GENERAL ANALYTICAL METHODS FOR MILK AND MILK PRODUCTS

WHAT IS MILK?

- Definition - Food drug and administration
  - ✓ Milk is the lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows
- Generally,
  - ✓ Milk is a pale whitish liquid produced by the mammals and it is the primary source of nutrition for infant mammals before they are able to digest other types of food

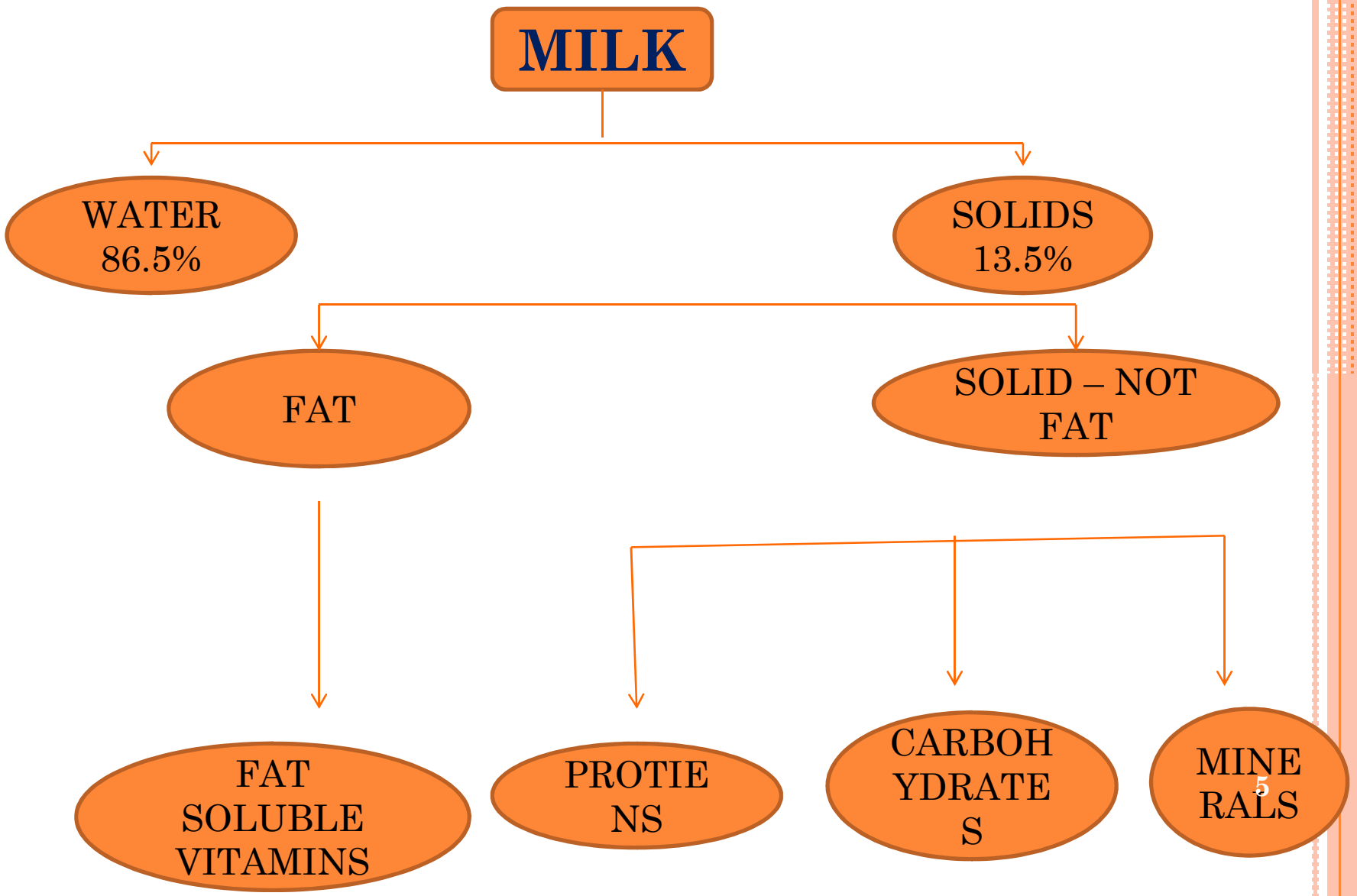
PROPERTIES OF MILK

- It is usually white in colour and sometimes ranges from bluish white to yellowish white
- It has characteristic odour
- It is slightly sweet in taste
- Fresh milk has pH range from 6.4 – 6.8
- It has viscosity of about 1.640 centipoise

