UNIT 1

Composition of Milk

Structure

1.1 Definition of milk, PFA designated milks
1.2 Composition of milk of different species
1.3 Factors affecting composition of milk

Learning Objectives

After studying this unit, the student will be able to

• Define Milk
• PFA standards and milks.
• Composition of Milk and different species.
• Factors affecting the composition of milk.

1.1 Definition of milk, PFA designated milks

Definition

Milk may be defined as the whole, fresh, clean lacteal secretion obtained by the complete milking of one or more healthy milchy animals, excluding that obtained within 15 days before or 5 days after calving, or such periods as may be necessary to render the milk practically colostrum free, and containing the minimum prescribed percentage of milk fat and milk - solids- not fat. in india, the term ‘milk’ when unspecified, refers to cows or buffalos milk or a combination of both.
The term market milk refers to fluid whole milk that is sold to individuals usually for direct consumption. It excludes milk consumed on the farm and that used for the manufacture of dairy products.

**PFA Designated milk**

According to Prevention of Food Adultration (PFA) rules 1976 the standards for different classes and designation of milk in India are given in the table.

| Class of milk    | Designation                                      | Locally                                                                 | Minimum
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% milk fat</td>
</tr>
<tr>
<td>Buffalo milk</td>
<td>Raw, pasteurized, boiled, flavoured sterilized.</td>
<td>Assam, Bihar, Chandigarh, Delhi, Gujarat, Haryana, Punjab, Uttar Pradesh, West Bengal.</td>
<td>(6.0)</td>
</tr>
<tr>
<td></td>
<td>-do-</td>
<td>Andaman and Nicobar, Andhra Pradesh, Dadra and Nagar - Haveli, Goa, Daman and Diu, Kerala, Himachal Pradesh, Lakshadeep, Tamil Nadu, Madhya Pradesh, Manipur, Karnataka, Nagaland, Orissa, Pondicherry, Rajasthan, Tripura,</td>
<td>(5.0)</td>
</tr>
<tr>
<td>Cow milk</td>
<td>-do-</td>
<td>Chandigarh, Haryana, Punjab.</td>
<td>(4.0)</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
<td>-------------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>-do-</td>
<td>-do-</td>
<td>Andaman &amp; Nicobar, Andhra Pradesh, Assam, Bihar, Dadra and Nagar - Haveli, Delhi, Gujarat, Goa, Daman and Diu, Himachal Pradesh, Kerala, Madhya Pradesh, Maharashtra, Tamil nadu, Karnataka, Manipur, Rajasthan, Nagaland, Pondichery, Rajasthan, Tripura, Uttar pradesh, West Bengal, Lakshadeep.</td>
<td>(3.5)</td>
</tr>
<tr>
<td>-do-</td>
<td>-do-</td>
<td>Orissa</td>
<td>(3.0)</td>
</tr>
<tr>
<td>Goat or Sheep milk</td>
<td>Raw, pasteurized boiled, flavoured and sterilized.</td>
<td>Chandigarh, Haryana, Kerala, Madhya Pradesh, Maharashtra, Tamil nadu, Karnataka, Manipur, Rajasthan, Nagaland, Pondichery, Punjab, Uttar Pradesh.</td>
<td>(3.5)</td>
</tr>
<tr>
<td>-do-</td>
<td>-do-</td>
<td>Andaman and Nicobar, Andhra Pradesh, Assam, Bihar, Dadra and Nagar - Haveli, Delhi, Goa, Daman and Diu, Gujarat, Himachal Pradesh, Lakshadeep, Tamil nadu, Karnataka, Manipul, Nagaland, Pondichery, Orissa, Rajasthan, Tripura and West Bengal.</td>
<td>(3.0)</td>
</tr>
</tbody>
</table>
### 1.2 Composition of milk from different species

<table>
<thead>
<tr>
<th>Name of the species</th>
<th>Percentage</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>fat</td>
</tr>
<tr>
<td>Cow (foreign)</td>
<td>86.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Buffalo</td>
<td>84.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Ewe (sheep)</td>
<td>79.4</td>
<td>8.6</td>
</tr>
<tr>
<td>Goat</td>
<td>86.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Ass</td>
<td>90.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Camel</td>
<td>86.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Elephant</td>
<td>67.8</td>
<td>19.6</td>
</tr>
<tr>
<td>Mare</td>
<td>89.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Sow</td>
<td>89.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Whale</td>
<td>70.1</td>
<td>19.6</td>
</tr>
<tr>
<td>Dog</td>
<td>75.4</td>
<td>9.6</td>
</tr>
<tr>
<td>Ginne pig</td>
<td>82.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Cat</td>
<td>84.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Liama</td>
<td>86.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Human Milk</td>
<td>87.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>
**Water** : Water constitutes the medium in which the other milk constituents are either dissolved or suspended. Most of it is free and only a very small portion is in bound form, being firmly bounded by milk proteins phospholipids etc.

**Total solids** : Total solids constitutes lipids (Fat) and solid not Fat.

**Milk Fat (Lipids)** : The bulk of the fat in the milk exists in the form of small globules, which average approximately 2 to 5 microns in size. This an oil - in - water type emulsion. The surface of these fat globules is coated with an adsorbed layer of material commonly known as the fat globule membrane. This membrane contains phospholipids, and proteins in the form of a complex and stabilizes the fat emulsion. In other words, the membrane prevents the fat globules from coalescing and separating from one and another. The emulsion may, however, be broken by agitation (at low temperature), of heating, freezing etc.

Chemically, milk fat is composed of a number of glycerol - esters of fatty acids milk fat on hydrolysis gives a mixture of fatty acids and glycerol.(The milk fat is a mixture of true fats in established from the fact that it has no sharp melting point). The fatty acids are saturated or unsaturated fatty acids. Saturated fatty acids are relatively stable.

The fat associated substances are phospholipids, cholesterol, carotene and fat soluble vitamins (A, D, E, K).

**Phospholipids** : Three types of phospholipids, exists ie. Lecithin, Cephalin and Sphingomylin. Lecithin, which forms an important constituent of the fat globule membrane, contributes to the richness of flavour of milk and other dairy products. It is highly sensitive to oxidative changes, giving rise to oxidized / metallic flavours. Phospholipids are excellent emulsifying agents, and no doubt serve to stabilize the milk fat emulsion.

**Cholesterol** : This appears to be present in true solution in the fat, as a part of fat globule membrane complex and in complex formation with proteins in the non - fat portion of milk.

**Fat Soluble Pigments** : Carotene is fat soluble and responsible for the yellow colour of milk, cream, butter, ghee, and other fat rich products. Carotene acts as antioxidant and also as a precursor of Vitamin A. One molecule of B - carotene gives two molecules of Vitamin A, where as carotene give one.

**Fat Soluble Vitamins** : Milk is rich in fat soluble Vitamin ie. A, D, E and K. Solid - not - fat content contains lactose, proteins and mineral contents.

**Milk Sugar or Lactose** : This exists in milk only. it is in true solution in milk serum. On crystallization from water, it forms hard gritty crystals. It is one
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- sixth as sweet as sucrose. Lactose, on crystallization is responsible for the defect known as sandiness in ice-cream or condensed milk. It is fermented by bacteria to yield lactic acid and other organic acids and is important both in the production of cultured milk products and in the spoilage of milk and milk products by souring.

Milk Proteins: The proteins in milk consists mainly of casein, lactoglobulin, lactalbumin, milk serum albumin, immuno globulins etc. Casein forms more than 80% of the total proteins of the milk. Casein exists only in milk and is found in the form of calcium caseinate phosphate complex. It is present in colloidal state. It may be precipitated by acid, rennet, alcohol, heat and concentration. Casein compose of a, b and gamma fraction. a - casein micelle in milk. b and gamma forms constitutes 22 and 3 percent respectively. a - casein constitute two fractions as is calcium sensitive which is coagulated by calcium ions and another form is K-casein which is called calcium insensitive casein fraction, not precipitated by calcium ion. K-casein is the richest repository of carbohydrates as against other casein fractions. It is the site for rennin action.

Lactalbumin and lactoglobulin are known as ‘whey or serum proteins’. They are also present in colloidal state and are easily coagulated by heat. Milk serum albumin is same as blood serum albumin of the blood. Immunoglobulins are present only in colostrum and gives immunity to the calves.

Non protein nitrogenous compounds: Eg: Ammonia, aminocids, proteose - peptones, urea, uric acid etc.

Mineral Matter or Ash: The mineral matter or salts of milk although present in small quantities, exert considerable influence on the physico-chemical properties and nutritive value of milk. The major salt constituents i.e. those present in appreciable quantities, includes potassium, sodium, magnesium, calcium, phosphate, citrate, chloride, sulphate and bicarbonate. The trace elements includes all other minerals and salt compounds. The mineral salts of milk are usually determined after ashing.

Although milk is acidic, ash is distinctly basic. Part of the mineral salts occur in true solution, while a part are in colloidal state.

Other Constituents

Pigments: Water soluble pigments are Riboflavin and Xanthophyll. Riboflavin besides being a vitamin, is a greenish yellow pigment which gives characteristic colour to whey. Earlier it is Known as lactoflavin or lactochrome.

Dissolved Gase: Milk contains gases like O₂, CO₂, N₂ etc.,
**Vitamins**: Water soluble vitamin B complex and vitamin ‘C’.

**Enzymes**: These are biological catalysts. Milk contains Amylase, Lipase, Phosphatase, protease, peroxidase and catalase enzymes.

**Details Composition of Milk**

**Constituents or Group of Constituent** | **Approx. (Wt per Litre of milk)**
--- | ---
Water | 860-880 g.
Lipids in emulsion phase | 
Milk fat | 30-50 g.
Phospholipids | 0.30 g.
Sterols | 0.10 g.
Vitamin A, D, E, K | 

**Proteins in Colloidal Dispersion**

- Casein (a,b,g) | 25 g.
- Lactalbumin | 3. g.
- Lactoglobulin | 0.7 g.
- Albumin, pseudoglobulin etc | 

**Dissolved Materials**

- Lactose | 45-50 g.

Inorganic and organic ions and salts

- Calcium | 1.25 g.
- Phosphates | 2.1 g.
- Citrates | 2.0 g.
- Chlorides | 1.0 g.

**Trace Minerals**

- Cu, Fe, I |
1.3 Factors Affecting Composition of Milk

Milk differs widely in composition. All milks contain the same kind of constituents, but in varying amounts. Milk fat shows greatest daily variation, then comes proteins, followed by ash and lactose.

The various factors that affect the composition of milk are

1. **Species**: Each species yield milk of a characteristic composition as shown in chapter 1.2.

2. **Breed**: In general, breeds producing the largest amount of milk, yeild milk of a lower fat percentage vice versa. Holstein - Friesian gives less fat where as Jersey gives high fat in cow breeds.

3. **Individual Variation**: Each cow tends to yeild milk of a composition that is characteristic of the individual.

4. **Season**: Both fat and SNF show slight but well defined variation during the course of year. A variation in fat percent when graphed is of ‘U’ shape with maximum values in January and November and minimum in June. For non-fat - solids graph will be ‘W’ shaped showing highest values in January and December. Lower values occurs in April and August but during these two months little increase .

5. **Age**: Fat percent increase up to 3rd lactation and after wards decrease. SNF will be high in the first lactation and slightly decrease as locations increased.

6. **Milk Interval**: When milking is done at longer intervals, the yield is more with a corresponding decrease in fat and vice versa. It has not much effect on - solid - not - fat content.

7. **Completeness of Milk**: Fore milk contains less fat and strippings (last milk) contains high fat. If the milking is not complete, it tests less fat. Not much effect on SNF.

8. **Irregularity in Milking**: Frequent changes in the milking timings and frequent changes in milking intervals results in less fat and not much effect on SNF.

9. **Yield**: With increase in yield per milking the percentage of lactose increases, while fat and non - fatty solids decrease.
10. **Lactation Effect**: The first secretion after parturition namely colostrum high in globules and chlorides and low lactose content. The yield increase and attains maximum within 2-4 weeks and then slowly decrease. When the yield is more, fat and SNF decrease and vice versa.

11. **Exercise**: More exercise increase fat in milk as body fat is metabolized - no effects on SNF.

12. **Excitement**: Sexual or freight excitement caused decrease in fat, and has no - effect in SNF.

13. **Hormones**: Prolactin and thyroid hormone which are essential for milk synthesis increase the fat percentage. Oestrogen has stimulating and depression effect, optimum levels causes increase in fat and higher doses decreases the fat percent.

14. **Udder Diseases**: Mastitis and other udder diseases causes low lactose and casein %, increase in chloride content. Subnormal SNF is the characteristic of mastitis.

15. **Physiological Condition**: The condition of cow at the time of parturition has effect on fat and SNF content. Healthy cows gives high fat and SNF content.

16. **Pasture Feeding**: Pasture feeding increase both fat and SNF.

    Pasture feeding increases unsaturated fatty acids in milk.

17. **Feeding**: Feeding oils such as palm oil, coconut oil increase fat percent where codliver oil decreases the fat percentage. Starvation increases unsaturated fatty acids in milk.

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**Summary**

The definition of milk and various PFA designated milks discussed. The composition of various species milk and the detailed composition of milk explained. The various factors affecting the composition of milk mentioned. The nutritive and energy value of milk explained.

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**Short Answer Type Questions**

1. Define milk as per PFA.

2. What are fat and SNF standards for Toned and Double Toned Milks?

3. Mention fat and SNF levels in recombined and standardized milk.

4. Give the composition of buffalo milk.
5. What are the various types of proteins present in milk?
6. What is the effect of mastitis on fat and SNF of milk?
7. Mention the energy values of cow and buffalo milk.

**Long Answer Type Questions**

1. Briefly write about PFA designated milks.
2. Give the composition of cow, buffalo, sheep, goat and human milks.
3. Draw schematic diagram of detailed composition of milk.
4. Explain in detail the detailed composition of milk.
5. What are the various factors affecting the composition of milk?
2.1 Colour and flavour
The colour of an opaque object is the colour it reflects. The yellow object. Yellow light is reflected to the eye. White object reflects the entire colour, while black absorbs all the colours. The characteristic white opalescent colour of milk is due to scattering of light by the colloidal particles which it contains. The yellow colour of carotene is confined to the fat phase and therefore, becomes prominent in cream layer and more so in butter. The colour of the milks are

**Buffalo Milk** : Creamy White

**Cow’s Milk** : Yellowish creamy white
Skim Milk : Bluish

Whey : Greenish yellow (This is due to pigment Riboflavin)

The intensity of yellow colour of cow milk depends on various factors such as breed, feeds, size of fat globules, fat percentage of milk. Certain breeds of cow imparts deeper colour of yellow than other. The greater intake of green feed, the deeper the colour of cow milk. The larger the fat globules and the higher the fat percentage, the greater the intensity of yellow colour.

Flavour is a property difficult to define but the help of taste and smell is taken in its judgment. The sweet taste of lactose is balanced by the salty taste of minerals, specifically chlorides and both are damped down by proteins. Some workers attribute the characteristic rich flavour of dairy products to phospholipids. Hence of flavour of milk, is delicately balanced property. With advance in lactation, lactose decreases and so milk becomes salty. Similar dislocation occurs sometimes due to udder disturbances.

The flavour of milk is shifted from normal sometimes due to passage in to milk the odour of certain plants as mint and garlic. Whether odiferous feed effect the flavour of milk due to passage through blood to milk or due to the remarkable absorbing capacity of the milk from atmosphere is not yet definite. Both the possibilities can be avoided by the feeding such feed immediately after milking so that if former possibility exists tainting material may be excreted by the next milking and if the latter possibly exists, all traces of food would have removed by the next milking time. The freshly drawn milk has certain ‘cowy’ odour which passes off by the time reaches the consumer. With the development of lactic acid the flavour also changes to ‘sour’. This is due to lactic acid, butyric acid and diacetyl.

Sulphydryl compounds significantly contribute to the cooked flavour in heated milks.

2.2 pH and Acidity

The hydrogen ion concentration of milk is about 10^-6 gms per litre that is in terms of pH value it is 6.6. Freshly drawn milk is amphoteric to litmus i.e. it turns red litmus to blue and blue litmus to red. The pH of cow milk is 6.4 – 6.6 and buffalo milk 6.7 – 6.8. Higher pH values in freshly drawn milk indicates mastitis.

When the milk is drawn from the udder of the cow, it is acidic in nature, which is called natural acidity or apparent acidity. This is mainly due to the presence of casein, acid phosphates and citrates and to a lesser degree to albumin, globulin and carbon dioxide. The acidity is estimated by titrating 10 ml of milk
against N/9 sodium hydroxide solution in the presence of phenolphthalein indicator, and expressed as percentage of lactic acid. In titration the standard alkali required to shift the pH of milk to 8.4 PH, which change in colour of phenolphthalein becomes perceptible.

Colostrum has high natural acidity due to its high protein content. In early lactation also the value is above average by the value is above average but the value falls about normal in second month and then remain fairly steady until the last month of lactation, when further decline occur. The natural acidity of individual, stage of lactation, physiological condition of the udder etc. the high the solid not fat content of the milk, the higher the natural acidity and vice versa.

When the milk is kept for some time, the bacteria will multiply and utilize lactose and converts in to lactic acid, there by increasing the acidity and decreasing the pH value. This acidity is known as developed or real acidity, the sum of natural acidity and developed acidity is known as titratable acidity.

Milk having titratable acidity more than 0.18 % is not suitable to prepare heat treated products as the milk coagulates on heating.

The dilution of milk will decrease the acidity and increases the pH. Heating of milk increases acidity due to conversion of colloidal casein in to soluble casein and formation of acids by degradation of lactose.

### 2.3 Specific Gravity of Milk

The density of a substance is its mass (weight per unit volume). Specific gravity is the ratio of density of the substance to the density of standard substance (water). Since the density of a substance varies with temperature, it is necessary to specify the temperature when reporting specific gravities or densities. The gravity of a substance (when referred to water at 4°C) is numerically equal to the density of that substance in the metric system. The specific gravity of milk is usually expressed at 60 °F (15.6°C).

The density of specific gravity of milk may be determined by either determined by either determining the weight of a known volume or the volume of a known weight. The weight of a known volume may be determined either with a pydnometer or with hydrostatic balance; while the volume of a known weight is determined by using lactometer; the scale of which is calibrated not in terms of volume but as a function of either density or specific gravity. The Common types of lactometers are zeal, quevenne etc.)

Milk is heavier than water. The average specific gravities are

**Cow Milk :** 1.028 to 1.030
Buffalo Milk: 1.030 to 1.032

Skim Milk: 1.035 to 1.037

The specific gravity of milk is influenced by the proportion of its constituents (e.g., composition), each of which has different specific gravity approximately as follows.

- Water: 1.000
- Fat: 0.930
- Protein: 1.346
- Lactose: 1.666
- Salts: 4.12
- SNF: 1.616

As the milk fat is lighter constituent, the more there is of it the lower the specific gravity will be and vice versa. However, although buffalo milk contains more fat than cow milk, its specific gravity is higher than the latter, this is because buffalo milk contains more solids-not-fat as well, which ultimately results in a higher specific gravity.

The specific gravity of milk is decreased by

- Addition of water
- Addition of cream (fat)
- Increased temperature.

The specific gravity of milk is increased by

- Addition of separated milk
- Removal of fat
- Reduction of temperature.

The specific gravity of milk is calculated by the following formula.

\[
\text{Sp. Gravity of milk} = 1 + \frac{\text{CLR}}{1000}
\]

When CLR = Corrected Lactometer Reading. As the temperature of expression is 60°F, if the temperature is more than 60°F, add 0.1 for every 1°F above 60°F to the lactometer reading or subtract 0.1 for every 1°F below 60°F from the lactometer reading to get corrected lactometer reading Richmond has given formula for SNF% and total solid % in milk as given below.
% SNF in milk = CLR/4 + 0.2F + 0.14

% of total solids = CLR /4 + 1.2f + 0.14

Recently Richmond’s formula has been recalculated for the use with modern improved density hydrometer.

% SNF = 0.25 D + 0.2 F + 0.66

% total solids = 0.25 D + 1.2 F + 0.66

D = Density hydrometer reading at 20°C

i.e. (1000 x Density – 1)

F = Fat%

The specific gravity of milk should not be determined for at least one hour after it has been drawn from the udder; else a lower value than normal value will be obtained. This is due to dissolved gases which will escape afterwards. Another theory is conversion of liquid fat (light) to solid condition (heavy). The specific gravity of freshly drawn milk tests less.

2.4 Off – Flavours

Among foods, milk is particularly susceptible to off flavours. In this regard, the animal constitutes the initial problem by acting as a condenser for odour substances in feeds, weeds, and barn air. In a matter of minutes following inhalation by the dairy animal, strong odors may be reflected in the flavour of milk. Off flavours in milk and its products are divided into three aspects. Chemical flavour deterioration and absorbed flavour.

1. Chemical Flavour Deterioration: These flavours are produced by heat, light, air etc. They are oxidized flavour, rancid flavour, sunlight flavour, heated flavour.

Oxidized Flavour: Phospholipids in the milk serve as the origin of oxidized flavour in fluid milk. Sweet cream butter which is particularly high in phospholipids is very susceptible to oxidized flavour development. Phospholipids oxidation is accompanied by a flavour termed called board or cappy. Numbers of factors are essential for the development of oxidized flavour in fluid milks. The first important is atmospheric oxygen. The copper and sun light acts as a catalysts. The primary substrates from which the flavour compounds are formed are highly unsaturated fatty acids. Oxygen is known to attack the methylene group adjacent to the double bonds of unsaturated fatty acids resulting in formation of hydroperoxides. These are unstable leading to secondary oxidation products like $\alpha - \beta$ unsaturated aldehydes.
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CH₂ - O - R
CH - O - R
CH₂ - O - C - (CH₂)₇ - CH = CH - CH₂ - CH = CH - (CH₂)₄ - CH₃
CH₂OR
CHOR
CH₂O - C - (CH₂)₇ - CH₂ - CHO + OHC - CH = CH - (CH₂)₄ - CH₃

Copper, even when present in milk at levels of ppm is very potent catalyst. Using of processing temperature will activate Sulphydryl (-SH) groups of Lactaglobulin and acts as effective in preventing oxidized flavour.

(b) **Rancid Flavour (hydrolytic Rancidity)**

The lipase enzyme in the presence of water hydrolyses triglycerides liberating free acids. Butyric acid is the principle acid responsible for rancid flavour in dairy products.

Pasteurization destroys the lipases in milk. Homogenization increases the surface area of fat globules, so favour rancid flavour.

(c) **Sun Light Flavour** : Also known as burnt or cabbage flavour. The amino acid methionine appears to serve as the specific origin of sun light flavour. Riboflavin is directly involved in the development of sun light flavour. The principle off flavour compound is 3-mercapto-methylpropionaldehyde (methionol).

(d) **Heated Flavours** : When heat treatments in excess of those employed for pasteurization are used, a distinct cooked flavour develops. This flavour arise from –SH groups activated by heat denaturation of β-Lactaglobulin. The flavour is specifically due to volatile sulfids and hydrogen sulfide CH₂S in particular. When heat treatment is prolonged cooked flavour slowly gives way to caramelized flavour. The lactose is responsible for coconut flavour.

2. **Microbiological flavour deterioration** : The growth of different microorganisms in the milk and milk products give rise to different flavours.

<table>
<thead>
<tr>
<th>Off Flavours</th>
<th>Organism Responsible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malty flavour</td>
<td>Streptococcus Lactic var maltigenes</td>
</tr>
<tr>
<td>Unclean flavour</td>
<td>E-Coli, Aerobacter aerogenes</td>
</tr>
<tr>
<td>Potato flavour</td>
<td>Pseudomonas graveolens</td>
</tr>
<tr>
<td>Medicinal flavour</td>
<td>Aerobacter aerogenes</td>
</tr>
</tbody>
</table>
### Off Flavour

<table>
<thead>
<tr>
<th>Off Flavour</th>
<th>Organism Responsible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishy flavour</td>
<td>Pseudomonas Fluorescens</td>
</tr>
<tr>
<td>Phenol flavour</td>
<td>Bacillus Circulans</td>
</tr>
<tr>
<td>Amyl alcohol flavour</td>
<td>Micrococcus caseolyticus</td>
</tr>
<tr>
<td>Putrid</td>
<td>Pseudomonas putrefaciens</td>
</tr>
<tr>
<td>Fruity</td>
<td>Pseudomonas fragi.</td>
</tr>
</tbody>
</table>

For the development of microbiological off flavours different chemical reactions will occur. For example, malty flavour is produced by *S. lactis* var. *maltigenes*. This is due to action of organism on amino aid leucine to produce isovaleraldehyde: \((\text{CH}_2)_2 - \text{CH} - \text{CH}_2 - \text{CH} \cdot \text{NH}_2 - \text{COOH} \longrightarrow (\text{CH}_3)_2 \text{CH} - \text{CH}_2 + \text{NH}_3 + \text{CO}_2\) even 0.5 ppm of isovaleraldehyde gives characteristic malty flavour.

### 3. Absorbed Flavours:

Flavourful substances may enter milk either before or after milking. There are two path ways by which flavours and odour substances may gain entry into milk via cow. One is by nose or mouth to the lungs, to the blood stream, to the udder cells and into the milk. The other is from the digestive tract to the blood, to the udder cells and into the milk. For example, when a cow either eats or smells onions or garlic, the odour is noted in the blood within a very few minutes and flavour is detected in the milk within 20-30 minutes. Off flavours that are absorbed into milk through cow are classified as feed, weed, cowy, barny and unclean. The most problematic among these are feed and weed off flavours.

<table>
<thead>
<tr>
<th>Sources of feed</th>
<th>Flavours Sources of Weed Flavours</th>
<th>Feed with little effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onions</td>
<td>Garlic and chives</td>
<td>Sugar beets, dried beet pulp.</td>
</tr>
<tr>
<td>Fermented silage</td>
<td>French weed</td>
<td>Soya beans</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>Mustard</td>
<td>Carrots</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Bone set</td>
<td>Pumpkins</td>
</tr>
<tr>
<td>Turnips</td>
<td>Buck horn</td>
<td>Soy beans hay</td>
</tr>
<tr>
<td>Rape</td>
<td>Pepper glass</td>
<td>Potatoes</td>
</tr>
<tr>
<td>Kale</td>
<td>Tar weeds</td>
<td>Mangoes</td>
</tr>
<tr>
<td>Sources of Feed</td>
<td>Flavours Sources of Weed Flavours</td>
<td>Feed with little effect</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Beet tops</td>
<td>Alanthus shots</td>
<td>Oats</td>
</tr>
<tr>
<td>Green barley</td>
<td>Rag Weed</td>
<td>Rye</td>
</tr>
<tr>
<td>Clover hay</td>
<td>Wild tansy</td>
<td>Peas</td>
</tr>
<tr>
<td>Distillers grains</td>
<td>Dog fennel</td>
<td>Corn, Clover &amp; grass</td>
</tr>
<tr>
<td>Brewers grains</td>
<td></td>
<td>Timothy hay</td>
</tr>
<tr>
<td>Musty hay or silage</td>
<td></td>
<td>Most concentrates</td>
</tr>
<tr>
<td>Citrus pulp</td>
<td></td>
<td>Tankage</td>
</tr>
</tbody>
</table>

Milk will never absorb odour or flavour from the barn or stable air during the course of customary milking. The off flavours are also derived from cow breathing under improper ventilation. In this connection, it is of interest that morning milk frequently carries a Bany taint, when cows are stable under conditions of poor air circulations.

Cowy or Bany flavour is particularly prevalent in raw milk supplies during the winter months. The sources are (a) tainted stable air (b) abnormal silage fermentation either due to temperature or age of silage (c) ketosis, a disease in cattle involving the endogenous energy metabolism. Ketosis in dairy animals is fairly common especially in the late winter or early spring ad particularly after calving. The animal blood show high concentration of acetone bodies and milk from such animals contains some what lower concentration of these substances.

Direct relation occurs between the concentration of acetone in milk and degree of cowy flavour.

**Summary**

The various import physicochemical properties of milk, i.e. Colour flavour, pH acidity, specific gravity, freezing point, viscosity, surface tension, B.R. Reading, electrical conductivity, oxidation - reduction potential Buffering of milk were discussed in detail. The various factors that affects the different physico - chemical properties of milk explained in details.

**Short Answer Type Questions**

1. Mention the colour of cow and buffalo milks.
2. Mention the colour of skim milk and whey.
3. What are Ph and acidity values of cow and buffalo milks?
4. What are the specific gravity values of cow, Buffalo and skim milk?
5. What are the factors that affect the specific gravity of milk?
6. Mention the Richmond’s formulae for SNF and total solids estimation in milk.
7. How freezing point of milk is helpful to detect adulteration of milk with water.
8. Define viscosity.
9. What are the factors that affect viscosity of milk?
10. What are the constituents responsible for buffering action in milk?
11. What are the normal values of viscosity, surface tension and potential of milk?

**Long Answer Type Questions**

1. Briefly write about colour and flavour of milk.
2. Describe in detail about pH and acidity of milk.
3. Explain about specific gravity of milk.
4. Briefly explain about viscosity and surface tension of milk.
5. What is viscosity? However, viscosity of milk is affected.
6. Explain the buffering action in milk.
**Structure**

3.1 Adulterants in milk – their detection  
3.2 Preservatives in Milk – their Detection  
3.3 Adulteration of Buffalo Milk with Cow Milk Hamsa Test  
3.4 Effects of adulterants and Preservatives on human health

**Learning Objectives**

After studying this unit, the student will be able to

- Understand about Adulterants and preservatives in Milk  
- Understand about Adulteration of Buffalo Milk and Cow Milk.  
- Understand about effects of Adulterants.

**3.1 Adulterants in milk – their detection**

Adulterants of milk may be defined as addition of any material to the milk or removal of any constituent of milk. As per PFA adulteration of milk is not allowed and it is punishable with a fine and imprisonment. The common adulterants in milk are

1. Addition of water  
2. Removal of fat  
3. Addition of cane sugar
4. Addition of starch/ cereal flour
5. Addition of skim milk powder
6. Addition of gelatin
7. Addition of urea
8. Addition of Ammonium sulphate
9. Addition of glucose

The common adultration of milk is addition of water. By water adultration of milk constituents are diluted (Fat and SNF). The water adultrated milk tests less lactometer readings and less SNF content. To mask this other compounds listed above are added so that milk shows required lactometer reading. The water adultrated milk will be thin and nonviscous. To mask this also the various substances are added, so that the adultrated (water) milk will have normal consistency.

Detection of Adultrants of milk

1. Added water: Many methods are used for detection of milk adultration with water.

(a) By estimation of SNF: Estimate the solid not fat content of the sample of milk and calculate the percent of added water using the following formulae

\[
\% \text{ added water} = \left( \frac{S - s}{S} \right) \times 100
\]

Where \( S = \text{Standard SNF (9.0 for Buffalo milk, 8.5 for cow milk)} \)

\( S = \text{SNF of sample milk.} \)

This method is not appropriate, as the people will make up SNF with addition of other adultration listed from 3 to 9 as given above.

(b) Detection of Nitrate: Natural water supplied usually contain nitrates, whereas milk contains no appreciable traces, therefore the presence of nitrates in milk may be taken as evidence of watering the milk. The disadvantage of this method is some public water supplies are free of nitrates.

(c) Freezing point test: Freezing point of milk is its most constant property. By using hortvet cryoscope freezing point of milk is estimated. Addition of water will dilute the dissolved constituents so that the freezing point of milk on adultration with water causes less depression. The normal freezing point
depression of cow milk is 0.547°C and buffalo milk is 0.549°C. This method cannot detect addition of fat separated milk, as skim milk has the same freezing point will be normal, as acid will give soluble ions to depress the freezing point.

(d) Spectrometric method: Recently spectrometer has been suggested as a means of detecting addition of water to milk. This method will detect 10% of adultration of milk with water. This method cannot detect pure water as rain water or may upland surface water incomplete.

2. Removal of Fat: Fat being the costly ingredient of milk, some portion of fat is removed. Removal of fat also comes under adultration of milk. Detect the fat Percentage of the sample of milk and calculate the percent of fat removed using the formulae

\[
\% \text{ of fat removed} = \frac{F - f}{F} \times 100
\]

Where F = Standard fat or fat in pure milk.

f = Fat percent in the sample of milk.

3. Addition of Cane Sugar

- Take 10 ml of milk in a test tube
- Add 1 ml of concentrated hydrochloric acid and mix
- Add 0.1 g of resorcinol powder and mix thoroughly.
- Place the test tube in a boiling water bath for 5 minutes and observe for colour

Red colour obtained with resorcinol indicates adultration of milk with cane sugar

4. Addition of starch / Cereal flour

- Take 3 ml of well mixed sample of milk in a test tube
- Boil the milk over a Bunsen burner
- Cool and add one drop of 1 percent Iodine solution ad observe for colour change.

Iodine solution gives intense blue colour with starch due to formation of an unstable complex starch – iodo compound. So development of blue colour indicates adultration of milk with starch / cereal flour.
5. Addition of skim milk powder.

- Take 50 ml in each of two centrifuge tubes and balance properly in the centrifuge.
- Centrifuge at 3000 RPM for 30 mts.
- Decant the supernatant liquid carefully.
- Dissolve the residue in 2.5 ml of concentrated nitric acid.
- Dilute the solution with 5 ml of water.
- Add 2.5 ml of liquid ammonia and observe for colour development.

Skim milk powder being highly proteinacious in nature gives orange colour with nitric acid. While unadulterated milk being low in protein content gives only a yellow colour.

6. Addition of Gelatin

- Take 10 ml of milk in test tube
- Add an equal amount of mercuric nitrate solution mercury is dissolved twice of its weight of nitric acid of sp. G. 1.422. Before use this solution is diluted with distilled water to 25 times of its volume.
- Shake and add 20 ml of distilled water shake again and allow to stand.
- Filter after 5 minutes.
- Add to a part of the filtrate an equal volume of picric acid reagent (saturated solution of picric acid solution) and observe.

White cloudiness shows the presence of gelatin in the milk. Yellow Precipitate indicates a large amount of gelatin added. Transparent yellow solution indicates absence of gelatin.

7. Addition of Urea

- Take 5 ml of milk sample in 50 ml of conical flask.
- Add 5 ml of sodium acetate buffer or 24% Trichloroacetic acid solution and heat for 3 min in boiling water bath (no heating if Trichloroacetic acid is used).
- Filter the precipitates through a whatman no 42. Filter paper and collect 1 ml of filtrate in a test tube.
• Add one ml of sodium hydroxide solution (2% solution) to the filtrate, followed by 0.5 ml of sodium hypochloride solution (2% solution), mix thoroughly and finally add 0.5 ml of 5% (W/V) phenol solution and observe.

A characteristic blue or bluish green colour in the filtrate from the milk with extraneous urea indicate the presence of urea. Colourless indicate no urea added. This will detect even 0.1 percent of urea addition.


• Take 1 ml of milk in a test tube.
• Add 0.5 ml of sodium hydroxide (2%) solution and 0.5 ml of sodium hypochloride solution (2%) and mix thoroughly.
• To the solution add 0.5 ml of phenol solution (5%) and heat for 20 seconds in a boiling water bath, and observe.

A bluish colour immediately forms, which turns deep blue after wards, in the sample of milk having added ammonium sulphate. In case of pure milk only a salmon pink colour forms which gradually changes to bluish in course about 2 hours, even 0.1 % addition of ammonium sulphate can be detected by this method.

9. Addition of Glucose

• Take 1 ml of milk sample in a test tube.
• Add 1 ml of Bar foed’s reagent.
• Heat the mixture for 3 minutes in boiling water bath and cool for 3 min under tap water.
• Add one ml of phosphomolybdic acid reagent to the turbid solution and observe.

Immediate formation of deep blue colour indicates the presence of extraneous glucose, which is stable for 24 hours. In case of pure milk only faint bluish colour due to diluted barfoeds reagent appears. But this methods as low as 0.05% extraneous glucose in milk can be detected.

Note

**Barfoeds Reagent** : dissolve 24 gms of cupric acetate in 450 ml of boiled distilled water. (If precipitate forms do not filter) add immediately 25 ml of 8.5% lactic acid to the hot solution. Shake to dissolved precipitate, cool and dilute 500 ml and after sedimentation filter the impurities.
**Phosphomolybdic acid reagent**: To 35 gms of ammonium molybdate add 5 gms of sodium tungstate. Add 200 ml of 10% (W/V) sodium hydroxide solution and 200 ml of distilled water. Boil vigorously for 20 – 60 minutes so as to remove nearly the whole of ammonia. Cool, dilute to about 350 ml and add 125 ml concentrated (85%) phosphoric acid. Dilute to 500 ml.

### 3.2 Preservatives in Milk – their Detection

Micro organisms are susceptible to the action of chemicals which either check their growth or destroy the organisms and then keep the milk for a longer time. There are many substances of this sort and are known as preservatives. Antiseptics, disinfectants, germicides etc. boric acid, borax, formalin, benzoic acid, salicylic acid, hydrogen peroxide, β – naphthol potassium nitrate and other chemicals have been used from time to time for the purpose of prolonging the keeping quality of milk sold for human consumption. This practice is reprehensible as well as illegal.

The use of various chlorine preparations for the sterilization of dairy equipment and milk bottles etc is legitimate application of the principles of chemical preservation or sterilization as there is no direct addition of chemical to milk. Moreover chlorine solution will not destroy the micro organisms in milk unless they are present in enough quantities that the milk itself is changed so much in favour and appearance that it will be unacceptable as food. Sodium carbonate or sodium bicarbonate are some times added to milk to reduce the acidity of milk (lactic acid formed as a result of lactic fermentation).

Addition of chemicals is illegal, therefore, common methods to detect their addition is important.

**1. Boric Acid or Borax**

- Take 5 ml of milk in a test tube.
- Add 1 ml of concentrated hydrochloric acid and mix well.
- Dip a strip of turmeric paper in the acidified milk.
- Dry the filter paper immediately and note the change in colour.

Turmeric paper turns red if boric acid or its salts are present.

**2. Carbonates / Bicarbonates**

- Take 10 ml of milk in a test tube.
- Add 10 ml of alcohol and shake well.
- Add 3 drops of aqueous solution of rosalic acid (1%)
• Mix well and observe the change of colour.

Rose red colour indicates presence of carbonate / bicarbonate in the milk only brownish colour indicates absence of carbonate / bicarbonate.

3. Formalin

There are two tests

I. Hehnes Test

• Take 10 ml of milk in a test tube.
• Add 0.5 ml of 1% ferric chloride solution.
• Add carefully about 5 ml of concentrated sulphuric acid down the side of the test tube in such a way that it forms a separate layer at the bottom without mixing with milk.
• Observe the colour of the ring formed at the junction of the two liquids.

II. Leech Test

• Take 5 ml of milk in a test tube
• Add to it equal volume of concentrate hydrochloric acid containing 1 ml of 10% ferric chloride solution to each 500 ml of the acid.
• Heat over a flame for 5 minutes.
• Rotate the tube to break up the curd and observe the colour.
Violet colour indicate presence of formaldehyde.

4. Hydrogen Peroxide

• Take 10 ml of a sample of milk in a test tube.
• Add 2 drops of paraphenylene diamine hydrochloride solution, mix thoroughly and observe.
Development of an intense blue colour indicates presence of hydrogen peroxide.

5. Salicylic Acid

Mercuric nitrate is added to the milk and milk is filtered. If much salicylic acid is present the filtrate attains a red colour after some time.
6. Benzoic Acid

About 20 gms of milk is treated with equal volume of concentrated hydrochloric acid until the curd dissolves. It is now allowed to cool. About 25 ml of a mixture of either and petroleum ether is added to the mixture of milk and precipitated with a drop of ammonium hydroxide in the presence of benzoic acid.

7. β–Naphthol

Milk is extracted with chloroform and heated with potassium hydroxide for 5 min. If a deep blue colour appears it indicates the presence of β–naphthol.

3.3 Adulteration of Buffalo Milk with Cow Milk Hamsa Test

Buffalo milk is richer than cow milk in almost all the constituents. Hence watered buffalo milk is used as an adulterant of cow milk. Hamsa test is used to detect this type of adulteration.

Materials: Hamsa Test serum

• Clean glass slides
• Ordinary pipettes which delivers about 20 drops to a milli litre.
• Tooth picks or any clean thin sticks.
• A few glass test tubes of 15 – 20 ml capacity.
• Pure cow milk (a few ml)
• Pure buffalo milk (a few ml)

Procedure: In two test tubes place 9 ml each of tap water.

• In one tube, market ‘C’ add one ml of pure cow milk and mix well.
• In another test, mark ‘B’ add one ml of pure buffalo milk and mix well.
• Place one drop of diluted cow milk from tube ‘C’ and one drop from tube ‘B’ separately on a glass slide.
• Now place one drop of Hamsa test serum on each of these drops and mix well with tooth pick.
• Start a stop watch. At the end of 30 seconds, observe big coagulated and like particles in B while C will remain milky.
If the unknown sample is tested, if curd particles appear, the sample is contaminated with buffalo milk. If it is milky and opaque, no buffalo milk is added.

This test is affected by any preservatives added to the milk in normal concentrations. The hamsa test serum is effective, if it is stored at 3 – 5°C at all times.

### 3:4 Effects of adulterants and Preservatives on human health

The National Survey on Milk Adulteration 2011, a snap shot survey, was conducted to check the contaminants in milk, especially liquid milk, throughout the country. The study found that due to lack of hygiene and sanitation in milk handling and packaging, detergents (used during cleaning operations) are not washed properly and find their way into the milk. Other contaminants like urea, starch, glucose, formalin along with detergent are used as adulterants. These adulterants are used to increase the thickness and viscosity of the milk as well as to preserve it for a longer period. The study notes that the consumption of milk with detergents in hazardous to health. About eight per cent samples were found to have detergents.

Milk is most commonly diluted with water - this not only reduces its nutritional value, but contaminated water can also cause additional health problems.

The other adulterants used are mainly starch, sodium hydroxide (caustic soda), sugar, urea, hydrated lime, sodium carbonate, formalin, and ammonium sulfate.

The Indian Council of Medical Research has reported that “milk adulterants have hazardous health effects. The detergent in milk can cause food poisoning and other gastrointestinal complications. Its high alkaline level can also damage body tissue and destroy proteins. Other synthetic components can cause impairments, heart problems, cancer or even death. While the immediate effect of drinking milk adulterated with urea, caustic soda and formalin is gastroenteritis, the long-term effects are far more serious.”

Urea can lead to vomiting, nausea and gastritis. Urea is particularly harmful for the kidneys, and caustic soda can be dangerous for people suffering from hypertension and heart ailments.

Formalin can cause more severe damage to the body like liver damage. The health impact of drinking milk adulterated with these chemicals is worse for children. Caustic soda harms the mucosa of the food pipe, especially in kids. The chemical which contains sodium, can act as slow poison for those suffering
from hypertension and heart ailments.

To avoid these dangers, it is best to buy milk from a renowned source. For those who can, buying milk sold by reputed companies in tetra packs is also a good option.

**Water, most common adulterant**

Water turned out to be the most common adulterant in milk. It reduces the nutritional value of milk. If contaminated (with pesticides, heavy metals), water poses a health risk to consumers.

Of the total non-compliant samples, the highest, nearly 46 per cent, belonged to the category of low Solid Not Fat (SNF) and this was due to dilution of milk with water. About eight per cent samples were found to have detergents.

Skimmed milk powder was present in nearly 54.8 samples, out of which 477 samples contained glucose.

**Summary**

The methods to detect various adulterants in milk i.e. water removal of fat cane sugar, starch, skim milk powder, gelatin, urea, glucose and ammonium sulphate were explained in detail. The various preservatives used in milk and the technique to detect them were explained. The hansa test used to detect adulteration of buffalo milk with cow milk explained.

**Short Answer Type Questions**

1. Mention the various adulterants used in milk.
2. Give the formulae used to calculate the % of water added in milk.
3. What are the different method of detection of adultration of milk with water?
4. How gelatin in milk is detected?
5. What is starch test?
6. How ammonia in milk is detected?
7. What are the chemicals generally used to preserve the quality of milk?
8. Mention the tests used to detect formalin in milk.
9. What is Hansa test?
Long Answer Type Questions

1. How water adulteration in milk is estimated.
2. Explain the methods used to detect starch, sugar, skim milk powder in milk.
3. Explain briefly about detection of preservatives in milk.
4. Explain in details about Hansa test.
4.1 Types of Micro-Organisms present in Milk

Genus streptococcus organisms are Gram positive, spherical or ovoid and non motile. Carbohydrate fermentation is homofermentative with dextrorotatory lactic acid as the dominant end product. Carbon dioxide is produced either in very small quantities or not at all from sugar fermentation. Ethanol, acetic acid and formic acid may be produced in appreciable quantities from glucose, of allowed to ferment in alkaline media. Many of the streptococci oxidizes a number of alcohols, glycols and short chain fatty acids.

All streptococcian are fastidious with respect to their nutritional requirement as they require a number of B vitamins and amino acids for growth. Sherman (1937) has divided streptococci into four groups viz., pyogenic group, viridians group, entrococcus group and lactic group. All streptococci except viridians group possess a serologically active, group specific ‘C’ substance.
(polysaccharide). The following table gives certain characteristics on the basis of which the streptococci can be placed into one of the four groups as pyogenic (A B C D E F C H antigens) viridans (no group specific antigens demonstrated), enterococcus (D antigens) and lactic group (N antigens)

<table>
<thead>
<tr>
<th>Test</th>
<th>Pyogenic</th>
<th>Viridans</th>
<th>Enterococcus</th>
<th>Lactic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at -10°C</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at -45°C</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 6.5% NaCl both</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth at pH 9.6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 0.1% methylene blue</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ammonia from arginine</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reduction of litmus before curding</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine decarboxylation</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Survival at 60°C for 30 min</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Haemolysis</td>
<td>β</td>
<td>α or γ</td>
<td>α, β or γ</td>
<td>γ</td>
</tr>
</tbody>
</table>

**Morphological, cultural and biochemical identification of pyogenic group of streptococci**

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Pyogenes</th>
<th>S. agalactiae</th>
<th>S. dysgalactiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>G + ve cocci in pairs or chains</td>
<td>G + ve cocci</td>
<td>G + ve cocci</td>
</tr>
<tr>
<td>Size</td>
<td>0.6 - 1.0 μ</td>
<td>0.6 - 1.2 μ</td>
<td>&lt; 2 μ</td>
</tr>
<tr>
<td>Motility</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Agar Colonies</td>
<td>Mucoid malt and glossy variants.</td>
<td>Mucoid malt</td>
<td>Mucoid malt</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Litmus milk</td>
<td>Acid</td>
<td>Acid followed by curdling</td>
<td>Acid reaction with coagulation</td>
</tr>
<tr>
<td>Getatin Liquefaction</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 10% bile</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 40% bile</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Optimum temp</td>
<td>37°C</td>
<td>37°C</td>
<td>37°C</td>
</tr>
<tr>
<td>Haemolysis</td>
<td>β</td>
<td>α β</td>
<td>α</td>
</tr>
<tr>
<td>Polysaccharic antigen acid from</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>- Glycerol</td>
<td>-</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>- Lactose</td>
<td>=</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>- Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Mannitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Salicin</td>
<td>+</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>- Sorbitol</td>
<td>-</td>
<td>-</td>
<td>V</td>
</tr>
<tr>
<td>- Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VP reaction</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>-</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>camp test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bile solubility</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Positive reaction - = Negative reaction V = Variable reaction
Morphological, cultural and biochemical identification of varians groups of species.

<table>
<thead>
<tr>
<th>Test</th>
<th>S. bovis</th>
<th>S. thermophilus</th>
<th>S. uberis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Staining</td>
<td>Gram +ve cocci in pairs or chains</td>
<td>Gram + ve cocci in pairs or long chains</td>
<td>G + ve cocci in pairs or chains</td>
</tr>
<tr>
<td>Size</td>
<td>0.8 - 1.0µ</td>
<td>0.7 - 0.9µ</td>
<td>Moderate</td>
</tr>
<tr>
<td>Hermolysis</td>
<td>α</td>
<td>Weak γ</td>
<td>α / γ</td>
</tr>
<tr>
<td>Litmus Milk</td>
<td>Acid</td>
<td>Acid curding</td>
<td>No Acid</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 10% bill</td>
<td>?</td>
<td>-</td>
<td>?</td>
</tr>
<tr>
<td>Growth in 40% bill</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Optimum temperature</td>
<td>37°C</td>
<td>40 - 50°C</td>
<td>35 - 37°C</td>
</tr>
<tr>
<td>Polysaccharide Antigen</td>
<td>D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid from</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Arabinose</td>
<td>V</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>- Glycerol</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>- Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Mannitol</td>
<td>V</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>- Salicin</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>- Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Trehalose</td>
<td>V</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>- Maltose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hippurate Hydrolysis</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Camp Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bile Solubility</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Morphological Cultural and Biochemical Identification of enterococcus group of streptococci

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Faecalis</th>
<th>S. Durans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Staining</td>
<td>G + ve cocci, ovoid pairs or chains</td>
<td>G + ve cocci, spherical to avoid, pairs or chains.</td>
</tr>
<tr>
<td>Size</td>
<td>0.5 - 1.0 μ</td>
<td>0.5 - 1.0 μ</td>
</tr>
<tr>
<td>Haemolysis</td>
<td>α</td>
<td>β</td>
</tr>
<tr>
<td>Motility</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 40% bill</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Litmus milk</td>
<td>Acidified Curdled</td>
<td>Acidified, Curdled</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 0.04% tellurite</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Optimum</td>
<td>37°C</td>
<td>37°C</td>
</tr>
<tr>
<td>Acid from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>V</td>
</tr>
<tr>
<td>Mannitol</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aesculin Hydrolysis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>V</td>
<td>V</td>
</tr>
</tbody>
</table>
### Distinctive characters of varieties of S. faccalis

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Faecalis var faecalis</th>
<th>S. Faecalis var liquefaciens</th>
<th>S. Faecalis var Zymogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin liquefaction</td>
<td>-</td>
<td>+</td>
<td>-/+</td>
</tr>
<tr>
<td>Litmus milk</td>
<td>Acidified curdled</td>
<td>Acidified curdled peptonization</td>
<td>Acidified, curdle no peptonization</td>
</tr>
<tr>
<td>Haemolysis</td>
<td>α/β</td>
<td>α or β</td>
<td>β</td>
</tr>
</tbody>
</table>

### Morphological cultural and biochemical identification of lactic group of streptococci

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Lactis</th>
<th>S. Lactis subsp diacetylactis</th>
<th>S. Cremoris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Staining</td>
<td>G + ve cocci ovoid cells in pairs or short chains</td>
<td>G + ve cocci ovoid cells in pairs or short chains</td>
<td>G + ve cocci spheres or ovoid in long chains</td>
</tr>
<tr>
<td>Size</td>
<td>0.5 - 1.0 μ</td>
<td>0.5 - 1.0 μ</td>
<td>0.6 - 1.0 μ</td>
</tr>
<tr>
<td>Harmolysis</td>
<td>Weak α or γ</td>
<td>Weak α or γ</td>
<td>α or γ</td>
</tr>
<tr>
<td>Litmus milk</td>
<td>Reduced before curdling</td>
<td>Reduced before curdling</td>
<td>Reduced before curdling</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Optimum temperature</td>
<td>30°C</td>
<td>30°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Co₂ and diacetyl from citrate</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Acid from</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Maltose</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>- Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
4.1.1 Lactobacillus Group

These are gram positive rods typically, non-motile, non sporulating and non acid fast. Lactobacilli are aerobic and facultatively anaerobic, catalase negative, and grow best at pH 6.0. The carbohydrates and poly alcohols are changed by homofermentation to lactic acids or by hetero fermentation to lactic and acetic acids, alcohols and carbondioxide. Surface growth is enhanced on enriched media and under anaerobic conditions with added Co₂ (5 – 10%). The genus lactobacillus is subdivided into three groups. i.e. Thermobacterium, Strep to bacterium and Beta Bacterium.

Differential characters of the three groups of lactobacillus.

<table>
<thead>
<tr>
<th>Test</th>
<th>Thermobacterium</th>
<th>Streptobacterium</th>
<th>Beta bacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 5°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 15°C</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 45°C</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Gas from glucose</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acid from</td>
<td>-</td>
<td>v</td>
<td>v</td>
</tr>
</tbody>
</table>
### Morphological and cultural identification of lactobacillus subgenus *Thermobacterium*

<table>
<thead>
<tr>
<th>Test</th>
<th><em>Thermobacterium</em></th>
<th><em>Streptobacterium</em></th>
<th><em>Beta bacterium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Arabinose</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Melezitose</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>- Salicin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Voges-proskauer reaction</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate Reduction</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fermentation</td>
<td>Homofermentation</td>
<td>Homofermentation</td>
<td>Homofermentation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Lactis</th>
<th>L. Bulgaricus</th>
<th>L. Helviticus</th>
<th>L. Acidophilus</th>
<th>L. Thermophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>G + ve rods long, singly or in pairs</td>
<td>G + ve rods slender with rounded ends in chains</td>
<td>G + ve singly or in pairs</td>
<td>G + ve rods singly, pairs short chains</td>
<td>G + ve rods</td>
</tr>
<tr>
<td>Size</td>
<td>0.5 - 0.8 x 2.9μ</td>
<td>0.5 - 0.8 x 2.9μ</td>
<td>0.7 - 0.9 x 2 - 0 to 6.0μ</td>
<td>0.6 - 0.9 x 1.5 - 6.0μ</td>
<td>0.5 x 3.0μ</td>
</tr>
<tr>
<td>Lactic acid form produced</td>
<td>D (-)</td>
<td>D (-)</td>
<td>D, L</td>
<td>D, L</td>
<td>D, L</td>
</tr>
<tr>
<td>Litmus milk</td>
<td>Acid followed by coagulation</td>
<td>Coagulation at 37°C no</td>
<td>Acid with coagulation may</td>
<td>Coagulates from bottom up</td>
<td>Acid Small colonies</td>
</tr>
</tbody>
</table>
### Biochemical Identification of Lactobacillus subgenus thermobacterium species

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Lactis</th>
<th>L. Bulgaricus</th>
<th>L. Helviticus</th>
<th>L. Acidophilus</th>
<th>L. Thermophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agar colonies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rough, 1-3 mm in diameter, non pigment, white to grey</td>
<td>Rough</td>
<td>Flat, 2-3 mm</td>
<td>Rough to Rhizoid 2-3 mm in diameter</td>
<td>Rough with no pigment</td>
<td>Small colonies</td>
</tr>
<tr>
<td><strong>Growth at 15°C</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Growth at 45°C</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Opt. Temp.</strong></td>
<td>40 - 45°C</td>
<td>40°C</td>
<td>40-42°C</td>
<td>35- 38°C</td>
<td>50- 62.8°C</td>
</tr>
<tr>
<td><strong>Aetachromatic granules</strong></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Biochemical Identification of Lactobacillus subgenus thermobacterium species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Lactis</th>
<th>L. Bulgaricus</th>
<th>L. Helviticus</th>
<th>L. Acidophilus</th>
<th>L. Thermophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas from glucose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid from - Arabinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Morphological and cultural identification of lactobacillus subgenus streptobacterium species

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Lactis</th>
<th>L. Bulgaris</th>
<th>L. Helviticus</th>
<th>L. Acidophilus</th>
<th>L. Therphilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Maltose</td>
<td>+</td>
<td>v</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Mannitol</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Malibiose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>-</td>
</tr>
<tr>
<td>- Melezitose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Reffinose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td>- Salcin</td>
<td>v</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>- Sorbitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Trehalose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arginine hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Casei</th>
<th>L. Plantarum</th>
<th>L. Curvatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>G + ve rods short or long chains of short or long rods.</td>
<td>G + ve rods, singly or in chains with rounded ends.</td>
<td>G +ve rods single in pairs</td>
</tr>
<tr>
<td>Size</td>
<td>0.7 - 1.1 x 2.0 - 4.0μ</td>
<td>0.7 - 1.0 x 3.0 - 8.0μ</td>
<td>0.7 - 0.9 x 1.0 - 2.0 μ</td>
</tr>
</tbody>
</table>
Biochemical identification of Lactobacillus subgenus thermobacterium species

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Casei</th>
<th>L. Plantarum</th>
<th>L. Curvatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid produced (from)</td>
<td>L(+), D (-)</td>
<td>D, L</td>
<td>D, L</td>
</tr>
<tr>
<td>Litmus milk</td>
<td>Acid coagulation in 3-5 days, may become slimy.</td>
<td>Acid coagulated</td>
<td>Acid coagulation</td>
</tr>
<tr>
<td>Agar colonies</td>
<td>White to light yellow</td>
<td>White to light or dark yellow</td>
<td>Smaller but similar to L. plantarum</td>
</tr>
<tr>
<td>Growth at 15°C</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 45°C</td>
<td>v</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Opt. Temperature</td>
<td>30°C</td>
<td>30 - 35°C</td>
<td>30 - 37°C</td>
</tr>
</tbody>
</table>

Biochemical identification of Lactobacillus subgenus thermobacterium species

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Casei</th>
<th>L. Plantalum</th>
<th>L. Curvatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas from glucose</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid from</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Arabinose</td>
<td>-</td>
<td>v</td>
<td>v</td>
</tr>
<tr>
<td>- Galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Lactose</td>
<td>v</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Mannitol</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>- Melezitose</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>- Melibiose</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Raffinose</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Salicin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>- Sorbitol</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Morphological and cultural identification of lactobacillus subgenus beta bacterium species

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Casei</th>
<th>L. Plantalum</th>
<th>L. Curvatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Trehalose</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aasculin hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate Reduction</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arginine hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gram Staining</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>0.7 - 1.0 x 2.0 - 4.0 μ</td>
<td>0.5 - 1.0 x 0.3 - 15.0 μ</td>
<td></td>
</tr>
<tr>
<td>Lactic acid produced (from)</td>
<td>D, L</td>
<td>D, L</td>
<td></td>
</tr>
<tr>
<td>Litmus milk</td>
<td>Acid but no clot</td>
<td>Unchanged</td>
<td></td>
</tr>
<tr>
<td>Agar colonies</td>
<td>Flat, rough, often translucent</td>
<td>Flat, circular or irregular to rough, often translucent</td>
<td></td>
</tr>
<tr>
<td>Growth at 15°C</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Growth at 45°C</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Optimum temperature</td>
<td>30°C</td>
<td>41 - 42°C</td>
<td></td>
</tr>
</tbody>
</table>
Biochemical identification of lactobacillus subgenus beta bacterium species

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Brevis</th>
<th>L. Fermentum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas from Glucose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Arabinose</td>
<td>+</td>
<td>v</td>
</tr>
<tr>
<td>- Galactose</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td>- Lactose</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td>- Maltose</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td>- Mannitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Melizitose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Melibiose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Raffinose</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td>- Salicin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Sorbitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Trehalose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate Reduction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arginine hydrolysis</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

V = Variable Reaction  + = Positive Reaction  - = Negative reaction.

4.1.2 Leuconostoc Group

The genus consists of gram positive cocci in pairs or short chains which are micro aerophillic and heterofermentative i.e. glucose is fermented with production of D (-) lactic acid, ethanol and CO₂. Certain types grow with a characteristic slime production in sucrose media. They generally grow on ordinary culture media, but growth is enhanced by the addition of yeast. Tomato or other vegetable extracts. These species are generally found in milk and plants juices.
Differential Characterization of leuconostoc species

<table>
<thead>
<tr>
<th>Test</th>
<th>Leuco. Dextranicum</th>
<th>Leuco. mesenteroides</th>
<th>Leuco. Paramesenteroides</th>
<th>Leuco. lactis</th>
<th>Leuco. cremoris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran production from sucrose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid from</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>- Trehalose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Arabinose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Survival at 55°C/30min</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Amino acid requirement</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth in salt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 3%</td>
<td>+ (slow)</td>
<td>+</td>
<td>+ (slow)</td>
<td>+ (slow)</td>
<td>-</td>
</tr>
<tr>
<td>- 6.5%</td>
<td>-</td>
<td>+ (slow)</td>
<td>+ (slow)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

4.1.3 Characteristics of other Bacteria in Milk

(Genera Escherichia)

The presence of this organism in food is of considerable public health significance, as the occurrence indicates faecal contamination. Escherichia coli are rods occurs singly, in pairs and in short chains. The agar colonies are white, entire to undulate and moist. Litmus milk gets coagulated with rapid acid production and gas. Ferments glucose, lactose, manitol and xyloze and do not ferment sucrose, salicin and glycerol. Citric acid and salts of citric acid are not
utilized by this organism. The optimum growth is at 30 – 37°C, but also grow at 10°C and 45°C as well. The Imvic test (Indole production, methylred, voges proskar and citrate utilization) is + ++ - -. The selective media used for isolation is Eosin-Methylene blue (EMB) agar or violet red Bile agar (VBRA).

**Genus Enterobacter**

These are non faecal origin. There are two species i.e. Enterobacter Aerogenes and Enterobacter cloacae. The Imvic test is - - + + . All other characters are just like escherichia.

**Genus Shigella**

These are extremely pathogenic type and causes acute food borne illness like desentry. The organisms are Shigella dysenterae, sh. Flesneri ad sh. + Sonnei. These are gram negative rods.

**Genus Salmonella**

These organisms will cause a variety of food- infections and illnesses. These are gram negative rod, motile by peritrichous flagella, unable to liquify gelatin and hydrolyse urea, Indole negative, methyl red positive and voges proskauer negative. The various organisms are Salmonella typhosa, paratyphi, S.typhimurium, S. Enteridis, S.Schottmuellri, S.Hirschfeidii.

**Genus Yersinia**

The various organisms are Yersinia enterocolitica, Y. pestis, Y.pseudotuberculosis

**Genus Klebsiella**

Gram negative rods which are plump with rounded ends and are non motile. These are encapsulated in mucoid stage. The organism is Klebsiella pneumonia and causes pneumonia. The optimum temperature for growth is 37°C.

**Genus Proteus**

Straight rods which are motile. The species are proteus mirabilis and P. Valgaris which produce amoeboid colonies that show a swarming phenomenon on solid media. Urea is hydrolyzed and glucose and other carbohydrates are fermented with production of acid and gas.

**Genus Pseudomonas**

These organisms develop florescent, diffusible pigments of greenish, bluish, violet, lilac, yellow or other colour. The important pathogenic species is
pseudomonas aeruginosa which forms greenish blue pigment (pyocyanin). The other species are ps. Fluorescence, ps. Fragi, ps. putida, ps. Putrefaciens are associated with certain defects in milk and milk products.

**Genus Vibrio**

These are curved, comma shaped rods, which do not attack cellulose. They grow well and rapidly on the surfaces of standard culture media and are heterotrophic organisms. Vibrio cholera is invariably associated with cholera.

**Genus Aeromonas**

These are short rod shape of gram negative cells and are heterotrophic. Carbohydrates are fermented with the production of $H_2$, $CO_2$ and 2,3 butylene glycol. They grow best at 25 – 30°C.

**Genus Chromo Bacterium**

These produce violet pigment soluble in alcohol. They grow at 37°C.

**Genus Flavobacterium**

These are gram negative motile rods. These produce yellow, orange, red or yellow brown pigmentation due to water soluble carotinoid pigment.

**Genus Brucella**

The important species are Brucella abortus, B. Melitensis and Brucella suis. These are aerobic, Gram negative, non motile rods. Gas is not produced from carbohydrates and gelatin is not liquified. They grow at 37°C. the agar colonies are small, circular and convex.

**Genus Micro Cocci**

These cells are in irregular and some are motile. Their growth in agar is abundant. The species are micro coccus luteus and M. varians. Voges proskauer’s test is negative. These are Gram positive cocci.

**Genus Staphylococci**

These are spherical cells occurring singly in pairs, in tetrads and in irregular clusters. The cocci are rather smaller than the micro cocci. They are non motile. The species are staphylococcus aureus, staph, Epidermis. These are Gram positive cocci occurring singe. Pairs or short chain, non motile.
Genus Listeria

These are small rods, motile by peritrichous flagella, Gram positive, grow freely on ordinary media, and produce acid and no gas from glucose. The species is Listeria monocytogenes. It causes food poisoning. Optimum growth temperature is 37°C. It can survive in 20% NaCl Solution at 4°C for eight weeks.

Genus Bacillus

These are saprophytes and found in soil and widely distributed in nature. The important species are Bacillus cereus and B. anthracis causes milk borne illness. Other organisms are B. Stearothermophilus. B. Megaterium which causes spoilage or some changes in milk. These are gram positive rods and spore formers. Optimum temperature of growth in around 30 – 35°C except B. Stearothermophilus which grows between 55 – 65°C.

Genus Clostridium

These are mostly found in soil and the intestinal tract of man and other animals. These are anaerobic spore formers and cause problem in canned milk products. The important organisms are clostridium butycricum and Cl. Tyrobutyricum. These are thermophiles. These two organisms may cause late blowing in cheese. Other organisms like Cl. Botulinum, Cl- perfringens are classical food pathogens causing gastro intestinal disturbances and neurological disorders. These are gram positive, non, motile and the spores are produced located centrally or eccentrically giving bulging appearance to the cells. These are also responsible for butyric and saccharolytic fermentations.

Genus Mycobacterium

These are acid fast, slender rods, straight or slightly curved, classical test for these organisms is demonstration of acid fastness by carbol fuchsin or ziel neelsen method. Cells are non motile but aerobic. The organisms are M. tuberculosis, and M. Bovis, the former is human bacilli and the latter is bovine bacilli. These are gram positive and optimum growth art 37°C ranging from 20-40°C. These are pathogenic and cause tuberculosis disease.

Genus Microbacterium

These are small rods with round ends, non-motile, granulour and grow media supplemented with milk or yeast extract. These are thermoduric
saprophytes. Optimum temperature for growth is 32°C the important organism are M. Lacticus.

**Genus Brevibacterium**

These are gram positive, varying from a quite short coccus to long straight and unbranched rods. The organism is B. Linens. The optimum temperature is 21°C.

**Genus Propionibacteriaceae**

These are gram positive, irregularly shaped cells and non motile. These grow under anaerobic conditions. These are catalase positive. Organisms are propionibacteria freudenreichii and p. shermanii.

**Genus Coxiella**

Organism is Coxiella berneti causes ‘Q’ fever. This organism is heat resistant and now this organism as index organism for pasteurization.

**Genus Actinomyces**

The organism is Actinomyces bovis, gram positive, non acid fast, non-proteolytic. Optimum temperature is 37°C. Litmus milk is not coagulated.

### 4.1.4 Yeasts and Moulds

**Yeast**

Yeast are gram positive, unicellular, non motile, ovoid or elliptical cells, whose size is bigger than bacteria. The common method of reproduction is budding although some produce ascospores. The growth temperature ranges from 25 – 40°C. They can tolerate high acidities(pH 3.5) and are fermentative or oxidative in their metabolism of carbohydrates. Few species are lipolytic in nature. The yeasts commonly associated with milk and milk products are given below.

1. **Saccharomyces species / kluyveromyces species**

The common species are Saccharomyces cerevisae, S. fragilis, S.Lactis, S. delbrueckii etc. S.fragilis and L. Lactis are also known as Kluyveromyces fragilis and kluyveromyces lactis.

S. cerevisae is generally used in brewing and baking industries. Cells may appear as spherical, ovoid, cylindrical in malt extract broth and occur as
single or in pairs. Ascospores are formed. It ferments glucose but it is unable to ferment lactose. Kluyveromyces fragilis is an ovoid to elongated organism and forms white glistening colonies on malt extract agar. It occurs singly, in pairs, or in short chains. Lactose is fermented to alcohol and carbondioxide which forms the bases of its use in the manufacture of kumiss and kefir. It produces acid and gas in litmus milk but does not peptonize it. The optimum temperature of growth is 37°C and does not grow at 5°C or 43°C. Kluyveromyces lactis forms spherical, cylindrical cells in single, pairs or clusters. It is capable of fermenting galactose and lactose. It has been isolated from cheese and milk.

2. Candida Species

Different species of candida have been isolated from butter, margarine, cheese, kefir and sweetened condensed milk. Some of the species cause yeasty or gassy cream with a high acidity, foaming and yeasty odour. The important species are C. pseudotropicalis, c. Lipolytica, C. mycoderma, C.kefir etc. These are bio chemically oxidative in nature rather than fermentative. The cells are usually cylindrical and form pellicle on the surface of liquid media as they are strictly aerobi. C. pseudotropicalis forms ovoid to elongated cells, ferments lactose with the production of alcohol and carbondioxide and produces gas in litmus milk. Optimum temperature of growth is 37°C.

C. Lipolytica also forms ovoid to elongated cells. It can hydrolyze fat and produces lipolytic enzymes but is unstable to ferment sugar. C. kefir is capable of fermenting glucose and lactose.

3. Torulopsis Species

The various species are T. Holmii, T. Lactis ondensi, T. spherical and t. gibso. Cells are ovoid in shape. These species can usually ferment glucose but not lactose.

Moulds

Commercial application of moulds in food and chemical industry is going on. However moulds are capable of producing extremely toxic components in foods including milk and milk products, which can pose serious problems to the consumer. Moulds are multicelullar, aerobic organism capable of growth over wide range of pH and temperature. The colonies appear cottony or wooly and are generally white, creamy, green or black, mold spores are reproductive. Generally these are found in soil, barn dust, feeds, manure and unclean utensils. Moulds can grow on malt extract, potato dextiose or Rose Bengal chloram phenicol agar and can be identified on the basis of morphological characters.
The important moulds in dairy industry are.

1. The Penicillium Species

The penicillium species usually form blue-green spreading colonies eg. P. roqueforte, which is used in the manufacture of Roqueforte cheese and blue veined cheese. It is mainly responsible for characteristic flavour and appearance of these cheeses. Another species namely p. camemberti is used in the manufacture of camembert and bric cheeses. Its colonies are pale grayish-green in colour Penicillium species bears conidiospores which are globose and smooth in shape.
2. Rhizophus Species

Rhizophus species are present in food stuffs like bread and dairy products. The mycelium produces stolons and at points of attachment of stolons, rhizoids (downward) and sporangiphores (upwards) arise. Sporangia are white at first which changes to black on ripening. R.stoleniferis one of the common species.

3. Aspergillus Species

Like penicillium, this mould also forms conidiospores but these are borne on sterigmata attached to swollen vesicles. Some species (A.Flavus, A. parasiticus) are able to produce aflotoxins (G1, G2, B1 and B20 in dairy products. The aflotoxins are elaborated in animal feed as well which consequently get secreted as M1 and M2 in milk.

4. Geotrichum Candidum

G. Candidum is known to be responsible for yeasty flavour in dairy products. It is not saccharolytic in nature. Colonies are white in colour and appear yeast like and butyrous. The organism grows on the surface of sour cream as a white mass and oxidizes lactic acid to carbondioxide and water. Arthospires which are cylindrical in shape with rounded ends are formed.

5. Alternaria Species

These species are involved in the discolouration of butter. They bear conidiospores of dark brown colour.

6. Cladosporium Species

Cladosporium is recognized by deep olive green to black colour. The spores usually two celled are produced from mycelium. These are also involved in surface discoloration of butter.

4.2 Milk Borne Diseases (Pathogens)

A variety of micro organisms may gain access into milk and milk products from different sources and causes different types of food borne illnesses. Milk and milk products may carry organisms as such as their toxic metabolites (poisons) called toxins to sensitize consumers. Ingestion of toxins already synthesized in the food (pre-formed) brings about poisoning syndrome and called ‘food intoxication’ and the toxins affecting gastrointestinal tract are called enterotoxins.

On the other hand the ingestion of viable pathogenic bacteria along with the food leads to their lodgements and are termed as ‘food infections’. The other organisms infect intestines when ingested along with food and produce toxins in situ to bring about symptoms of poisoning and called Toxic-infection.
**Common milk borne infection, intoxications and toxic infections.**

<table>
<thead>
<tr>
<th>S.no</th>
<th>Types of milk borne disease</th>
<th>Causative agents</th>
<th>Disease/Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Food infection</td>
<td>Salmonella typhi and related sps.</td>
<td>Typhoid, salmonellosis (Food poisoning)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shigella dysenterae</td>
<td>Shigellosis (Dysentery)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stroptococci sps (enteroccci)</td>
<td>Septic sore throat, scarlet fever, food poisoning.</td>
</tr>
<tr>
<td>B</td>
<td>Food intoxication</td>
<td>Staphylococcus aureus, clostridium botulinum, Escherichia coli, vib cholerae</td>
<td>Food poisoning Botulism (Food poisoning)</td>
</tr>
<tr>
<td></td>
<td>(i) Bacterial</td>
<td>Aspergellus flavus other toxigenic molds</td>
<td>Food poisoning</td>
</tr>
<tr>
<td></td>
<td>(ii) Fungal</td>
<td>Bacillus cereus Clostridium perfringens</td>
<td>Gas gangrene</td>
</tr>
<tr>
<td>C</td>
<td>Toxic infection</td>
<td>Aeromonas Spp proteus Spp Klebsiella Spp Pseudomonas Spp Citrobacter Spp</td>
<td>Food poisoning Food poisoning Food poisoning Food poisoning Food poisoning</td>
</tr>
<tr>
<td></td>
<td>Other milk borne disorders (uncertain pathogenesis)</td>
<td>Listeria monocytogenes Yersinia enterocolitica Campylobacter jejuni Vibrio parahaemolyticus Mycobacterium tuberculosis</td>
<td>Listeriosis Diarrhoeal diseases Diarrhoeal diseases Diarrhoeal diseases</td>
</tr>
<tr>
<td>D</td>
<td>New emerging pathogens</td>
<td>Brucella abortus Corynebacterium diphtheriae Bacillus anthracis Coxiella bennettii Enteroviruses Infectious hepatitis virus Tick borne encephalitis virus</td>
<td>Tuberculosis Brucellosis Diphtheria Anthrax ‘Q’ Fever Enteric fever Infectious hepatitis Tick born encephalitis</td>
</tr>
<tr>
<td>E</td>
<td>Other milk borne diseases</td>
<td>Foot and mouth disease virus</td>
<td>Foot and mouth disease</td>
</tr>
<tr>
<td></td>
<td>(i) Bacterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(ii) Rickettsial</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(iii) Viral</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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A. Milk Borne Infections

1. Salmonellosis: the causative organisms are

Salmonella typhi : Typhoid
Salmonella paratyphi : Paratyphoid
Salmonella Enteritidis : Food poisoning
Salmonella weltiverdin : Food poisoning

The sources of salmonella organisms into the milk are water, milk handlers, and suffering animals. Typhoid and paratyphoid are non-pathogenic to animals.

The main symptoms of typhoid are

• Continued fever
• Inflammation of intestine, and formation of intestinal ulcers
• Enlargement of spleen
• Characteristic rose spot eruptions on the abdomen and toxanemia.

Symptoms of paratyphoid are

• Resembles typhoid but it is milder.

Salmonella food poisoning symptoms are

• Nausea, vomiting, abdominal pain
• Diarrhoea, chills, head ache,
• Prostration, muscular weakness, drowsiness
• Moderate fever, restlessness

Incubation period varies from 7-14 days for typhoid and 1 – 7 days for paratyphoid. The specific diagnostic test for Salmonellosis is Widal test for typhoid fever.

Prevention and Control

• Adequate treatment of water supply
• Infected individual should not be allowed to handle milk.
• Hygienic conditions during production, processing and all stages.
• Pasteurization of milk.
2. Bacillary dysentery (Shigellosis)

The causative organisms are Shigella dysenteries, Sh. Sonnei, sh. Flexneri.

Sources of organism are through contamination with infected materials like utensils, water flies and milk handlers.

**Important Symptoms are**

- Diarrhoea with blood, pus or mucous
- Fever, abdominal cramps and tenesmus.

Incubation period 1-7 days.

**Prevention and Control measures**

- Rigid sanitary discipline
- Control of flies.

3. Streptococcal infections

Causative agents are

- (a) Streptococcus pyogenes- Scarlet fever, septic sore throat, tonsillitis, septicemia
- (b) Str. Agalactiae – Mastitis in animals (non pathogenic to human)
- (c) Group D streptococci (enterococci): Food poisoning.

**Sources of infection are**

- Animals infected with S. Agalactiae
- Persons concerning care and milking of animal
- Milking machines
- Human carriers of S. pyogenes

**Symptoms**

Septic sore throat – high and irregular fever, and sudden onset of fever

- Inflammation and swelling of lymphnodes of throat and some times absess around tonsils.

Scarlet fever – Acute febrile disease of throat accompanied by scarlet rash.
• Scarlet rash is due to release of toxin

Food poisoning: Resembles staphylococcal food poisoning syndrome which will be milder.

Incubation period is 1–3 days.

**Prevention and Control**

• Adequate heat treatment of milk
• Regular health check of dairy worker.
• Avoiding faecal contamination of milk.

### B. Milk Borne Milk Intoxications

#### 1. Staphylococcal poisoning: Staphylococcus aureus

**Elaborates different types of toxins like**

• Haemolysin (alpha, beta, gamma and delta)
• Leucocidin
• Necrotizing factor
• Enterotoxin
• Coagulase

Among all the above toxins the important toxin in entero toxin, this is heat stable and not destroyed even after boiling for 15 minutes. Sources of organisms are milch animals and human handlers.

**Symptoms are**

• Nausea, Vomiting, abdominal cramps.
• Diarrhoea, sweating, headache and prostration

**Incubation period**: varies 1 – 16 hours.

Diagnosis is by biological, serological methods. Coagulase test and thermonuclease test are also employed.

**Prevention and Control**

• Adequate heating of milk destroys only organisms but not enterotoxin. So heating immediately after production before toxin production is necessary.
• Post pasteurization contamination should be avoided.
• Infected handlers should not be allowed to handle milk
• Mastitis animals should be isolated.

2. Botulism

The botulism poisoning is the severest of all food poisoning as it affects the nervous system and is often extremely fatal. The causative organism is clostridium bitulinum. Several types of toxins are produced i.e. A to G but A, B, E and F affect the human being.

The sources of organisms is soil and water

**Symptoms**

• Nausea, Vomiting, fatigue, dizziness
• Head ache, dryness of skin, mouth and throat.
• As it acts on central nervous system, it leads to paralysis of muscles, double vision and respiratory failure resulting finally into death.

Incubation period is 12-96 hours.

Mortality rate is high.

**Prevention and control**

• Adequate heating of milk and milk products
• Hygienic milk production
• Chilling of milk immediately after production.

3. E. Coli Poisoning

Escherichia coli is known to be associated with Enteritis in infants and adults as well as travellers diarrhoea and food poisoning. Produces two types of toxins i.e. heat labile at and heat stable (ST).

**Sources**

• Water supplies, contaminated with faecal matter
• Unhygienic practices by the handlers
• Infected animals.

**Symptoms**

• Symptoms resembles cholera by ingestion of LT toxin. Massive watery diarrhea.
In ST type of toxin diarrhoea with and without vomiting which is non bloody. Fever in children and not in adults.

**Prevention and control:** Control of Sources

4. Cholera

This is one of the acute diarrhoeal diseases caused by vibrio cholerae. It occurs as massive epidemics and unhygienic practices appears to be chiefly responsible for out break. This is mainly water borne illness. Adulteration of milk with water may be one of the causes for this disease.

Incubation period is few hours to five days.

**Symptoms**

- Diarrhoea, Vomiting
- Rice water stools, abdominal pain
- Thirst, dehydration symptom
- Death even within 12 hours after the appearance of symptoms

**Prevention and Control**

- Proper pasteurization of milk
- Sanitary disposal of human excreta
- Isolation of patient and carrier.

5. Fungal Intoxication

I. Aflotoxicosis: Produced by common mould Aspergellus flavus and A- parasiticus. The toxin is known as Aflotoxin. The toxins B1, B2 and B2a and G1, G2 and G2a. These toxins are heat stable and also corcinogenic.

Symptoms are – Liver hyperplasia, Tissue haemorrhage,

- Anorexia, hepatitis, finally death

II. Other Mycotoxicosis, other moulds produce toxins as follows

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roquefortin</td>
<td>Penicillium roqueforti</td>
</tr>
<tr>
<td>Camembertin</td>
<td>Penicillium camemberti</td>
</tr>
<tr>
<td>Citrinin</td>
<td>P.Citrinin</td>
</tr>
</tbody>
</table>
Penicillic Acid  P. Martensii  
Rubratoxin  P. Rubrum

C. Milk Borne Toxic Infections

1. Clostridium perfringens (welchii) poisoning

   It causes gas gangrene. It is anaerobic organism. Sources are soil, faeces and water supplies.

   Symptoms are Diarrhoea, nausea, abdominal pain

   Incubation period is 8–22 hours

2. Bacillus cereus poisoning

   Bacillus cereus is sporulating aerobic organism. Toxins produced are Haemolysin, lecithinase and enterotoxin. Only enterotoxin causes food poisoning.

   Symptoms are: Nausea, diarrhoea, abdominal pain, incubation period is 6–12 hours.

D. Other Milk Borne Disorders

1. Proteus infection: caused by Proteus vulgaris causes summer diarrhoea. It is easily destroyed by pasteurization.

2. Acromonas Infection: Acromonas hydrophilia causes food poisoning through contaminated water supplies.

3. Citrobacter infection: Produces entertoxins.

4. Klebsiella infection: Causes gastro intestinal illness. Caused by K. pneumoniae which produce heat stable and heat labile toxins which are comparable to E.coli.

5. Pseudomonas infection: Organisms are Ps. Putrefaciens, Ps. Fragi, Ps. Viscose and Ps. Aeruginosa. Of these Ps. Aeruginosa causes food poisoning and also causes urinary tract infection, eye infection, ear infections, abscess meningitis and enteritis in human beings.

E. New Emerging Pathogens

1. Listeriosis: Listeria monocytogenes is G +ve non sporulating, rods, capable of growing at a wide range of temperature (1–45°C). The heat resistance is more and sometimes it survives pasteurization, as these organisms are ingested by leucocytes and gives protection.

   Symptoms: Acute meningitis, with or without septicaemia.
• Fever, nausea, head ache, vomiting, followed by delirium, coma, collapse and shock resulting in death.

Prevention : Avoid human carriers in handling of milk
• Culling of infected animal
• Proper storage condition
• Proper heat treatment in milk.

2. Campylobacter jejuni poisoning (Campylobacteriosis)

Sources : infected water and infected animals and persons.

Symptoms : severe abdominal pain and diarrhoea.

3. Yersiniosis

Organism is Yersinia entercolotica

Symptoms : Abdominal pain, fever, vomiting and diarrhoea.

4. Vibrio para haemolyticus poisoning.

Source is contaminated water supply.

Symptoms : Gastroenteritis, Abdominal cramps, nausea, vomiting, headache, chills and fever.

Incubation period 12 – 24 hours.

F. Other Milk Borne Diseases

1. Bacterial diseases.

(a) Tuberculosis

Organism is Mycobacterium tuberculosis, human type produces pulmonary type of tuberculosis. Bovine type produces non pulmonary type of tuberculosis.

Sources
• Milch animals
• Milk handlers
• Wash water
• Environment
Symptoms: Parenchymal pulmonary infiltration

• Cough, fever, fatigue, loss of weight.

Prevention and Control

• Handlers and animals infected should be screened.
• Proper heat treatment of milk
• Avoid over crowding of animals
• Avoid infected persons in handling of milk.

(b) Brucellosis

Organisms are Br. melitensis (goats) br. Abortus (Cattle) br. Suis (pigs). All these species can infect human beings.

Sources: Diseased animals, secrete organism in milk. Persons handling the milk.

It causes undulant fever in humans.

(c) Diphtheria

Causative organism is Corynebacterium diphtheriae.

Sources of infections are milk handlers and infected animals.

Symptoms: Febrile infection of nose, throat and tonsils followed by inflammation of throat.

• Diphtheria toxin affects kidney, heart muscles resulting death.

(d) Anthrax

Organism is Bacillus anthracis which is spore forming organism. Sources of infection are infected animal and environment. Causes two type i.e. Contaneous and pulmonary types. It causes carbuncle disease in human (contaneous type). Pulmonary type causes pneumonia which may be fatal.

2. Rickettsial disease

(Q) Fever: Organism is Coxiella breneti which is more heat resistant organism. It survives some times pasteurization temperature also.

Symptoms: High fever, head ache, weakness, malaise, severe, sweating and pneumonia.
3. Viral Diseases

(a) Enteroviruses

Causative viruses are a group of viruses causing severe epidemic summer diarrhoea in infants and children.

Symptoms: Gastroenteritis
- Headache, fever, muscle stiffness and paralysis.

(b) Infectious hepatitis

Causes jaundice, which is one of the serious diseases in human beings through contaminated water. Sources are contaminated water, milk handlers and environment.

Symptoms: Nausea, vomiting, lethargy, abdominal pain, diarrhoea fever anorexia, sore throat, bile in urine and jaundice.

(c) Tick borne encephalitis

Caused by arbo virus. Caused through bite of ticks and mites.

Symptoms: Biphasic meningio encephalitis

(d) Foot and Mouth disease.

Causes fever and difficulty in swallowing in human beings.

Microbial Standards of Raw and Pasteurized Milk

The different national and international organizations have given various standards to milk and milk products.

1. Raw Milk

I.S.I. (BIS) Standards

(a) Direct Microscopic Count (DMC)

<table>
<thead>
<tr>
<th>Count per ml</th>
<th>Bacteriological grade</th>
<th>quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5, 00,000</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>5 Lakhs – 4 millions</td>
<td>Fair</td>
<td></td>
</tr>
<tr>
<td>4 – 20 millions</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>&gt;20 millions</td>
<td>Very poor</td>
<td></td>
</tr>
</tbody>
</table>
(b) Standard Plate Count (SPC)

<table>
<thead>
<tr>
<th>Count per ml</th>
<th>Quality / Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2, 00,000</td>
<td>Very Good</td>
</tr>
<tr>
<td>2 Lakhs – 1 million</td>
<td>Good</td>
</tr>
<tr>
<td>1 – 5 millions</td>
<td>Fair</td>
</tr>
<tr>
<td>&gt;5 millions</td>
<td>Poor</td>
</tr>
</tbody>
</table>

(c) Methylene blue Reduction time (MBRT)

<table>
<thead>
<tr>
<th>MBRT hours</th>
<th>Quality / Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 and Above</td>
<td>Very Good</td>
</tr>
<tr>
<td>3 to 4</td>
<td>Good</td>
</tr>
<tr>
<td>1 or 2</td>
<td>Poor</td>
</tr>
<tr>
<td>½ and below</td>
<td>Very poor</td>
</tr>
</tbody>
</table>

(d) One hour Resazurin Test (RRT)

<table>
<thead>
<tr>
<th>Disc No.</th>
<th>Quality / grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 and above</td>
<td>Good</td>
</tr>
<tr>
<td>3 ½ - 1</td>
<td>Fair</td>
</tr>
<tr>
<td>½ and 0</td>
<td>Poor</td>
</tr>
</tbody>
</table>

(e) 10 mts Resazurin rest (RRT)

<table>
<thead>
<tr>
<th>Disc No.</th>
<th>Quality/grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 - 5</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>3 ½ -1</td>
<td>Doubtful</td>
</tr>
<tr>
<td>½ and 0</td>
<td>Unsatisfactory</td>
</tr>
</tbody>
</table>

(f) Thermoduric Count

<table>
<thead>
<tr>
<th>Count/ml</th>
<th>Quality / grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10,000</td>
<td>Good</td>
</tr>
<tr>
<td>10,000 – 30,000</td>
<td>Fair</td>
</tr>
<tr>
<td>&gt;30,000</td>
<td>Poor</td>
</tr>
</tbody>
</table>
(g) Coliforms

Absent in 0.001 ml – Satisfactory

(h) Leucocyte Count

<table>
<thead>
<tr>
<th>Count per ml</th>
<th>Quality / grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5,00,000</td>
<td>Normal Milk</td>
</tr>
</tbody>
</table>
| >5,00,000    | Mastitis or early or late lactation milk.

**USDA/FDA Standards**

<table>
<thead>
<tr>
<th></th>
<th>(SPC) (max) ml</th>
<th>(Coliform (max) ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Milk (pick up)</td>
<td>1,00,000</td>
<td>-</td>
</tr>
<tr>
<td>Raw milk (Co- mingled)</td>
<td>3,00,000</td>
<td>-</td>
</tr>
</tbody>
</table>

**Military Federal Purchases Standards**

- **Raw Milk DMC**: 5,00,000 to 30,00,000/ ml
- **Fresh milk SPC**: 20,000/ ml and Coliform 10 ml

**Suggested Standards**

- Total Bacterial count <2,50,000/ml
- Coliform <100/ml
- E.coli (Faecal type) Absent 1 in 0.01 ml
- Thermoduric < 1000 / ml
- Spores < 10 / ml
- B. Cereus spores < 1 / ml
- Staphylococcus aueus < 100 / ml
- MBRT (at 37°C) Not < 5 hours
- RRT Not < 3 hours
- Somatic Cell count < 7,50,000 / ml

2. Pasteurized Milk

(a) SPC count / ml

<table>
<thead>
<tr>
<th>Quality/grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satisfactory</td>
</tr>
</tbody>
</table>
Coliforms. Absent in 1 : 10 Dilution satisfactory
USPHS Standards (United State Public Health Society)

(a) SPC – Grade ‘A’ milk not more than 20,000/ ml
Certified milk not more than 500 /ml
(b) Coliform Grade A > 10 /ml
Certified milk > 1 / ml

Suggested Standards
Total bacterial count < 50,000 / ml

3. Sterilized milk
BIS Standards
Spore count max 5 /ml
Turbidity test negative

Summary
The characteristic of various important dairy microbes i,e, streptococci, lactobacillus and others were discussed in detail. The important dairy yeasts and moulds were described. The various milk borne diseases including food poisoning containing the causative organisms, symptoms and prevention were explained in details.

Short Answer Type Questions
1. Name various important groups of organisms under streptococcus group.
2. Mention the various organisms that comes under pyogenic group of streptococci.
3. What are the organisms that comes under viridans group of streptococci ?
4. Give the varieties of organisms under Str - faecalis.
5. Mention various organisms under Leuconostoc group.
6. What are the morphological characteristics of Escherichia ?
7. Mention the names of various important dairy moulds.
8. Define food intoxication.

9. Name the organisms that causes food infection.

10. Mention salmonella food poisoning symptoms.

11. Give some examples for milk borne toxic infections.

12. Name the various toxins from staphylococcus organism.

13. What are the different new emerging milk pathogens?

**Long Answer Type Questions**

1. Write about morphological cultural and biochemical characteristics of pyogenic group of streptococci.

2. Discuss in details the various characteristics of lactic group of streptococci.

3. What are the differential characters of three groups of lactobacillus group?

4. Mention the various differential characters of Leuconostoc species.

5. Briefly write about important characters of genus Escherichia.

6. Explain briefly important yeast of dairy industry.

7. Discuss in details about dairy moulds.

8. Classify milk borne diseases with suitable examples.


10. Explain in details about botulism food borne intoxication.

11. Write about fungal milk intoxication.
Estimation of Microbes in Milk

Structure

5.1 MBRT Test
5.2 Direct Microscopic Count (DMC Test)
5.3 Standard Plate Count
5.4 Coliform Count.
5.5 Yeasts and Mould Count

Learning Objectives

After studying this unit, the student will be able to understand

• Methylene Blue Reduction test
• Resazurin Reduction test
• Direct microscopic count test and Standard Plate count test

5.1 MBRT Test

Dye reduction tests are indirect methods of estimation of total bacterial content of milk. Instead of counting bacteria directly, a correlation is made between the time required to reduce dyes to colourless in milk. Generally the time required for reduction of dye is inversely proportional to the number of bacteria present in the milk. The milk will have dissolved oxygen content and so initially it is oxidation side and when the bacteria multiply utilizes the oxygen and after some time total oxygen content in milk is exhausted and the milk is reduced
stage. The dyes used will have colour in oxidized stage and become colourless in reduction stage.

**Methylene Blue Reduction Time (MBRT)**

The Methylene blue concentration used is 1 part of dye in 3,00,000 parts of milk. Presently tablet forms are available. One tablet dissolved in 200 ml of hot, distilled water produces the stock dye solution for addition to the milk. Although this solution is stable when it is refrigerated and protected from light, it is safer to prepare the solution weekly.

Essentially the procedure is, add 10 ml of milk sample to 1 ml of Methylene blue dye solution in test tube. The tube is incubated at 37°C in a constant temperature bath. After the tempering period, the tubes are inverted gently 3 times to redistribute the cream, and this point is used as the starting time of the test. The initial observations for reduction are made after 30 minutes and hourly intervals afterwards. At each observation those tubes which show reduction are removed and recorded and the remaining ones are inverted 1 time and reincubated. Those reduced at the initial 30 minutes observation are recorded as reduced in 30 minutes, those reduced between 0.5 and 1.5 hours time, recorded as 1 hour those between 1.5 and 2.5 hours recorded as 2 hours and so on.

The inversion of the tubes at the specified intervals has aided materially in over coming the earlier objectional features of creaming. Bacteria normally are carried to the top as the cream rises, making the reduction of the irregular throughout the tube. These defects are minimized by the present technique. The inversion of the tubes must be gentle, otherwise the incorporation of oxygen in the milk will cause oxidation of the dye. Since the change from blue (oxidized form of the dye to the colourless (reduced) form is reversible, unnecessary agitation of the sample will cause an extended reduction time and will give results indicative of a higher quality than actually exists. The exposure of the surface of the milk to the oxygen in the air above it will cause that part of the milk to remain blue for some time after the reminder is reduced. For this reason the reduction time is taken to be the time required to reduce the colour in four fifth of the milk.

Milk as it exists in the udder has a sufficiently low oxidation-reduction potential to reduce Methylene blue immediately. The incorporation of oxygen into the milk during milking, cooling, dumping raises the potential to above + 0.3 volts. At this potential, methylene blue will exist in the oxidized form, i.e. have a blue colour. As the bacteria in the milk grow during progress of the test, the O.R. potential is lowered and the methylene blue is reduced to the colourless form when the oxidation reduction potential reaches approximately + 0.06 to –
0.01 volt. Oxygen is removed from the milk by the respiratory process of the bacteria.

This results in a shift of the oxidation – reduction potential, since the oxygen ordinarily maintains a positive potential. As the potential falls hydrogen presumably is transferred from milk constituents and bacterial metabolites to methylene blue, causing its reduction. Bacteria such as streptococcus lactis and Escherichia coli lower the potential rapidly, others lower it much more slowly. Although the dye is reduced at a high oxidation – reduction potential at lower pH values, the ability of organism to produce acid and to reduce methylene blue are not necessarily correlated.

Methylene blue test has found many uses in grading of raw milk for pasteurization and of milk to be used as evaporated milk. Its simplicity and the rapidity with which data are obtained are definitely in its favour. There are however definite limitations, such as --

1. The 37°C temperature of incubation is not favourable for the metabolism of all the bacteria contained in milk.

2. The different bacteria have varying abilities with regard to lowering the oxidation – reduction potential of milk.

Thermoduric bacteria frequently are relatively inactive in the test for the above two reasons. This is probably the most important objection to the method, for the thermoduric bacteria constitute a very important problem for the process. Psychrophillic and thermophillic bacteria would show little or no activity in this test. Inhibitory materials in milk also prevent the growth of many bacteria and will cause the test to give an indication of higher quality than may actually exist. For these reasons, counting methods will reveal certain bacteria, which would not be detected by MBRT.

On the other hand there may be circumstances where in the dye reduction technique would be more indicative of the quality than actual counts by the plate method.

Involved here would be the ability of certain bacteria to grow in millions, but their mobility to form visible colonies on the standard plating medium. Further more, the individual cells of a clump, which would form just one colony by the plate count, would be more evident by the dye reduction test, since each cell would be metabolizing and the cumulative effect of all the cells would be noted.

The milk is graded as given below using methylene blue reduction test (MBRT).
<table>
<thead>
<tr>
<th>Time required for reduction (hrs)</th>
<th>Grade/Quality of milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 and above</td>
<td>Very Good</td>
</tr>
<tr>
<td>3 and 4 hrs</td>
<td>Good</td>
</tr>
<tr>
<td>1 and 2</td>
<td>Fair</td>
</tr>
<tr>
<td>0.5 and below</td>
<td>Poor</td>
</tr>
</tbody>
</table>

**Resazurin Reduction Test (RRT)**

This test is similar to the methylene blue test, but it uses the indicator Resazurin to measure the bacteriological quality of milk. Resazurin has certain characteristics that make its use as an indicator very useful. The colour Resazurin at the normal pH of milk is blue. This compound is reduced to resorufin, which is pink, the colour changes gradually during the reduction process. From initial blue through shades of purple and lavender to the full pink colour. This phase of reduction is not reversible and the change to the resorufin occurs at an oxidation. Reduction potential between +0.2 and 0.05 volts. The resorufin is then reduced to hydroresorufin, which is white. This reaction is reversible, the change occurring between +0.15 and 0 volt. In conducting the test, however the first reaction is of chief concern.

In performing the test milk is added to a screw capped vial plus resazurin to give a concentration of about 1 part of dye in 1,80,000 parts of milk. Standard tablets of resazurin are available, one tablet dissolved in 50 ml of boiled, cooled distilled water make 0.005% solution which can be directly used in the test. The tubes are then incubated in a water bath at 37°C. after the samples reach this temperature, they are gently inverted three times and returned to the bath, then the time of incubation begins.

**This test is used in three types.**

- 1 hr RRT
- 10 minutes RRT

In the 3 hour RRT comparisons of the tubes at intervals of one hour are made with a standard lavender colour (munsell colour standard p 7/4) and grading is done on the basis of time required to each this colour. Munsell colour standards are.

Grade 1-Colours from initial to PBP 7/5.5 (Purple shade)
Grade 2- Colours from PBP 7/5.5 to PRP 7/8 (Lavender shade)
Grade 3- Samples showing pink colour
Grade 4-Samples decolorizing dye completely.

The three hour triple reading test is more commonly used earlier. After the first hour of incubation, those tubes having a colour of 7/4 (munsell colour standard) are removed and recorded. The remainder of the tubes are inverted once again and reincubated. At the end of second, third hour of incubation the comparison to the colour standard of 7/4 and so on. The time taken for resazurin reduced to colour of 7/4 will be taken for judging the quality. This test is more indicative than 1 hour test. Since some bacteria may not be so rapid in initiating growth. Grade 1 (munsell Standard) would correspond with MBRT not less than 5.5 hours.

For 1 hour and 10 mts RRT, test samples are compared with control tubes (milk without dye and incubated at 37° C) is a levibond comparator with resazurin disc. The disc will have 6 discs (Starting 0-6 with colours ranging from blue and shades of purple, lavender and pink). The disc number matching is recorded and the quality of milk is graded as follows.

1 Hour RRT test

<table>
<thead>
<tr>
<th>Disc no</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 or 6</td>
<td>Good</td>
</tr>
<tr>
<td>3 ½ -1</td>
<td>Fair</td>
</tr>
<tr>
<td>0.5 and 0</td>
<td>Poor</td>
</tr>
</tbody>
</table>

10 minutes RRT Test

<table>
<thead>
<tr>
<th>Disc No</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 or 6</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>3 ½ -1</td>
<td>Doubtful, requires further examination</td>
</tr>
<tr>
<td>0.5 and 0</td>
<td>Unsatisfactory</td>
</tr>
</tbody>
</table>

Since, the colour changes that are used as end products of the test occurs at higher oxidation – reduction potentials than those for the methylene blue test, the resazurin test can be done in a shorter period of time. Further more, colostrum and milk from cows that have diseased udders or from cows that are being fried up reduce the resazurin very quickly. In such milk the oxidation reduction zone of dye reduction is shifted more to the positive side than is required for normal milk. It is generally assumed that leucocytes or substances associated with leucocytes are responsible for this shift. Reduction in such instances is not always associated with high bacterial count but ability of the test to detect such milk is certainly a point in its favour.
Even though the milk of this nature may be unsafe its flavour but is undesirable and the quality of normal milk should be maintained by excluding abnormal milk from it. Knowledge of the effect of milk from diseased udders on resazurin has been employed to advantage in locating mastitis cow, the RRT being used as a screening test for mastitis milk from individual quarters.

**Advantages of Dye reduction tests**

1. Dye reduction tests are useful for estimating the suitability of milk for liquid consumption.

2. These tests are cheaper and also the time required is less.

3. In case of standard plate count, clumps of organisms are regarded as one colony; whereas the rate of decolourization of dyes is due to the combined effect of each bacterium in the clump.

4. With the help of these tests, the activity is measured rather than the numbers of bacteria. Hence, it is a better estimate of the rapidity with which milk will sour as bacteria which sour milk quality, will reduce dye rapidly.

5. Unlike the artificial media used in SPC, here milk is used which is the natural environment for microbes.

6. In case of the dye reduction tests, particularly RRT, the stage of reduction can be measured in shorter and result expressed.

7. Colostrum and milk from diseased udders reduce resazurin quickly and thus RRT is also used as a screening test for mastitis.

**Disadvantages**

1. The rate of reduction of dye varies considerably and is related to species and the rate at which different organisms grow at a particular temperature. Most of the thermodurics are less active in reducing the dyes than many other common contaminants and these are less readily detected by the dye reduction test. The same is true of psychrotrophs. However, leucocytes reduce such dyes at a faster rate. Coliforms are the most rapid reducers followed by S. Lactis, faecal streptococci, staphylococci, micrococi and aerobic spore formers.

2. Somatic cells at levels of about $1 \times 10^6 / \text{ml}$ reduce resazurion at rate not dissimilar to that resulting from the same number of bacteria.

3. Inhibitory substances like penicillin and other antibiotics prevent the growth of bacteria and this increase the reduction time.

4. Dye reduction tests are not suitable for classifying milk with low bacteria counts of less than $10^5 / \text{ml}$. 
5. Some of the bacteria capable of reducing dye may not develop colonies on the medium used in SPC.

6. Reduction capability may vary because of the variation in proportion of bacteria carried in to cream layer by the rising fat globules.

7. These tests do not give indication for the type of organisms present.

8. Temperature of incubation used during these tests is not the optimum for majority of the organisms present in milk.

9. The dye reduction tests are not suitable for pasteurized milk because of the low number of organisms.

10. These tests require attention until reduction takes place.

5.2 Direct Microscopic Count (DMC Test)

Direct microscopic count (DMC) is one of the several methods used in quality control laboratories for direct enumeration of microorganisms in milk sample. It consists of examining fixed and stained smears of a known volume of milk and milk products under a compound microscope. This method provides a rapid indication of quality of milk or liquid milk products. It also helps us in keeping a permanent record of the quality of the product. However accuracy and reproducibility of the results achieved by this method depends largely on the expertise and training of the personal in the quality control laboratory.

A small quantity (0.01 ml) of the sample is spread over the outlines area of one square (100 mm² on a grease proof microscope slide with the help of a breed’s pipette.) After making a uniform smear, it is air dried, fixed, stained with new-man’s stain and then examined under the microscope. The number of organisms per field is counted and average number per field is determined after examining at least 10-20 fields. Total number of organisms (viable as well as non viable) per ml are then calculated by multiplying the average number of organisms per field by the microscopic factor.

For determining the microscopic factor (MF) of a given compound microscope, the following formula is used.

\[
MF = 100 \times \frac{100}{\pi r^2}
\]

Where ‘r’ is equal to radius of the microscopic field \( \pi \) (pi) is a constant having a value of 3.14. In this formula in order to convert area of one field from Sq. mm to sq.cm field area in Sq mm has been divided by hundred. Similarly, to determine the number of such fields in one cm square, one cm square is divided by the area of one field in mm followed by multiplication with hundred to
determine the number of organisms per ml sample. The number of such fields to be counted depends upon the average number of organisms per field as given below.

<table>
<thead>
<tr>
<th>No of Organism per Field</th>
<th>No of Fields to be counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 –3</td>
<td>64</td>
</tr>
<tr>
<td>4 – 6</td>
<td>32</td>
</tr>
<tr>
<td>7 – 12</td>
<td>16</td>
</tr>
<tr>
<td>13 – 25</td>
<td>08</td>
</tr>
</tbody>
</table>

The average number of organism per field multiplied by the MF – yields the number of organisms per ml of milk product. It is better to count clumps instead of individual cells because clump count agrees most with SPC.

**Advantages**

1. This is rapid method as the results are obtained on the spot.
2. The stained smears of the sample can help in the identification of different sizes, shapes and arrangement of bacteria and somatic cells.
3. Actual counts of clumps of bacteria and individual somatic cells are obtainable.
4. It is less expensive.
5. Microscopic preparations gives a permanent record.
6. Preservatives can be used with samples intended for microscopic examination because the individual cells will also be included in the count.
7. It helps to locate the source of contamination depending on the predominance of organisms.

**Results Interpretation**

<table>
<thead>
<tr>
<th>Count / MI</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 5,00,000</td>
<td>Good</td>
</tr>
<tr>
<td>5,00,000 – 40,00,000</td>
<td>Fair</td>
</tr>
<tr>
<td>4,000,0000 – 20,000000</td>
<td>Poor</td>
</tr>
<tr>
<td>Over 20,000,000</td>
<td>Very poor</td>
</tr>
</tbody>
</table>
Disadvantages

1. There will be tremendous strain on the eye of the operator.
2. Both the viable and non viable are counted, hence not very reliable.
3. The method is not suitable for low count raw milk samples and the pasteurized milk.
4. Great amount of skill and expertise is needed for getting consistent results.
5. Results are not reproducible because organisms are unevenly distributed in the smear.

The observations of bacteria shapes and arrangements will give type of contamination.

1. Presence of cocci particularly when in clumps of varying sizes indicates that the sample under observation has been handled improperly cleaned utensils.
2. Rod forms of bacteria indicate dusty or dirty environment.
3. Bacteria in pairs or short chains (usually streptococcus lactis or streptococcus cremoris) indicate improper cooling of milk.
4. Presence of leucocytes indicate mastitis or udder disease.

5.3 Standard Plate Count.

The standard plate count or pour plate method is used for estimating the viable micro organisms in milk and milk products. In view of a wide range of bacterial population in dairy products, their number can be counted only by making appropriate dilutions. An aliquot of 0.1 ml or 1 ml of the diluted sample is poured in sterilized plates and mixed with liquefied sterilized agar medium. After solidification of agar, the plates are incubated at a specific temperature and for suitable period of time depending on the type of bacteria being suspected in the food sample.

After incubation, bacterial cells grows in to distinct and isolated colonies. Each colony develops from a single bacterial cells) which can be counted with the help of a colony counter. The plates with 30–300 colonies are selected for counting to obtain plate counts or colony forming units (cfu) per ml or g of the product. In order to calculate the total number of viable bacteria/g or ml of the sample, the number of the colonies developed on each plate are multiplied by the dilution factor. The dilutions will be 1 : 10, 1 : 1000, 1 : 10000, 1 : 100000, 1 : 10,00000 etc. This is carried out as shown in the figure.
Fig 5.1 Protocol for preparing dilutions of a milk sample, indicating volumes to be added to dilution blanks and petri dishes

The type of medium and incubation temperature and period should be indicated while expressing the results.

Introduction of automation in counting the number of colonies in SPC has considerably improved the efficiency of this method. Most of the colony counters consists of a television camera or screen to detect the colonies on the illuminated petri dish and a small electronic computer. One such counting device is electronic micro-colony counter using particle counts. The various factors which affect SPC include temperature of incubation period of incubation, composition of plating medium, existence of bacterial clumps etc.

**Advantages**

1. Enumeration of viable microorganisms is possible.
2. Differential counts can also be determined
3. The cultural and morphological differentiation based on colony characteristics is possible.
4. This method is suitable for determination of quality of milk. Samples with low bacterial numbers, pasteurized milk and high grade raw milk.

**Disadvantages**

1. The method gives only a rough estimate of microbial population in the given sample, hence is not very accurate.
2. It requires complex standardization conditions for specific counts.
3. It is a time consuming, laborious and cumbersome method.

4. It requires huge quantities of reagents, chemicals and glassware at a time.

5. It is not a rapid method as at least 24 hours are required to get the result.

6. This method does not give accurate counts as
   (a) The SPC medium is not suitable for the growth of all the species of bacteria present in milk.
   (b) Temperature of incubation may not be the optimum for the growth of all types of bacteria.
   (c) The amount of sample may not be representative of the whole lot.

7. Pathogenic organisms are not detected because certain organisms like M. tuberculosis cannot grow under the conditions of the test.

8. Specific information regarding the type of micro flora is not obtained.

In spite of all the limitations, SPC method is still being routinely used in most of the quality control laboratories for assessing the micro biological quality of milk and milk products.

**Interpretation of Results**

<table>
<thead>
<tr>
<th>Count / ml</th>
<th>Quality / Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 200,000</td>
<td>Very good</td>
</tr>
<tr>
<td>2,00,000 – 1 million</td>
<td>Good</td>
</tr>
<tr>
<td>1 – 5 million</td>
<td>Fair</td>
</tr>
<tr>
<td>Over 5 million</td>
<td>Poor</td>
</tr>
</tbody>
</table>

**5.4 Coliform Count.**

The members of the Coliform group of bacteria eg. Escherichia and Aerobacter aerogenes are commonly found in dairy products, produced and handled under insanitary conditions. Their presence in milk and milk products is indicative of possible faecal contamination although some species (Aerobacter aerogenes) may be derived from feeding materials and soil. As these organisms are capable of growing rapidly at room temperature (30 - 45°C) and produce acid, gas and objectionable taints in the products they are considered to be very undesirable contaminants.
The estimation of Coliform bacteria in milk is, therefore, very important in quality control work. Since these organisms are generally destroyed during pasteurization treatment their presence in pasteurized milk is considered to indicate post pasteurization contamination. The test for Coliform organisms is based on the principle that the members of this group are capable of producing acid and gas from lactose in the presence of bile salt: A small amount of milk (1.0, 0.1 or 0.01 ml) is added to liquid or solid media containing lactose and bile salt with a suitable indicator.

Production of acid and gas in liquid media and appearance of typical colonies of Coliform on the plates is taken as evidence of Coliform on the plates is taken as evidence of Coliform contamination. A few other bacteria such as those belonging to the genus clostridium and genus bacillus and certain yeasts also produce acid and gas under these conditions giving rise to false positives.

However even these organisms are desirable in milk and their interference with the test therefore is not considered to be of much significance. Hence the test commonly employed to detect the presence of Coliform bacteria in milk is called presumptive test and in cases of doubt the completed test is considered to confirm the presence of coliform.

**Liquid Media test**

Transfer 1 ml portion of milk and its dilutions (1/10 and 1/100) into macconkey’s broth tubes in triplicate. Incubate the tubes for 24 hours at 37°C and observe for acid and gas production. The production of acid is indicated by change of colour of medium from purple to yellow in case of bromocresol purple and orange to pink in case of andrade’s indicator. Production of gas is observed in the Durham’s tubes which may be particularly or completely filled with gas. If no change is observed incubate for another period of 24 hours and record the observation.

**Solid Media Test**

Incubate 1 ml portion of the required dilutions into sterile petridishes (in duplicate). Add to each plate 10-15 ml of macconkey’s agar previously melted and cooled to 45°C, Mix the content thoroughly by tilting and rotating the plates. Allow the agar to solidify. Pour additional layer (3–4 ml) of the medium completely over the surface of the solidified medium invert and incubate the plates at 37°C for 24 ours. After incubation examine for typical colonies of coliform bacteria. Presence of dark red colonies measuring at least 0.5 mm in diameter constitute a positive test count such colonies only and express the results as coliform per ml of milk.
Confirmation Test for Coliforms

Select positive acid and gas tube for the above experiments and subject them to confirm the above tests. Two solids media are generally used in the test, namely eosine methylene blue (EMB) agar medium and Endoagar medium. Typical colonies of coliform organisms will appear pink with dark centre and metallic sheen on EMB agar. Endo agar produce coliform colonies which appear red in colour and the growth will darken the medium to deep red.

Pour 10 – 15 ml of melted EMB or end agar in to petridish and allow the media to set. Make three sectors on lower dish by marking with glass marking pencil by inverting the petridish. Move the inoculating needle slightly curved so that to streaking should ensure the presence of well isolated colonies. Introduce the needle to the depth of ½ m below the surface of positive presumptive tubes. Place the curved section of the needles on the agar surface in one segment and streak gently to avoid tearing of the medium. Similarly streak a loopful of the culture of E-Coli in second sector. Streak the third sector with the culture of aerogenes. Invert the plates and incubate at 37°C for 4 hours and record the result. (Standard is absent in 0.001 ml is satisfactory).

5.5 Yeasts and Mould Count

For certain dairy products the yeasts and moulds count is used as an index of proper plant sanitation and high quality raw products.

Yeasts and moulds counts can be made by using potatodextrose agar or malt agar with a pH adjusted to 3.5 + 0.1. At this pH bacterial growth is inhibited although most yeasts and moulds are uninhibited of owing their preference of an acid reaction. The pH is adjusted with a predetermined amount of sterile 10% tartaric acid after the medium is melted and tempered and then plates are poured in the usual manner explained under SPC method. The medium should not be acidified before sterilization or melting for the acid will hydrolyse the agar and destroy its ability to solidify. Extended holding of the acidified melted agar will prove undesirable for the same reason. The plates are incubated at 21°C or 25°C. For 5 days and the count is reported as yeasts and moulds plate count per ml of milk or butter.

When examining butter, one should place a quantity of the product in a sterile jar and should warm this in a bath at 40°C until the butter melts. Then 1 : 10 dilution is prepared by adding 11 ml of the melted to 99 ml of water blank from which after dilutions can be made. It is well to have all glassware and dilution blank tempered to 45°C, until just before use to facilitate the handling of
the sample and to prevent any solidification. The pipeting of diluted sample should be done immediately subsequent to shaking while the fat droplets are evenly distributed. This will aid in preventing errors caused by the coalescing of the fat and by uneven distribution of organisms adhering to the fat droplets.

Summary

The indirect methods of microbial estimation i.e. Methylene blue reduction test and resazurin reduction test were explained in detail. The direct enumeration methods like DMC, standard plate count, coliform test were explained. The estimation of various pathogens in milk explained in detail. The enumeration of yeast and mould explained.

Short Answer Type Question

1. What is the principle involved in dye reduction tests?
2. Mention the quality standards of milk as per MBRT test.
3. What are Munsell colour standards?
4. Mention the quality standards as per 10 minutes RRT.
5. What is the microscopic factor for DMC method?
6. How you will interpret the SPC results?
7. What is confirmation test for coliforms?
8. Give the isolation process for staphylococcus aureus.

Long Answer Type Questions

1. Mention the advantages and disadvantages of dye reduction tests.
2. Explain in details MBRT test.
3. Briefly write about Direct microscopic count method.
4. What are the advantages and disadvantages of SPC method?
5. Discuss in detail SPC method.
6.1 Milk Collection and Transportation

Milk Collection

In almost all developed dairying countries, production of milk is confined to rural area, while demand is mostly urban in nature. Hence the milk has to be collected and transported from the production points in the milk shed areas to processing and distribution points in cities.

(a) The common system of collection (assembling) of milk areas follows.
1. **By cooperative organizations**: formed by individuals or collective milking societies. Suits procedures best as no profit making middle men are involved.

2. **By contractors**: Less returns to producers.

3. **By individual producers**: Practical for those situated nearer processing dairies.

**Note**

Milk shed is the geographical area from which a city dairy receives its fluid supply. The allocation of definite milk shed to individuals dairies for the purpose of developing the same is now being considered in India.

(b) Milk Collection - chilling centres / dipos: Normally attached to city diaries.

**Objectives**

1. To preserve the quality of raw milk supply.
2. To provide easy transport to the processing dairy.

**Location**

**This is guided by**

1. Adequate milk production
3. Proximity to a good road or railway station.
4. Electric supply.
5. Sewage disposable facilitation


**Operational Procedures**: Essentially this is the same as in a small dairy. On arrival the milk is graded for acceptance/ rejection, weighed, sampled for testing, cooled and stored at a low temperature until dispatched to the processing dairy.

**Transportation**

Under Indian conditions, milk has to be regularly collected and transported twice a day (Morning and Evening).
**Methods of transport**: These depend upon the carrying load, the distance of collection and local conditions.

<table>
<thead>
<tr>
<th>Mode</th>
<th>Optimum load (K G)</th>
<th>Optimum distance (KMS)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Head load</td>
<td>15 - 25</td>
<td>3 - 4</td>
<td>Generally employed for small loads and distances - important in hilly areas.</td>
</tr>
<tr>
<td>2. Shoulder sling</td>
<td>Upto 40</td>
<td>3 - 6</td>
<td>Means of heavier loads but for shorter distance than head load.</td>
</tr>
<tr>
<td>3. Pack animal</td>
<td>Upto 80</td>
<td>6 - 10</td>
<td>Ponies, horses and donkeys usually employed.</td>
</tr>
<tr>
<td>4. Bullock cart</td>
<td>300-400</td>
<td>10 - 12</td>
<td>Rather slow</td>
</tr>
<tr>
<td>5. Tongos</td>
<td>250 - 300</td>
<td>12 or more</td>
<td>Larger quantities transported, faster than head load, should sling and pack animal.</td>
</tr>
<tr>
<td>6. Bicycle</td>
<td>40 or more</td>
<td>15 or more</td>
<td>Quick and handy, easily accessible to milk producers.</td>
</tr>
<tr>
<td>7. Cycle rickshaw</td>
<td>150-200</td>
<td>10 or more</td>
<td>More carrying capacity than bicycle.</td>
</tr>
<tr>
<td>8. Boat</td>
<td>40 - 200</td>
<td>2 - 8</td>
<td>Only means of transport when rivers, etc have to be crossed.</td>
</tr>
<tr>
<td>9. Auto Rickshaw</td>
<td>250-500</td>
<td>15 or more</td>
<td>Greater carrying capacity and faster than cycle rickshaw.</td>
</tr>
<tr>
<td>10. Motor truck</td>
<td>$\frac{1}{2}$ -3 tons</td>
<td>15 or more</td>
<td>Increasingly in use with more road building and improvement.</td>
</tr>
</tbody>
</table>
Road Vs Railways transport (Advantages)

**Road**

1. Loading and unloading possible directly at godown of seller and buyer.
2. Cheaper than rail over short distances.
3. Less time consuming.

**Railway**

1. Cheaper than road over long distances.
2. Larger quantity of milk can be handled at a time.

<table>
<thead>
<tr>
<th>Mode</th>
<th>Optimum load (KG)</th>
<th>Optimum distance (KMS)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Rail wagon</td>
<td>11 tons or more</td>
<td>80 or more</td>
<td>Great scope in future.</td>
</tr>
<tr>
<td>12. Tankers (Road Rail)</td>
<td>5 tons or more</td>
<td>80 or more</td>
<td>Great scope in future.</td>
</tr>
</tbody>
</table>

Fig 6.1 Milk transport in Railway

Can Vs Tanker Transport. (Advantages).

**Can**

Handling of small quantities possible.
Tankers
1. Quicker mode of transport
2. Lower Costs
3. Better temperature control
4. Less Risk of contamination.
5. More time and labour Saving.
6. Over all savings in detergents etc.,

Types of Containers Used.
1. Baked Earth
2. Wood or Bamboo
3. Metal (Generally Brass)
4. Galvanized Iron (GI)
5. Second hand tins(mainly vegetable oil or ghee)
6. Tinned iron and aluminum alloy (used by organized dairies.)
7. Polypropylene cans.

The problems in relation to milk collection and transportation are
1. Milk is liquid, perishable & bulky.
2. Small and scattered production of milk.
3. Tropical climate
4. Lack of transport facilities.
5. Lack of countrywide organization for milk collection and transport.
6. Vested interests among milk merchants.

6.2 Methods of milk Preservation

Milk is highly perishable item. The keeping quality of fresh milk is only 5-6 hours unless proper steps are taken to preserve the quality. The major cause for spoilage of milk is due to the action of micro organisms on lactose yielding lactic and other acids, causing increased acidityof milk. The milk with high acidity can't tolerate heat and so coagulates on heating .when the milk acidity reaches 0.6 % acidity, milk coagulates at room temperature with out heating.
The principle involved in the preservation of milk is only to destroy the microorganisms or obstructing the microbial growth, so that acidity development is stopped or slowed down, the various methods are.

1. **By Cooling the Milk**: The most of the microorganisms present in milk are mesophilic i.e. they grow well at 20 – 40°C. By cooling the milk to refrigeration temperature i.e. 5–10°C, the multiplication of microorganisms can be restricted. Only psychrophils will grow. So the acidity development is at slower rate. This table shows that the bacterial growth factor in the milk at different temperature.

<table>
<thead>
<tr>
<th>Temp °C</th>
<th>Bacterial Growth Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>1.05</td>
</tr>
<tr>
<td>10</td>
<td>1.80</td>
</tr>
<tr>
<td>15</td>
<td>10.00</td>
</tr>
<tr>
<td>20</td>
<td>200.00</td>
</tr>
<tr>
<td>25</td>
<td>1,20,000.00</td>
</tr>
</tbody>
</table>

 Multiply initial count with the above factor to get final microbial count. That is why milk should be cooled to 5°C to maintaining quality.

2. **By heating**: By heating the milk, the microorganisms will be killed. The various microorganisms are destroyed at different temperatures. Pasteurization temperatures kill cent percent pathogenic microorganisms and 98–99% of spoilage microorganisms. Boiling milk will kill all the microorganisms, except spores. The effect of heat is discussed well in chapter 3.

3. **By addition of Chemicals**: Preservatives are the chemicals, which when added to milk at small concentrations will inhibit the microbial multiplication by interfering the metabolism pathway of microorganisms or by neutralizing the acids produced.

The various preservatives are sodium carbonate, sodium bicarbonate, formaldehyde. Boric / benzoic acids, salicylic acids etc.

Antibiotics will also inhibit microbial growth. Microbial antibiotics like nisin, acidophillin etc will also inhibit microbial growth.

Lactoperoxidase System: it is also known as cold sterilization. This system contains three components i.e. thiocyanate, Hydrogen peroxide- Lactoperoxidase. Milk contains natural Lactoperoxidase enzyme. Thiocyanate
and hydrogenperoxides are added at 30 : 70 ppm level to activate the Lactoperoxidase system.


Most of the dairy plants receive milk in cans. The equipment used at reception sections are chain conveyer, weighing balance can washers. Milk reception should be so planned and the equipment so chosen that intake operations are expedited. This is especially important where larger volumes of milk are received.
Delays permit deterioration of milk awaiting dumping, increases labour costs and may increase the operating cost of the can washer. The deliveries of milk should follow a schedule. If the milk is received continuously during the schedule period, operations in the plant will not be interrupted and employees in the various sections will be fully occupied. The aim should be to complete milk reception within 3-4 hours, especially in tropical countries.

Unloading: The motor truck carrying the filled milk cans is backed up (or brought aside) to the unloading platform. These milk cans are unloaded manually. If the level of the truck surface is in line with the platform, the unloading requires the least effort (No lifting up or down but only pulling a level surface). Then the milk cans are assembled for grading in a definite order, according to each supplier viz, the contractor or patron.

The reception of milk from large rail or road tankers is primarily a matter of providing a covered area under which emptying and subsequently cleaning can take place. Road/Rail milk tankers are mainly used for receiving milk in feeder, feeder balancing dairies, Mother dairies and city milk plants. As the tanker arrives to the dairy the milk is tested for smell, taste and appearance. thoroughly mixed manually using a plunger or by mechanical or air agitation. The temperature of milk is measured and composite sample is taken for chemical and microbiological tests.

After getting the report from the laboratory, the reception of milk is to be started. The tanker outlet must be connected to sanitary piping. The milk may be removed by the milk pump, suited at a lower level than the tanker, or a compressed air line may be connected to the top of the tanker and milk forced out by air pressure. Washing and sanitization of the tanker should follow immediately, after emptying is completed. The measurement of milk delivered by tankers may be done either by using a weigh bridge or flow meter.

Weighing

This is an essential step in accounting for milk receipts and disposal, making payment for milk etc. the milk in cans is dumped into the weight tank, either manually or mechanically. The tank is mounted on scales and the scale dial set at zero when the empty tank is on the scale, thus enabling the operator to make a direct reading of the weight of the milk. Automatic printing of the weight is also now becoming common. (Weighing is facilitated by the use of dial reading or some other indicating scale, rather than a beam scale.

There are two ways of measuring the quantity of milk received at the dock. 1. By weighing 2. By volumetric measurement. The weighing system is as follows. The gross weight of the tankers on Weigh Bridge is recorded then the
milk is emptied and weight of the empty taker is taken. The difference between the two readings gives the net weight of milk received by the dairy. The volumetric measurement is by taking the level of the milk in the tanker and translating into unit of volume. Other method is to pass the milk through a flow meter and record its reading, which is multiplied by density of milk to get weight of the milk received.

**Weight Vs Volume**

**By Weight**

1. Gives Accurate reading, regardless of foam or temperature.
2. Involves considerable initial expenses for both apparatus and its installation.
3. Involves problems with maintenance.

**By Volume**

1. Not so accurate, as affected by foam and temperature, both influencing density.
2. Lower initial expenses.
3. Presents maintenance problems.
4. Definitely a factor to be considered in the overall picture of sanitation.

**Sampling**

The importance of securing an accurate and representative sample of milk for subsequent chemical and bacteriological examination cannot be over emphasized, while strict precautions regarding sterility of the stirrer, sampler, container etc are required for obtaining a bacteriological sample. Dryness and cleanliness of the above equipment should suffice for a chemical sample.

The first prerequisite of sample is thorough mixing of the sample. This can be done with a plunger or stirrer (agitator), operated manually or mechanically in the milk— in cans or tankers, as the case may be. With the former, a representative sample may also be taken after quick dumping of the milk into the weigh tank, where by it gets mixed thoroughly that a representative sample may be taken without further mixing.

Samples may be individual, composite, (mixture of two or more individual lots of milk), drip (representing the entire days supply) etc. Samplers may be dipper, proportionate (also known as milk thief), automatic vaccum, drips etc., whose characteristics are given below.
<table>
<thead>
<tr>
<th>Type</th>
<th>Principle</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dipper</td>
<td>Secures 10 - 15 ml milk.</td>
<td>1. Fairly fast and easy to work with.</td>
<td>Inaccurate when wide variation exist in milk lots, both in quality and quality.</td>
<td>Most commonly used. most useful for cream.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Quite accurate when milk is mixed adequately before sampling.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Proportionate</td>
<td>Secures aliquot of proportion of milk.</td>
<td>Most Accurate</td>
<td>1. Cumber some to use.</td>
<td>Not so commonly used (not so useful for cream)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Larger sample bottle needed.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Very accurate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Drip</td>
<td>Milk collects in drops in the sample bottles (which is kept under refrigeration)</td>
<td>Helpful in fat and SNF accounting of the total intake.</td>
<td>Not useful for individual sampling.</td>
<td>Useful in large product factories.</td>
</tr>
</tbody>
</table>
If the composite sample is to be successful, the milk must be kept sweet while the sample is being assembled. This is accomplished by use of a preservative. It is a good plan to place the preservative in the empty bottle milk is added. A wide mouthed glass bottle with a rubber stopper has been found to be the most reliable and practical container for keeping composite samples of milk or cream. The common preservatives used are:

1. **Mercuric chloride or corrosive sublimate**: This is very poisonous. It may be added in the form of tablets, which are coloured to prevent the milk being mistaken for food.

2. **Formalin**: This is a 40% solution of formaldehyde. Being liquid form, it is very convenient to handle; however it interferes with fat test.

3. **Potassium dichromate**: This is not effective as the above two, but it is easy to handle in dairy plants because, it is available in tablet form. The composite samples should be stored in a cool place away from direct sunlight. Each bottle should be properly labeled.

**Grading**

This refers to the classification of milk on the basis of quality, for price fixing purpose. It is well known that the quality of the finished product depends on that of the raw material user. The milk grader is the key man for the proper selection of milk. The principle of grading is based on organoleptic (sensory) tests, such as those for smell (odour), taste, appearance and touch, sediment etc. These are included under platform tests. The term ‘platform tests’ includes all those tests which are performed to check the quality of incoming milk on the receiving platform, so as to make a quick decision regarding its acceptance/rejection. They are performed on each can/tanker of milk with the object of detecting the milk of inferior or doubtful quality. So as to prevent it being mixed with high-grade milk. Some times the term “Rapid platform” is used to refer mainly to the organoleptic or sensory tests, which take very little time to perform.

The technique of grading milk may be described as under.

(a) **Milk tanker (Road/Rail)**: Actually the grading should have been done at the milk collection – cum-chilling centre. As the milk should be cold (5°C or below), it is not possible to detect off-odours. Only the appearance can be noted, as testing of raw milk is usually avoided. After thoroughly mixing it for 5-10 minutes., a sample is taken for laboratory testing.

(b) **Milk cans**: The main tests applied to each can of milk consists of smell, appearance and temperature (touch), other tests such as taste (seldom carried out with raw milk) and sediment might be used to substantiate the initial
findings. Tests involving time, laboratory facilities and special techniques are best done by the quality control technician. For which a sufficiently large sample is properly taken as the milk is being received (even if cans of milk have been dispatched from chilling centre, it is wise to inspect each can separately).

The various Platform tests are discussed as below

1. Smell (odour): This furnishes an excellent indication of the organo optic quality of milk. It can be ascertained very quickly (in just few seconds). In making the test, the cover of each can be removed, inverted and raise to the nose. The odour / smell will be representative of that in the can. The top of the milk in the can may simultaneously be noted for smell. By replacing the lid and shaking the can vigorously, the rest may be repeated. An experienced milk grader with a trained nose usually relieves to a great extent in the acceptance / rejection of the intake milk on the odour test alone. The milk should be free from any off flavour.

2. Appearance: By regularly observing the milk, each can after the odour test has been made, any floating extraneous matter, off-colour or partially churned milk may be noted. The milk should be normal in colour, free from churned fat globules and reasonably free from any floating extraneous material.

3. Temperature: The temperature at which milk is delivered is often an indication of its quality; a daily check on the temperature of milk is helpful in grading the milk on the receiving platform. With practice, the grader can tell with a high degree of accuracy whether or not the milk is sufficiently cold by touching the side of the can. A temperature of 5°C or below is satisfactory.

4. Sediment: This test shows the visible foreign matter contained in the milk. It need not be made daily, but should be made sufficiently often to ensure a clean milk supply, for this purpose a reliable sediment test (such as an off- the bottom sediment tester), by which the work may be expedited, should be selected, for intensity of discolouration and sediment on the pad will depend to some extent upon the manner in which the test is taken. A low sediment is desirable.

5. Acidity: The natural or apparent acidity of milk does not make the milk to taste sour, nor does it affect the normal properties of milk or jeopardize its quality or its behavior towards processing heat. However developed or real acidity does adversely affect the quality of milk. It is always well to have a certain acidity above which milk should not be accepted. Milk suppliers are freely adding neutralizers to milk to reduce its acidity, which is highly objectionable.

6. Lactometer Reading: The addition of water to milk results in the lowering of its lactometer reading. Hence this test is applied for detection of
adulteration of milk with water. As it does not take much time, it is often used as a platform test in the milk collection/chilling centres. However, this test has its own drawbacks.

7. **Dumping**: After the weighing of milk, it is operated manually to release each batch of milk in the dump tank immediately below. The milk is then pumped to a chiller and then to storage tank.

### 6.4 Milk Chilling: Methods and Storage

Milk Cooling: Immediately after receiving the milk in cooled to 4 or 5°C and stored cool till used. The various methods of milk cooling are.

1. **Surface Coolers**: In surface cooler, a series of small diameter horizontal tubes are welded one above another terminating in a header at each end. The header connects the tubes together for series of parallel flow. The cooling operation may be completed in two or more stages by mounting one unit above another. Milk is distributed over the coil by means of distribution pipe or trough and the milk drops the lower coil into the collection trough, from which it is removed by gravity or pump. The cooling medium flows through the coil. There are units available, called cabinet or fan type coders in which two or more surface type coils are used served by common distributor and collecting troughs, and one mounted within a cabinet having hinged doors.

![Surface Pipe cooler](image)

**Fig 6.4 Surface Pipe cooler**

2. **Plate coolers**: For continuous cooling, commonly used in the dairy industry, especially for large scale handling. It consists of a number of thin, flat, grooved, stainless steel plates, sealed at the edges with a gasket and clamped tightly within a press. The spaces between the plates are occupied alternatively
by the milk and the cooling medium (chill water/brine), thus one side of each plate is exposed to milk and the other side to the cooling medium.

Fig 6.5 Plate cooler

Plates may be added to provide increased capacity at nominal cost.

**Advantages**

(a) Cooling (heat exchanges) is quick and efficient.

(b) Not exposed to airborne contamination.

(c) No evaporation losses.

(d) Cleaning and sanitization are easy.

3. **Internal Tubular Cooler**: For continuous cooling. It consists of a stainless steel tube about 2.5 to 5.0 cm in diameter surrounded by a similar tube, forming a concentric cylinder. Several such tubes may then be connected in series to obtain sufficient cooling. The cooling medium flows counter to the milk flow.

**Advantages**

(a) Cooling is quite, efficient

(b) Not exposed to airborne contamination.

(c) No evaporation losses.
Disadvantages

(a) Cooling efficiency is lower than the plate cooler.

(b) Larger floor space is required.

4. Jacketed Vat/Tank: For batch cooling especially of small quantities. It consists of a tank within a tank, with the space between the two being used for circulation of the cooling medium, by either pump or main pressure. An agitator is provided to move the milk (which is in the upper tank) for rapid cooling.

Disadvantages

(a) Cooling efficiency is rather low.

(b) Too much agitation is required. Which causes churning and impairs the creaming property of milk.

Milk Storage

Raw cooled milk is stored in storage tanks until required for further processing. Modern milk plants hold raw and pasteurized milk, which is equal
to one day intake. This allows a more nearly uniform work day for processing and packing. Storage tanks are used for the storage of raw, pasteurized or processed products. The storage tanks must be designed for ease in sanitation, preferably by the circulation-cleaning method. In addition, the tanks should be insulated or refrigerated, so that they can maintain the required temperature throughout the holding period. Agitation should be adequate for homogenous mixing, but gentle enough to prevent churning and incorporation of air.

**Objects**

1. To maintain the milk at a low temperature so as to prevent any deterioration in quality prior to processing /product manufacture.
2. To facilitate bulking of raw milk supply, which will ensure uniform composition.
3. To allow for uninterrupted operation during processing and packing.
4. To facilitate standardization of milk.

**Types**

1. **Insulated /Refrigerated**: In the former there are 5 to 7.7 cm of insulating material between the inner and outer lining in the latter, the space between two linings is used for circulation of the cooling medium.

2. **Horizontal or Vertical**: While the former requires more floor space and less head space, the latter requires less floor space and more head space.

3. **Rectangular or cylindrical or Oval**: of these, the first suffers from the disadvantages of having dead corners during agitation while the other two do not.

4. **Built for gravity flow, air pressure or vacuum operation**: The first is the most common. However air pressure is sometimes used to evacuate the products. This requires special construction of the storage tank for greater strength than necessary for normal operations under gravity flow.

A relatively recent innovation in the storage of milk is the silo storage tank. It is vertical, cylindrical tank. Which is insulated outside the building due to its appreciable height. They have the capacity upto around 1,00,000 litres. The silo tanks in general, requires the same operational fittings and controls as in the other types.

Normally storage tanks are located on an upper floor. The milk is pumped from the receiving room to the floor above. It then flows by gravity.
Parts of Storage Tank


Summary

Milk collection systems and various types of transportation of milk from production site to chilling/processing centres discussed. The various methods of milk preservation discussed. The various operations at reception of milk i.e. unloading, weighing, sampling, grading, dumping etc, explained in detail. The different methods of milk chilling and storage of milk described in scientific way.

Short Answer Type Questions

1. What are common systems of collection of milk?
2. Name different modes of milk transport.
3. What are the problems involved in milk collection?
4. What is sampling of milk?
5. What are the different preservatives used in milk samples?
6. What do you mean by platform tests?
7. What is advantage of lactometer reading?
8. Define dumping of milk.
9. What is the principle used in surface cooler?
10. Name the different parts of storage tank.

Long Answer Type Questions

1. Explain different modes of milk transport.
2. Briefly write about weighing of milk.
3. Describe in detail about various milk samples.
4. Discuss in detail about platform tests.
5. Explain different methods of milk cooling/chilling.
6. What are the objectives of milk storage and briefly write about type of milk storage tanks?
7.1 Milk Filtration

Objective: To improve the aesthetic quality of milk by removing visible foreign matter which is unsightly and may therefore least cause consumer complaints.

Principle: Filtration removes suspected, foreign particles by the straining process.
Types of Filters: Two types
(a) Those that operate with cold milk and
(b) Those operating with warm milk – most widely used worldwide.

The advantages of cold filters are
(a) No need for pre heating
(b) Less likelihood of soluble dirt going into the solution.

Disadvantages of Cold filters
(a) The flow of milk is low.

Advantages of warm filters
(a) The flow of milk is fast

Disadvantages of Warm Filters
(a) The milk should be preheated
(b) Possibility of soluble dirt going into the solution.

General Construction of Filter
The important features are
(a) A filter cloth or pad of desired pore size, which can retain the smallest particle.
(b) A frame or support to compress and hold the margins of the cloth or pad so that milk can pass through the pores.
(c) A metal or other support with perforations for supporting the cloth or pad which will not tear or break under pressure of the milk.
(d) An enclosure to confine both the unfiltered and filtered milk in a closed system fitted suitably with inlet and outlet connections for sanitary piping.
(e) A means of distributing the incoming steam of milk so that it does not damage or tear any part of the cloth or pad by vigorous washing.
(f) A design so planned that filter cloths or pads can be changed quickly and all parts are easily accessible for washing.

Where continuous operation is essential or where large volumes of milk are processed, two or more filters are used so that operations need not be interrupted when it becomes necessary to change the filter cloth. The frequency
with which the cloth is changed will depend upon the temperature of the milk, the amount of foreign matter in it etc. It is the best to use filter cloths only once; a washed cloth, besides being a source of contamination results in inefficient filtration.

Filtration tends to decrease the depth of the cream layer that will form on the milk and this effect becomes more pronounced as the processing temperature increases. Filtration will not improve the keeping quality of milk. Milk should not be filtered after pasteurization.

The location of filters in the processing line may be in the raw milk line before milk enters pasteurization or in the regeneration section.

### 7.2 Milk Clarification

A high speed centrifuge known as clarifier is used to remove the insoluble soluble solids from a liquid by centrifugal means. It is just like filtration process, but using centrifugal force instead of filters.

Just like filters two types of clarifiers are available i.e. Those working with warm milk and those working with cold milk. The insoluble solids may be larger bacteria, body cells and contaminants, which may get into the milk during or after milking. The density of these materials is greater than the liquid.

If a liquid containing solids with a greater density is fed into a rotating bowl, the solids will move towards the bowl. If any outlet is provided for the liquid near the centre of rotation, then those particles of solids, which reach the bowl wall, remain in the bowl. Those particles which do not reach the bowl wall be carried out in the liquid. The fractions remaining in the bowl and the faction passing out in the liquid will be controlled by the feed rate i.e. the dwell time in the bowl. When the outer space is filled with sludge the operation has to be interrupted, the bowl opened and sludge removed. Disc bowl centrifuges have larger movement of inertia than tubular bowl centrifuges and they therefore take longer shut down times. Disc bowl machines have solid capacities in the range of 20-30 kg and are only suited to clarify feeds with less than a few percent by weigh of solids.

If a larger amount of sludge is to be discharged, a conical shaped unit, having holes or nozzles in the outer periphery, from which the sludge is discharged continuously, is used. The separated sludge slides down the incline formed by the conical upper and lower bowl and collects at the corner.

A nozzle discharged (self cleaning) centrifuge is shown in figure 7.1
This type of centrifuge is of disc-bowl type but the bowl is biconical in shape. A number of holes of the order of 3-4 mm diameter are spaced around the bowl at its larger diameter. The solids removed from the liquid are continuously discharged, in the form of thick slurry, into an outer casing. Feeds containing up to 25% solids can be handled in this type of clarifier.

In general the appearance and construction, clarifiers are quite similar to centrifugal cream separator. However the major differences are: a. In clarifiers, there is only one outlet, while in separator there are two (one of cream and another for skim milk) b. the discs in the clarifier bowl are smaller in a diameter (so as to provide a large space for the accumulation of slime) than separators c) the milk distribution holes are at the outer edge of the discs in clarifiers, but near the centre in the separators.

The clarifier may be located in one of the following places in the processing line.

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of Clarification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between reception and storage tanks</td>
<td>Cold</td>
</tr>
<tr>
<td>Between storage tank and Pasteuizer</td>
<td>Cold</td>
</tr>
<tr>
<td>Between Pre-heater and Pasteuizer</td>
<td>Warm</td>
</tr>
<tr>
<td>Between regeneration and heating section of HTST</td>
<td>Warm</td>
</tr>
<tr>
<td>Between heating section and holding tube of HTST</td>
<td>Warm</td>
</tr>
</tbody>
</table>
Clarification removes sediment much more efficiently than filtration. Clarifier remove still finer particles that escape filters. The slime that accumulates in the clarifier bowl consist of foreign matter, milk proteins, leucocytes, fragments of the secreting cells from the udder, fat, calcium phosphate and other ash, bacteria and occasionally red blood corpuscles. The amount of clarifier slime is influenced by the amount of foreign matter, the condition of udder, the stage of lactation, the bacterial count and acidity of milk, run through the bowl (or the length of time the bowl is run). The average composition of clarifier slime is water 63.3% total solids 32.7% fat 1.1%, protein 25.9% ash 3.6% and lactose 2.1%.

The clarification tends to decrease the depth of cream layer that will form on the milk and this effect becomes more pronounced as the processing temperature increases. Clarification will not improve the quality of milk, the milk should not be clarified after pasteurization.

7.3 Cream Separation – Methods

Principle: The basic principle of cream separation, whether by gravity or centrifugal methods, is based on the fact that milk fat is lighter than the skim milk portion. At 16°C (60°F), the average density of milk fat is 0.93 and skim milk 1.036. Hence, when milk, which may be considered to be a mixture of fat (as cream) and skim milk, is subjected to either gravity or a centrifugal force, the two components, viz, cream and skim milk by virtue of their differing densities, stratify or separate from one another.

Methods: Principally there are two methods

(a) Gravity Method and

(b) Centrifugal method.

Fig 7.3 Effect of forces acting on a fat globule
(a) **Gravity Method**: In a gravitational field or earth, a fat globule in milk, is subjected to gravitational force. Since the density of the globules is lower than the surrounding of the medium, it rises to surface.

The fat globule will remain in suspension if the densities are equal; i.e. its speed is zero in the medium. The rate in rising of fat particles is given by stroke’s law.

\[
V = \frac{2G}{9} \frac{(d_s - d_f) r^2}{n}
\]

Where \(V\) - velocity of rate at which fat globule rises
\(G\) = Acceleration due to the gravity
\(d_f\) = Density of skim milk
\(d_s\) = Density of fat Globule
\(r\) = radius of fat globule
\(n\) = Viscosity of skim milk

from the strokes law, it will be observed that theoretically, velocity is increased by
(i) Increase in radius of the globule
(ii) Increase in difference in densities of skim milk and fat
(iii) Decrease in viscosity of skim milk

However, in practice the important factors affecting the rate of the rise of cream by gravity are

1. **Size of fat Globule**: As the size of fat globules increases, the rate at which cream rises increases (Thus in buffalo milk, gravity creaming occurs faster due to the larger size of fat globules than those in cow milk.)

2. **Temperature**: As temperature increases, Viscosity decreases, and hence velocity increases.

3. **Clumping**: A clump or cluster acts like a single globule in so far as movement through skim milk is concerned. There by the effective “\(r\)” is increased, which in turn increases velocity.

4. **Addition of Adhesive**: Ultimately helps in increasing the rate at which fat globules rises.
(b) Centrifugal Method

**Principle**: When milk enters the rapidly revolving bowl of the cream separator, it is immediately subjected to a tremendous centrifugal force, which is 3000 – 6000 times greater than gravitational force. While both the fat and skim milk is subjected to the centrifugal force. The difference in the density affects the heavier portion (i.e. skim milk) more intensely than the lighter portion (i.e. cream). Thereby skim milk is forced to the periphery, while the fat portion moves towards the centre. The skim milk and cream both from vertical walls within the bowl and are separated by being led through separate outlets. The cream outlet is at higher level than skim milk outlets, both being near the axis of rotation.

The strokes law applied to separation is

\[
V = \frac{r^2 d_s - d_f}{n} N^2 R K
\]

Where \(V\) = velocity of movement of fat globule

- \(r\) = radius of the globule
- \(d_s\) = Density of skim milk
- \(d_f\) = Density of fat
- \(N\) = Speed of bowl
- \(R\) = Distance of fat globule from the axis of rotation
- \(K\) = constant
- \(n\) = viscosity of skim milk

It is seen from the above that, rate of cream separation is increased by

(i) Greater radius of fat globule
(ii) Greater difference in densities between skim milk and fat
(iii) Greater speed of the bowl
(iv) Greater size of the bowl
(v) Lower viscosity of skim milk
### Difference between Gravity and Centrifugal methods

<table>
<thead>
<tr>
<th>S.no</th>
<th>Particulars</th>
<th>Gravity methods</th>
<th>Centrifugal method</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>Direction of movement of fat and skim milk particles.</td>
<td>Vertical</td>
<td>Horizontal</td>
</tr>
<tr>
<td>4.</td>
<td>Bacteriological quality of skim milk and cream.</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>5.</td>
<td>Fat % of cream</td>
<td>10 - 25</td>
<td>18 - 85 (can be controlled)</td>
</tr>
<tr>
<td>6.</td>
<td>Skim milk fat %</td>
<td>0.2 or above</td>
<td>0.1 or below</td>
</tr>
<tr>
<td>7.</td>
<td>Scale of operation</td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>8.</td>
<td>Fat % recovered in cream</td>
<td>Not more than 90</td>
<td>99 - 99.5</td>
</tr>
</tbody>
</table>

### 7.4 Cream Separator – Parts and Arrangement of Parts

**Parts**

1. Supply can
2. Fallcet or milk regulator
3. Regulator chamber
4. Milk float
5. Cream Outlet (sprout)
6. Skim milk outlet (sprout)
7. Bowl Shaft
8. Rubber ring
9. Milk distributor
10. Bottom disc
11. Intermediary discs
12. Top disc with cream or skim milk screw
13. Bowl nut
14. Spindle
15. Set of gear
16. Crank handle

Fig 7.4 Centrifugal Milk Separator
7.5 Factors Affecting Efficiency of Cream Separator

The various factors influencing the efficiency of cream separator are

1. Position of the cream screw (or skim milk screw): Cream screw in or skim milk screw out, higher fat percentage in cream and vice versa. The cream screw/outlet consist of a small, threaded hollow screw pierced by a circular orifice through which cream emerges. This screw can be driven IN or OUT, thus bringing it nearer to or away from the centre of rotation. Similarly the skim milk screw/outlet is for the removal of skim milk. Once the skim milk or cream is adjusted, the cream separator delivers under normal conditions. A definite ratio of skim milk and cream, which is usually 90:10 (or 85:15) by volume.

By altering the position of the screws, the ratio of skim milk to cream changes. Thus when the cream screw is moved IN towards the axis of rotation, a higher fat percentage of cream is obtained and vice versa. This is because the force tending to discharge the cream through the orifice is decreased. A similar proportion of cream is therefore discharged. Which containing the same quantity of fat, shows a higher fat percentage. Screwing OUT the cream screw produces thinner cream. Upto 50% fat in cream, there is greater loss of fat in skim milk. Above 60% fat in cream, still higher fat losses in skim milk at low temperature.

2. Fat % in milk: The higher the fat percentage in milk, the higher the fat percent in cream and vice versa, since particularly all the fat in the milk is contained in the cream, the cream from the separation of high fat milk has a higher fat content than that from low fat milk.

3. Speed of the bowl: The higher the speed of the bowl, the higher the fat percentage in cream and the lower the speed the higher fat loss in skim milk. The higher the speed, the greater will be the centrifugal force, and the more rapidly will the skim milk leave the bowl. An increase in the bowl speed therefore increases the capacity of skim milk discharge. This means less cream is discharged with the same fat amount, a higher fat % in the cream. The below rated speed there will be more fat loss in the skim milk because insufficient centrifugal force is generated and above rated speeds. Skimming efficiency will not increase greatly, therefore optimum speed is good.

4. Rate of Milk flow: The higher the rate of milk flow, the lower the fat percentage in cream and the higher fat loss in skim milk vice-versa. When the rate of inflow increases, the discharge from the cream outlet increases, as the skim milk discharge remains constant, more cream containing the same amount of fat results in a lower fat % in cream, when the flow of milk is high through the
bowl too rapidly to allow for complete separation, thereby results a higher fat loss in skim milk.

5. **Temperature of the Milk**: The lower the temperature of the milk the higher the fat% in the cream and vice-versa. Lowering of temperature increase viscosity of both cream and skim milk, but that of cream increase (proportionately) more than skim milk, hence the quantity of cream discharge is decreased (due to clogging of the bowl) there by resulting a higher fat % in cream. The lower the temperature. The higher the fat loss in skim milk and vice versa. This is due to clogging of the bowl due to higher viscosity of cream results in greater fat loss in skim milk. As the temperature is increased, efficiency increases. But after 40°C, no increase in efficient, so the optimum temperature of milk is efficient separation is 40°C.

6. **Mechanical Condition of Separator**: Unsatisfactory mechanical condition of the separator causes greater fat loss in skim milk. These include.

   (a) **Vibration of the Separator**: This reduces the efficiency of separation by disturbing the counter currents of cream and skim milk (vibration is caused by installation on an insufficiency firm foundation, the bowl being out of balance, bearing being worn out, the axis of rotation not exactly vertical).

   (b) **Condition of Discs**: Discs in an unsatisfactory condition suffer a loss of skimming efficiency due to the uneven flow of the counter current stems of cream and skim milk between them. (An unsatisfactory disc is one which is out of shape, dirty scratched of rough).

   (c) **Amount of Separator slime in bowl**: If too much slime accumulates, the fat loss in skim milk increases, this not only by a disturbance in the even flow of the currents of cream and skim milk, but by reduction in the centrifugal force beacuse of decrease in effective diameter of the bowl).

   Separator slime consists of slimy mass which accumulates inside the bowl shell and it is made of foreign matter, milk proteins, lucocytes, and fragments of secreting cells from the udder, fat, calcium phosphate and other minerals, bacteria and occasionally red blood corpuscles.

7. **Amount of Water or skim milk added to flush the bowl**: The greater the quality of water or skim milk added to flush the bowl, the lower the fat % in cream and vice-versa. The addition of more water or skim milk will cause an increase in the amount of the cream, with the same amount of fat and will show a lower fat content.
8. Other Miscellaneous Factors

(a) **Size of fat Globules**: The greater the number of fat globules of less than 2 microns size, the greater fat loss in skim milk and vice-versa. Fat globules less than 2 microns are not subjected to sufficient centrifugal force and so enters into skim milk.

(b) **Presence of Air in the milk**: The greater the amount of air, the higher the fat loss in skim milk. This is due to disturbance of counter-current streams of cream and skim and milk and lowers the efficiency.

(c) **Acidity of the milk**: The higher the acidity, the lower the efficiency of separation. The higher the acidity, the lower the stability of casein particles, which in turn get precipitated and clog the bowl, thereby lowering the efficiency.

(d) **Degree and Temperature at which milk is agitated before separation**: The higher the degree and temperature of agitation, the greater the fat loss in skim milk and vice-versa. Agitation of hot milk causes the disintegration of fat globules into smaller ones which escape the effect of centrifugal force, thereby leading to more fat loss in skim milk.

### 7.6 Milk Standardization for FAT and SNF Procedure

**Definition**: Standardization of milk refers to the adjustment i.e. either raising or lowering of fat and/or solids – not-fat percentages of milk to a desired value, so as to confirm to the legal or other requirements prescribed.

**Procedure**: Milk is standardized by the addition of milk or cream with a higher or lower fat percentage than that of the material to be standardized. Sometimes the addition of skim milk will do. To solve the problem, it is necessary to find the relative amounts of the original material and the standardizing material to be mixed together to make a product with the desired fat content. Once these relative amounts/proportions have been determined, it is easy to calculate the exact number of each which must be mixed together to give a certain weight of the finished product or the exact amount of standardizing material needed to use up a given weight of milk or cream. A simple scheme, the Pearson’s square, can be used to calculate the relative quantities of the materials involved in a standardization problem. It should be remembered that all measurements based on these calculations are by weight and not by volume.

The Pearson’s square method is as follows. Draw a square and place in the centre of it the fat percentage desired. Place at the left-hand corners of the square, the fat percentage of the materials to be mixed. Next subtract the number in the centre from the larger number at the left-hand side of the square and place the remainder at the diagonally opposite right-hand corner. Subtract
the smaller number on the left-hand side from the number in the centre and place the remainder at the diagonally opposite right hand corner. The numbers on the right hand side now represent the number of the parts of each of the original materials that must be blended to make a product with a fat test given by the number in the middle of the square. The number at the upper right hand corners, and the number at the lower right corner refers to the parts of the material, whose fat test was placed at the lower left represents the parts of the finished products, with the fat test given by the number obtained in the middle of the square.

**Problem 1:** How many parts by weight of 35% fat cream and 4% fat milk must be added to make milk testing 5% fat milk?

```
35          1.0

5

4  30.0
```

Hence 1 part of 35% fat cream when mixed with 30 parts of 4% fat milk will give 31 parts of 5% milk.

**Problem II**

How many kgs each of 28% cream and 3% milk be required to make 500 kgs of a mixture testing 4% fat.

```
28          10

4

3  24.0
```

To make 25 parts of mixture testing 4% fat it requires 1 part of 28% fat cream.

To make 500 kgs of mixture how much 28% fat cream is required.
C = 500 x 1/25 = 20 kgs

Milk 3% fat required is = 500 – 20 = 480 kgs.

Proof

500 kg of 4% standard milk contains 500 x 4/100 = 20 kg fat
20 kg of 28% fat cream contains 20 x 28/100 = 5.6 kg fat
480 kg 3% fat milk contains 480 x 3/100 = 14.4 kg fat
So 5.6 + 14.4 = 20 kg fat which is equivalent to fat present in 500 kgs of 4% milk.

7.6.1 Standardization of cream

Definition: This refers to the adjustment of the fat level in cream to the desired percentage, confirming to standard requirements.

Procedure: The fat percentage in the cream is usually adjusted to the prescribed level by the addition of calculated amount of skim milk. For this Pearson’s square method described in chapter 2.6 is followed.

Problem: Given 1000 kg of cream testing of 50% fat. How much skim milk testing 0.1% fat must be added to obtain 40% fat in the standardized cream?

\[
\begin{array}{c|c|c}
50.0 & 40 & 39.1 \\
0.1 & 10.0 & 49.1 \\
\end{array}
\]

It is seen that 39.1 parts of 50% fat cream when mixed with 10 parts of 0.1% fat skim milk will give 49.1 parts of standardized cream testing 40% fat.

To prepare 49.9 kgs of standardized cream. Skim milk required is 10 kgs. To prepare 1000 kgs of standardized cream how much skim milk is required?

i.e. \(1000 \times 10/49.9 = 200.4\) kgs.

For 1000 kgs of cream with 50% fat 200.4 kgs of skim milk with 0.10, fat should be added to prepare 1200.4 kgs of standardized cream with 40% fat.
Summary

Filtration of milk is done to improve aesthetic quality of milk, and types of filters explained. The principle and operation of clarification process narrated. Gravity and centrifugal methods of cream separation described and mentioned the difference between these methods. The cream separator parts are shown with the help of sketch diagram. Various factors affecting the efficiency of cream separation are fully explained. The fat percentage adjustment in milk and cream is explained by solving the problems which helps in commercial formulations.

Short Answer Type Questions

1. What is the objectives of filtration of milk?
2. Give the principle of clarification.
3. Mention the different location points for cold and warm clarifiers.
4. Give the strokes law formulae of gravity and centrifugal cream separation.
5. What is the principle in centrifugal cream separation?
6. Name the two outlets in a cream separation.
7. How the position of cream screw will change the fat % in cream.
8. Define standardization of milk.
9. What is the effect of temperature of milk in cream separation?
10. What are the factors that will increase cream separation rate in gravity method?

Long Answer Type Questions

1. What are the advantages and disadvantages of cold and warm filters
2. Briefly write about construction details of a filter.
3. Explain about clarification process of milk.
4. Briefly write about gravity methods of cream separation,
5. What are the major differences between gravity and centrifugal methods?
6. Draw a sketch diagram of cream separator and label the parts.
7. Explain the various factors affecting cream separation.

9. How many kgs of 6.2% fat milk and 0.4% fat skim milk are required to prepare milk testing 3.5% fat.

10. Given 15250 kgs of cream testing 42.5% fat. How much of skim milk is required to decrease the fat % in cream to a level of 30%.
Structure

8.1 Pasteurization – Definition – Objectives, Advantages and Disadvantages
8.2 Types of Pasteurization
8.3 HTST Pasteurization
8.4 UHT Pasteurization
8.5 Sterilization of milk
8.6 Homogenization Definition – Advantages and Disadvantages
8.7 Packing of milk (Prepack) and storage

Learning Objectives

After studying this unit, the student will be able to

- Understand the concept of heat treatment to milk by Pasteurization/sterilization.
- Understand the concept of Homogenization and Prepack

8.1 Pasteurization – Definition – Objectives, Advantages and Disadvantages

Pasteurization term has been coined after the name of Louis Pasteur of France, who in 1860-64 demonstrated that heating wine at a temperature between 122 to 140°F (50 to 60°C) killed the spoilage organisms and helped in
preservation. The application of this term “pasteurization”, although, Louis Pasteur pioneered studies on heat treatment for preservation, pasteurization of milk was first attributed to Dr. Soxhlet of Germany in 1886.

**Definition**: The term pasteurization as applied to market milk today refers to the process of heating every particle of milk to at least 63°C (145°F) for 30 minutes or 72°C (161°F) for 15 seconds or to any temperature – time combination which is equally efficient in approved and properly operated equipment and immediately cooling to 4°C.

As per international Dairy Federation (IDF) pasteurization is defined as a process applied to a product with an object of minimizing possible health hazards arising from pathogenic microorganism associated with milk by heat treatment, which is consistent with minimal chemical, physical and organoleptic changes in the products.

**Objectives**

1. To render the milk safe for human consumption by destruction of cent percent pathogenic microorganisms,

2. To improve the keeping quality of milk by destruction of almost all spoilage organisms (85 to 99%).

**Need**: As it is difficult to exercise strict supervision over all milk supplies, it becomes necessary to pasteurize milk so as to make it safe for human consumption. Any impairment of nutritive value is of the slightest extent.

**Objections**

1. Pasteurization encourages slackening of efforts for sanitary milk production.

2. It may be used to mask low quality milk.

3. It diminishes significantly the nutritive value of milk.

4. It reduces the cream line or cream volume.

5. Pasteurized milk will not clot with rennet.

6. Pasteurization may be carelessly done; it gives false sense of security.

7. It fails to destroy bacterial toxins in milk.

8. In India, pasteurization is not necessary; as milk is invariably boiled on receipt by the consumer.
Formulation of Standards

The following considerations were involved in the formulation of standards for pasteurization.

**Bacterial Destruction**: Cent percent for pathogens. Mycobacterium tuberculosis being considered the most heat resistant among pathogens, was chosen as the index of organism “Coxiella bernette” was considered the heat resistant organism among pathogens. Any heat treatment (i.e. temperature – time combination), which kills T.B/ Q fever organism, also destroys all other pathogens in milk.

**Cream Line reduction**: The Cream line or cream volume is reduced progressively with increase in temperature – time of heating. The consumer judges the quality of milk on the basis of the cream line.

**Phosphatase inactivation**: The complete destruction of phosphatase by pasteurization. (The phosphatase test is used to detect inadequate pasteurization).

Thus the standards of pasteurization were such as to ensure 1. complete destruction of pathogens 2. Negative phosphatase test and 3. Least damage to the cream line. As T.B. Germs are destroyed by a heat treatment slightly lower than that for phosphatase inactivation, pasteurization is carried out at a heat treatment temperature above that for phosphatase inactivation and at below that for cream-line reduction as shown below.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>30 minutes</th>
<th>15 seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>To kill T.B. Germs</td>
<td>138°F / 58.9°C</td>
<td>158°F / 70°C</td>
</tr>
<tr>
<td>To inactivate phosphatase</td>
<td>142°F / 61.1°C</td>
<td>160°F / 71.1°C</td>
</tr>
<tr>
<td>Pasteurization Requirement</td>
<td>143°F / 61.7°C</td>
<td>161°F / 71.7°C</td>
</tr>
<tr>
<td>Creamline Reduced</td>
<td>144°F / 62.2°C</td>
<td>162°F / 72.2°C</td>
</tr>
</tbody>
</table>

**Types of Pasteurization**

1. Batch Pasteurization
2. High Temperature Short Time pasteurization (HTST)
3. Ultra – high Temperature pasteurization(UHT)
4. Vacuum Pasteurization
5. Stassanization.
8.2 Types of Pasteurization

This is also called low temperature – long time (LTLT) method. The milk is heated to 63°C / 145°F for 30 mts and promptly cooled to 5°C or below. In this method heating and cooling of the product is done through a metal wall. When the product is heated or cooled gentle agitation is done for rapid heat transfer. Agitation must not be so rapid that whipping or churning occurs. For continuous processing 3 to 5 tanks may be connected in series. Depending upon the method of heating the batch process may be classified into four types.

1. **Water Jacketed Vat or Flooded tank system**: This is double walled around the sides and bottom in which hot water or steam under partial vacuum circulates for heating and cold water, for cooling. The outer wall (lining) is usually insulated to reduce heat loss. The heat exchange takes place through the wall of the inner lining. The difference between the temperature of the heating water and the milk is kept to a minimum. The milk is agitated by slow moving (revolving) paddles / propellers. When heating, the vat cover is left open for escape of off flavours and when holding, the cover is closed. During the holding period, an air space/ foam heater (steam or electrically heated) prevents surface cooling of milk.

   **Advantages**

   (a) Flexibility in use (It is also known as a multipurpose or multi process vat).

2. **Water Spray type**: It consists of an inner tank for product surrounded by an outer tank to form space between the two. A film or hot water is sprayed from a perforated pipe over the outer surface of the tank holding the product. The product is agitated. A rapidly moving continuous film of hot water provides rapid heat transfer. The temperature of hot water is kept about 72°C to heat the product to 62°C. The speed of the agitation is 45 to 50 rpm. The overall heat transfer coefficient of water spray heat exchanges is approximately 1000 k cal / hm²°C.

   **Advantage**

   (a) Flexibility in use (b) It provides quicker control

3. **Coil- Vat type**: In this method, the heating or cooling medium is pumped through a coil place in either horizontal / or vertical position, while the coil is turned through the product. The turning coil at a speed of about 30 rpm agitates the product. The coil and walls of the tank is constructed of stainless steel.
The side and bottom of the tank is insulated. Steam or hot water may be used as heating medium. The overall coefficient about 1000 k cal / hm²°C

**Disadvantages:** Coils are difficult to clean, which accounts for decline in their use.

4. **High Velocity Liquid type:** A heating or cooling medium is pumped at a high velocity over the outside surface of the tank through pipes surrounding the tank. Vat pasteurization is well suited for small plants and for low volume products. It can handle a variety of products with a wide range of physical characteristics.

But vat pasteurization is a batch operation and is slow. It requires manual controls and constant attention must be given to prevent over heating and over holding. Regenerated heating is not possible so heating and cooling of products is relatively expensive.

**Agitation of Liquids.**

![Fig 8.1 Batch Pasteurization](image)

In food industry the purpose of agitation may be promoting heat transfer, uniform heating or cooling, preventing separation of various elements of the product being processed; through mixing of products or maintenance of homogenous distribution and equalization of concentration and temperatures. The agitators may be impellers of self pacing control as sown in the fig: No:8.3
Fig 8.2 Batch Pasteurization Unit

Fig 8.3 Self-acting Control

Fig 8.4 Milk Pump
8.3 HTST Pasteurization

The HTST system usually employs plate heat exchangers for heating regeneration and cooling. In this method milk is heated to 72°C for 15 seconds. An HTST unit consists of a balance tank, a timing pump, a regeneration tank, a heating section, a holding section, a cooling section a flow diversion value (FDV) and controls as shown in the Fig 3.5.

HTST pasteurizer was first developed by A.P.V.Co, in the U.K. in 1922.

Milk Flow: The following steps or stages are involved as milk passes through the HTST pasteurization system Balance tank, pumps, regenerative heating, heating – holding, regenerative cooling and cooling by chill water or brine. An arrangement for incorporation of the filter/clarifier, homogenizer etc., in the circuit is also made when desired.

![Fig 8.5 High temperature short-time pasateurizer](image)

Raw milk from storage tank will enter in to float control balance tank (FCBT) which controls the flow rate by sinking or floating in the milk. Centrifugal milk pump with a flow control device to ensure constant output is used and after FCBT a rotary positive pump between regeneration and heater.

Plates: The heat exchanger plates will be about 1.25 to 3 mm thick. The plates are used for heating of milk to temperatures which are below the point of boiling. The plate heat exchanger is a compact, simple, easily cleaned and inspected unit. Its plates may be used for heating/cooling, regeneration and holding. These plates will have pors openings to permit transfer of fluid through
the plates, which are gasketed in such a manner that during operation, milk and medium cannot mix and no leakage can occur. The gap between the plate is about 3-5 mm. These plates are supported in a press between a terminal block in each heating and cooling section. The heat moves from warm to a cold medium through stainless steel plates. These plates are numbered and must be properly assembled.

They are tightened in to place and are so designed as to provide a uniform but not excessively turbulent flow of products with rapid heat transfer. Raised sections (corrugations) on the plates in the form of knobs, diamonds and channels, help to provide the turbulent action required. Usually the ports are provided in appropriate places, both at the top and bottom of heat exchanger plates, to permit the products and heating cooling medium flow in alternative passages without mixing.

**Regenerative Heating:** The raw cold incoming milk is partially and indirectly heated by the hot outgoing milk (milk to milk regeneration). This adds to the economy of the HTST process, as the incoming milk requires less heating by hot water to raise its temperature for holding.

**Filters:** Various shaped filter units to connect directly to the HTST system are placed after the preheater or regenerative heating section at 43°C for warm siltation. Usually 40 – 90 mesh cloth, usually in cylindrical shapes are used. Usually two filters are attached but they are used one at a time. This permits continuous operation, the flow being switched from one to the other while replacing a filter.

The warm raw milk is forced by a pump through the final heating section, which raises the temperature of milk by using hot water or vacuum steam to 72°C and then through holding section it takes at least 15 seconds to traverse.

**Flow Diversion Value (FDV):** It routes the milk after heat treatment. If the milk has been properly pasteurized, it flows forward through the unit; that which is unpasteurized (i.e. in which the temperature does not reach the legal limit) is automatically diverted back to the FCBT for reprocessing. It is usually operated by air pressure working against a strong spring, should the temperature fall, air pressure is released and the valve snaps shuts immediately. Then the temperature is regained, air pressure builds up and the valve opens to forward flow.

The system is so arranged that any failure of air or electricity moves the valve in the diverted position. The flow of unpasteurized milk can also be stopped with a ‘pump stop’ which automatically stops the milk pump motion of the product temperature drops below the desired level. When the proper temperature is
reached the pump stop restarts the operation and allows the flow of milk to continue.

**Regenerative Cooling**: The pasteurized hot outgoing milk is partially and indirectly cooled by incoming cold milk (milk to milk regeneration). This again adds to economy of HTST process. In fact when precooled (raw) milk is received the high degree of regeneration (72 to 85\%) allows water cooling to be dispensed with, entirely.

From regenerator down the milk goes to the final cooling section. When chilled water cools the milk usually to 4°C flow rates of hot water and cooling water are about 4-8 and 2.5 to 4 times that of milk respectively.

As mentioned above, the final heating may be achieved by hot water or vacuum steam. In the hot water system, the water is circulated through the pasteurizer. Steam injection in a compact unit usually mounted on the pasteurizer. Steam injection is controlled by a diaphragm valve operated via a pneumatic relay. Which is actuated by thermosensitive bulb placed in the hot water pipe or in the milk line. Variations in the water temperature produce an immediate response in the diaphragm valve and consequently in the amount of steam injected.

The water temperature is then maintained within very narrow limits. In the vacuum steam heating system, the heating section of the pasteurizer is put under vacuum by a vaccum outfit, which consists of centrifugal pump, section, water tank, cooling coil or three water injectors. This also evacuates condensate. Steam is fed to the pasteurizer through steam valve and expands in under pressure prevailing in the heating section. A damping device supplies condensate to the steam, preventing over heating of the later.

**Control Panel**: It contains instruments, controls, FDV – mechanism and holding system, all centralized in one moisture proof panel. The lower halt of the panel forms and air-insulated chamber which carries the holding tube.

**I. Automatic control Device**: This include 1. Steam pressure controller: Maintains a constant hot water temperature for heating of milk accurately to the required pasteurization temperature. (Acts as a reducing valve in the steam supply line, so as to give a constant steam pressure).

**II. Water Temperature Controller**: Regulates the amount of steam entering the hot water circulating system.

**III. Milk Temperature Recorder**: Records the temperature of milk leaving the holding tube / plate. This is an electric contact instrument that operate either a FDV or a milk supply pump, automatically preventing milk from leaving
the holding section at sublegal temperature. Both the frequency and duration of
the flow diversion and the temperature of milk leaving the holder are recorded
on the thermograph (recording chart) by means of two separate pens. The
check thermometer is placed near the milk temperature recorder.

**Pressure in the System**: The normal pressure maintained in the HTST
system are

- **Pasteurized milk**: 15 PSI
- **Raw Milk**: 14 PSI
- **Heating / Cooling medium**: 12 to 13 PSI

**Holding time test**: The holding time of a HTST pasteurizer is the flow
time of the faster particle of milk of milk at a prescribed temperature through the
holding section. The holding time is calculated between the points at which the
heated milk leaves the heating section and reaches the FDV. The efficiency of
pasteurization in the HTST system depends as much on the correct maintenance
of temperature as on the holding time. Hence the later should be checked
periodically.

**Advantages of HTST System**

1. Capacity to heat treat milk quickly and adequately, while maintaining
   rigid quality control over both the raw and finished product.
2. Less floor space required.
3. Lower initial cost.
4. Milk packing can start as soon as pasteurization begins, thus permitting
   more efficient use of labour for packing and distribution.
5. Easily cleaned and sanitized (system adapts well to CIP cleaning).
6. Lower operating costs.
7. Pasteurizing capacity can be increased at nominal cost.
8. Reduced milk losses due to closed system.
9. