</td>
<td>Mediums</td>
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<td>---------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>KRAISEP Agar</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus Medium No. 110</td>
</tr>
<tr>
<td>Streptococci</td>
<td>Azide Blood Agar Base</td>
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<td></td>
<td>Edewards Medium (modified)</td>
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<tr>
<td></td>
<td>L-S Differential Medium</td>
</tr>
<tr>
<td></td>
<td>Tryptose phosphate Broth</td>
</tr>
<tr>
<td><strong>Total count of viable organisms</strong></td>
<td><strong>L-S Differential Medium</strong></td>
</tr>
<tr>
<td></td>
<td>Milk Agar</td>
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<tr>
<td></td>
<td>Milk Agar (roll tube)</td>
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<tr>
<td></td>
<td>Nutrient Broth No. 2</td>
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<tr>
<td></td>
<td>Plate count Agar</td>
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<td></td>
<td>Plate count Agar (roll tube)</td>
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<tr>
<td></td>
<td>Standard Plate Count Agar (APHA)</td>
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<tr>
<td>Yeasts and moulds</td>
<td>Buffered Yeast Agar</td>
</tr>
<tr>
<td></td>
<td>O.G.Y.E. Agar</td>
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<tr>
<td></td>
<td>Potato Dextrose Agar</td>
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<td></td>
<td>Rose-Bengal</td>
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<td>Chloramphenicol Agar</td>
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</tbody>
</table>

**FROZEN FOODS**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Mediums</th>
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</thead>
<tbody>
<tr>
<td><strong>Anaerobes</strong></td>
<td>Iron Sulphite Agar</td>
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<tr>
<td></td>
<td>Liver Broth</td>
</tr>
<tr>
<td></td>
<td>Perfringens Agar (OPSP)</td>
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<td></td>
<td>Reinforced Clostridial Medium</td>
</tr>
<tr>
<td></td>
<td>Schaedler Agar</td>
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<tr>
<td></td>
<td>Thioglycollate Medium (USP)</td>
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<tr>
<td><strong>Coliforms</strong></td>
<td>Brilliant Green Bile (2%) Broth</td>
</tr>
<tr>
<td></td>
<td>Buffered Peptone Water</td>
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<tr>
<td></td>
<td>Endo Agar Base</td>
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<tr>
<td></td>
<td>MacConkey Broth (purple)</td>
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<tr>
<td></td>
<td>Violet Red Bile Agar</td>
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<tr>
<td><strong>Salmonella and Shigella</strong></td>
<td>Mannitol Selenite Broth Base</td>
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<tr>
<td></td>
<td>Muller-Kauffman</td>
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<tr>
<td></td>
<td>Tetrathionate Broth Base</td>
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<td></td>
<td>Bismuth Sulphite Agar</td>
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<td></td>
<td>Brilliant Green Agar</td>
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<tr>
<td></td>
<td>DCLS Agar</td>
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<tr>
<td></td>
<td>Deoxycholate Citrate Agar</td>
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<tr>
<td></td>
<td>Hektoen Enteric Agar</td>
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<tr>
<td>Organism Description</td>
<td>Nutrient Media</td>
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<td>----------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>Baird-Parker Medium, DNase Agar, Giolitti Cantoni Broth, KRNREP Agar Base, Mannitol Salt Agar</td>
</tr>
<tr>
<td>Streptococci (particularly enterococci)</td>
<td>Azide Blood Agar Base, MacConkey Agar No. 2, Slanetz and Bartley Medium</td>
</tr>
<tr>
<td>Total Viable Count</td>
<td>Nutrient Agar, Plate Count Agar, Standard Plate Count Agar (APHA), Tryptone Soya Agar</td>
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<tr>
<td>Yeasts and Moulds</td>
<td>Malt Extract Agar, O.G.Y.E. Agar, Potato Dextrose Agar, Rose-Bengal, Chloramphenicol Agar, Wort Agar</td>
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</tbody>
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**Sugar Alcoholic and Other Beverage**

<table>
<thead>
<tr>
<th>Organism Description</th>
<th>Nutrient Media</th>
</tr>
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<tbody>
<tr>
<td>Anaerobic spore forming organisms e.g. Cl. Nigrificans</td>
<td>Cooked Meat Medium synthetic, Iron Sulphite Agar, Liver Broth</td>
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<tr>
<td>Coliforms</td>
<td>Lactose Broth, MacConkey Agar, MacConkey Broth (purple)</td>
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<tr>
<td>Lactobacilli</td>
<td>MRS Agar, Rogosa Agar, Tomato Juice Agar</td>
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<tr>
<td>Thermophilic ‘flat-sour’ Microorganisms and Total Viable Count</td>
<td>Dextrose Tryptone Agar</td>
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<tr>
<td>Total contaminating bacteria (in yeast)</td>
<td>‘Acididone’ Agar</td>
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<td>Yeasts and Moulds</td>
<td>Lysine Medium (wild yeasts), Malt Extract Agar, O.G.Y.E. Agar, Rose-Bengal Chloramphenicol Agar, W.L. Nutrient Agar, W.L. Nutrient Broth, Worth Agar</td>
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**Water**

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<td>Clostridium welchii</td>
<td>Perfringens Agar (OPSP), Reinforced Clostridial Medium, Brilliant Green Bile (2%) Broth</td>
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<td>Coliforms</td>
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<tr>
<td>---------------------------------</td>
<td>--------------------------</td>
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<tr>
<td></td>
<td>Endo Agar Base</td>
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<tr>
<td></td>
<td>Lauryl Tryptose Broth</td>
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<tr>
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<td>MacConkey Agar</td>
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<tr>
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<td>MacConkey Broth</td>
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<tr>
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<td>MacConkey Membrane Broth Tablets</td>
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<td></td>
<td>Membrane Enriched Teepol Broth</td>
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<tr>
<td></td>
<td>Minerals Modified Glutamate Medium</td>
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<td>Resuscitation Membrane Broth Tablets</td>
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<td>Violet Red Bile Agar</td>
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<td>Faecal streptococci</td>
<td>Azide Blood Agar Base</td>
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<td>MacConkey Agar No. 2</td>
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<td>Slanetz and Bantley Medium</td>
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<td>Salmonella and Shigella</td>
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<td>Brilliant Green Agar</td>
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<td>DCLS Agar</td>
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<td>Desoxycholate Citrate Agar (Hynes)</td>
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<td>Tetraionate Broth Base</td>
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<td>XLD Medium</td>
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CHAPTER THREE
METHODS FOR CULTURING MICRO-ORGANISMS

1. Pure culture technique
   a. Clonal populations:
      i. **Pure culture technique** is a method of culturing microorganisms in which all of the individuals in a culture have descended from a single individual.
      ii. This is done so as to:
          1. inhibit evolutionary change within cultures
          2. allow the characterization of types microorganisms without the confounding presence of other, different types of microorganisms
   b. Colony isolation:
      i. The basis of pure culture technique is the isolation, in colonies, of individual cells, and their descendants, from other colonies of individuals.
      ii. This is usually done by culturing methods employing petri dishes such as:
          1. Streaking
          2. pour plate
          3. spread plate
   c. Isolation from the wild:
      i. When isolating microorganisms from complex mixtures it is always a good idea to repeat the isolation procedure at least once (e.g., restreak an isolated colony) to make sure that an isolated colony is truly derived from only a single cell (i.e., closely overlapping colonies can be indistinguishable from colonies founded from single cells).
      ii. Following their isolation from the wild, microorganisms may be characterized by inoculation into differential medium to determine what type of nutrients they require or can use, and what types of by-products they produce. This aids in identification.

Before food samples can be microbiologically examined there is need to dilute the samples in order to reduce the population of the microorganisms in the sample. This will aid in proper enumeration and identification of the microorganisms. A **serial dilution** is the stepwise dilution of a material (microorganisms or substance) in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. Serial dilutions are used to accurately create highly diluted solutions for experiments. Serial dilutions are widely used in experimental sciences, especially in microbiology. The illustration below shows how a serial dilution can be prepared:
The technique used to make a single dilution is repeated sequentially using more and more dilute solutions as the "stock" solution. At each step, 1 ml of the previous dilution is added to 9 ml of distilled water or other suitable diluents. Each step results in a further 10-fold change in the concentration from the previous concentration.

The values shown in the tubes are the amount (in ml) of the stock solution present in each ml of the dilute solution.

The dilution of the original stock solution is shown below the tubes:
Note: As the sample is further diluted the number of microorganisms reduces.

For solid food samples the stock sample is prepared by weighing 1.0g of the food sample and transferring it to 9.0ml of the diluents. This will give a 1:10 dilution. From this, further dilutions can be obtained by taking 1.0ml from the stock sample and transferring to another 9.0ml of diluents to give 1:100 dilution.

1. Pouring a plate
   a. A pour plate is a method of melted agar inoculation followed by petri dish incubation.
   b. Steps include:

Material:
- Mixed culture of bacteria or other microorganisms
- Inoculating loop
- Sterile pipette
- Nutrient agar liquefied
- Sterile Petri dish

Procedure:
1. Label the bottom of the sterile Petri dish with the source of the culture and turn the plate
2. Prepare a serial dilution of the microorganisms you want to plate out.
3. Prepare the agar of choice as directed by the manufacturers.
4. Introduce 1.0ml of the microbial cell suspension into the sterile petri dish with a sterile pipette.
5. Cool the agar to a temperature of about 45°C and then aseptically pour about 17ml of the liquefied agar into the petri dish.
6. Swirl the plate gently for proper mixing of agar and cell suspension in the plate. Please do not spill or splash the agar on the body of the petri dish.
7. Allow the agar to solidify on a flat surface.
8. Then incubate at the appropriate temperature for 24 to 48 hours. Most bacteria are incubated at 37°C while yeasts and moulds are incubated at 30°C for up to 5 days.

   c. Pour plates are useful for quantifying microorganisms that grow in solid medium.
   d. Because the "pour plate" embeds colonies in agar it can supply a sufficiently oxygen deficient environment that it can allow the growth and quantification of microaerophiles.

2. Spreading a plate
   a. Quantification technique:
      i. Spreading a plate is an additional method of quantifying microorganisms on solid media.
      ii. Instead of embedding microorganisms into agar, as is done with the pour plate method, liquid cultures are spread on the agar surface using a device that looks more or less like a hockey stick.
   b. An advantage of spreading a plate over the pour plate method is that cultures are never exposed to 45°C melted agar temperatures.

Procedure:
1. Label the bottom of the sterile Petri dish with the source of the culture and turn the plate
2. Prepare a serial dilution of the microorganisms you want to plate out.
3. Prepare the agar of choice as directed by the manufacturers.
4. Cool the agar to about 45°C and pour about 17 ml of the molten agar into the sterile petri dish and allow the agar to solidify on a flat surface.
1. Introduce 0.1ml of the microbial cell suspension onto the surface of the solidified agar in the sterile petri dish with a sterile pipette.

2. Spread the drop of cell suspension on the surface of the agar with a sterile bent glass rod. When spreading you should be careful so that you don’t cut into the agar.

3. Then incubate at the appropriate temperature for 24 to 48 hours. Most bacteria are incubated at 37°C while yeasts and moulds are incubated at 30°C for up to 5 days.

4. Stabbing
   a. Cultures are stabbed deeply into agar using an inoculating needle.
   b. The stabs are incubated until visible cultures form, then sealed and stored at room or lower temperature.

5. Slant method
   a. Cultures may be streaked onto the surface of the solid medium in a slant tube.
   b. The slants are incubated until visible culture formation then sealed and stored at room or lower temperature.

6. Streaking a plate
   a. Colony isolation:
      i. A petri dish is streaked in manner such that individual colonies may be isolated.
      ii. See illustration below.

7. Illustration streaking a plate

Using a Loop to Streak a Plate

a plate (petri dish; top view)

---

a loop

note the culture on the tip of the loop

following incubation, there is colony formation.
note the isolated colonies toward the bottom. can you spot the likely contaminants?

a properly streaked plate
CHAPTER FOUR

PROCEDURE FOR IDENTIFICATION OF BACTERIA

The cultural characteristics of bacteria colonies, their forms of growth on Agar slants, the way they grow on liquification of gelatin and their surface growth on nutrient broth, all these are used in the identification of unknown bacteria. But these are not as important as separating bacteria into their gram stain characteristics, morphological characteristics and biochemical reactions. Charts 1-5 and table 1 summarize gram reactions of bacteria, their morphology and their biochemical reactions.

The use of different stains enables us to differentiate microorganisms into different types (e.g. the gram stain technique) and to see their shape and structures associated with them. Before staining of bacterial cells, the cells have to be smeared on a slide.
1. Place a drop of distilled water on a clean slide and mix it with small amount of solid growth from the culture to be examined.
2. Smear the drop over the surface of the slide and fix by holding the slide well above a Bunsen flame so that the water evaporates and the cells are not distorted, or leave the smear to dry on the slide at room temperature. This however takes time. See Fig. 5.

A. Simple Stains
Place the heat-fixed smear on a staining rack over the sink and flood with any of the staining solutions given below. After the time indicated, rinse the slide gently in water and blot dry with clean blotting paper or filter paper.
Examine under the microscope
(a) Crystal violet: stain for 1 min
(b) Loeffler's methylene blue: stain for at least 5 min as this stain is weaker in action than crystal violet
(c) Carbol fuchsin (dilute): stain for 30 sec only

B. Gram Stain
This test is important in the study and identification of bacteria since they are generally described as gram positive or gram negative. However, some bacteria are gram variable since the staining properties of bacteria often vary with the age of the cell. It is important to be able to use this technique, since it will be needed for characterizing isolates and pure cultures used in experiments.

Principles
The gram stain procedure separates bacteria into two groups.
Gram positive – organism retaining the first stain below after washing with alcohol
Gram negative – organisms which lose the first stain after washing but retain the second stain used.

Procedure
1. Make smears of the various bacterial cultures provided. (Do not make smears too thick or the staining action will not work correctly).
1. Stain the smears for one minute with gram stain I (Crystal violet).
2. Wash off stain with tap water and flood slide with gram stain II (iodine) for one minute.

**Note:**
The iodine acts as a mordant—a substance which increases the affinity of the bacterial cell for the dye. After using a mordant, stain is much more difficult to remove.

3. Wash in tap water, blot dry carefully, decolorize for 30 seconds in 95% alcohol with gentle agitation. Blot dry carefully.
4. Stain smear for 10 seconds with gram stain III (safranin).
5. Wash in tap water and dry. Observe smears under oil immersion.

**Result**
Gram positive = blue
Gram negative = red

Record the results of your staining and determine the gram stain reaction to unknown cultures.

---

**A. Ziehl–Neelsen Method for staining Acid-Fast Bacteria**

Members of the general *Mycobacterium* and *Nocardia* can be differentiated from many other organisms by this staining technique. The Ziehl-Neelsen method consists firstly of staining the organism with a hot, concentrated dye. Once stained, the cells resist decolorisation with acid; they are thus “acid-fast”. Decolorisation is effected with suitably strong acid and the smear is then counterstained with methylene blue solution. Acid-fast bacteria stain red, other bacteria and the background stain blue.

**Procedure:**

1. Cover the slide with strong Ziehl-Neelsen’s carbol fuchsin and heat the underside of the slide with a lighted alcohol-soaked swab. Stop heating when the slide steams. Keep the slide hot and replenish the stain if necessary. Taking care not to allow the smear to become dry. Heat for 5 min not allowing the staining solution to boil.
2. Wash well.
3. Decolorize with acid-alcohol or with 1.5 or 20 percent sulfuric acid. The excess stain is removed as a brownish solution, and the smear will become brown. Rinse in water, when the film will appear pink once more. Apply more acid and repeat the rinsing several times until the film appears faintly pink upon washing.
4. Wash well.
5. Counterstain with Loeffler’s methylene blue for 5 min.
6. Wash well and carefully remove the stain deposits from the back of the slide with filter paper. Blot dry and examine.
D. Flagella Staining of Bacteria
Since flagella are so small we cannot see them under the light microscope normally. Using this stain we increase the diameter of the flagellum by treating the bacterial cells under test with a mordant, a colloidal solution which forms thick layer of stainable material over the whole surface of the flagellum. The flagellum is now thick enough to be visible by the light microscope and a stain is applied to show up the material on the flagellum.

1. Use glass slides which are perfectly clean
2. Place a drop of 24 hour broth culture on the slide, and allow drop to run slowly over the slide surface to give a thin smear.
3. Allow the smear to dry in the air.
4. Cover the smear with the mordant for 3-5 minutes and then wash off with sterile distilled water.
5. Pick up the slide in forceps, and whilst holding over a Bunsen flame (not too near) add stain II (The Fontana silver solution) and keep it almost boiling for 3-5 minutes.
   An alternative way is to place the slide on a small beaker of boiling water to add stain II.
6. Wash again with sterile distilled water and blot dry carefully.
7. Observe under the microscope using oil immersion.
Flagella will appear as fine wavy threads, usually much larger than the cells they originate from.
To see flagella, focus microscope carefully and reduce the illumination. This technique is used in conjunction with motility agar.

B. The Capsule Staining
Procedure
1. Place a loopful of Indian ink on a very clean microscope slide.
2. Mix into the Indian ink a little of the bacterial culture of suspension.
3. Place a coverslip on the mixture, avoiding air bubbles, and press firmly with blotting paper until the film of liquid is very thin.
4. Examine with high power dry objective or the oil immersion objective.
   The capsule will be seen as a clear area around the bacterium.

C. Spore Staining
Bacteria in the general Bacillus and Clostridium produce endospores, which are highly resistant to high temperature, lack of moisture, and toxic chemicals. The endospores are also resistant to bacteriological stains and, in a smear stained by Gram's method, they can be seen as colourless areas in the vegetative organisms which stain Gram-positive. However, once stained, the spores tend to resist decolourisation.

Procedure
1. Prepare a smear in the usual way, but heat-fix very thoroughly by passing through a Bunsen flame 20 times.
2. Stain for 10 min with a saturated aqueous solution of malachite green.
3. Wash gently with cold water for 10 sec.
4. Counterstain with a 0.25 percent solution of safranine for 15 sec.
5. Wash with water and blot dry.
6. Examine under the oil-immersion objective.
Chart 2: Biochemical Reactions of Gram Positive Rods
Chart 3: biochemical reactions of Gram negative bacteria
<table>
<thead>
<tr>
<th>Organism</th>
<th>Glucose</th>
<th>Phenylalanine</th>
<th>ONPG</th>
<th>Indole</th>
<th>Methyl Red</th>
<th>Voges Proskauer</th>
<th>Lysine Decarboxylase</th>
<th>H₂S</th>
<th>Lysine Decarboxylase</th>
<th>Urease</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Mannitol</th>
<th>Maltose</th>
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<td>0</td>
<td>91.987</td>
<td>65.972</td>
<td>39</td>
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<td>413-355</td>
<td>343-991</td>
<td>6-21</td>
<td>000</td>
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<td>90.559</td>
<td>61</td>
<td>33</td>
<td>966-1000</td>
<td>969-989</td>
<td>0</td>
<td>02</td>
<td>937-937</td>
<td>547-546</td>
<td>703</td>
<td>941-499</td>
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<td>609-816</td>
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Some cultural characteristics that are useful in identification of bacteria are shown below:

i. Margin of colonies

entire  undulating  erose  lobate  filamentous

ii. Elevation of colonies

flat  raised  convex  pulvinate  umbonate

PROCEDURE FOR IDENTIFICATION OF MOULD CULTURES

1. Note:
   (a) Age
   (b) Temperature of incubation
   (c) Medium

2. Examine the colony for the following characteristics using naked eye and or the low power of the microscope.
   (a) Colony size (diameter)
   (b) Amount and type of growth
   (c) Colour of surface and reverse
   (d) Presence of pigment, water soluble or not
   (e) Exudation droplets
   (f) Odour (be circumspect in smelling cultures)
   (g) Presence of reproductive structures if asexual. note. if possible, the way in which the spores are borne.

3. Prepare a slide of a suitable portion of the colony for detailed examination under the x-40 objective. The material may either be mounted in water, or better in lactophenol cotton blue. The following procedure should be adopted.
   (a) Place large drop of lactophenol-picric acid or lactophenol-cotton blue on a clean slide.
(a) Pick off a portion of the growth with a needle
(b) Tease it out in a drop of lactophenol-cotton-blue stain on the slide
(c) Cover with a clean cover slip, taking care to exclude air bubbles
(d) Examine the prepared slide under the microscope:
   i. First using the low power objective (x 10)
   ii. And then using the x 40 (high power dry) objective for a closer examination of
      selected field.

1. Examine the preparation for:
   (1) Hypae septate or non-septate
   (2) Mycelium clear or dark (smoky)
   (3) Mycelium coloured or colourless
   (4) Whether sexual spores are produced and the type of spores, zygospores, or
        ascospores.
   (5) Type of asexual spores: sporangiospores, conidia or arthrospores (oidia)
   (6) Characteristics of the spore head:
       (a) Sporangia: size, colour, shape and location
       (b) Spore heads bearing conidia: single conidia, chains, budding conidia, or masses;
           shape and arrangement of sterigmata or phialides; gumming together of conidia.
   (7) Appearance of sporangiophores or conidiophores: simple or branched, and if
       branched, the type of branching. Size and shape of columella at tip of
       sporangiophore. Whether conidiophores are single or in bundles.
   (8) Microscope appearance of the asexual spores especially of conidia shape, size,
       colour, smooth or rough, one-, two-, or many-celled.
   (9) Presence of special structures (or spores) stolons, rhizoids, foot cells, apophysis,
       chlamydospores, sclerotia, etc.

A clear, labeled diagram should be made, under the x 40 objective to give as much of the above
information as possible.

EXAMINATION OF YEASTS

Yeasts are identified on the basis of morphological and cultural characteristics and ascospore and
ballistospore production, but biochemical tests are also of great importance.

Cultural Characteristics

Examine
1. Growth as a film over surface of a liquid (film yeasts) or growth throughout medium.
2. Colour of macroscopic growth.

Microscopic Examination of Yeast

Yeast culture to be examine under the microscope can be stained with any of the following stains:
1. Wet mount on Gram's iodine
2. Wet mount on Loeffler's methylene blue.
1. Gram stained heat fixed smear
The preparation is examined for the following which form the principal bases for identification and classification of general of yeasts:
1. Whether or not ascospores are formed
2. If they are spore-forming.

A. The method of production of ascospores:
(1) Produced without conjugation of yeast cells (parthenogenetically).
   Spore formation may be followed by
   a. Conjugation of ascospores, or
   b. Conjugation of small daughter cells.
(2) Produced after isogamic conjugation (conjugation cells appear similar)
(3) Produced by heterogamic conjugation (conjugating cells differ in appearance).

B. Appearance of ascospores: shape, size and colour. Most spores are sphroidial or ovoid, but some have odd shapes, such as those of most species of *Hansenula*, which look like derby hats (figure 12).

C. The usual number of ascospores per ascus: one, two, four or eight.

3. Appearance of vegetative cells: shape, size, colour, inclusions.

4. Method of asexual reproduction:
   (a) Budding
   (b) Fission
   (c) Combined budding and fission
   (d) Arthrosopes (oidia)

5. Production of a mycelium, pseudomycelium, or no mycelium

How can yeast be differentiated into species?
This could be achieved by determined the physiological characteristics of the yeast culture, e.g.
   a. Nitrogen and carbon sources
   b. Vitamin requirements
   c. Oxidative, or fermentative e.g. film yeasts are oxidative; other yeasts may be fermentative or fermentative and oxidative.
   d. Lipolysis, acid production or formation of starch like compounds.
CHAPTER FIVE

FACTORS AFFECTING THE GROWTH OF MICRO-ORGANISM

The aim of the following experiments is to use micro-organisms to demonstrate what we mean by the term "growth" and the various parameters that affect it.

Growth of an organism can be defined as an increase in the amount of cellular constituents and structures. This applies to all types of organisms, and so microorganisms can be used as models for observing the growth of an organism.

When growth occurs, an organism either increases in size e.g., length or weight increase and/or in number either of cells, tissues etc. or of actual organisms e.g., the growth of bacteria. When we use micro-organisms to demonstrate growth, we usually mean the growth of a population of cells, e.g., bacteria in a broth, and when these populations grow, they increase in number.

Population growth (i.e., increase in cell numbers) can be followed using yeasts or bacteria since they grow rapidly, producing more cells, which can then be counted.

ESTIMATION OF MICROBIAL GROWTH

Bacteria and yeast grow rapidly and as a result, they are used to follow growth studies in microbes. We can estimate growth of microbes by estimating viable numbers or by turbidimetric method.

Experiment 2.1: Estimation of growth by viable or colony count method

In this method, we either use spread/pour plate or drop plate method to follow the growth of microbial populations under different conditions. Only pour plate method will be outline here.

The steps involved include:

a. Mix the sample
b. Making serial dilution of the sample
c. Transfer of 1mL of the dilution [e.g., 1/10 dilution] into the sterile petri-dishes.
d. To each plate, add 10 molten sterile agar at 45°C and mix by a combination of to-and-fro shaking and circular movement for 5-10 secs. Incubate at optimum temperature.
e. Incubate the chosen dilution you plated e.g., 1/10 dilution. Repeat this experiment from (c) every 30 minutes for up to 7 hours.
f. Count the number of colonies in each petri-dish and find the mean count from zero hour for up to 7 hours.
g. Plot Log10 number of viable organisms against time of incubation.

QUESTIONS

1. At what time (in minutes) from the beginning of the experiment does the organism begin to increase in number?
2. When does this increase in number begin to decline?
Experiment 2.2: Estimation of growth of bacteria by turbidimetric method.

You will need:
A flask of sterile nutrient broth (150ml in a 250-ml flask), culture of *E.coli* or other bacterium, or yeast (provided), colorimeter and colorimeter tubes, and 10ml sterile pipettes.

Procedure

i. Inoculate the flask of nutrient broth with 10ml of culture of *E.coli*. Mix well, place in incubator at 37°C.

ii. Note the time: remove a sample and determine the turbidity with the colorimeter. Do this quickly.

iii. After every half an hour, remove 10ml of sample with a 10ml sterile pipette and determine the turbidity with the colorimeter. Place the sample then into disinfectant. Remember to use a sterile pipette each time you sample the culture.

iv. Continue following the growth over a period of 6-7 hours. Arrange amongst yourselves a system for doing the turbidity.

Plot a graph of colorimeter reading against time.

QUESTIONS

1. At what time (in hours) from the beginning of the experiment does the organism begin to increase its growth? Does the organism then grow at a constant rate?
2. When does this last rate of growth begin to decline and when does it stop?
3. The rapid increase in growth is due to the cells growing, dividing, separating and then the new cells growing. From this information, say how the population of cells increases. This type of growth is called exponential growth. How long does it last (in hours) for your organism under test?
4. The rate at which the organism grows exponentially is called the *generation time or doubling time*. From your graph determine the doubling time for your organism (i.e. the time taken for the turbidity to double its value). The faster generation times are about 10 minutes for some bacteria, whereas most take 30-60 minutes to double in number. At the other extreme, some organisms have a long generation time, e.g., slow growing protozoa and algae show generation times of 24 hours or more.
5. What would you expect to happen to the generation time if we had used nutrient broth and glucose as a growth medium, or the nutrient broth diluted a hundred times?
6. What would happen if we inoculate our cells growing in each medium to a poor one?
7. What would happen to the answer to (1) if we had subjected our cells to the following pre-treatments (a) heated to 100°C for 10 minutes, (b) subjected to a non-lethal dose of radiation, (c) heated to 50°C for 10 minutes?
8. From your answer to (2) above, explain why the cells do not continue to grow and eventually begin to die out.
9. At some stage on your curve, growth remains stationary – the cells have stopped growing. How do you know? This is the maximum growth of cells. On what factor(s) will it depend?
The experiment we have done can be used to follow many effects on the growth of populations of cells. Devise experiments to test the following:

i. Does the amount of oxygen available affect the growth rate?

ii. Temperature, pH, different nutrients, all may affect growth. Test this hypothesis:

iii. Do different microorganisms grow to the same extent under the same conditions?

iv. Why can't we follow growth of fungi such as Aspergillus spp. or Mucor, whereas we can with fungi such as yeast?

**Experiment 2.3: Effect of Temperature on the growth of microorganisms.**

You will need:

Six tubes of nutrient broth cultures of *Serratia marcescens* or *Sarcina lutea* or *E. coli*, inoculating loop, colorimeter and colorimeter tubes.

**Procedure**

i. Label the tubes with the temperature at which they are incubated e.g. 40°C, 5°C, 30°C.

ii. Inoculate each tube aseptically with a loopful of culture.

iii. Incubate the tubes at the temperature adopted for 72 hours.

iv. After incubation, measure the amount of growth in each tube using the colorimeter. Mix up the contents of each tube before estimating the growth produced in each tube.

v. Note the readings for the various growth tubes and plot a graph of colorimeter reading (i.e. amount of growth) against the incubation temperature used. You can use different cultures to see the effect of temperature on a range of organisms.

**QUESTIONS**

1. What is the optimum temperature for the growth of the organisms you have used?

2. If you wished to grow these bacteria for use in an experiment, at which temperature would you grow them?

3. Does the optimum temperature of growth vary with different organisms?

4. Why is there no growth or very poor growth at high temperatures?

5. If growth is not obtained at low temperatures, does this mean that the organism has died or been killed?

**FOOD SOURCES AND GROWTH**

Higher animals and many microorganisms exhibit the same type of nutrition. They are both classed as **heterotrophs** i.e. obtaining their food materials in a complex organic form derived from other organisms which manufacture their own food materials from inorganic simpler materials. Organisms manufacturing their own organic compounds are termed **autotrophs** e.g. green plants, algae, photosynthetic bacteria.

Some microorganisms are also able to obtain energy from the breakdown of simple inorganic materials such as sulphur, iron or even hydrogen. These are called **chemosynthetic organisms**. So we have autotrophic and heterotrophic types of nutrition. Chemosynthetic bacterial belonging to the first group. Examples of these are:

- Autotrophs: any algae and nitrogen fixing bacteria.
- Heterotrophs: all bacteria and fungi.
**Experiment 2.4: Sources of Nitrogen for the Growth of Organisms**

You will need:
- Plates labeled with various nitrogen compounds
- *Penicillium chrysogenum* culture
- Inoculating loop

**PROCEDURE**

1. Inoculate each plate with fungus from the stock culture in the centre of the plate.

   **NOTE:** Take care in inoculating, as you only want one colony to develop on the plate.

2. Incubate the plates at 25 - 30°C.

3. After 2 days, estimate the diameter of the colony on each plate and repeat this every few days up to about 14 days incubation.

   **NOTE:** Measure the diameter of the colonies using the underside of the plate; do not take the lid off.

4. Plot a graph, from your results, of diameter in millimeters against incubation time in days, for each nitrogen source used.

**QUESTIONS**

1. Does the fungus use all the sources of nitrogen for growth? How do you know?
2. Which was the best, and which was the poorest source of nitrogen for growth of the organism?
3. Would you classify the fungus as a heterotrophy or autotroph in respect of its nitrogen source for growth?

You can modify this experiment to compare the ability of different fungi to use different nitrogen sources, as well as devising experiments to show how bacteria and other unicellular organisms are affected by different nitrogen sources for their growth.

**SALTS AND GROWTH**

The process of osmosis is familiar from earlier work in school and since microorganisms possess a semi-permeable membrane in the form of their cell membranes, the presence of substances such as salts or sugars in high concentration in their environment can affect them. Since microorganisms normally live in dilute nutrient environments and contain high concentrations of salts etc. in their cytoplasm, we can appreciate what would take place concerning water movement in these organisms if they were placed in more concentrated salt surroundings.

For example:

(a) What would happen if we place microbial cells in an environment where the concentration of salts is much greater outside than inside the cell?

(b) How is the above principle utilized in the food industry?
EXPERIMENT 2.5: SALT AND ITS EFFECTS ON GROWTH
You will need
Ten tubes of peptone water medium containing various amounts of salt (You will be told the salt concentration of the various numbered tubes).
Inoculating loop, culture of E. coli or yeast, colorimeter and colorimeter tubes.

Procedure
1. Inoculate the tubes with a 0.2ml of E. coli culture or yeast culture (whichever you are provided with).
2. Incubate the tubes for 48 – 72 hours at 37°C.
3. After incubation, measure the growth of the organisms in each tube, using the colorimeter with sterile 1% peptone water as a blank to zero the colorimeter. Note the reading for each tube.
4. Plot a graph of colorimeter reading (i.e. amount of growth) against salt concentration.

QUESTIONS
1. Do both organisms grow well in peptone water and salt?
2. What is the optimum salt concentration for each organism to grow in according to your results?
3. Are there any salt conditions in which either organisms does not grow at all? Explain the probable reason for lack of growth.
4. If placed in a highly salty environment, which organism would you expect to survive?
5. Is plain peptone water the best medium to grow these organisms in? From your results, suggest how you would alter the medium composition if at all?

Experiment 2.6: Effect of pH on the growth of organisms
You will need:
Seven tubes of nutrient broth, each at a different pH.
Universal indicator paper.
Colorimeter and colorimeter tubes.
Culture of E. coli or Saccharomyces cerevisiae (thick suspension).

Procedure
i. Inoculate each tube with a loopful of culture of the organisms under test
ii. Incubate the tubes at 37°C for 48 hours.
iii. After incubation, determine the amount of growth in each tube using the colorimeter and colorimeter tubes. Use sterile nutrient broth to zero the instrument.
iv. Determine the pH value of each tube after assessing the amount of growth. Plot a graph of growth produced against the pH of the growth medium.

QUESTION
1. At what pH does the E. coli grow best?
2. Are there any pH values where no growth is produced?
3. Are other organisms you have cultured affect by pH?
4. Most natural environments have pH values between 5 and 9. 
What can you say then about the optimum pH for growth of microorganisms found in nature?

5. Since E. coli is a bacterium, and saccharomyces is a fungus, are there differences between fungi and bacteria in their preference for pH conditions for growth?

6. Table 1 shows the pH values of various types of habitats for microorganisms. Which examples do you think would be colonized by fungi and bacteria in general?

7. Is the pH value of each growth tube approximately the same at the end of the experiment as it was at the beginning? If there has been a change, what do you think could be responsible for it?

8. If there is no change, how has the pH been kept constant?

9. How is the pH of fluids, such as blood, kept constant in higher organisms?

10. Name two organisms you think would survive best in a salted food?

11. What is the effect of salts on proteins and why is this information useful with regards to the growth of organisms in salty conditions?

12. Some organisms need lots of salt to grow normally and these are termed halophiles. Name two habitats where halophiles are found.

<table>
<thead>
<tr>
<th>Table 1:</th>
<th>pH Values of Various Types of Habitat for Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. of H' (g/litre)</td>
</tr>
<tr>
<td>Increasing acidity</td>
<td>10^-1</td>
</tr>
<tr>
<td></td>
<td>10^-2</td>
</tr>
<tr>
<td></td>
<td>10^-3</td>
</tr>
<tr>
<td></td>
<td>10^-4</td>
</tr>
<tr>
<td></td>
<td>10^-5</td>
</tr>
<tr>
<td></td>
<td>10^-6</td>
</tr>
<tr>
<td>Neutral</td>
<td>10^-7</td>
</tr>
<tr>
<td></td>
<td>10^-8</td>
</tr>
<tr>
<td></td>
<td>10^-9</td>
</tr>
<tr>
<td>Increasing alkalinity</td>
<td>10^-10</td>
</tr>
<tr>
<td></td>
<td>10^-11</td>
</tr>
<tr>
<td></td>
<td>10^-12</td>
</tr>
<tr>
<td></td>
<td>10^-13</td>
</tr>
<tr>
<td></td>
<td>10^-14</td>
</tr>
</tbody>
</table>

**INORGANIC NUTRITION OF ORGANISMS**

Minerals of various sorts are needed for the successful growth and development of organisms, especially plants. In plants, the lack of certain minerals may cause reduced growth, and affect yields of fruits etc. Since bacteria and fungi have similar needs and organisms grow rapidly, we can use them to demonstrate the need for minerals, and see what effects the deficiency of a particular mineral has on growth.
Experiment 2.7: Mineral deficiencies and fungal growth

You will need:
Plates of agar (the name of the deficient mineral in the medium should be noted on the lid, e.g. Mg means minus magnesium):
Culture of *Penicillium chrysogenum* or *muco* sp. or *Aspergillus niger*
Inoculating needle.

Procedure
i. Inoculate the plates by adding 0.5 ml of microbial suspension.
ii. Incubate at 25°C. After 2 days, determine the colony diameter for each plate as the fungus grows. Replace in the incubator and repeat the measurements after various days of incubation.
iii. From your results, plot a graph of growth rate (i.e. colony diameter) against incubation time for each mineral deficiency.
iv. Remove material from each plate and observe the colonies and mycelium carefully to see if any effects of the deficient minerals can be seen in the actual growth of fungus. Note any differences.

(b) Alternatively, we can look at the effects of mineral deficiency on growth by using dry weight as an estimate of growth. We shall be measuring cell, mass and dry weight of fungus.

Procedure
i. Inoculate each flask with culture from the fungal stock. Do this aseptically. The deficient ions in each flask can be seen by comparing the flask number of Table 2.

Table 2: Mineral Deficiencies in Liquid media

<table>
<thead>
<tr>
<th>Flask Number</th>
<th>Deficient ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None (full nutrients)</td>
</tr>
<tr>
<td>2</td>
<td>Minus Nitrogen (N₂)</td>
</tr>
<tr>
<td>3</td>
<td>Minus Potassium (K)</td>
</tr>
<tr>
<td>4</td>
<td>Minus Phosphate (PO₄)</td>
</tr>
<tr>
<td>5</td>
<td>Minus magnesium (Mg)</td>
</tr>
<tr>
<td>6</td>
<td>Minus sulphur (S)</td>
</tr>
<tr>
<td>7</td>
<td>All (no nutrient)</td>
</tr>
</tbody>
</table>

ii. Incubate the flask for 7 days at 25°C and then make observations on the state of growth after this period and any differences between the growths under the different mineral conditions.
iii. Determine the dry weight of filter papers numbered 1 to 7 and then set up the filter apparatus.
iv. Filter the contents of each flask through the correspondingly numbered filter paper. Make sure you transfer all the mycelium material and wash out the flask with distilled water. Filter the washings.
v. Remove the filter paper and mycelium mat, reweigh and then dry the material and filter paper overnight on a watch glass, in an oven at 105°C.

vi. Remove the dried material and watch glass, and cool down in the dessicator. When cool, reweigh. Note the weight.

vii. Repeat the procedure for the other flasks. Table 3 shows a typical class result.

Table 3
Growth of Aspergillus niger in Liquid Medium Deficient in various ions (class result)

<table>
<thead>
<tr>
<th>Culture deficient ion solution</th>
<th>Dry weight of fungal mycelium after seven days (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flask 1</td>
</tr>
<tr>
<td>1 Full Nutrient</td>
<td>0.195</td>
</tr>
<tr>
<td>2 Minus Nitrogen</td>
<td>0.005</td>
</tr>
<tr>
<td>3 Minus Potassium</td>
<td>0.016</td>
</tr>
<tr>
<td>4 Minus Phosphate</td>
<td>0.008</td>
</tr>
<tr>
<td>5 Minus Magnesium</td>
<td>0.123</td>
</tr>
<tr>
<td>6 Minus Sulphur</td>
<td>0.056</td>
</tr>
</tbody>
</table>

QUESTIONS
1. Determine the dry weight as a percentage of the wet weight for each flask growth material. Is this value constant?
2. Why are we using dry weight as indicative of the amount of growth produced in each flask, instead of using the fresh weight?
3. How did the cultures grow in each flask? Were there any difference in growth or appearance of the growth, under different mineral conditions?
4. Which mineral deficiency gave the poorest growth yield and which gave the highest yield?
5. Make a list of the order of minerals that have the greatest effect on the growth of the fungus. Does the order compare with using the colony diameter as an indicator of the growth of the fungus?
6. Give the functions for the various ions to the fungus in the media used. How can the deficiencies produced by the absence of certain minerals in plants be remedied, and how could normal growth be restored to the fungus?
7. Calcium is a major component of the spores of bacteria. Has spore production been affected in the fungus used here under conditions of ion deficiency?

VITAMINS
All types of organisms need small amounts of growth factors called vitamins. Some organisms can make their own, others obtain them in their food materials. Some organisms need only a few, whereas others, e.g. *Lactobacillus* (a rod shaped bacterium), need many complex vitamins for growth. Many aquatic microorganisms, e.g. bacteria, and algae, need vitamin B, for successful growth whereas some fungi do not need it and never make it. Ruminants, such as cows, depend on rumen microorganisms for vitamins and amino-acids necessary for their growth and metabolism.

Experiment: To show that vitamins are needed for the successful growth of an organism.
You will need:
One plate each of MRS medium, nutrient agar and CPS inoculating needle: culture of *Lactobacillus plantarum.*

Procedure
i. Using the streak plate method, streak some culture of *Lactobacillus* on each plate.
ii. Make a smear of the *Lactobacillus* used and gram-stain it.
Note its characteristics. Produce smears of any colonies developing on the three media and compare with the characters of the original *Lactobacillus* inoculum.

QUESTIONS
1. Has the organism grown on all the media? If not, which are the one that have not allowed growth?
2. Compare the composition of each given medium and relate this with the growth of *Lactobacillus* on these media. Is there any relation between the composition of the medium and growth?
3. How could you increase the possibility of *Lactobacillus* growing on the two media on which it has not grown?

Experiment: To show that some fungi produce vitamins and others need them for growth.

Your will need:
Two plates of GMK medium cultures of *Aspergillus regius* and *Sordaria fimicola* and inoculating needle.

Procedure:
1. Inoculate the *sordaria* at one side of the plate and the *Aspergillus* at the other.
2. Inoculate the other plate with *sordaria* only.
3. Incubate the plates for 8-10 days at 20-250°C. Observe the plate.

QUESTIONS
1. Has the *sordaria* grown on both plates?
2. The fruiting bodies of *sordaria* are small blackish bodies. Can you see any on the plates?
3. If these fruiting bodies or perithecia are present whereabouts are they found in relation to the growth of the *Aspergillus*?
4. Remove a single fruiting body, place on a slide in lactophenol and press gently with a needle. The reproductive structures inside with their spores will burst out, and you can observed these under the microscope. Remove the fruiting body remains before placing the coverslip on. Look for fruiting bodies on the other plate as well.
5. How many spores can be seen in each ascus or spore case which has come out of the perithecia.
6. It is known that *Sordaria fimicola* needs biotin – a vitamin for the production of fertile perithecia. Since there is no biotin in the agar, how has the *sordaria* produced fertile perithecia?
7. How do you know that perithecia are fertile?
CHAPTER SIX

MICROORGANISMS IMPORTANT IN FOODS

A. MOULDS

Molds may spoil foods under conditions that would inhibit the growth of most bacteria and yeast. On the other hand, growth of molds is encouraged in some foods, e.g., blue cheese and tempeh, and they may be used for the production of enzymes to be employed in food processes.

Experiment 3.1: Identification of Moulds

**Procedure**

Examine the general of molds selected by the instructor, for example: Rhizopus, muced thamnadium, penicillium, aspergillus, cladosporium, alternaria, geotrichum, fusarium, monilia (Neurospora), Botrytis, and Trichotheccium.

With low power (16mm objective) of microscope, examine edges and upper surface of mycelial growth of the mold in the culture provided. Look especially for isolated fruiting heads. Note the arrangement of parts of the mold.

Experiments 3.2: Staining and Examination of Moulds

**Procedure**

i. Moisten a wireloop with 10% glycerol and carefully transfer some of the mold mycelium and fruiting parts to a drop of 10% glycerol on a glass slide. Cover with a cover slip and examine microscopically for detailed structure.

ii. (a) Place large drop of lactophenol-picroi acid or lactophenol cotton blue on clean slide.
   (b) Pick off a portion of the growth with dissecting needles.
   (c) tease out in a drop of Lactophenol-cotton-blue stain on the slide.
   (d) Cover with a clean coverslip, taking care to exclude air bubbles.
   (e) Examine the prepared slide under the microscope:
      (i) First using the low power objective (x10)
      (ii) And then using the x40 (high power dry) objective lens for a closer examination of selected field.

Record the following observations:

A. Mycelium: Whether septate or nonseptate; hyaline (and whether colored or not) or dark; smoky (thin or full); type of branching of hyphae.

B. Asexual spores: Whether conidia, sporangiospores, smooth, rough or spiny; whether 1, 2 or many celled.

C. Fruiting heads:
   1. If sporangia, their size, color, shape and location.
   2. If bearing conidia, whether one or many conidia per conidiophores: shape and arrangement of stigmata: arrangement of conidia: whether conidia are gummed together or not.

D. Special structures and location: stolons, rhizoids, foot cell, columella, apophysis, vesicle, sclerotia, chlamydospores, sexual spores, etc.
Experiment: Examination of Mouldy Foods

Procedure:
Examine samples of moldy foods (fruits, bacon, cheese, etc) as provided. Attempt to ascertain genus of mold by direct examination of a piece of mycelium. If unsuccessful, transfer some mycelium to a slant of malt agar, and incubate at room temperature until spores are formed. Then examine in the tube by focusing on the thin edge of the slant, or in 10% glycerol on a slide. Draw parts and indicate source of mold and genus name.

Experiment: Effect of temperature, pH, and NaCl on mould growth

Procedure:
Inoculate with spores of Aspergillus, penicilliun and Cladosporium:
A. Three malt agar slants and incubate one at 5°C for 7 days, the second at room temperature for 3-4 days, and the third at 45°C for 3-4 days.
B. Two malt agar slants, one adjusted to pH 3.0 and the other to pH 8.0. Incubate for 5-4 days at room temperature.
C. Two malt agar slants, one with 10% NaCl added, and the other with none. Incubate for 3-4 days at room temperature. Examine cultures, noting relative amounts of growth and of sporulation. Record results and explain them.

Experiments: Plate Count of Molds and Yeast

A. Moulds

Procedure
Plate one of the following foods, using the dilutions indicated by instructor, with potato dextrose agar cooled to 46°C and then adjusted to pH 3.5 by addition of 1ml of sterile 10% tartaric acid solution per 100ml of agar: juice from sauerkraut or pickle fermentation, fresh fruit juice, or old butter. (NOTE: With butter, place 15-20g in a large sterile test tube; place tube into a water bath at 45°C for 10min; mix and transfer 10ml of melted butter with a warm pipette to a sterile 90-ml blank of buffered water at 45°C. Plate 0.1 and 0.01)

B. Yeasts

Yeast fermentation is important in the preparation of foods and beverages. Yeast may be grown for food or feed or for the preparation of enzymes, and they may be concerned in the spoilage of certain foods.

Experiment 3.6: Morphological Characteristics of Yeast
Examine microscopically methylene blue stained smears (or wet mounts) of fresh palm wine make drawings showing differences in shape, size, and budding of yeast cells.

Experiment 3.7: Examination for Ascospores and Conjugation in Yeasts

Procedure:
Inoculate one orange juice agar slant with S. cerevisiae and one with a Zygosaccharomyces spp. Incubate on the laboratory bench and examine each laboratory period for conjugation and ascospore formation. Make drawings.
Experiment 3.8: Detection of Oxidizing (film) yeast.
Procedure:
Inoculate a tube of grape juice with C. Krusei and a second tube with S. cerevisiae. Determine the pH of the juice by means of a pH meter. Incubate tubes for 14 days at room temperature, inclining the tube with film yeast to give a maximal surface to the juice. Note the appearance of growth and colour in the two tubes; determine the pH of the juice in each tube.

Experiment 3.9: Detection of Fermenting Yeasts:
Procedure:
Inoculate a fermentation tube of glucose broth, one of lactose broth and a tube of litmus milk Tornalopsis spheroica, and tubes of the same media with S. cerevisiae. Incubate tubes at room temperature for 2-5 days and report evidences of growth: gas, cloudiness, and sediment in broths: gas and change of pH of milk. Record the odor of tubes.

Experiment 3.10: Detection of Leavening Power
Procedure:
Mix 50g of flour, 1g of compressed yeast, and 30ml of distilled water, first making a thin sponge of the yeast, water, and a little flour. Mix in the remaining flour and knead vigorously for 5 min. Mixing and kneading may be done on a piece of waxed paper. Roll the dough into a narrow cylinder and drop it into a greased (Vaseline) 100-ml graduated cylinder and press down: read volume. Do not fill cylinder over the 25-30 ml mark. Incubate at 30°C, and read volume every 30 min for 90 min. Express results as percentage increase in volume (based on original volume) per 30 min.

Experiment 3.11: Detection of Sugar and salt-tolerant yeasts
Procedure
Inoculate single tubes of 5, 20, 40, and 65% sucrose (w/w) broths with Zygosaccharomyces sp., and a similar set with S. cerevisiae. Incubate tubes at room temperature for 5-7 days; then observe for cloudiness, sediment, and gas production as evidences of growth. Inoculate single tubes of 2, 5, 10, 15, and 20% sodium chloride (w/w) in plate count broth with 0.1 ml of a suspension of Debaryomyces sp. And a similar set with S. cerevisiae. Incubate tubes at room temperature for 5-7 days then observe for growth.

C. BACTERIA
Included in the Gram-negative rods, are many of the bacteria important in the spoilage of foods; many of the psychrotrophs. Pseudomonas and Achromobacter species concerned in the spoilage of fresh foods and some dairy foods; the coliform bacteria which are both spoilage organisms and indicator bacteria for contamination from intestinal sources; and some of the intestinal pathogens, e.g., Salmonella sp.

Aseptic technique is essential in this experiment in handling a potentially pathogenic. Salmonella strain. All Salmonella cultures and slides must be segregated and sterilized before the glassware is turned in for washing.
Pay particular attention to instructions for handling cultures and slides!
Experiments 3.12: Reactions of some gram-negative bacteria important in food on some media.

Procedure:
Inoculate each of the gram-negative organisms provided *Pseudomonas* sp, *Escherichia coli*, *Proteus* sp, *Alcaligenes* sp, *Aerobacter aerogenes*, and *Salmonella* sp — into the underlisted media:

(a) Triple sugar iron (TSI) agar, stab butt, streak slants
(b) Urea broth
(c) Lactose, sucrose, and glucose broths
(d) Lysine agar
(e) Tryptophane broth
(f) Litmus milk
(g) Smith or Durham fermentation tube of lactose broth: inoculate only with *E. coli* and *A. aerogenes*.

Incubate the *Pseudomonas* and *Alcaligenes* cultures for 2 days at 25°C; all others at 37°C.

Make the following observations with respect to the above listed media, as in A–G.

A. TSI agar: note reaction in butt and slant; yellow indicates acid production; red alkaline reaction. Blackening of medium indicates H₂S. Also record gas production.

B. Urease activity: red color indicate alkalinity from ammonia.

C. Yellow indicate acid production in sugar broths.

D. Lysine decarboxylase activity: purple indicates alkalinity from amine production.

E. Indole production from tryptophane: add 3-5 drops of Kovac's reagent shake tube; red color is positive.

F. Observe changes in litmus milk.

G. Gas production: compare amounts of gas from *E. coli* and *A. aerogenes*.

Experiment 3.13: Gram Staining

Procedure:

i. Make smears of the various bacterial cultures provided. (Don't make smears too thick or the staining action will not work correctly).

ii. Stain the smears for one minute with gram stain I. (Crystal Violet).

iii. Wash off stain with tap water and flood slide with gram stain II (iodine) for one minute.

iv. Wash in tap water; blot dry carefully, decolorize for 30 seconds in 95% alcohol with gentle agitation.

v. Stain smear for 10 seconds with gram stain III (safranin). Wash in tap water and dry. Observe smears under oil immersion.

RESULT

Gram positive: blue

Gram negative: red

Record the results of your staining and determine the gram stain reaction to unknown cultures.
Experiment 3.14: Differential Test for Gram Negative Bacteria

Procedure:

A. For coliforms, streak a plate of eosin methylene blue agar with each culture to obtain isolated colonies. After incubation for 2 days at optimum temperatures, note differences in appearances of colonies, and record.

B. For coliforms: IMViC reactions. Inoculate E. coli and A. aerogenes, into duplicate tubes of proteose broth containing 1% glucose and into single tubes of Koser citrate medium. Incubate at 37°C for 2 days except for the tube for the methyl red test, which should be incubated for 5-7 days.

1. Voges-Proskauer test. To 1 ml of the 2-day culture in proteose broth add 0.6 ml of 5% alpha naphthol in absolute alcohol and 0.2 ml of 40% aq KOH. Shake tube for aeration. Development of a pink to cherry-red color within 2 hr indicates a positive test for acetone.

2. Methyl red test. To 5 ml of the 5-7 day culture in proteose broth add 5 drops of methyl red solution. Red indicates a positive test, yellow a negative test.

3. Citrate as carbon source. Examine tube of Koser medium for increased cloudiness as evidence of the utilization of citrate as sole carbon source.

4. Indole production from tryptophane: add 3-5 drops of Kovac's reagent. Shake tube; red colour is positive.

State the IMViC reaction (e.g. + + - - ) for each organism.

C. For Pseudomonas. Prepare streak plates of E. coli and Pseudomonas sp. on Pseudomonas agar. Incubate 32 days at 25°C. Remove covers of dishes and compare the appearance of colonies under U-V light.

QUESTIONS

1. How can you estimate the number of Pseudomonas organisms in a food sample? Coliform bacteria? Enterococci?

2. Why is Pseudomonas the predominant spoilage organism in unprocessed meats, poultry, and fish?

3. Does the finding of coliform bacteria in a food sample always indicate fecal contamination? Explain.

PHYSIOLOGICAL GROUPS OF BACTERIA

Food organisms may be grouped on the basis of one common characteristic. So the thermophiles grow at high temperature. ....... In general, are more heat-resistant than mesophiles. Lipolytic organisms can be active in fatty food, proteolytic organisms in protein foods, acid-forming bacteria in foods containing sugar etc.

Experiment 3.15: Detection of Lipolytic Bacteria

Procedure:

Gently shake 3 tubes of melted and cooled fat agar and pour into sterile petri dishes. Incubate the center of the solidified agar in one plate with Pseudomonas fluorescens to produce a giant colony, a second plate with Alcaligenes viscolactis, and the third plate with Escherichia coli.
After incubation for 1 weak at 32°C, flood the fat agar plate with a saturated solution of copper sulfate. After 5 min, pour off the excess solution and examine the agar for the presence of greenish-blue fat globules in the vicinity of each colony, especially under the colony. Record the ability of the organisms to hydrolyze fat.

**Experiment 3.16: Detection of Slime and Rope Forming Bacteria**

**Procedure:**
Inoculate single tubes of litmus milk with *Aerobacter aerogenes* and *Alcaligenes viscolactis*, and a plate of sucrose gelatin agar with *Leuconostoc mesenteroides*. Incubate for 24 hrs at 20°C and examine for slimmess or ropiness. Introduce a sterile wire loop into each medium and withdraw it to pull out the slime. Prepare a capsule stain of *A. viscolactis* or observe a demonstration. Describe the slimm material and note its location in the medium.

**Experiment 3.17: Detection of Salt-tolerant and Halophytic Organisms**

**Procedure:**
A. Inoculate tubes of Trypticase (or Tryptic) Soy Broth containing 15 and 20% NaCl with 0.1 ml of a suspension of each of the following microorganisms: *Halobacterium sp.*, *Micrococcus sp.*, *Staphylococcus aureus*, *Pediococcus cerevisiae*, and *Debaryomyces sp.* Incubate 7-9 days at room temperature and examine for growth characteristic of the organism. Indicate relative amounts of growth by numbers of + marks. **NOTE**: use aseptic precautions with *S. aureus* culture and TURN IT OVER TO THE INSTRUCTOR AFTER EXAMINATION!

B. Prepare 3 petri plates of milk-salt agar. By means of a sterile spatula scatter a little solar salt over the agar surface of one plate. Over a second agar surface, spread 0.1 ml of brine from a cheese brine tank, and over the third agar surface, 0.1 ml of ham or bacon curing brine. Incubate plates agar side down, at room temperature and examine periodically for colony formation. Prepare and examine stained preparations from different types of colonies.

**Experiment 3.18: Detection of Psychrotrophs**

**Procedure:**
Pour duplicate plates with plate count agar of dilution (indicated by the instructor) of one of the following:
A. Pasteurized or raw market milk that has been kept in the refrigerator for at least 5 days.
B. Hamburger that has been kept in the refrigerator for several days. Weigh 11 g into a sterile blender, and 99 ml of sterile water; and blend for 2 min with a mechanical blender before making dilutions.
C. Raw fish. Treat like the hamburger.
   Incubate plates for 7 days at 7°C and count colonies. Express results as numbers of psychrotrophs per ml or g. Make and examine gram-stained preparations from different types of colonies and report results.
CHAPTER SEVEN

THE MICROBIOLOGICAL EXAMINATION OF SPECIFIC FOODS/WATER

MEAT AND MEAT PRODUCTS
Experiment 4.1: Examination of Meat and Meat Products

Procedure:
Prepare decimal dilutions to $10^n$ and carry out the following:
(a) General viable counts on plate agar, incubated at 5, 25 (or 20) and 37 (or 35)$^\circ$C for 7, 3 and 2 days respectively.
(b) Coliform counts either by a pour plate procedure using violet red bile agar, or by a multiple tube count (if small numbers of coliforms are expected).
(c) An examination for the presence of Salmonella.
(d) Viable counts of anaerobic bacteria using a dilution series prepared with reinforced clostridial medium as a diluent. Use plate count agar, and blood agar; incubate aerobically at 37$^\circ$C for 2 days.
(e) Examination for the presence of Clostridium perfringens.

Recommended Standards
ICMSF (1974) recommended that the general viable count at 35$^\circ$C (or at 20$^\circ$C in the case of chilled meats) should be less than $10^7$ per gram, and that Salmonella should be detected in not more than one of five 25g samples.

Experiment 4.2: Examination of Raw Sausages, Hamburgers and similar Meat Products

Procedure
Surface counts of sausages may be performed, but more usually homogenates of decimal composite samples are used for the preparation of serial decimal dilutions up to $10^n$. Counts should be made on:
(a) Plate count agar for general viable counts, incubated at 5, 25 and 37$^\circ$C for 7, 3 and 2 days respectively.
(b) Violet-red bile agar for coliform counts (or use a multiple tube technique if small numbers are expected).
(c) Acetate agar (or Rogosa agar) with Acti-dione (to inhibit microfungi). As double layered pour plates for Lactobacillus, incubated at 30$^\circ$C for 5 days.
(d) Selective media for fecal streptococci.
(e) Davis's yeast salt agar, acidified to pH 3.5 for counts of yeast and moulds, incubated at 25$^\circ$C for 5 days.
(f) Examine for such pathogens as appropriate.

Experiment 4.3: Examination of Fish and Shellfish

Procedure:
Carry out surface counts and tissue counts in a manner similar to that described for meat, using:
(a) Plate count agar for general viable counts, incubated at 5, 25 and 37$^\circ$C for 7, 3 and 2 days respectively.
a. Plate count agar + 15 per cent NaCl for counts of extreme halophiles and salt-tolerant organisms, incubated at 5, 25 and 37°C for 7, 3 and 2 days respectively. In this case, the diluent should be 15 per cent NaCl solution.
b. Coliform counts by the multiple tube count method.
c. Baird-Parker's medium for Staphylococcus aureus (surface counts) at 37°C

**Experiment 4.4: Examination of Smoked Fish**

**Procedure:**
Same as for raw fish, but with the addition of a qualitative examination for moulds by streaking the 10^7 dilution (or a higher concentration) across the surface of Davis's yeast salt agar or Czapek-Dox agar.

**Recommended Standard:**
ICMSF (1974) recommended that cold-smoked fish should have a general viable count of less than 10^6 per gram, a faecal coliform count of less than 400 per gram, and a Staphylococcus aureus count of less than 2000 per gram.

**Experiment 4.5: Examination of Shell Eggs**

(A) **Contents of Shell Eggs:**

**Procedure:**
Scrub the eggs with warm soapy water and stiff brush, then rinse well and drain. Immerse in alcohol for 10min, then allow to drain well and flame quickly. Cut a hole in the end, opposite the air sac (which is located at the blunt end) using a small carborundum disc on an electric drill, or using a sterile scalpel. Remove the contents aseptically and homogenize using a blender or the Colworth Stomacher. Counts can be carried out on single eggs or on the bulked contents of a number of eggs. Prepare serial decimal dilutions in the usual way. Carry out general counts on plate count agar at 25 and 37°C, presumptive coliform counts and an examination for the presence of Salmonella.

(B) **Frozen Whole Egg**

**Procedure:**
Sample the product while still in the frozen state. Clean the lid and top of the tin, swab with alcohol, flame and then remove the lid. With a sterile auger or other suitable instrument, remove two cores, one from the centre of the can and one at the edge, extending from the top surface to as deep a level as possible with the instrument used. Transfer these to a sterile container and examine as soon as possible. Allow the frozen samples to soften slightly, and while still very slightly frozen, blend thoroughly. Prepare serial decimal dilutions to 10^7 and carry out the following:
(a) General viable counts on plate count agar at 25 and 37°C.
(b) Presumptive coliform counts using either violet red bile agar (pour plates) or the multiple tube technique.
(c) A direct microscopic count by the Breed's smear method, on the 10^5 dilution.
(d) Examination for the presence of Salmonella.
LIQUID MILK

Experiment 4.6: General Viable Counts on Milk:
Media suitable for this purpose are plate count and yeast extract milk agar. For official tests on milk samples in England and Wales and in Scotland, yeast extract milk agar is the approved medium and the prescribed incubation period is 3 days at 30°C. Psychrotrophic counts and thermophilic counts can be determined by incubating sets of plates at 5 and 55°C for 7 and 2 days, respectively. If low numbers of thermophiles are expected, they may be counted by the multiple tube technique either by using tryptone glucose yeast extract broth, with microscopic examination after incubation, or (for milk-spoiling thermophiles only) by detecting changes in the milk or in inoculated litmus milks. With the increasing emphasis on refrigerated cooling of milk supplies, the use refrigerated bulk tanks, and alternate day collection, the psychrotrophic count becomes of great significance.

Experiment 4.7: Methylene Blue Test:
The method described here is based on the work of Wilson (1935) and follows the specifications for the Milk (Special Designation) Regulations, 1963.

Procedure:

i. Prepare a stock solution of methylene blue. Add one standard tablet (B.D.H., Ltd.) to 200ml of cold, sterile, glass-distilled water in a sterile flask; shake until completely dissolved, and make up to 800ml with more distilled water. This solution can be stored in a cool dark place, preferably a refrigerator, for up to two months. Transfer aseptically each day's requirement into a sterile container and discard at the end of the day.

ii. Thoroughly mix the sample to be tested and pour the milk aseptically into a sterile test-tube up to the 10-ml mark.

iii. Add 1ml of methylene blue solution and, after a lapse of 3 seconds, blow out the remaining drops. The same pipette may be used for a series of tubes provided that it does not contact the milk or the wetted side of the tube.

iv. Close the test tube with a sterile rubber stopper and invert the tube slowly twice to mix the contents.

v. Within 5 min. place the tube in a water bath at 37.5 ± 0.5°C and note the time. The level of the water in the bath should be above that of the milk in the tubes, and the bath should be fitted with a lid to exclude light.

vi. Set up a control tube with each bath similar in colour and fat content to the milk under test. Pour 10ml milk into a sterile stoppered test-tube, add 1ml of tap water and place in boiling water for 3 min. then cool and place in the water bath. The control tube will help to determine when decolorisation is completed.

vii. Examine the tubes after half an hour. The milk is regarded as decolorized when the whole column of milk is completely decolorized or decolorized to within 5mm of the surface. A trace of colour at the bottom of the tube may be ignored provided that it does not extend upwards for more than 5mm.

viii. When the test is to proceed beyond the half-hour period, the tubes should be examined at half-hourly intervals for the duration of the test. Tubes which have decolorized should be removed from the water bath; tubes in which decolorisation has begun should remain in the bath without inversion until decolorisation is completed. All other tubes in the water bath should be inverted once to redistribute surface cream within the milk and then replaced.
ix. Excessive inversion should be avoided since it results in reoxidation of the methylene blue and consequently will invalidate the test result.

Experiment 4.8: Examination of Ice Cream
Procedure
Invert the sample bottle three times to mix the sample. Using a sterile 10ml pipette, weigh out 10g of melted ice-cream into a container. Add 90ml of sterile diluents and invert three times. This constitutes the $10^1$ dilution. Prepare further dilutions up to $10^7$ or as required. Carry out counts on the following:

a. Plate count agar or yeast extract milk agar incubated at 5 and 30°C for 5 and 3 days respectively for psychrotrophs and mesophiles.
b. Violet-red bile agar incubated at 30°C for 24h for coliform-aerogenes organisms (This conforms to the British standard 1970). Alternatively, use a multiple tube technique for the detection of smaller numbers (the International Diary Federation 1971b). Specified the use of brilliant green lactose bile broth in this case. Positive cultures can be confirmed as coliforms or as *Escherichia coli* as described.
c. Davis's yeast salt agar (pH 3.5) for yeast and moulds. If moulds are suspected, incubate at 25°C for 5 days.
d. Baird-Parker's medium (surface counts) incubated at 37°C for 24h, for *Staphylococcus aureus*.
e. Thermolabile bacteria can be counted using the $10^1$ dilution in a laboratory pasteurization test as described for milk.

Experiment 4.9: Examination of Fruit Juices and Squashes
Procedure:

a. Prepare serial dilutions and carry out the following:
   i. A general viable count of yeast and moulds on orange serum agar, or Davis's yeast salt agar (pH 3.5) or with antibiotics.
   ii. A general viable count of yeast, moulds and bacteria on malt extract agar, pH 5.4.
   iii. An osmophilic count on osmophilic agar or on orange serum agar containing 20 percent sucrose adjusted to pH 5.4 or pH 3.5, plates should be incubated at 25-30°C for 7 days and examined after 3 and 7 days.

b. Spore count: Transfer aseptically 10ml of the sample to each of two sterile, plugged test-tubes into one of the tubes, insert a thermometer through the cotton-wool plug so that the thermometer bulb is completely immersed in the sample. Place both tubes in a water bath at 80°C and allow the tubes to remain in the bath for 15min after the temperature in the control tube had attained a maximum temperature (usually just below the temperature of the water-bath). An alternative time-temperature combination which may select a slightly different thermolabile population is obtained by holding at 100°C for 5min. Remove the tubes and cool quickly in cold water. Carry out pour plate counts or multiple tube counts from the sample and its dilutions and incubate duplicate sets at 50 and 55°C, for the mesophilic spore count and the thermophilic spore count, respectively, using the media specified.
Microscopic count: Carry out a direct microscopic count for yeast by placing a known volume (1/100ml) of the sample or a low dilution of the sample on a slide, adding one drop of 0.01 percent erythrosine solution and mixing well. Spread the mixture over lem² of the slide and allow to dry. Alternatively, the sample-stain mixture may be examined as a wet preparation using a counting chamber or haemocytometer slide. The yeast and mould fragments can be seen and counted by due to the high solids content of most fruit juices and squashes, bacteria cannot usually be distinguished. A modification of Howard mould count method may also be adopted using wet mounts with a suitable counting chamber or haemocytometer slide.

Experiment 4.10: Examination of Flour
Procedure:

i. Prepare decimal dilutions in 0.1 percent peptone water for aerobic counts, and in reinforced clostridial medium for anaerobic counts. Use a Colworth “Stomacher” or other homogenizer to ensure adequate mixing.

ii. Carry out the following counts.
   (a) General and thermophilic viable counts on plate count agar incubated aerobically and anaerobically at 25, 37 and 55°C for 5 days.
   (b) A coliform count at 30°C using violet red bile agar, or by a multiple tube technique.

iii. Using the appropriate 10⁻¹ dilutions, perform both aerobic and anaerobic spore counts on plate count agar at 30 and 55°C.

Recommended Standard
Flour to be used in soups and meat products should contain not more than 15 thermophilic spores per g, not more than 10 flat sour spores per g, and less than 1 Clostridium spore per gram.

Experiment 4.11: Examination of Frozen Foods
Procedure:

i. While the food is frozen, remove it from its pack, with full aseptic precautions, into a sterile container. Allow the frozen sample to soften slightly and weigh 10g aseptically. Homogenize the weighed sample with 90ml of sterile diluents to give the initial 10⁻¹ dilution, preferably using a Colworth “Stomacher”. Prepare serial decimal dilutions as require, and carry out the following tests:
   a. Plate counts on plate count agar, incubated at 25 and 37°C for 3 and 2 days respectively.
   b. A presumptive coliform count using either violet red bile agar layer plates incubated at 35°C for 24hrs., or a multiple tube technique. Confirm presumptive coliforms at Escherichia coli by the Eijkman test.
   c. A direct microscopic count on the 10⁻¹ dilution, or if that is not possible, the 10⁻² dilutions staining the Breed’s smear by Gram’s method or with Loeffler’s methylene blue as appropriate.
d. An anaerobic viable count on pre-cooked meat products and an examination for the presence of *Clostridium perfringens* using for these examinations, a dilution series prepared in reinforced medium.

e. A count of *Staphylococcus aureus* on Baird-parker's medium (surface counts), incubated at 37°C for 24h.

ii. Store further packets of the frozen food at the maximum storage temperature recommended by the manufacturer (for example, if it is recommended that the frozen food can be stored for 3 days in refrigerator or for 12h at room temperature, carry out the keeping test by storing for 12h at room temperature), and repeat the tests after storage.

**Experiment 4.12: Examination of Canned Foods**

**Procedure:**

Select a representative number of cans from each batch. Examine the cans for physical defects that include faulty end seams, perforations, rust or other corrosion, dents, and bulging ends. Bulging at one or both ends may be due to bad denting or to the multiplication in the food of micro-organisms which ferment sugars with the production of gas. If any of the cans selected show such defects, examine these separately and remove further cans from the batch to make up the number of normal cans examined. Since most cans are partially evacuated, opening the can provides an opportunity for contamination of the contents with air-borne organisms. Therefore, cans should be opened in an inoculating chamber which should preferably ensure a sterile atmosphere by the use of forced ventilation of sterile air, ultraviolet light sterilization or other means, or at the very least, provide completely draught-free conditions.

a. Examine half the normal cans selected as follows: swab the top of a can with alcohol and then flame it. Open the can with a sterile can opener (sterilize this also by swabbing with alcohol and flaming). If the food product is liquid, remove a sample with a sterile pipette to a sterile container. In the case of a solid sample, use a sterile cork borer to remove a core of the food from the centre of the can. Also remove a core from the immediate vicinity of the side seam.

Carry out a microscopic examination of a smear of the food, stained by Gram's method if possible. Inoculate five tubes each of tryptone soya broth, and glucose tryptone broth with portions of the food to examine for the presence of viable bacteria, and "flat-sour" organism respectively. Inoculate five tubes of freshly prepared liver broth (oxoid) and incubate anaerobically either by sealing with 2 percent agar or by placing in an anaerobic jar. Incubate these media at 25°C for 3 days. Similarly inoculate two further sets of media and incubate at 37 and 55°C for 3 days. After incubation, examine the tubes for the growth of microorganisms and prepare Gram-stained smears from tubes which show growth. In the case of the glucose tryptone broth, "flat-sour" organisms will produce acid from the glucose and cause the medium to change colour from purple to yellow.

b. Incubate the remainder of the normal cans for one week at 37°C and then examine the cans for evidence of blowing. Sample and examine the contents of the cans as described above. Examine blown cans by opening the cans aseptically with precautions to prevent the high pressure in the cans causing the contents to be scattered. For example, after
a. sterilizing the top of the can with alcohol and by flaming, invert a sterilized funnel over the
top of the can. Insert a sterile metal punch through the hole in the funnel and puncture the top
of the can. When the pressure has been released, open the can with a sterile can opener and
examine the contents. As well as the tests indicated above, inoculate media as follows:

1. Lauter tryptose broth or violet-red bile agar 
   To detect coliform bacteria
2. Robertson’s cooked meat medium or anacrobically incubated liver broth (oxid)
   To detect *Clostridium* (mesophilic or thermophilic)
3. Tryptone soya broth 
   To detect *Bacillus*.

**MICROBIOLOGICAL WATER ANALYSIS**

**INTRODUCTION**

Water is indispensable to life and it is given priority in sitting of settlements, industries and farms
(Ekpo, 1990). It is necessary that water supply for human consumption should be free from
unpleasant or harmful contaminants.

Nigeria has pipe-borne water supply, but because of incessant interruption of water supply,
Nigerians resorts to other sources of water supply more often than not. These are shallow wells,
streams, surface tank reservoirs of rainwater.

The frequent outbreak of dysentery, gastroenteritis and typhoid fever in recent times has become
a concern to the country and the health authorities, since the causative agents of typhoid fever
and other gastrointestinal disease can be transmitted to their hosts through food and water
(Harrigan and McCance, 1976).

**Experiment 4.13: Microbiological Water Analysis**

**Collection of Water Samples**

Water samples from different sources: well, reservoir, steam, tap, were collected and used for
presumptive and confirmatory tests for coliform. Tables 4 and 5 summarise the protocol used
and Tables 6 and 7 show results and its interpretations.

**Results**

The number of bottles showing presumptive coliform positive and negative reactions from each
of the three batches were read from a probability table (Table 10), which indicated the estimated
number of coliform *E. coli* present in 100ml of water corresponding to various combinations of
positive and negative results in the quantities used for test (tables 6 & 7).
**TABLE 4**
Number of Bottles, Quantities of MacConkey Broth and Water Samples put in each Universal Glass Bottle Used for Presumptive Coliform and *E. coli* Count.

<table>
<thead>
<tr>
<th>Number of Bottles</th>
<th>MacConkey Broth incubated at 37°C</th>
<th>MacConkey Broth incubated at 44°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 batches of .5 bottles in each batch.</td>
<td>3 batches of 5 bottles in each batch</td>
<td></td>
</tr>
</tbody>
</table>

| Quantity of MacConkey Broth | Batch one contain 10ml double strength/bottle, batches two and three contain 5ml/bottles | Batch one contain 10ml double strength/bottle, batches two and three contain 5ml/bottle. |

| Quantity of Water Sample | Batch one above contain 10ml/bottle, batches two and three above contain 1ml and 0.1ml per bottle respectively | Batch one above contain 10ml/bottle, batches two and three above contain 1ml and 0.1ml per bottle respectively. |

**TABLE 5**
Amount of Brilliant Green Bile Broth, Peptone Water and Inoculum From Presumptive Coliform Positive Bottles

<table>
<thead>
<tr>
<th>Number of Bottles used</th>
<th>Brilliant Green Bile broth at 37°C</th>
<th>Brilliant Green Bile Broth at 44°C</th>
<th>Peptone Water at 44°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>According to number of bottles showing presumptive coliform positive at 37°C</td>
<td>According to number of bottles showing presumptive coliform positive at 37°C</td>
<td>According to number of bottles showing presumptive coliform positive at 37°C.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quantity of Medium</th>
<th>5ml per bottle</th>
<th>5ml per bottle</th>
<th>5ml per bottle</th>
</tr>
</thead>
</table>

| Quantity of inoculum from presumptive coliform positive bottles | Loopful per bottle | Loopful per bottle | Loopful per bottle |
Table 6
Most probable number (MPN) of *Coliform* and *E. coli* of water samples using MacConkey broth incubated at 37°C and 44°C respectively.

<table>
<thead>
<tr>
<th>Source of Water</th>
<th>Weakly Water Samples</th>
<th>Number of Positive Bottles at 37°C</th>
<th>MPN of Coliform/100 ml of Water</th>
<th>No. of Positive Bottles at 44°C</th>
<th>MPN of E. coli/100 ml of Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>1</td>
<td>5.5.5</td>
<td>1800⁷</td>
<td>5.1.1</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.5.5</td>
<td>1800⁷</td>
<td>4.2.1</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.5.5</td>
<td>1800⁷</td>
<td>5.1.3</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.5.4</td>
<td>1600</td>
<td>4.2.2</td>
<td>30</td>
</tr>
<tr>
<td>Reservoir</td>
<td>1</td>
<td>1.1.0</td>
<td>4</td>
<td>0.0.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>0</td>
<td>0.0.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.2.0</td>
<td>50</td>
<td>0.0.0</td>
<td>0</td>
</tr>
<tr>
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Table 7
Confirmatory Test for *Coliform* and *E. coli* using Brilliant Green Bile Broth (BCBB) and Peptone Water incubated at 37°C and 44°C respectively.

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**Keys:**
- + *Coliform confirmed*
- ++ *Thermotolerant Coliform confirmed*
- ++* *E. coli* type 1 confirmed*
- E *E. coli* absent
- ** No subculture to brilliant green bile broth because presumptive coliform test was negative

**PROBABILITY TABLES**
These tables indicate the estimated number of bacteria of the coliform group present in 100ml of water, corresponding to various combinations of positive and negative results in the quantities used for test. The tables are basically those originally computed by McCrady (1918), with certain amendments due to more precise calculations by Swarop (1938); a few values have also been added to the tables from other sources, corresponding to further combinations of positive and negative results which are likely to occur in practice. Swarop (1951) has tabulated limits within which the real density of coliform organisms is likely to fall, and his paper should be consulted by those anxious to know the precision of these estimates.
### Table 8

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Most probable number of coliform organisms in 100ml of the original water.

### Table 9

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Most probable number of coliform organisms in 100ml of the original water.
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Most probable number of coliform organisms in 100ml of the original water.
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Table 10 continued

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| Most probable number of coliform organisms in 100ml of the original water.
QUESTION

1. Define the term *Coliform*.
2. Interpret the following results obtained from microbial analysis of water:
   (a) *Coliform* organisms were present.
   (b) *E. coli* was present.
   (c) *Coliform* was absent while spores of *Clostridium perfringens* were present.
   (d) Presence of *S. faecalis* in presence or absence of coliforms.
3. Why is it necessary to use indicator organism to show that water is polluted by human faeces?

EXAMINATION OF FROZEN FOODS

Bacteriological standards have been recommended for frozen foods and have been adopted by some agencies. These standards may include limitations on total numbers as estimated by the plate count, limits on numbers of coliform bacteria, and absence of detectable numbers of enteric pathogens and *staphylococci*.

Experiment 1: Frozen Vegetable

a. For peas, lima and green beans, cut corn, etc. break content without opening package, sample with sterile spoon or knife from different part of package.

b. For spinach, asparagus, cauliflower, broccoli: Defrost at room temperature for 2 hrs, sample from different part package.

c. Weigh 50g into 450ml sterile water in a sterile mechanical blender cup and blend 2 min, then let stand 2 min. Re-suspend and pipette 1ml into a 99ml water blank. (If blender is not available weight 11g of vegetables into a sterile mortar with sterile sand, grind thoroughly, transfer to sterile 99ml water blank).

d. Total count: plate 1:100 and 1:1000 dilutions in duplicate with plate count agar. Incubate 3-4 days at 32°C. Count colonies and express as numbers per g.

e. Direct microscopic count: blend 50g of vegetable plus 100ml of sterile distilled water for 2 min. With broad pipette spread 0.01ml over 1sq cm on slide: dry; fix with heat or methanol. Stain with North's aniline oil methylene blue stain. Rinse dry, and count 100 fields. Report as micro-organisms per g.

Experiment 2: Frozen Foods

a. Preparation of sample: After defrosting for 2hr at room temperature, sample proportional parts of fruit and syrup from different parts of package. Then proceed as in frozen vegetables.

b. Total count: proceed as in frozen vegetables.

c. Direct microscopic count: proceed as in frozen vegetable.

Experiment 3: Precooked Frozen Foods

a. Preparation of sample: proceed as in frozen vegetable except without the thawing. With precooked frozen meals each part of the meal must be sampled, in whole or in part.
a. Total count: proceed as in frozen vegetable
b. Yeast and mold count: Plate recommended. Dilutions in duplicate with acidified potato dextrose agar and incubate at 21°C for 3-5 days. Express as yeast and mold colonies per g of product.
c. Direct microscopic count: proceed as in frozen vegetables.

**Experiment 4: Frozen Fish or Meat (raw or precooked):**

Follow procedure as described for spinach in frozen vegetable.

**Experiment 5: Effect of thawing and holding at room temperature:**

Examine microscopically a stained preparation (Gram method) from each of two samples of frozen food, one of vegetable and one of meat or fish, half of which has been held for 24hr at room temperature and the other half has been held for 24hr at refrigerator temperature.

**RECORD RESULTS IN TABULAR FORM**

**QUESTION**

1. What is the significance of a high bacterial count of frozen food?
2. What kinds of microorganisms survive the freezing process?
3. What kinds are most likely to grow on thawing?
4. How does long storage in the frozen condition affect the numbers of bacteria in frozen food?
5. Can pathogens survive freezing, cold storage, and thawing?
6. What precautions should be taken in the thawing of frozen foods?
7. Which foods can be refrozen and which cannot?

**SPOILAGE OF COMMERCIAL CANNED FOODS**

Biological spoilage of thermally processed canned foods results from growth of microorganisms that have survived the heat treatment or have entered thereafter through leaks.

**Experiment 1: Spoilage of Low Acid And Medium Acid Canned Foods**

**A. Spoilage caused by Thermophiles**

1. Flat-sour spoilage. Inoculate a can of corn or peas with a culture of the flat-sour thermophile, *Bacillus stearothermophilus*. Seal with solder. Reserve another can for a control and incubate both cans 2-5 days at 55°C, open and:
   a. Examine microscopically, staining with crystal violet. Determine the pH of the test can and compare it with the pH of the control.
   b. Plate for flat-sour bacteria: remove 10ml of corn or pea juice to a 90ml water blank by means of a sterile 10ml pipette or a sampling tube. Prepare bottle plates of petri plates of the 1:100 and 1:1000 dilutions with dextrose tryptone bromocresol purple agar pour rich plates. Incubate at 55°C for 2-5 days and study the typical flat-sour colonies.
Experiment 2: Spoilage cause by “T.A.” bacteria, or thermophilic anaerobes not producing $H_2S$.
Inoculate a can of corn or pumpkin with 5ml of a culture of Clostridium thermosaccharolyticum and seal with solder. Reserve another can for a control and incubate both cans at 55°C for 2-3 days. Wrap cans well in cheesecloth before incubation; examine periodically and remove swollen cans before bursting. Examine contents macroscopically and microscopically.
Inoculate duplicate tubes of thioglycolate medium; seal tubes of medium with 3% agar; incubate one tube at 37°C and the other at 55°C for 2-3 days, note changes.

Experiment 3: Sulfide Spoilage
Examine demonstration cans of corn spoilage bacterium (Clostridium nigrificans)
Remove 10ml of corn juice sampling tube. Inoculate 1ml of the dilution into a tube of melted sulfite agar at 40°C and transfer 1ml from this first tube to a second tube of sulfite agar. Transfer from tube to tube through a series of 5 tubes. Seal tubes with 3% agar, and incubate for 2-3 days at 55°C. Examine for black colonies.

A. Spoilage caused by Mesophiles
Experiment 4: Spoilage by putrefactive anaerobes.
Examine demonstration cans of peas and meat spoiled by putrefactive anaerobes. Note appearance of unopened cans and odor and appearance of contents. Make stained preparations from the spoiled foods. Subculture into duplicate tubes of thioglycolate medium, seal with 3% agar, and incubate one set of tubes at 37°C and the other at 55°C for 2-3 days. Observe changes produced.

Experiment 5: Spoilage caused by leakage
Examine swollen cans of vegetables, discarded by canners or wholesalers. Open cans, taking precautions. Describe changes in the product and the organisms found in a Gram-stained smear from it.

SPoilAGE OF ACID CANNed FOODS
Experiment 6: Non-biological spoilage: hydrogen swells.
Examine swollen cans of sauerkraut, cherries, tomatoes, or other acid foods. Collect the gas and test for carbon dioxide and hydrogen. Empty the can and examine the interior for corrosion, discoloration, and perforations.
FOOD CHEMISTRY AND NUTRITION
LABORATORY MANUAL

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY
FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI
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**CHAPTER SIX – MINERAL CONSTITUENT OF FOODS**

1.1 Potassium and Sodium

**CHAPTER SEVEN – FRUIT JUICE**

7.1 Ascorbic Acid
CHAPTER ONE

PROXIMATE COMPOSITION

1.1 MOISTURE DETERMINATION USING OVEN METHOD

• Wash thoroughly the aluminium or plastic dishes and dry in the oven. Put inside the desicator to cool. Weigh each dish.
• Mix the food sample, take the laboratory sample and put into the weighed dish and take the weight plus weight of undried sample (in duplicate)
• Dry in the moisture oven at 70–80°C for 2 hours and at 100–135°C (usually 105°C) for the next 4 hours or until weight is constant.
• Cool the sample in the desiccator and take the dry weight of sample plus dish.
• Calculate the moisture content as described in the equation below

\[
\text{% Moisture} = \frac{W_1 - W_2}{W_1 - W_3} \times 100
\]

Where:
- \( W_1 \) = Initial weight of empty crucible
- \( W_2 \) = Weight of crucible + food before drying
- \( W_3 \) = Final weight of crucible + food after drying.
- \% = Total solid (dry matter) – 100 - % moisture

1.2 ASH DETERMINATION

• Weigh accurately 2-5g finely ground, dry sample (for high moisture foods or liquid samples) evaporate to dryness or small volume on a water bath or in an oven at 100°C, into a tared silica or porcelain crucible.
• Char sample on a heater or Bunsen flame inside a fume cupboard, to drive off most of the smoke.
• Transfer sample into a pre-heated muffle furnace at 550°C. Leave at this temperature for 2 hours or until a white or light grey ash results. When the residue is black in colour, moisten with small amount of water to dissolve salts, dry in an oven and repeat the ashing process.
• Cool in a desiccator and reweigh.

\[
\text{% Ash (dry basis)} = \frac{\text{Weight of ash}}{\text{Weight of original food}} \times 100
\]

\[
= \frac{W_1 - W_2}{W_1 - W_3} \times 100
\]

Where:
- \( W_1 \) = Weight of empty crucible
- \( W_2 \) = Weight of crucible – food before drying and/or ashing
- \( W_3 \) = Final weight of crucible + ash.

1.3 CRUDE FIBRE DETERMINATION

• Defat about 2g of material (food stuff) with petroleum ether (if the fat content is more than 10%).
• Boil under reflux for 30 minutes with 200ml of a solution containing 1.25g of H₂SO₄ per 100ml solution.
• Filter the solution through linen or several layers of cheese cloth on a fluted funnel.
• Wash with boiling water until the washings are no longer acid.
• Transfer the residue to a beaker and boil for 30 minutes with 200ml of a solubon containing 1.25g of carbonate free NaOH per 100ml.
• Filter the final residue through a thin but close pad of washed and ignited asbestos in a Gooch crucible.
• Dry in an electric oven and weigh
• Incinerate, cool and weigh.

The loss in weight after incineration x 100 is the percentage crude fibre.

1.4 FAT DETERMINATION
• Dry 250ml clean boiling flask in oven at 105 - 110°C for 30 minutes.
• Transfer into a desiccator and allow to cool.
• Weigh about 2g of samples accurately into labelled thimbles.
• Weigh correspondingly labelled, cooled boiling flasks.
• Fill the boiling flasks with about 300ml of petroleum ether (boiling point 40 - 60°C).
• Plug the extraction thimble tightly with cotton wool.
• Assemble the soxhlet apparatus and allow to reflux for about 6 hours.
• Remove thimble with care and collect petroleum ether in the top container of the set-up and drain into a container for re-use.
• When flask is almost free from petroleum ether, remove and dry at 105°C - 110°C for 1 hour.
• Transfer from the oven into a desiccator and allow to cool; then weigh

% Fat = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100

Note: Do not heat the petroleum ether at a temperature above its boiling point (40 - 60°C). Ensure that the joints of the “set-up” are tight to avoid fire.

1.5 PROTEIN DETERMINATION USING MICRO KJELDAHL METHOD
1.5.1 Protein Digestion
✓ Weigh about 2g of sample into a kjeldahl flask.
✓ Add 5g of anhydrous sodium sulphate or 4 tablets of kjeldahl catalysts.
✓ Follow this up with the addition of 1g copper sulphate and a speck of selenium or 1 tablet of kjeldahl catalyst (each tablet contains 1g Na₂SO₄ + 0.05g selenium).
✓ Into the mixture, introduce 25ml conc sulphuric acid and 5 glass beads (glass beads prevents bumping during heating).
✓ In the fume cupboard, heat very gently at first and then increase heat with occasional shaking until solution assumes a green colour (temperature of digest is above 420°C for about 30 minutes).
Cool and wash down any black particle showing at the mouth and neck of the flask with distilled water. Re-heat gently at first until the green colour disappears. Then allow to cool.

After cooling, transfer the digest with several washings into a 250ml volumetric flask and make up the mark with distilled water.

Distil using markham distillation apparatus.

1.5.2. Protein Distillation

Before use, steam through the markham distillation apparatus for about 15 minutes

Under the condenser, place a 100ml conical flask containing 5ml of boric acid indicator such that the condenser tip is under the liquid.

Pipette 5ml (or other known quantity) of the digest into the body of the apparatus via the small funnel aperture, wash down with distilled water followed by 5ml of 60% NaOH solution.

Steam through for about 5-7 minutes to collect enough ammonium sulphate.

Titrate solution in the receiving flask using (0.01N) hydrochloric or sulphuric acid and calculate the nitrogen content and hence the protein content of the food.

Note: Always run a blank through along with the sample (a) using HCl

\[
\% \text{ Nitrogen} = \frac{V_i - V_b \times N\text{acid} \times 0.01401 \times 100}{W}
\]

Where: 
- \(V_i\) = Vol (ml) of acid required to titrate sample.
- \(V_b\) = Vol (ml) of acid required to titrate blank.
- \(N\text{acid}\) = Normality of acid
- \(W\) = Weight of sample in grams

\[
\% \text{ Crude protein} = \frac{N \times \text{Conversion factor}}{(100/\% \text{ Nitrogen in protein}) - \text{Conversion factor i.e. 100/16 = 6.25}}
\]

\(N\) = Nitrogen


1.6 CARBOHYDRATE DETERMINATION

* Determination of carbohydrate content of the food stuff by 'difference'
* \(100\% - (a + b + c + d + e) \times \% \text{ carbohydrate.}\)

Where:
- \(a\) = \% moisture
- \(b\) = \% ash
- \(c\) = \% crude fibre
- \(d\) = \% fat
- \(e\) = \% crude protein
CHAPTER TWO

2.1 POLARIMETRY METHOD

Principle:

Racemic sugars are optically active; hence they possess the property of rotating the plane of polarized light. The extent of rotation depends on the sugar, its concentration and the light path. The rotation also varies with the sugar. Optical rotation is defined as the angle through which the plane of polarization of light is rotated when the polarized light passes through a layer of liquid. Unless otherwise specified, the measurement is carried out with sodium light and a path length (layer of liquid) of 1 dm (10 cm) at a temperature of 20°C.

The specific rotation which is a basic property of a carbohydrate is expressed as:

\[
\text{Specific rotation} = \frac{100a}{1c}
\]

Where:
- \(a\) = observed optical or angular rotation
- \(l\) = path length in decimeters
- \(c\) = concentration of solution in g/100ml

Application:

Polarimetry method can only be used for solutions containing only one carbohydrate. It finds important application in the quantitative and qualitative analysis of sugar solutions.

![Polarimeter Diagram](image)


Materials and Apparatus: Polarimeter, sodium vapour lamp with filters and sugar solutions (glucose, fructose, maltose, sucrose). The basic part of a polarimeter are listed above (Fig. 1).

Procedure

1. With the polarimeter switched ON, and the tube empty, adjust the analyser to obtain a uniform field through the eye piece.
2. Place in the tube the reference sample (distilled water) and adjust the analyser again until a uniform light field is attained. Do this several times and take the average reading. Note this reading.
3. Remove the water from the tube.
4. Rinse with the sugar sample solution, and fill the tube with the solution.
5. View the field, and observe a dark sector of the field through the eye piece.
6. Adjust the analyzer (laevo dextro) until a uniform (light) field is attained again, i.e. the dark sector is removed.
7. Read the angle of rotation from the scale. Repeat the measurement and take the average reading.
Calculation:
Observed angle of rotation - Rotation caused by the solution - rotation caused by the reference sample

Length of the tube or the light path = \( T \)
Concentration of the sugar solution = \( C \)
Specific rotation of the particular sugar solution = \( SR \)
(Unknown concentration of the sugar solution) = \( C = \frac{100 \times a}{1 \times SR} \)

2.1 QUALITATIVE DETERMINATION OF SUGAR USING THE POLARIMETRY

Procedure:
1. Prepare a serial dilution of known concentrations of the sugar.
2. Measure the angular rotation of each concentration. Readings should be in duplicate.
3. Plot the graph of angular rotation against the concentration and obtain a straight-line passing through the origin.
4. Calculate the specific rotation of the sugar sample from the graph.
5. Use this value to identify the sugar.

Note: \( C = \frac{100}{1 \times SR} \times a \) OR \( a = \frac{1SRc}{100} \)

where: \( Y = mx \)
\( Y = a' \)
\( m = \frac{1SR}{100} \)
\( x = 'C' \)

Therefore:
Specific Rotation = \( \frac{100 \times \text{Slope}}{100} \)

2.1.1 Dinitrosalicylic Acid Method

Principle:
Reducing sugars from colored complexes in the presence of dinitrosalicylic acid in an alkaline medium. The intensity of the complex is proportional to the concentration of the sugar and the concentration can be determined using the colorimetric method.

Reagents:
Solution A: Dissolve 225g of potassium sodium lactate in a mixture of 800ml of 1% dinitrosalicylic acid and 300ml 4.5% KOH.
Solution B: Add to 22ml of a 10% solution of sodium hydroxide, 10g of crystalline phenol in 100ml volumetric flask and make up to the mark with distilled water.
Solution C: Dissolve 69g of sodium metabisulphite in 69ml of solution B.
Standard Sugar Solution: Prepare a series of glucose solution varying in concentration from 0.1 - 0.5 mg/ml with distilled water.
Dinitrosoyldene acid (DNS) Solution. Mix solutions A and C until all the compounds dissolve and keep the mixture in a brown bottle of 4c.

**Procedure:**
1. To 1ml of each of the standard and test samples, add 3ml of DNS solution.
2. Boil the mixture for 15min.
3. Measure the colored complex formed at 540nm.
4. Use 1ml of distilled water for the blank.

**2.1.2 Lane and Frynon Method**

Principle sugars which possess in their structure free aldehydic or ketonic groups are termed reducing sugars. These properties are used to estimate sugars by the measurement of the reduction of CuII in CuII Fehlings solution consists of alkaline cupric tartrate which is converted to insoluble sugars oxide when boiled with solution of a reducing sugar.

**Reagents:**
- Fehlings 1: Dissolve 69.3g of copper sulphate (CuSO₄.5H₂O) in water and make up to a litre.
- Fehlings 2: Dissolve 100g sodium hydroxide NaOH and 345g sodium potassium tartrate (KNaC₆H₄O₁₄.4H₂O) in water and make up to a litre. Keep both reagents separately in amber bottles until required.
- Fehlings Solution: When required pipette 50ml of Fehling's 1 into 50ml of Fehling's 2 and mix.

**Procedure I: Preparation of standard invert sugar solution**
1. Dissolve 23.750g pure sucrose in about 120ml water in a 250ml volumetric flask, add 9ml concentrated hydrochloric acid and stand for 8 days at room temperature. Make up to mark.
2. Check the inversion, if the inversion is complete, rotation in a 20mm tube will be 11.80 × 0.05°.
3. Transfer 200ml of the inverted solution into a 2 litre flask and add about 200ml of water, shake, add 71.4ml g NaOH (1% solution containing 4g benzoic acid).
4. To the mixture and 1 litre of water. Check the pH which should be approximately 3. If necessary adjust and make up to mark.
5. This solution is a 1% stock solution of invert sugar and can be diluted 1:4 to give the required 0.25% standard solution of invert sugar.

**Procedure II: Determination of percent reducing sugar**
1. Pipette 20ml of Fehlings solution into a 500ml conical flask and add 15ml of water.
2. Run in from a burette 39ml of 0.25% standard invert sugar solution. Add anti-bumping granules and boil over a flame.
3. Allow the mixture to boil steadily for 2 min.
4. Just before the 2min lapse add 3-4 drops of 1% methylene blue solution.
5. From the burette add drop wise the standard invert sugar solution to the boiling mixture until the blue colour disappears leaving red colouration.
1. Titration should be completed in 3 min from the boiling. Titre vale \( V_1 \) must be between 39 and 41 ml with a final volume of 75 ml in the boiling flask.

2. Place the test sample containing between 250 and 400 mg/100 ml of reducing sugars in the burette.

3. Repeat the titration as above using 20 ml of Fehling’s solution + 13 ml of water + 25 ml of test sample solution.

4. Do a rapid determination to get a rough titre value.

5. Carry out a final accurate titration with titre value \( V_2 \) using 1 ml less the volume of the sample solution used in the rough titration. Add enough water to give the final volume of 75 ml.

**Calculation:**

\[
\text{% reducing sugars as invert sugar in sample} = \frac{V_2 \times 25 \times f}{C \times V_1}
\]

Where \( f \) = correction factor

\( C \) = percent concentration of the test sample (V/V)

**Sucrose Content of g in \( V_2 \) f**

<table>
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<td>0.926</td>
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<tr>
<td>0.915</td>
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</table>

**Note:**

1. If on the addition of methylene blue to the mixture containing the test sample, no blue colour is formed, the reducing sugar content of the 25 ml sample solution was too large to reduce all the Fehling’s solution. Use a weaker sample solution.

2. If final solution exceed 50 ml a stronger sample solution should be used.

### 2.2 Sucrose Reagents:

1. Zinc acetate solution, 21.9 g crystallised zinc acetate, and 3 ml glacial acetic acid made up to 100 ml water

2. Potassium ferrocyanide – 10% aqueous solution

3. Dilute ammonia – 10 ml of 0.88 ammonia diluted with water to 100 ml

4. Dilute acetic acid – approximately equivalent to the dilute ammonia (equivalent amount determined) titration with bromothymol blue as indicator.

5. Dilute hydrochloric acid 0.35N.
Principle
A clear filtrate of the sample, without mutarotation by lactose, is prepared by treatment of the solution with ammonia followed by neutralization and clearing by the addition of zinc acetate solution and potassium ferrocyanide solution. A part of the filtrate is taken and hydrolysed (inverted) by mild treatment with acid — conditions which produce complete hydrolysis of sucrose, but almost none of lactose or other sugars. Rotation of the plane of polarisation of plane polarised monochromatic light by the filtrate, before and inversion is used to calculate the sucrose content of the sample.

Procedure
Weigh accurately about 40g of the well-mixed sample in a 100ml beaker. Add 50ml hot water and transfer the mixture quantitatively to a 200ml volumetric flask, rinsing the beaker with hot water until the volume is between 120ml and 150ml. Mix and cool to room temperature. Add 5ml of the dilute ammonia solution, mix and allow to stand for 15 minutes. Neutralise the ammonia by adding an equivalent quantity of dilute acetic acid. Add 12.5ml of zinc acetate solution and mix by gentle rotation. Add 12.5ml of the potassium ferrocyanide solution and mix in the same manner. Cool to 20°C and add distilled water at 20°C up to 200ml mark.

Up to this stage all mixing must be done by gentle rotation to avoid formation of air bubbles. Close flask and mix well by vigorous shaking. Allow to stand for a few minutes and filter through a dry fast paper. Reject the first 25ml and collect the rest of the filtrate. Determine the optical rotation of the filtrate 20° + 20°C using a 2 decimeter tube and sodium light.

Inversion
Pipe 40ml of the filtrate into a 50ml volumetric flask. Add 6.0ml of the 6.35 normal hydrochloric acid. Place the flask in a water bath maintained at 60°C for 15 minutes. Rotate the flask for the first 5 minutes in order to mix the contents. Cool to 20°C and make up to the 50ml mark. Allow the flask to stand at least for 1 hour and determine the rotation of this inverted solution at 20°C ± 2°C.

Calculation:
Percentage sucrose in sample = \[ \frac{D - 5/44k \times \frac{V - V_{x}}{V} \times \frac{V_{x} - V}{L \times W}}{Q} = S \]

Where:
- \( W \) = (1.08E - 155P)/100
- \( Q \) = Temperature of the inverted solution at which the rotation was taken.

In these formulae:
- \( S \) = Sucrose content
- \( W \) = Weight of sample taken
- \( F \) = Percentage of fat in sample
- \( P \) = Percentage of Protein in sample (N x 6.38)
- \( V \) = Volume in ml to which sample was diluted before filtration.
- \( V_{x} \) = Correction in ml for volume of precipitate formed during clarification.
- \( D \) = Direct polarimeter reading before inversion.
- \( I \) = Polarimeter reading after inversion.
- \( L \) = Length in decimetres of the polarimeter tube.
- \( Q \) = Inversion divisor factor given below.

For sodium light and polarimeter with angular degrees:
\[ Q = 0.8825 + 0.0006 (C - 9) - 0.0033 (1 - 20) \]

Where:
- \( C \) = Percentage total sugars in the inverted solution as polarised and
- \( T \) = Temperature of the inverted solution at which the rotation was taken.
CHAPTER THREE.
EXAMINATION OF OILS AND FATS

3.1 IODINE VALUE

Definition

The iodine value of a fixed oil or fat is the weight of iodine absorbed by 100 parts by weight of the oil, or fat, when determined by a suitable method.

Reagent: Wij's solution.
Dissolve 8g iodine trichloride in 200ml glacial acetic acid. Dissolve 9g iodine in 300ml carbon tetrachloride. Mix the two solutions and dilute to 100ml with glacial acetic acid.

Procedure:
1. Weigh 4-6 reasonably sized drops of oil from the end of a small rod.
2. Add 5ml of carbon tetrachloride (cylinder) to the weighed-out portion of oil or melted fat (re-melted by warming if necessary).
3. Then mix thoroughly by swirling round, and add 10mls of Wij's solution from dry pipette.
4. Swirl round and insert the stopper which should have been moistened previously with potassium iodide solution and placed in a dark place for 30 minutes.
5. Then add 10ml of 10% potassium iodide solution and 50ml of distilled water.
6. Shake and titrate carefully with 0.1M (0.1N) sodium thiosulphate solution.
7. From time to time during the titration insert the stopper and shake. When the aqueous layer becomes a very pale yellow after shaking, add starch solution and continue the titration.
8. Just before the endpoint is reached, insert the stopper after the addition of each drop and shake.
9. Note the number of mls of thiosulphate required.
10. At the same time, carry out a blank in the same manner, but without the substance being tested in a separate dry stoppered flask or bottle. It is especially important to deliver the Wij's solution from the same pipette and with exactly the same technique, i.e. allow the liquid to drain from the pipette for the same time and include the same number of "extra" drops in both cases. Note the number of mls or thiosulphate required for the blank-titration (b).

Calculation:

Iodine Value = \( \frac{(b - a) \times 0.01269 \times 100}{\text{Weight of substance taken (g)}} \)

Note: (1) If \((b - a)\) is greater than \(b/2\), the test must be repeated on a smaller quantity of the substance.

(2) It is advisable to place a plug of cotton wool (about \(\frac{1}{2}\) long) between the mark and the top of pipette used for delivering Wij's solution.

Ref. B.S. 684: 1958
3.2 SAPONIFICATION VALUE

Definition
The saponification value of an oil or fat denotes the weight of potassium hydroxide, expressed in milligrams, required to saponify one gram of the oil or fat.
The saponification value is related to the molecular weight of the fat and from it can be calculated the saponification equivalent, which is the amount of oil or fat saponified by 1 gram-equivalent of potassium hydroxide and is equal to 56.120 divided by the saponification value.

NB: Perform a blank at the same time.

Procedure:
1. Weigh accurately about 2g of the oil or fat into a flask resistant to the action of alkali.
2. Boil continuously for ½ hour under a reflux condenser with 25ml. accurately measured, of the *alcoholic potassium hydroxide solution
3. Swirl the content of the flask at frequent intervals.
4. Determine the excess of alkali, while the solution is still hot, by titration, with the 0.5M hydrochloric acid, using 0.5ml of 1% phenolphthalein.
5. Make a blank determination upon the same quantity of the potassium hydroxide solution at the same time under the same condition.

Calculation:

\[
\text{If } X = \text{ volume in ml. Of 0.5M acid required for the blank}
\]

\[
\text{Y = volume in ml. Of 0.5M acid required when the oil or fat is used.}
\]

\[
W = \text{ weight in g. Of oil or fat taken.}
\]

Then the saponification value \[ \frac{(X - Y) \times 28.05}{W} \]

Approximately 0.5M

3.3 FREE FATTY ACIDS AND ACID VALUE

Definition of Acid Value
The acid value is the number of milligrammes of potassium hydroxide required to neutralize the acidity of one gramme of the oil or fat. Although the acid value is quoted in certain specifications (including B.P. monographs), the results of the titration are more usually termed the F.F.A. and calculated as the percentage of oleic acid.

Procedure:
1. Mix 25 ml ether with 25ml alcohol and 1ml phenolphthalein (1%) and exactly neutralize with a few drops of 0.1M NaOH.
2. Dissolve a suitable quantity (2–5g) of oil or melted fat in the mixed cold neutral solvent.
3. Titrate with 0.1M NaOH (warning titration may be very small for fresh oil or fat), shaking constantly until a pink colour persists for 15 seconds.
4. If the titration exceeds 10ml, repeat the method using a smaller quantity of oil or fat.
**Calculation**

If \[ X = \text{volume in m1. of 0.1M NaOH required for the test.} \]
\[ W = \text{weight in g of oil or fat} \]

Then the acid value \[ = \frac{X \times 5.61}{W} \]

**Acid value** \[ = \text{FF} \times X \times 2 \]

The percentage of free fatty acids

(oleic acid) \[ = \frac{X \times 2.82}{W} \text{ (for general use)} \]

(palmitic acid) \[ = \frac{X \times 2.56}{W} \]

(lauric acid) \[ = \frac{X \times 2.00}{W} \]

The percentage of free fatty acids is calculated as:

- Lauric acid in crude and refined kernel oils of the palmae.
- Palmitic acid in palm oil.
- Oleic acid in all other oils.

**3.4 REICHERT, POLENSKE AND KIRSCHNER VALUES**

**Definitions**

a. The Reichert value is the number of millilitres of 0.1N aqueous alkali required to neutralise the water soluble fatty acids distilled from 5g of the oil or fat under precise condition specified in the method.

b. The Polenske value is the number of millilitres of 0.1N aqueous alkali required to neutralized the water insoluble volatile fatty acids distilled from 5g of the oil or fat under precise conditions specified in the method.

c. The Kirschner value is the number of millilitres of 0.1N aqueous alkali required to neutralize the water soluble volatile fatty acids which form water soluble silver salts, distilled from 5g of the fat under precise conditions specified in the method.

**Principle**

The fat is saponified. The fatty acids are then liberated from the soap and distilled in a standard apparatus under specified conditions. The water insoluble fatty acids are filtered off, dissolved in neutral ethanol and titrated with standard alkali. The Polenske value \( P \) is calculated from this titration value. The water soluble fatty acid in the filtrate are then titrated with standard barium hydroxide and the Reichert value \( R \), is calculated from this titration value.

The neutralised solution is then treated with silver sulphate powder and filtered. The filtrate, which would contain the water soluble silver salts of the soluble volatile fatty acids, is acidified and distilled in the standard apparatus under specified conditions. The distillate is titrated with standard alkali to determine the Kirchnar value \( K \).
Apparatus:
The method is empirical, hence, the standard micro or semi-micro apparatus as described in the Codex Alimentarius Commission Standard CAC/RS 32 – 1969 or in the Chemical analysis of Foods by Pearson, must be used.

Reagents:
1. Glycerol
2. Sodium hydroxide solution, 50% w/w free from carbonates.
3. Dilute sulphuric acid. Dilute 25ml concentrated sulphuric acid to one litre and adjust until 40ml was neutralised 2ml of the above 50% sodium hydroxide.
4. Pumice powder passing through B.S. sieve mesh 50 and retain on B.S. sieve mesh 90.
5. Phenolphthalein solution – 0.5% in 95% ethanol.
6. Ethanol 95% neutralized to phenolphthalein immediately before use.
7. Sodium hydroxide 0.1N solution or Barium hydroxide 0.5M solution.
8. Powdered silver sulphate.

Procedure
Measurements for the semi-micro method are given within brackets. Weigh 5 ± 0.01g (1.0g) of the fat into the Polenske flask. Add 20g (4ml of the glycerol and 2ml (0.4ml) of the 50% sodium hydroxide solution. Heat the flask over a naked flame, with continuous mixing, until the fat is completely saponified and the liquid becomes perfectly clear. Avoid overheating. Cover the flask with a watch glass.

Measure 93ml (20ml) of boiling water which has been vigorously boiled for 15 minutes into a 100ml graduated cylinder. When the soap is sufficiently cool to permit the addition of water without loss, but before the soap has solidified, add the water draining the cylinder for 5 seconds, and dissolve the soap. If the solution is not clear (including incomplete saponification or if it is darker than light yellow (indicating overheating) repeat the saponification with a fresh sample of fat.

Add 0.1g of powdered pumice and 10ml (10ml) of the dilute sulphuric acid, and connect them immediately to the distillation apparatus. Heat the flask, slowly at first to melt the insoluble acids, and rapidly and distil 110ml (22ml) in-between 19 and 21 minutes (10–12 minutes).

Remove the flask and mix the contents by 4 or 5 careful inversions so that the insoluble fatty acid does not wet the stopper. Filter through a fast filter paper. Reject the first few ml's and collect 100ml (20ml) of the distillate into a dry volumetric flask. Pour into a titration flask, add 0.1ml of phenolphthalein solution and titrate with 0.05M (0.01M) barium hydroxide solution, rinsing the volumetric flask with the nearly neutral solution, towards the end of the titration. Make a blank test in a similar way, but omitting the fat. Let titration values for the test and blank be $T_t$ and $T_b$ml respectively.

Detach the still head and wash the condenser with 3 successive 15ml (3ml) portions of cold water, pass each washing separately, through the cylinder, the 110ml (22ml) flask and the filter. Discard the washings. Dissolve the insoluble acids with three similar washings, with 10ml (3ml) portions of neutralised ethanol. Collect the washings in a titration flask and
titrate with 0.05M (0.01M) barium hydroxide solution to phenolphthalein and point. Let the
titration values for the test and blank be $T_1$ and $T_2$, respectively.

For the Kirchner value, add 0.5 (0.1)g of finely powdered silver sulphate to the neutralized
aqueous solution from the first titration, and allow to stand in the dark for one hour, with
occasional shaking. Filter in the dark through a dry filter. Transfer 100ml (20ml) of the filtrate
into a dry Polenske flask. add 35ml (0.7ml) of recently boiled distilled water, 10ml (2ml) of
dilute sulphuric acid and a loosely wound coil of aluminium wire or 0.1g pumice powder, and
distil 110ml (22ml) in 19 - 21 (10 - 21) minutes. Mix, filter and titrate 100ml (20ml) with 0.05M
(0.01M) barium hydroxide solution. Let the titration values of test and blank be $T_3$ and $T_4$, respectively.

Calculations
Reichert Value $= 1.10(T_1 - T_2)$
Polenske Value $= T_3 - T_4$
Kirchner Value for Macro Method $= \frac{1.21 (100 + T_5) (T_1 - T_2)}{100}$
Kirchner Value for Semi-Micro $= \frac{1.21 (20 + T_5) (T_3 - T_4)}{20}$

Note:
If Kirchner Value is not required, the titration for Reichert and Polenske values may be done with
0.1M (0.02) sodium hydroxide solution instead of the barium hydroxide solution.

RELATIONSHIP BETWEEN REICHERT, POLENSKE, AND KIRCHNER VALUES FOR GENUINE BUTTER FAT

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<th>KIRCHNER</th>
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CHAPTER FOUR
ADDITIVES

4.1 SULPHUR DIOXIDE
4.1.1 Iodimetric Method

Principle:
The sample is boiled with acid and the sulphur dioxide is titrated directly with iodine as it distils over.

Apparatus:
Round bottomed flask fitted with a dropping funnel and splash head. An 18 inch straight condenser fitted with an adaptor. A 600ml beaker as receiver.
Note: Use quick fit acceptance if available.

Reagents:
Concentrated Hydrochloric Acid, 0.05M Iodine solution, starch indicator (1% in water).

Procedure
Connect the round bottomed flask through the splash head, condenser to the adaptor, using the 600ml beaker as receiver. Place sufficient distilled water in the beaker to cover the outer bell of the adaptor.

Add 0.2 - 0.3ml of 1% starch solution and a few drops of 0.05M (0.05N) iodine to produce a pale blue colour. Where a higher percentage of sulphur dioxide is anticipated a large quantity e.g. 1 - 5ml of 0.05M (0.05N) iodine may be added. Place 200ml of water in the distillation flask, boil for about 5minutes, cool and add 50grams of sample. Insert the stopper with funnel and still head as rapidly as possible. Run 20ml of concentrated hydrochloric acid into the flask through the funnel.

Take care to avoid frothing or bumping and heat contents of flask to boiling as soon as possible by means of the naked flame of a powerful burner. The time taken to bring about boiling should not exceed 2minutes.

As the distillation proceeds, add 0.05M (0.05N) iodine from a burette over the receiving vessel so that the blue colour just remains. It is undesirable to add considerable excess of iodine.

The distillate should be cool, with the temperature not exceeding about 27°c. The end of the titration is the point when the colour due to 0.1ml of 0.05M (0.0N) iodine persists for 1 minute. Record volume V of iodine used.

The distillation should be completed within 10minutes boiling but if this time is exceeded even when the most powerful flame practicable is used, recourse should be made to steam distillation.

Calculation
\[ 1 \text{ml} 0.05 \text{M iodine} = 0.0016g \text{SO}_2 \]
\[ \text{Mg/litre (ppm)} = 1.6 \times V \times 100 \]
\[ W \]
Notes
1. In the case of starch, the same method is used except that the flask should be of 1 litre capacity, the amount W of sample taken about 100g, the amount of cone HCL 100ml and deaerated distilled water 400ml. The flask should be shaken at first to keep the starch in suspension. The time required for boiling should not exceed 5 minutes.
2. Materials requiring more than 10 minutes distillation e.g. gelatine, meats, dried fruits, etc. require steady distillation, preferably superheated but not above 150°C.
3. In the case of gelatine, 25ml cone HCL is required for 25g or gelatine.
4. In the case of steam distillation, it will be found impossible to distill in 10 minutes but every step must be taken to render the distillation as rapid as possible.
5. Hard fruits require from 20-30 minutes.

For spices and meat containing decomposed protein and materials containing sulphides, mustard, onion, etc. use the Shipton's method.

4.1.2 Hydrogen peroxide method (SHIPTON's METHOD)
Principle
The sample is boiled with acid, and the sulphur dioxide is swept into neutralised hydrogen peroxide by a stream of carbon dioxide or nitrogen. The hydrogen peroxide oxidises the sulphur dioxide to sulphuric acid, which is titrated with standard barium hydroxide solution. When further confirmation is needed, the precipitated barium sulphate is filtered, ignited and weighed.

Apparatus:
1. Carbon-dioxide or nitrogen cylinder or carbon-dioxide generator.
2. 250ml flat or round bottom flask fitted with a stopper carrying a reflux condenser and a tube to pass the inert gas. The top of the reflux condenser is fitted with a delivery tube leading to a bubbler.

Reagents:
1. Concentrated hydrochloric acid.
2. Hydrogen peroxide 3% solution (10 volume)
3. 0.4% solution of bromophenol blue.
4. Standard 0.1N barium hydroxide solution.

Procedure
Add about 3 drops of bromophenol blue to about 25ml hydrogen peroxide solution and neutralise it with alkali. Add the neutralised hydrogen peroxide to the bubbler and fit it to the delivery tube and reflux condenser.
Weigh 15.30g of the sample into the flask and add about 100ml of water. Add 5ml concentrated hydrochloric acid and immediately fit it to the reflux condenser assembly. Pass a stream of inert gas and heat the flask to boiling for about 30 minutes. Detach the delivery tube and bubbler.
Pour the hydrogen peroxide into a titration flask. Wash the bubbler and tube twice with distilled water and add the washings to the titration flask. Titrate with the stand alkali.
When confirmation is needed, acidify the titrated liquid with hydrochloric acid. Filter the precipitate of barium sulphate through a quantitative filter paper such as Whatman No. 42. Wash with distilled water until the washings are chloride free. Place the filter paper with the precipitate in a tared platinum or silica dish. Dry in an oven and char the filter paper over until completely white. Cool and weigh.

**Calculations**

\[
\begin{align*}
\text{Weight of sample} & = m \\
\text{Volume of 0.1N alkali needed} & = v \\
\text{Weight of barium sulphate} & = w \\
\text{Sulphur dioxide} & = v \times 3.2 \times 1000 \quad \text{mg/kg} \\
& = w \times 274.48 \times 100 \quad \text{mg/kg} \\
M & \end{align*}
\]

**Note**

1. Sufficient water must be added to the flask so that the tube leading the inert gas dips well into the liquid.
2. Instead of 0.1 barium hydroxide solution, a standard sodium hydroxide solution can be used. In this case the sulphate is precipitated with a solution of barium chloride.
3. In the case of gelatine 25ml of concentrated hydrochloric acid is needed for 25g of sample.
4. If cylinders of C0₂ or N₂ are not available, a Kipp’s apparatus can be used to generate carbon-dioxide. In routine cases a few marble chips added to the flask with an extra 10ml of hydrochloric acid will serve this purpose.

**4.2 COLOURING MATTERS**

**N/B** In general, the methods described on this sheet are applicable if permitted water soluble colours are present.

**4.2.1 General Procedure**

1. Extraction of the dye from the food.
2. Identification of the extracted dye by paper chromatography.
3. Chemical tests and spectrophotometry.

**Extraction of the Dye from the Food**

First boil pure white knitting wool in dilute ammonia and then in water.

a. For liquid foods solutions of soluble solid foods, e.g. soft drinks, lollies, boiled sweets, jam, marmalade, take about 20ml of the liquid food or its solution in water, add 1ml bench HCl (2N) add the pure washed white knitting wool and boil for 5 minutes to dye the wool with colouring matter.

Take the dyed knitting wool and wash thoroughly under tap water to remove all soluble sugar particles. Then extract the dye from the wool with a small volume of dilute ammonia (0.1N) the solution to small bulk on a boiling water bath.
b. Non-fatty foods which contain starch, e.g. custard powder: remove the colour by adding 3-4 times the weight of the material taken of ammonia. Allow for 10 minutes and decant off the supernatant liquid.

Acidity slightly with bench HCl (2N) and complete extraction of the dye as in (a)

Note:
1. It is assumed here that most of the permitted dyes are acidic dyes.
2. If the dye is basic, then basify with dilute ammonia before extracting with knitting wool and re-extract from the wool by acidiy with 0.1M HCl.
3. If the colour tends to be absorbed by the food and resists the above treatments, then (a) treat with hot 50-80% alcohol or acetone, or (b) use trichloroacetic acid in extracting such protein-bound colour.

IDENTIFICATION OF COLOUR BY PAPER CHROMATOGRAPHY

Principle
This is based on comparison of the unknown dye with standard known dyes, using their differential partitions between the hydrated shell of the paper cellulose and the mobile solvent expressed in terms of RF value.

Procedure
Draw a line (with pencil) parallel to the bottom of a piece of Chromatography paper and about 2cm away from it. Using a draw out capillary tube for each dye, place a spot of the concentrated solution of the unknown dye on the line together with a series of spots (2-3cm apart) of aqueous solutions of known dyes of similar colour and dry after each spot using a hot air blower.

Roll the paper into a cylinder (or use any other suitable modification). Clip, place in a 1cm layer solvent and cover (select solvent in relation to the relative RF value (see table).

After the solvent front has moved 10 – 15cm remove the paper, dry and compare the spots. If more than one colour appears to be present, run fresh chromatograms with other solvents and if necessary other standards to get a better separation and identification. If necessary, cut out spots, evaporate to dryness, re-dissolve in 2 drops of water and run further chromatograms.

PAPER CHROMATOGRAPHY SOLVENTS

<table>
<thead>
<tr>
<th>NUMBER</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1ml 0.88 ammonia + 99ml water</td>
</tr>
<tr>
<td>2</td>
<td>2.5% aqueous sodium chloride</td>
</tr>
<tr>
<td>3</td>
<td>2% NaCl in 50% ethanol</td>
</tr>
<tr>
<td>4</td>
<td>2:1:1 Vols ethanol: Isobutanol: water</td>
</tr>
<tr>
<td>5</td>
<td>20:12:5 Vols Butanol: Water: Glacial acetic acid</td>
</tr>
</tbody>
</table>
2. 2.5% aqueous sodium chloride
3. 2% NaCl in 50% ethanol
4. 2:1:1 Vols ethanol: Isobutanol: water
5. 20:12.5 Vols Butanol: Water: Glacial acetic acid
6. 2 Vols ethanol, 3 vols isobutanol, 2 vols water. Then to 99ml of this mixture add 1ml 0.88 ammonia
7. Phenol 80g, water 20g
8. Ethyl methyl Ketone – 350 vols
   Acetone – 150 vols
   Water – 150 vols
   Ammonia (0.88) – 1 vol
9. Ethyl methyl Ketone – 70 vols
   Acetone – 30 vols
   Water – 30 vols
10. Ethyl acetate – 11 vols
    Pyridine – 5 vols
    Water – 4 vols
11. Dilute 5ml of ammonia solution (0.88) with water to 100ml and dissolve 2g of trisodium citrate in the solution
12. Water
   Hydrochloric acid (sp.gr. – 1.18) 6.5 vols.

3. CONFIRMATORY TESTS

(a) General
   i. Confirm the results obtained by running fresh chromatograms with other solvents.
   ii. Examine spots under UV light and compare with standards.
   iii. Compare addition of acids and alkalis to sample and standard.

(b) Spectrophotometry
   i. Dilute the pure neutral dye solution to a suitable intensity of colour and determine the absorb curve on the spectrophotometer.
   ii. Use neutral 0.02% ammonium acetate medium.
   iii. Use acid (0.1M HCl) medium
   iv. Use alkaline (0.1M NaOH) medium

Compare maxima with those obtained with the known pure dye.

Ref: (1) JAPA December 1973
(2) Separation and identification of food colours permitted by the colouring matters - APA 1960 (Separation and Identification of Food Colours Permitted by the colouring matters in Food Regulations, 1957, London, APA
CHAPTER FIVE

EXAMINATION OF SPECIFIC FOODS

5.1 MILK
5.1.1 Sample Preparation
Mix the milk thoroughly before each determination by continuous slow inversion of the sample bottle or if the bottle is full, by transferring the milk to a larger receptacle, mixing and re-transferring to the original receptacle.

5.1.2 Total Solids
Weigh accurately about 5g of milk into a flat bottomed metal dish. Place on a boiling water bath for about 30 minutes, or until the liquid just evaporated leaving the solid. Transfer to an oven maintained at 100°C. After 2½ hours, transfer to a desiccators, cool and weigh. Heat in the oven again for 1 hour, cool and weigh. Continue this process until constant weight is attained. If the acidity of the milk exceeds 0.20% as lactic acid, neutralise the milk to phenolphthalein with 0.05M strontium hydroxide solution before determining the total solids and deduct 0.00428g for each ml of strontium hydroxide used, from the weight of the residue.

5.1.3 Fat
(a) Determine the fat by the Rose Gottlieb method using 10g of milk. (See Section 1.3.3);
(b) For routine purposes, the Gerber method may be used. (See Section 1.3.4).

5.1.4 Acidity
Total acidity as lactic acid.
Reagents:
1. Phenolphthalein indicator. Dissolve 1g of phenolphthalein in 110ml ethyl alcohol (95% v/v) and add 0.1N sodium hydroxide solution until 1 drop produces a faint pink colour. Make up to 200ml with distilled water.
2. Rosaniline acetate stock solution. Dissolve 0.12g of rosiniline acetate in 50ml ethyl alcohol (95% v/v) containing 0.5ml glacial acetic acid and make up to 100ml with ethyl alcohol (95% v/v). Store in the dark.
3. Rosaniline acetate working solution. Dilute 1ml of the above stock solution to 500ml with a mixture of equal volume of ethyl alcohol and distilled water.

Procedure
Pipette 10ml of milk into each of two flasks. Add 1ml of the Rosaline working solution to one of the flasks and stir. Add 1ml of phenolphthalein solution to the second flask and titrate with 0.1M sodium hydroxide solution until the colour matches that of the first flask. Then acidity of the milk = 0.09 x V/100 as lactic acid where V is the volume of the 0.1M in ml sodium hydroxide solution required.

5.1.5 Protein
(a) Determine the nitrogen by the Macro Kjeldahl on 10g of milk.
Protein content = N x 6.28
(b) Rapid approximate method by formal titration (See Section 1.3.5).
5.1.6 Ash
Weigh 10g of milk into a tared platinum or silica dish. Evaporate to dryness on a boiling water. Char on a low flame and ignite in a muffle furnace below 500°C until fully ashed. Cool in a dessicator weigh. Moisten the ash with distilled water, if black specks appear, evaporate on a water bath, ignite again 1 hour, cool and weigh.

5.1.7 Chloride
Boil the ash from the ash determination with about 50ml distilled water. Filter and wash filter with water. Cool, add a few drops of 5% solution of potassium chromate and titrate with 0.1M silver nitrate solution.

\[
\text{1ml 0.1M silver nitrate} \quad = \quad 0.003546\text{g Cl}
\]

5.1.8 Lactose
Reagents:
1) Zinc acetate solution. 21g of crystallized zinc acetate dehydrate and 3ml glacial acetic acid dissolved in water and made up to 100ml.
2) Potassium ferrocyanide solution. A 10.6% aqueous solution.

Procedure:
Weigh 25.6g milk into a 250ml volumetric flask. Add 50ml water and 5ml zinc acetate solution and mix. Precipitate the fat and proteins. Add 5ml of potassium ferrocyanide solution to precipitate the excess zinc. Mix make up to mark and mix again. Filter through a rapid filter paper and collect the filtrate after rejecting the first 10ml. Determine the lactose by Lane and Eynon titration.

5.1.9 Citric Acid
Citric acid is converted to the insoluble pentabromo acetone, filtered and weighed.

Reagents:
1. 2% potassium oxalate solution
2. Dilute sulphuric acid (1:1).
3. 20% Phosphotungstic acid solution.
4. 37.5% Potassium Bromide solution
5. 5% Potassium permanganate solution
6. Ferrous sulphate solution (20% FeSO₄.7H₂O in H₂SO₄).

Procedure:
Heat 150g of the milk in a 250ml volumetric flask to 50°-60°C. Add 25ml of the oxalate solution mix. Add 20ml dilute sulphuric acid solution, cool and add 10ml phosphotungstic acid solution. Make up mark, mix, allow to stand and filter. To 50ml of the filtrate, add 5ml of potassium bromide solution and the potassium permanganate solution from a burette until a light brown precipitate persists for one hour- sufficient ferrous sulphate solution to produce a pale yellow solution with a white precipitate. Keep refrigerated overnight. Filter through a sintered glass crucible (G4). Wash the precipitate on the crucible with 10ml. 5ml portions of cold distilled water.
Dry the crucible in a vacuum desiccators to constant weight. Dissolve the precipitate in the crucible with rectified spirits and then with 20, 10 and 10ml portions of diethylether. Dry in vacuum desiccators and weigh to get the weight of the crucible.

**CALCULATION:**

\[
\begin{align*}
\text{Weight of crucible} - \text{precipitate} &= W_1 \\
\text{Weight of crucible alone} &= W_2 \\
\text{Citric acid (anhydrous)} &= 0.424 (W_1 - W_2 + 0.00125)
\end{align*}
\]

5.2 **Margarine**

Preparation of sample:

Place sample in a clean, dry screw capped jar. Warm in an oven at 32° – 35°C. Shake vigorously from time to time to produce a homogeneous mixture.

5.2.1 **Water**

Weigh out 3-4g of sample into a previously dried and weighed metal dish containing a flat end rod. Heat on a boiling water bath for 30min. with constant stirring. Heat in an oven at 100°C to constant weight (about 3hr). Calculate the water loss.

5.2.2 **Fat**

Dissolve the residue from the water determination in petroleum ether (40-60) and filter through a filter paper into a dry tared flask. Wash dish and filter with more petroleum ether. Evaporate off the solvent, dry in an oven at 100°C for 1 hour. Cool in a desiccator and weigh.

5.2.3 **Butter Fat Content**

Determine the Reichert, Polenske and Kirschners values on the margarine fat by the method given in Section 3.4.

\[
\text{Percentage m/m butter (milk) fat in margarine fat} = K - 0.1P - 0.24
\]

Where \( K = \) Kirschners value and \( P = \) Polenske value.

When the atmospheric pressure is low, both Polenske value and Reichert value have been found to be low. Corrections are therefore necessary.

\[
\text{Reichert value} = (\text{Observed value} - 100) \\
\text{Corrected Reichert value} = (\text{Observed value} - 10) \log \frac{760}{\text{P}}
\]

\[
\text{Corrected Polenske value} = - \text{observed Polenske value} x \frac{760 - 45}{\text{P} - 45}
\]

Where \( P \) is the barometric pressure in mm of mercury.
5.2.4 Vitamin A
The fat is saponified and the unsaponifiable matter containing the vitamin A, is extracted with Diethyl ether. The ether is evaporated off in an inert atmosphere and the unsaponifiable matter taken up in benzene. The vitamin A is converted to anhydrovitamin A by the action of the catalyst, toluene p-sulphuric acid. The difference in the absorption at 399 nm, before and after conversion to anhydrovitamin A is used to calculate the amount of vitamin A in the fat.

Reagents:
1. Diethyl ether—freshly distilled over sodium hydroxide pellets.
2. Ethanol—absolute
3. Potassium hydroxide solution—60% w/v.
4. Hydroquinone
5. Catalyst solution—Dissolve 15mg toluene p-sulphuric acid monohydrate in 100ml redistilled benzene by heating under reflux. Distil off 10ml of the solvent to remove any water. Cool while protecting from moisture. Adjust volume to 100ml with dry benzene. Activate before use by repeating the distillation.
6. Sodium carbonate
7. Benzene

Procedure:
Weigh 10g margarine into a 300ml flask. Add 20mg hydroquinone, 40ml ethanol and 10ml potassium hydroxide solution and boil under reflux for 20 minutes. Transfer quantitatively, into a 500ml separator using 2 x 40ml distilled water. Extract once with 100ml and then three more times with each 50ml of diethyl ether. Combine the ether in an inert atmosphere, add 5ml absolute ethanol and evaporate again to remove the last traces of water.
Dissolve the residue in dry benzene so that 1ml contains 5-50 in vitamin A. Pipette 2ml of the solution into each of 2 small stoppered cylinders. To one add 8ml of dry benzene and to the other add 8ml of the catalyst solution. Mix well and allow to stand for 60 seconds. Deactivate the catalyst by shaking with 1g sodium carbonate for one minute. Centrifuge and determine the optical density of both tubes at 399 nm in a 1cm cell.
Let E₀ and Eₐ be the optical densities of the vitamin A solution respectively.
Vitamin A in IU per ml of solution of unsaponifiable matter = \( \frac{E₀ - Eₐ}{0.0122} \)

5.3 ICE CREAM
Preparation of sample:
The sample should preferably be analysed immediately after receipt. Otherwise, it should be kept in a screw capped jar at -15°C or below. First before any analysis, melt the sample in a water bath below 45°C, shake well and cool to room temperature.

5.3.1 Water
Determine the water content on a 5g sample using a metal dish containing sand and a flat ended glass rod.
5.3.2 Fat
Weigh 5g of ice cream into a fat extraction tube. Add 1.5ml of 0.88 ammonia and mix. Add 7ml water, mix again and maintain at 65°C in a water bath for 15 minutes. Add 10ml of ethanol and proceed as in the Rose Gottlich method.

5.3.3 Butter Fat
Heat the sample with ammonia and alcohol to boiling. Extract with a mixture of diethyl ether and petroleum ether. Evaporate off the solvents and determine the Kirschner value on the dry fat as described under butter. The amount of butter fat present can be estimated from the Kirschner value.

5.3.4 Sugars
Weigh 10g of sample into a 200ml volumetric flask. Dissolve in 150ml water. Clear with zinc acetate and potassium ferrocyanide solutions. Make up to mark and filter. Determine the sucrose by the Lane and Eynon titration before and after inversion. The Lactose may be calculated from the titration value before inversion and if the value is about half the value for milk solids not fat, it can be taken that no other reducing sugars are present.

5.3.5 Protein
Determine the nitrogen content on 5g of the sample by the Macro Kjeldahl method.
Protein = N x 6.25.

5.3.6 Milk Solids Not Fat
a. For the titration method: weigh 10g ice cream in a porcelain dish. Add 1ml phenolphthalein solution and titrate with 0.1M sodium hydroxide. Calculate as lactic acid. Add exactly 3.0ml of formalin, mix well and titrate again with 0.1M sodium hydroxide (x ml). Titrate separately, 3.0ml formalin (y ml).
Then Milk solids not fat = 5.67 (x - y)%
b. From calcium content. Ash 10g of the sample. Take up the ash in dilute hydrochloric acid. Determine the calcium ether by atomic absorption or by precipitating as oxalate, filtering and titrating the oxalate with standard permanganate solution, skimmed milk powder (milk solids not fat) contains about 1.5% calcium

5.3.7 Colouring Matter Done in the Usual Way

5.3.8 Overrun
Principle:
When ice cream is manufactured, air is whipped into it to increase the volume and improve the texture. Therefore, the volume of the solidified product is greater than that of the melted liquid mix. The percentage increase in volume is referred to as the overrun.
\[
\% \text{ overrun} = \frac{x}{y} \times 100
\]
where
\[x = \text{specific gravity of solid ice cream}
\[y = \text{specific gravity of melted ice cream.} \]
Procedure:
Freeze the ice cream in a freezing chamber or better in solid carbon-dioxide. Remove the container and weigh to the nearest gramme. Fill an overflow can (as described in the A.O.A.C. or any similar apparatus where the overflow can be collected) with kerosene cooled to about 5°C. Immerse the block of ice in the kerosene and collect the overflowing kerosene in a measuring cylinder.
The volume of the ice cream may also be determined by weighing the kerosene which overflowed and dividing the weight by its specific gravity. Determine the specific gravity of the melted ice cream in the usual way using a specific gravity bottle.
Calculate the percentage overrun using the formula given above.

5.4. WHEAT FLOUR
5.4.1 Moisture:
Weigh 2g of the sample in a weighed metal dish provided with a lid. Dry in an oven at 130°C for one hour. Cool the lid, cool in a desiccator and weigh.

5.4.2 Acid Insoluble Ash:
Add 4-5ml dilute hydrochloric acid to the ash. Evaporate to dryness on a water bath. Add 25ml of 2M hydrochloric acid. Heat on the water bath for 5 minutes. Filter through an ashless filter paper. Warm the residue again with dilute acid and transfer to the filter. Wash the filter with hot water until washings are chloride free. Place the filter paper in the original dish used for determination of ash, dry again, cool and weigh.

5.4.3 Fat
Weigh 2g of the flour in a fat extraction tube. Add 2ml of alcohol and then 7ml of concentrated hydrochloric acid and 3ml water. Heat in a water bath at about 80°C for half an hour. Add 10ml of ethanol, cool and extract the oil as in the Werber Schmid method. The oil may also be determined by direct solvent extraction with light petroleum.

5.4.4 Proteins
Determine the nitrogen on 1.5g of the sample by the Macro Kjedahl method.
Protein = N x 5.7

5.4.5 Gluten:
Weigh 20g of flour into a beaker. Add 15ml of water and knead to make a stiff dough. Allow to stand in the beaker of water for half an hour. Squeeze gently and knead the dough in a stream of running water until all the starch is washed away and the water squeezed out is quite clear. Press out the water as much as possible. Tear into very small pieces and transfer to a dried and weighed metal dish. Dry in an oven at 100°C to constant weight. Use a Simon Gluten washer if available, for more consistent results.

5.4.6 Crude Fibre
Principle:
The starch and the protein are dissolved by boiling the sample with acid and then with sodium hydroxide. The residue of cellulose and lignin is washed, dried and weighed. The residue is ashed and the weight of the ash subtracted from the weight of the fibre. The method
is empirical and therefore details of procedure must be strictly adhered to. Brown or whole wheat flour and brown or whole meal bread should contain at least 6% fibre on the dry matter.

**Reagents:**
1. Ethanol or industrial methylated spirits.
2. Diethyl ether
3. Sodium hydroxide solution 0.313M
4. Sulphuric acid 0.1275M (0.255N)

**Procedure:**
Weigh to the nearest mg about 2.7 – 3.0g of the sample.
1. Transfer to an extraction apparatus and extract with light petroleum
2. Alternatively, extract with light petroleum by stirring, settling and decanting three items.
   Air dry the extracted sample and transfer to a dry 1000ml conical flask.
   Take 200ml of the sulphuric acid into a measuring cylinder. Pour 20ml from this into the 1000ml conical flask containing the sample and fitted with a reflux condenser, with mixing to produce a cream. Bring the balance 180ml boiling sulphuric acid to boil in another flask.
   Add the 180ml boiling sulphuric acid, fit the reflux condenser and heat so that the liquid starts to boil in 1 minute. Continue the refluxing with occasional shaking to bring down any particles attached to the sides for exactly 30 minutes.

   In the meantime, fit a Buchner funnel with a filter paper of such quality as would not release any paper fibre - whatman 54 or 531 are suitable. Pour boiling water into the funnel and allow to remain until the funnel is hot. Drain the water by applying suction. At the end of the 30 minutes, allow the acid mixture to stand for 1 minute and then pour immediately into a shallow layer of hot water in the Buchner funnel. Adjust the suction so that the filtration of the bulk of 200ml is completed within 10 minutes.

   Wash the residue with boiling water until the washings are acid free. Then wash back into the original flask by means of a solution, measured at room temperature and brought to boiling point. Boil for 30 minutes, allow to stand for 1 minute and filter, taking the same precautions as before. Transfer the whole insoluble material to the filter with boiling water, then with 1% hydrochloric acid and finally with boiling water until the washings are free from acid. Then wash twice with ethanol and three times with ether. Transfer the insoluble matter carefully into a previously dried and weighed platinum dish, by means of a spatula or brush, taking care not to dislodge any fibre from the filter paper. In the alternative, the insoluble matter may be transferred to the platinum dish with boiling water and then the water evaporated off. Dry the platinum dish in an oven at 100°C for 1 hour, cool in a desiccator and weigh. Ash the residue and weigh again. Subtract the weight of the ash from that of the crude fibre.

5.4.7 Iron:
Ash 10g of the sample in a muffle furnace at a temperature below 600°C. Cool and moisten the ash with 1ml concentrated nitric acid. Evapourate to dryness and return to the muffle furnace for 1 hour. Cool and add 5ml of 5M hydrochloric acid. Heat on a water bath and filter through a hardened filter paper into a 100ml volumetric flask. Add 3ml dilute hydrochloric
acid (1 in 100) warm and pass through filter. Repeat this process four times. Then wash the basin or filter with hot water. Make the volume up to the mark. Determine the iron content by the thiocyanate method or any other suitable method.

5.4.8 Calcium:
Use an aliquot iron solution made for determination of iron. Dilute to about 150ml in a 400ml beaker.
Add 8-10 drops of bromoresol green indicator and enough 20% sodium acetate indicator to change the colour to blue. Cover with a watch glass and heat to boiling. Precipitate calcium slowly by the drop-wise addition of 3% oxalic acid solution until the colour changes to a distinct green shade (pH 4.4-4.6). Boil for 1 or 2 minutes and allow precipitate to settle overnight. Filter the precipitate through a quantitative filter paper and wash with about 50ml (1 in 50) ammonium hydroxide solution. Wash down the precipitate into a titration flask with a mixture of 5ml concentrated sulphuric acid and 125ml water at 80 – 90°C. This may be done by breaking the filter paper with a pointed glass rod. Titrate with 0.01M (0.05N) potassium permanganate solution. Add the filter paper and continue the titration if necessary.
1ml 0.05N permanganate = 1mg Ca.

Note:
The calcium content of wheat flour varies from 15mg (patent flours) to 30mg (wholemeal) per 100g. This must be subtracted from the determined calcium value, if added calcium is to be determined.

5.4.9 Acidity:
Shake 18g of flour with 200ml of carbon dioxide-free water in a conical flask. Place the flask in a water bath at 40°C for 1 hour. Filter and titrate 100ml to phenolphthalein with 0.05M sodium hydroxide. Calculate the acidity as potassium dihydrogen phosphate. 1ml 0.05M sodium hydroxide = 0.0068g KH₂PO₃

Chlorine:
Filter through a Buchner funnel to get a clear filter weight equivalent. Evaporate to small bulk and transfer hydroxide and evaporate to dryness. Ignite gently with water. Ignite the filter paper and again extract to about 50ml. Add 5ml 0.01M sodium chloride filter into flask. Wash filter with cold water solution using ferric alum as indicator. Calculate and titrate due to the added NaCl.

Calculation of Original Gravity of the Beer Wort:
Multiply the specific gravity of the extract by 1000 and add the equivalent to gravity lost to get the origin gravity.

Bitter Substance in Beer:
Acidify 50ml of beer with dilute sulphuric acid and extract with 50ml of diethyl ether. Wash the ether layer with 5ml of distilled water. Evaporate off the ether and taste the residue. A bitter taste indicates hops similar substances.
Isohumulones present in hops may be identified by paper chromatography, TLC or Gas chromatography.

5.5 Wines
5.5.1 Total Solids:
Evaporate 25ml sample in a metal or silica dish on boiling water bath. Dry in an oven at 100°C for 2 hour. Cool in a desiccators and weigh.

5.5.2 Ash
Ignite the dry solids from 25ml in a furnace between 500°C, and 500°C until completely ashed. Cool and weigh.

5.5.3 Total Acidity
Boil 25ml of the sample under reflux for 20 minutes to expel carbon dioxide. Wash down the condenser with water and titrate with 0.1M sodium hydroxide solution, using bromothymol blue as indicator.

\[
\text{Total Acidity} = \frac{4}{\text{Titration value in ml}} \times \text{milliequivalents per litre}
\]

5.5.4 Fixed Acidity
Evaporate 25ml sample to dryness on a boiling water bath. Add 10ml water and evaporate again. Repeat this process twice more. Take up the residue in 50ml water and titrate with 0.1M sodium hydroxide solution using bromothymol blue indicator. Calculate as for Total Acidity.

5.5.5 Volatile Acidity
Volatile Acidity = Total Acidity - Fixed Acidity.

5.5.6 Sugars
Exactly neutralise 100ml of the wine with sodium hydroxide. Evaporate to half the volume. Cool. 10ml each of zinc acetate and potassium ferrocyanide clearing agents described in Section 2.3.1.

5.5.7 Alcohol
Measure out 100ml of sample into a volumetric flask at 20°C. Wash into a distillation flask using 50ml water. Neutralise the acidity with 1M sodium hydroxide solution. Distil and collect in the same volume flask. Cool to 20°C and determine the specific gravity. Determine the alcohol strength from the following:

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<thead>
<tr>
<th>Specific gravity at 20°C</th>
<th>% Alcohol by weight</th>
<th>By volume</th>
<th>% Proof Spirit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7904</td>
<td>100.00</td>
<td>100.00</td>
<td>175.35</td>
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<tr>
<td>0.7936</td>
<td>98.98</td>
<td>99.37</td>
<td>174.25</td>
</tr>
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<td>96.85</td>
<td>98.02</td>
<td>171.88</td>
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</table>
5.5.8 Glycerol
(Dry wines with total solids less than 5%). Evaporate 100ml of the wine in a porcelain dish to 10ml on a water bath maintained at 85-90°C. Add 5g of fine sand and 5ml of lime (containing 15g CaO/100ml each) total solids. Evaporate almost to dryness. Treat the residue with 50ml of 90% ethanol. Rub to a paste and heat on a water bath to incipient boiling with constant stirring. Filter and wash the residue with 10ml portions of the hot 90% ethanol, until the filtrate totals 150ml. Evaporate the filtrate to a syrupy consistency. Transfer the residue to a stopped cylinder with 20ml absolute ethanol. Add 3 x 10ml portions of dry ether, shaking well after each addition. Allow to stand until clear and filter. Wash the cylinder with a mixture of 2 parts of absolute ethanol and 3 parts of dry ether and pass the washings through the filter. Evaporate the filtrate to a syrup, dry at 100°C for 1 hour and weigh. Ignite the residue and reweigh. The loss in weight gives the % glycerol.
Wines with total solids more than 5%. Heat 100ml to boiling and treat with successive small portions of milk of lime until the wine becomes darker, then lighter in colour, cool, add 200ml ethanol. Allow precipitate to settle and filter. Wash with ethanol proceed as for dry wines.

5.5.9 Tartaric Acid
To 100ml of the wine in a beaker, add 2ml glacial acetic acid solution, 0.5ml of 20% potassium acetate to promote the crystallization of potassium hydrogen tartrate. Allow to stand overnight, decant through a slurred glass crucible leaving most of the precipitate in the beaker. Wash the precipitate and filter 3 times with 5ml quantities of a mixture containing 15g potassium chloride, 20ml ethanol 100ml water. Place crucible and precipitate in beaker, boil with 50ml water and titrate with 0.1M sodium hydroxide solution (V) using phenolphthalein as indicator.
% Total tartaric acid (m/v) = 0.015 (V + 1.5)
This value includes free tartaric acid and combined tartaric acid in the form of cream of tartar and calcium tartarate.
CHAPTER SIX

MINERAL CONSTITUENT OF FOODS

Flame Photometric Method for the Determination of Potassium and Sodium

Principle:
Organic matter is destructed and oxidised by the action of boiling sulphuric acid and nitric acid. The sodium or potassium content is measured flame photometrically.

Reagents:
1. Nitric acid: Dilute nitric acid (sp. gr. 1.42) 1:1 with water.
2. Filter paper: Diameter 9 and 12.5 cm.
3. Acid washing liquid for glassware: Dilute nitric acid (sp. gr. 1.42) to 100ml with distilled water.
4. Distilled water: Good quality distilled or deionised water.
5. Sodium Stock Solution (100mg sodium/litre): Dry some sodium chloride at 105°C for 2 hours. Weigh 254.2 mg of the dried product, dissolve it in water and make up to 100ml. Keep this solution in a polyethylene bottle.
6. Potassium stock standard (100mg potassium/litre): Dry some potassium chloride at 105°C for 2 hours. Weigh 190.7 mg of the dried product in water and make up to 1000ml. Keep the solution in a polyethylene bottle.
7. Potassium diluting solution: Weigh 3.8 g of potassium chloride, dissolve in water and dilute to 1 litre.
8. Sodium working standard solution: Pipette 0.5, 10, 15, 20 ml of sodium stock solution into 100ml graduated flasks. Add 5 ml of potassium diluting solution to each and dilute to the mark with water. These solutions contain 0, 0.5, 1.0, 1.5 and 2.0 mg of sodium per 100 ml respectively.
9. Potassium working standard solutions: Pipette 0.5, 10, 15 and 20 ml of potassium stock solution into 100 ml graduated flasks. Add 5 ml of potassium diluting solution to each and dilute to the mark with water. These solutions contain 0, 0.5, 1.0, 1.5 and 2.0 mg of potassium per 100 ml respectively.

Apparatus:
1. Flame filter photometer or flame emission spectrophotometer.
2. Special glassware for trace metal analysis or wash all glassware before use thoroughly with dilute citric acid and after that with good quality distilled or deionised water.

Procedure:
(a) Preparation of the Sample:
1. Weigh to the nearest mg, 2 g of the sample on a filter paper (diameter 9 cm).
2. Fold up the filter paper and transfer into a 250 ml kjeldahl flask.
3. Add 20 ml of 1:1 dilute nitric acid.
4. Boil gently for about 10 minutes and cool to room temperature.
5. Filter the digest solution through a filter paper (diameter 12.5 cm) into a 100 ml graduated flask. Wash the kjeldahl flask and the filter paper three times each with 10 ml of water.
1. Make up to 100ml and mix (solution A).
2. Prepare a blank starting from instruction a.2 (solution B).

(a) **Dilution of the Solution for Sodium**
1. Pipette 50ml of solutions A and B and 5ml of potassium dilution solution into 100ml graduated flasks, make up to the mark and mix (solution C and D respectively). See note.

(b) **Dilution of the Solution for Potassium Determination**
1. Pipette 5ml of solution A and B into a 100ml graduated flask, make up to the mark, and mix (solution E and F).

(c) **Measurements:**
1. Measure the standard solutions for both sodium and potassium. Use the right filters and in case of a spectrophotometer the wavelengths 589.0nm and 766.5nm.
2. Correct the obtained values for the zero concentration standards.
3. Measure the solution C and D for sodium and solution E and F for potassium.

**Calculation**
1. Prepare a calibration (standard) graph for sodium and potassium.
2. Read the concentrations (mg/100ml) of solutions c, d, e and f from the calibration graph.
Let: the concentrations (mg/100ml) be c, d, e and f respectively. Weight (g) of the sample = W
Then: Sodium content (mg/100g product) = \( \frac{(c-d) \times 200}{W} \)
Potassium content (mg/100g product) = \( \frac{(e-f) \times 2000}{W} \)

**Note:**
Without addition of potassium to the sodium standard the results from sodium may be too high. This is caused by the inter-element effect of potassium present in the product on sodium. Additions of a surplus of potassium to standard and sample, level out this effect. The effect depends on the temperature of the flame. An acetylene – air flame gives a greater deviation than a natural gas air flame.

6.1 **DETERMINATION OF CALCIUM (DISODIUM-EDTA METHOD)**

**Principle:**
Disodium EDTA forms stable water soluble complexes (termed chelates) with many different metallic ions. This property permits the quantitative determination of a number of these ions by titration with a standardized volumetric solution of disodium EDTA.
Equipment:
1. Analytical balance
2. Titration Burettes
3. Pipette
4. Beakers and Flasks
5. pH meter

Reagents:
1. Disodium EDTA (Disodium Ethylenediaminetetra-acetate)
2. Hydroxy Naphthol Blue - indicator
3. 0.05 molar of Disodium EDTA
4. 1.0 Normal Potassium Hydroxide solution
5. Hydrochloric Acid.

Procedure:
1. Weigh out the calcium salt, containing approximately 85mg of calcium
2. Dissolve in water containing the minimum quantity of dilute hydrochloric acid, necessary to dissolve the sample.
3. Dilute with water to 150ml and adjust pH to 12-12.5 by adding 1.0N potassium hydroxide solution
4. Add 0.2 – 0.3 gram Hydroxy Naphthol Blue indicator. The solution will acquire a pink colour (indicator is equipped with self-measuring portion of chemical).
5. Titrate with 0.05 molar disodium EDTA solution to a clear blue colour. Each ml of disodium EDTA solution is equivalent to 2.004mg of calcium.

Note: this titration procedure can be used to assay a large number of calcium compounds, such as calcium: Acetate, carbonate, chloride, citrate, gluconate, Hydroxide, lactate, nitrate, oxide, etc.
The procedure is also useful for determining small amounts of calcium in other substances (in some cases at a level of a few parts per million) such as in water and in blood and other body fluids.
CHAPTER SEVEN
FRUIT JUICE

7.1 DETERMINATION OF ASCORBIC ACID IN FRUIT JUICE

Principle: 2, 6-dichlorophenol indophenol dye is reduced by ascorbic acid and its sodium derivative. Thus, these are used for the titrimetric determination of vitamin C in foods. Pure vitamin C (ascorbic acid) is assayed by titration with a 0.1 N solution of iodine. However, for the determination of traces of this vitamin in foods and juices, a 2,6-dichlorophenol titration is the method of choice.

Equipment:
1. Juice extractor
2. Erlenmeyer flasks
3. Graduated pipettes
4. Burettes
5. Volumetric flask

Reagents:
1. Metaphosphoric Acid – Acetic Acid solution (Dissolve 15gm glacial HPO₃ in 40ml acetic acid and 200ml water. Dilute to 500ml and filter into a glass bottle (can store up to 7 days in refrigeration).
2. Ascorbic acid standard solution. (Accurately weigh 50mg assayed, dried ascorbic acid and transfer to 50ml volumetric flask. Dilute to volume immediately with HPO₃ – HOAC solution prepared above)
3. Indophenol standard solution.
4. Dissolve 50gm 2,6- dichloroindophenol, sodium salt into 50ml water and 42mg NaHCO₃. Shake vigorously and after the dye has dissolved, dilute to 200ml with water. Filter through fluted paper into amber glass bottle.

Procedure:
1. Transfer three 2.0ml aliquots of ascorbic acid standard solution to each of 50ml Erlenmeyer flasks containing 5.0ml HPO₃ – HOAC solution.
2. Titrate each of these rapidly with indophenol solution until rose-pink colour persists.
3. Similarly titrate 3 blanks of 7.0 ml HPO₃-HOAC solution diluted with water to approximately the same volume as was used in the above titrations.
4. Average the results for the blanks, subtract from the average results of titration of the standard solution and express the concentration of the indophenols as milligrams ascorbic acid equivalent to 1.0ml reagent. (the indophenols solution should be standardized daily with freshly prepared ascorbic acid solutions).
5. Mix the juice thoroughly by shaking and filter through rapid paper.
6. Measure out into flasks. 3 aliquots of 100mls of juice and add equal volumes of HPO₃-HOAC solution. Designate total volume as Vml, mix and filter through rapid folded paper.
7. Add 3 aliquots of the above sample to three 50ml Erlenmeyer flask containing 5ml HPO₄²⁻-HOAC solution and titrate.
8. Similarly titrate 3 blanks of 7.0ml HPO₄²⁻-HOAC solution diluted with water to approximately the same volume as the samples.

Calculation:
Mg Ascorbic acid/ml sample = (X-b) x (F/E) x (V/Y)
Where X = average ml for sample titration
  b = average ml for sample blank titration
  F = mg ascorbic acid equivalent to 1.0ml indophenols solution
  E = initial assay solution volume
  Y = volume of sample aliquot titrated.