TYPES OF MILK

- Standardized milk
- Skimmed milk
- Homogenised milk
- Sterilized milk
- Flavoured milk
- Toned milk
- Double toned milk

COMPOSITION OF MILK



QUALITY CONTROL OF MILK

Sample Preparation

- Warm the sample at 37°– 40°C
- Water bath 40-45°C
- Stir for homogenization
- Mix well and make it free from lumps
- Allow sample to reach room temperature
- Sample is ready for analysis

DETECTION OF ADULTERANTS IN MILK

Detection of Cane sugar in milk (Modified Seliwanoff method)

- **Fructose + resorcinol in HCL ⇨ red colour**
- **Reagent: 1g resorcinol in 100 ml Hcl**

PROCEDURE

- **1 ml Hcl + 25 ml milk (allow it to stand for 10 min.)**

- **5 ml solution + 1 ml of the filtered milk serum (in test tube)**

- **Place test tube in boiling water for 1 min and observe colour.**

- **Appearance of red color indicates presence of aldose or ketose**

QUANTITATIVE DETERMINATION OF CANE SUGAR

- PRINCIPLE: The milk sample is curdled with zinc acetate and positive ferrocyanide (Carrez solution 1 and 2). Determine the sugar before and after inversion. The value before inversion indicates lactose and after inversion indicates total sugars.

PROCEDURE:

5 g milk + 5 ml of carrez 1 solution (shake for a minute)

Add carrez solution 2 and shake for 1 min

Filter and wash precipitate with water

using Lane and Eynon method determine sugar content

DETECTION OF STARCH IN MILK

Procedure

- Take about 5 ml of milk in a test tube.
- Bring to boiling condition and allow the test tube to cool to room temperature.
- Add 1-2 drops of iodine solution to the test tube.
- Development of blue colour indicates presence of starch which disappears when sample is boiled and reappears on cooling.

QUANTITATIVE DETERMINATION OF STARCH IN MILK

- Weigh approx 25 gm sample in a 250 ml beaker.
- Add 20 ml of alcohol to curdle the milk.
- Filter the ppt on a filter paper and wash the ppt with50% alcohol till the ppt is free from lactose/sugar i e when the washings give a negative test with resorcinol.
- Transfer the ppt to a 500 ml flask with about 200 ml water and add 10 ml Conc. HCl to hydrolyse the starch by refluxing in a boiling water bath for 2.5 hours.
- Cool and neutralise with 10 % Sodium Hydroxide and Sodium Carbonate towards the end using litmus paper.
- Make upto 500 ml with water. Shake well and filter if necessary.
- Determine reducing sugar by Lane and Eynons method.
- Calculate starch as follows
- % starch = % reducing sugar x 0.9

DETECTION OF CELLULOSE IN MILK

Cellulose in milk gives blue color with iodine—zinc chloride reagent.

10 g of milk + 50 ml of hot water

Stir thoroughly for about 2 min.

Pour the mixture on a nylon cloth, wash residue with 50 ml of hot water twice.

Scrape the residue with spatula and place it in a spotting plate.

Stain a part of residue with iodine-Zinc chloride reagent and add another part with iodine solution.

Blue color in iodine-zinc chloride reagent and absence of blue color in iodine solution confirms presence of cellulose.

TEST FOR DETECTION OF SODIUM CHLORIDE IN MILK

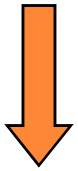
2.0 ml of milk + 1.0 ml of 5% K_2Cro_3 + 2.0 ml of 0.1N $AgNo_3$.

Red precipitate—absence of dissolved chloride in milk

Appearance of yellow color indicates presence of chlorides in milk

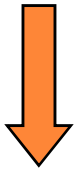
TEST FOR PRESENCE OF SACCHARIN

Milk sample + acetic acid (curdled)

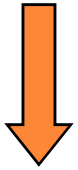


Shake and filter

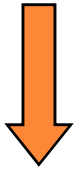
Clear filtrate + Conc Hcl



extract with two 25 ml of diethyl ether



Draw off aqueous layer and wash with 5 ml water thrice



Evaporate the ether extract on a water bath, add a drop or two of water, mix well with glass rod and taste a little.

sweet taste indicates the presence of saccharin

Confirm by heating with NaOH and detecting Salicylic acid.

Acidify 20 -25 ml of filterate + extract with three portions of ether.

Wash ether extract with two 5 ml portions of water

evaporate greater portion of ether in porcelain dish on steam bath

Evaporate spontaneously + add 1 drop of 0.5% (v/v) neutral $FeCl_3$ solution

Violet colour indicates Salicylic acid.

Test for Detection of Gelatin in Milk

10 ml sample +10 ml acid $Hg(NO_3)_2$ solution

↓ Shake mixture

Add 20 ml water, let it stand 5 minutes and filter.

↓

If gelatin is present, filterate will be opalescent and cannot be obtained quite clear.

↓

To portion of filterate + add equal volume of picric acid solution.

↓

Yellow precipitate indicates presence of gelatin, cloudiness indicates little amount of gelatin.

DETECTION OF PRESERVATIVES

1. Test for presence of Formalin in Milk (Hehner's Test)

A) 2 ml milk + 2 ml of 90 % H_2SO_4 with traces of $Fecl_3$ from the side of the test tube slowly.

Purple ring at the junction indicates formaldehyde is present in milk.

B) If sucrose is present,

distil the milk sample (25 ml)

Take 2-3 ml of distillate + 2 ml of formaldehyde free milk.

The violet coloration does not appear usually when relatively large quantities of formaldehyde are present.

2. Test for presence of Hydrogen Peroxide

Dissolve 1 gm V_2O_5 + 100 ml of 6% H_2SO_4

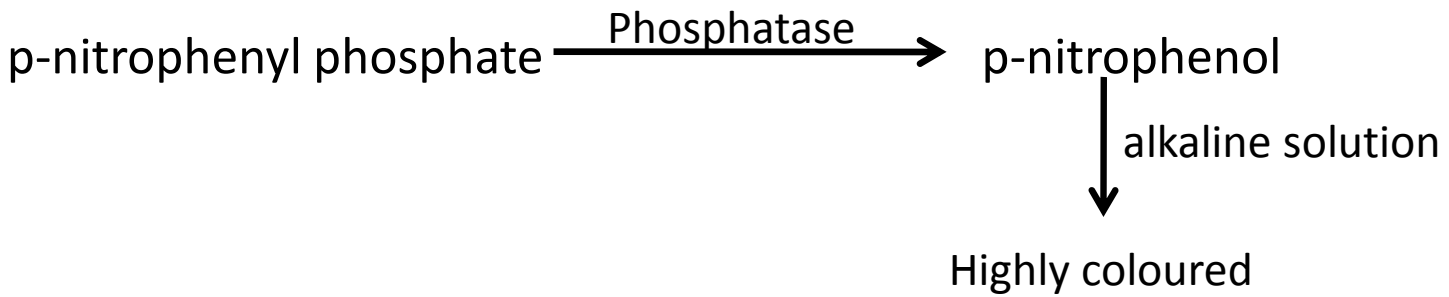
10 ml of sample +10- 20 drops of reagent and mix.

The develpment of pink or red colour indicates presence of H_2O_2

PHOSPHATASE TEST FOR PASTEURISATION IN LIQUID MILK

✓ **Phosphatase, an enzyme in milk is destroyed during pasteurization.**

✓ **The test is based on the above principle to judge the efficiency of pasteurization.**



❖ **The test does not apply to sour milk and milk preserved with chemical preservatives.**

Reagents

✓ All reagents should be of analytical grade.

a) Buffer solution - 1.5 g of sodium bicarbonate + 3.5 g of anhydrous sodium carbonate dissolved in water make up to one litre.

Store in a refrigerator and discard after 1 month

b) **Disodium p – nitrophenylphosphate**.

c) **Buffer-substrate solution** - 0.15 g of substrate (disodium pnitrophenyl phosphate) make up to 100 ml with buffer solution.

✓ The solution stored in refrigerator, protected from light.

✓ The solution must be discarded after one week.

5ml Phosphate-buffer solution at 37^{0}C

↓

Add 1ml milk and incubate for 2 hrs

↓

Simultaneously prepare blank and incubate

↓

After 2 hrs milk blank on left side of comparator balance

↓

Test Sample on right side

Record readings falling between two standards by affixing a plus or minus sign to the figure for the nearest standard.

Turbidity Test for Sterilised Milk:

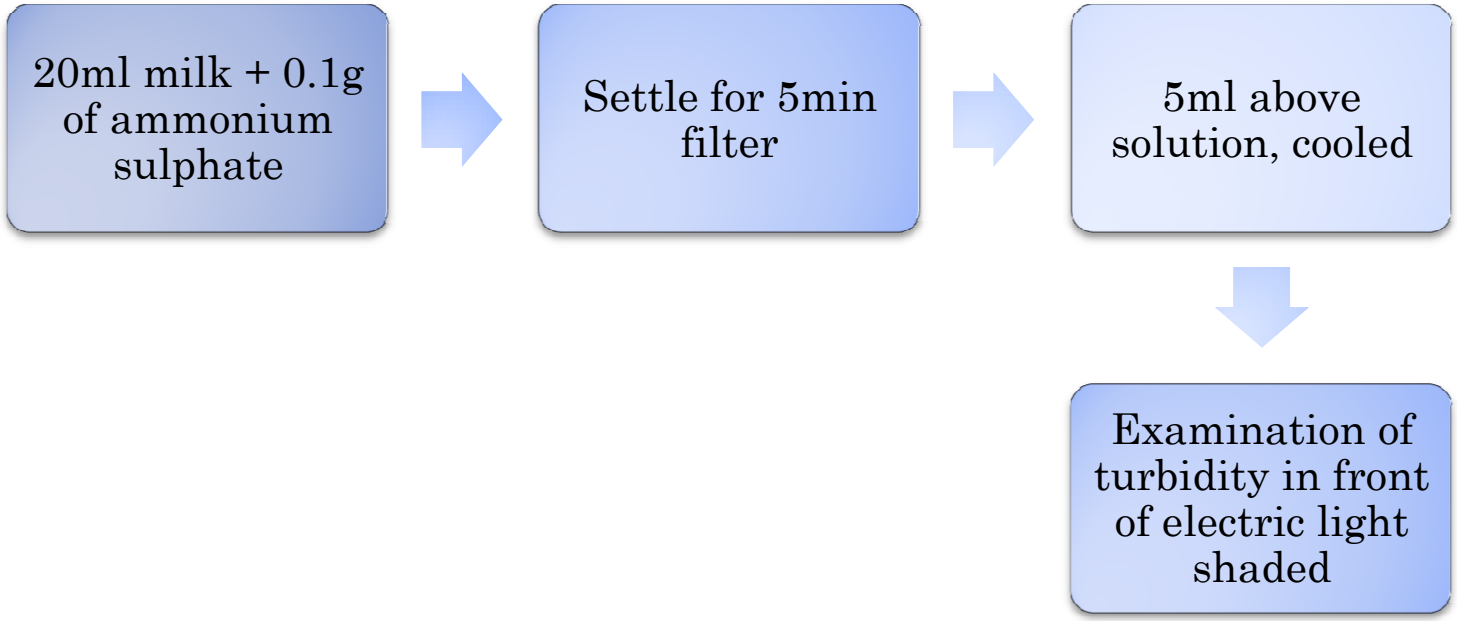
✓ **The turbidity test depends upon the denaturation of proteins of milk especially albumin after sterilisation.**

✓ **When solutions of inorganic salts or acids are added albumin separates with the casein.**

✓ **The sample on treatment with ammonium sulphate, filteration and heating of the filtrate shows turbidity due to presence of albumin on account of insufficient heat treatment.**

✓ **If milk has been sterilized properly all albumin will have been precipitated and no turbidity will be produced.**

Reagents: **Ammonium sulphate AR.**



The milk is considered sterilized when the filtrate shows no turbidity.

Determination of Total Solids (Gravimetric method):

Priniciple: Pre drying of a test portion on a boiling water bath and subsequent evaporation of the remaining water in a drying oven at a temperature of 102 ± 2 ^{0}C.

Preparation of sample

Warm sample slowly to 35^0 – 40^0 C on a water bath. Cool the sample quickly to room temperature.

Procedure:

Heat the dish with lid for1 hour. Place the lid on the dish and transfer it to a dessicator immediately.

↓

cool to room temperature (at least 30 mins) and weigh to the nearest 0.1 mg.

↓

Add 5 ml of prepared sample, place the lid on the dish and weigh again.

Heat the dish without lid on water bath , remove the dish and place it in oven for 2 hours.

Place the lid and transfer to the dessicator. Allow the dish to cool and weigh to the nearest 0.1 mg.

Again heat the dish with its lid in oven for 1 hour. Place the lid on the dish and immediately transfer to the dessicator.

Allow to cool and weigh again. Repeat the operation again until the difference in the two consecutive weighings does not exceed 1 mg. Record the lowest mass.

Calculation

$$\text{Total Solid Content} = \frac{m_2 - m_0 \times 100}{m_1 - m_0}$$

Where m_0 = mass in gm of dish + lid

m_1 = mass in gm of dish + lid and test portion

m_2 = mass in gm of dish + lid and dried test portion

Round the value obtained to nearest 0.01 % (m/m)

DETERMINATION OF FAT IN MILK

Gerber Method

Principle

Milk + sulphuric acid + alcohol

⇩ (mixed in a special Gerber tube)

Dissolution of the protein and release of fat.

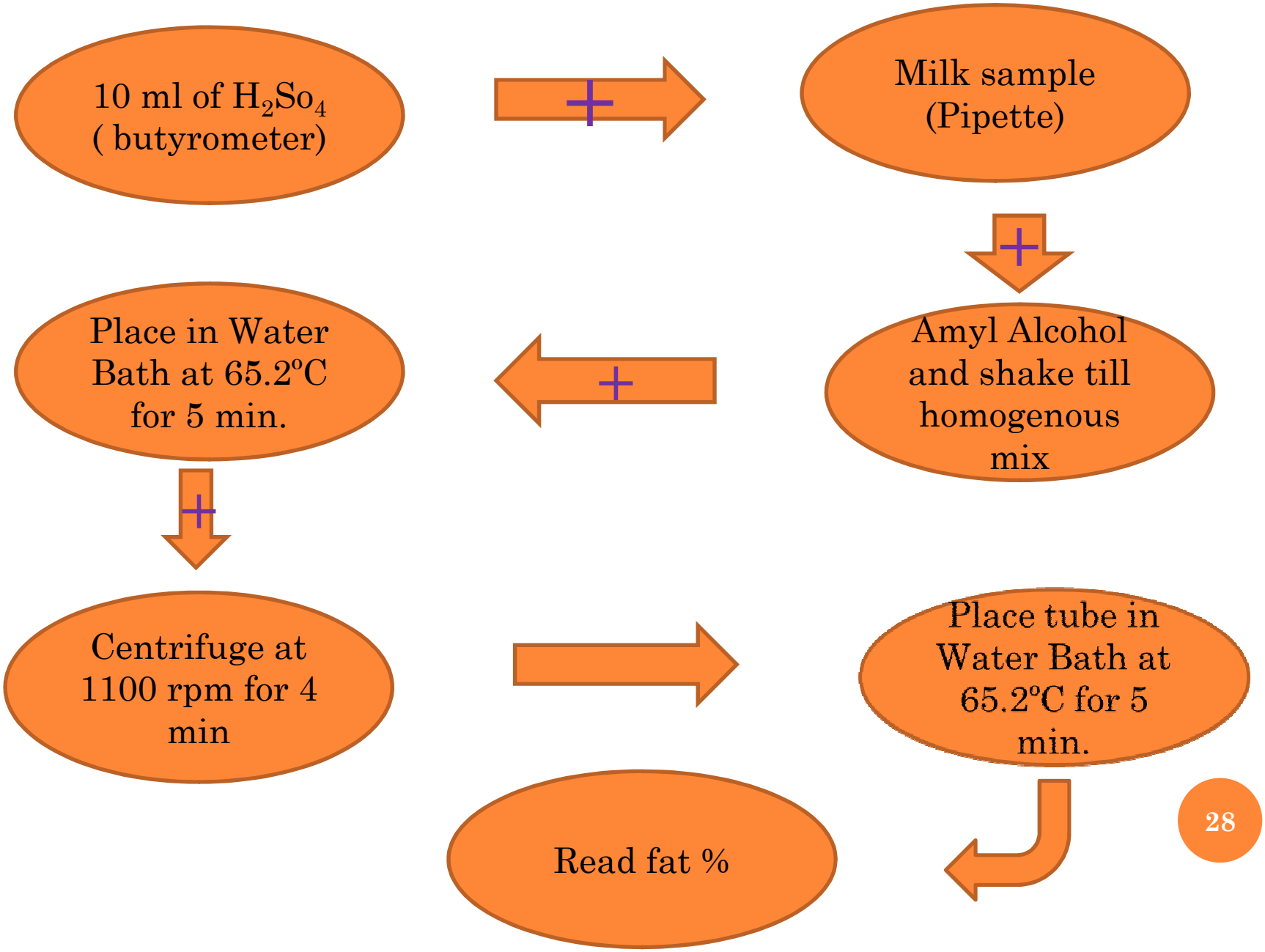
⇩

Centrifuge and measure fat %

It is a routine or screening test.

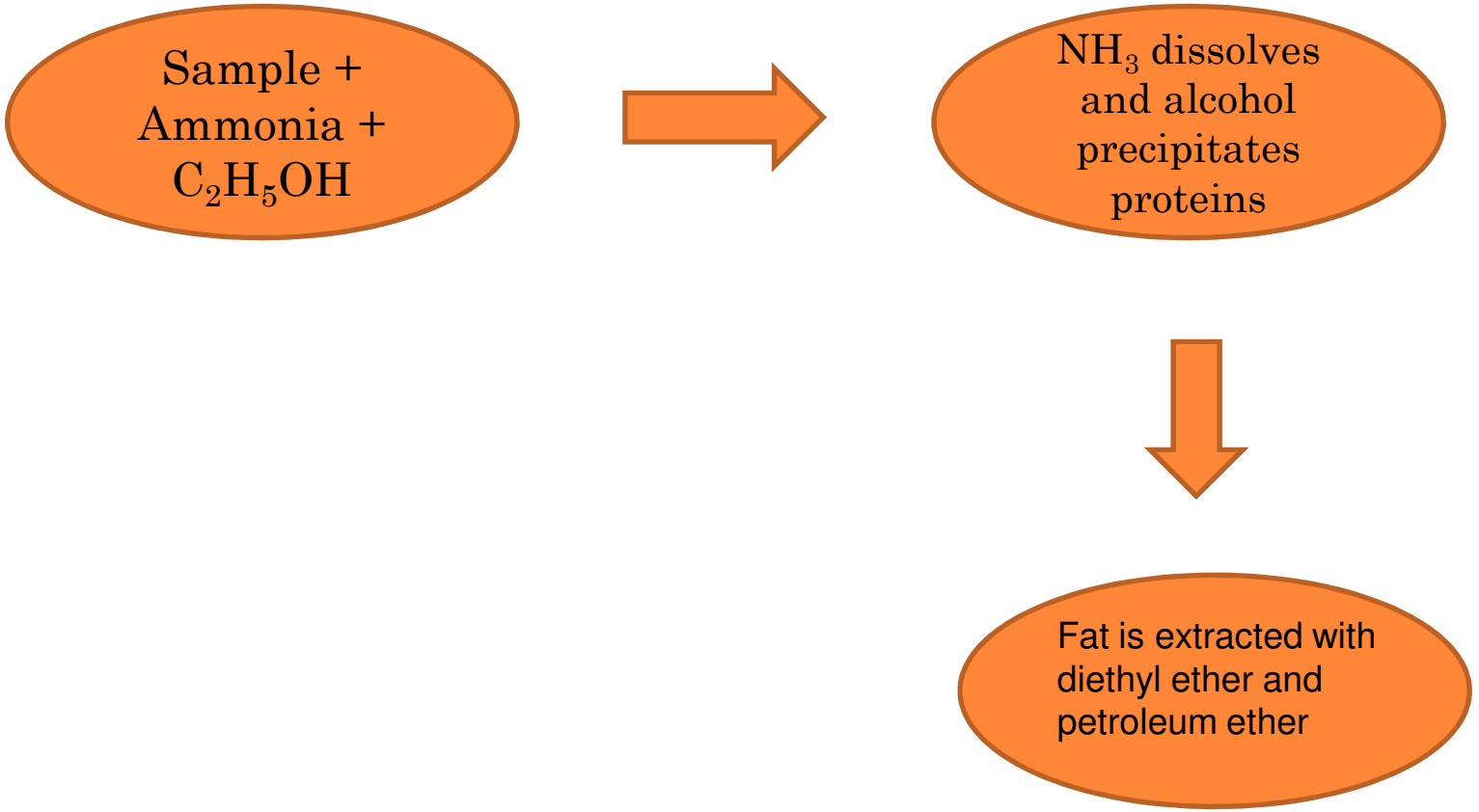
Reproducible results can be obtained if procedure is followed correctly.

PROCEDURE


10 ml of H_2So_4 (butyrometer)
Milk sample (Pipette)
Amyl Alcohol and shake till homogenous mix
Place in Water Bath at 65.2°C for 5 min.
Centrifuge at 1100 rpm for 4 min
Place tube in Water Bath at 65.2°C for 5 min.
Read fat %

Roese- Gottlieb Method:

Principle



PROCEDURE

10 g of sample + 1.25 ml of ammonia

mix and shake thoroughly

10 ml ethyl alcohol + 25 ml of diethyl ether

shake thoroughly for a minute

+ 25 ml petroleum ether and shake vigorously for half minute

Stand it still ethereal layer separates

Decant ethereal layer

Extract the remaining by repeating the above process twice

Evaporate obtained ether and dry the flask at 102 + 2°C for 2 hours

Cool in a dessicator and weigh

Heat the flask in oven for ½ an hour and cool in dessicator and weigh

Repeat the process until the difference between two successive weights does not exceed 1 mg

Wash out the fat from the flask with petroleum ether carefully leaving any insoluble residue in the flask. Dry the flask in the oven and reweigh

The difference in weights represents the weight of fat extracted from the milk.

Calculation

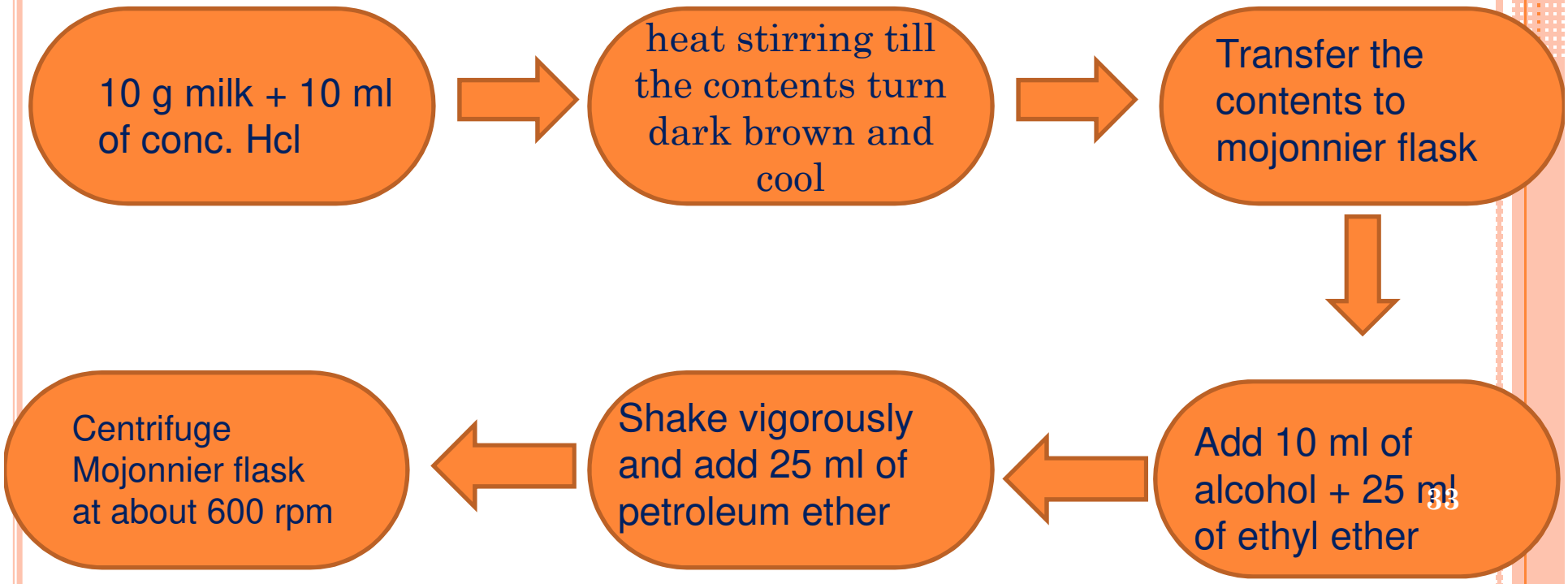
$$\text{Fat percent w/w} = \frac{\text{Weight of fat}}{\text{Weight of Milk}} \times 100$$

Acid Digestion Method (Werner Schmidt Method)

PRINCIPLE

Milk proteins are digested with conc. hydrochloric acid. Liberated fat is extracted with alcohol, ethyl ether and petroleum ether. Ethers are evaporated and residue left behind is weighed to calculate the fat content.

PROCEDURE



CALCULATION

$$\text{Fat percent w/w} = \frac{100\,(W1 - W2)}{W3}$$

Where

W1 = Weight in g of contents in the flask before removal of fat.

W2 = Weight in g of contents in the flask after removal of fat and

W3 = Weight in g of material taken for the test.

MILK POWDER

Preparation of sample

- Make homogeneous either by mixing or shaking or alternately rolling and inverting container.
- Avoid excessive temperature and humidity when opening sample container to prevent absorption of moisture.

Determination of Moisture

Principle

The sample is dried to a constant weight at 100 + 2°C and the loss in weight reported as moisture

Procedure

1. Accurately weigh approximately 5 g of the sample. Dry in the oven at 100 + 2oC for 5 hrs.
2. Place lid on the dish and cool it in a desiccator.
3. Quickly weigh the dish. Heat again in the oven for half an hour, cool in a desiccator and weigh again.
4. Repeat until succeeding weights do not differ by 0 .5 mg.

Calculation

$$\text{Moisture percent w/w} = \frac{W1 \times 100}{W}$$

W = Weight of powder taken

W1 = Loss in weight due to drying

DETERMINATION OF FAT

1. Weigh 1 g of the sample in a Mojonnier flask or suitable extraction flask.
2. Add 9 ml of warm distilled water, shake to dissolve the powder.
3. Add 1.25 ml of ammonia solution.
4. Shake well and proceed as per Rose Gottleib method

Determination of Total Carbohydrates

Total carbohydrates can be determined adding the mass of moisture, fat, protein and ash content and deducting it from 100 to give carbohydrate content by difference.

Total carbohydrate including sucrose, dextrose and dextrins, maltose or lactose percent by wt = 100 – (A+B+C+D)

Where A = Percent by mass of moisture
B = Percent by mass of total protein
C = Percent by mass of fat and
D = Percent by mass of Total ash

DETERMINATION OF ASH

- Weigh accurately in a previously heated and cooled silica / Pt dish about 10 g of the sample.
- Char it carefully on a heater or flame, heat the sample in a muffle furnace maintained at 525-550oC until white ash is obtained.
- The temperature of the muffle furnace should not exceed 550oC, otherwise lower values will be obtained due to evaporation of certain metal chlorides.

Calculation

$$\text{Total ash percent w/w} = \frac{W1}{W} \times 100$$

W = Weight of the sample

W1= Weight of the residue after ashing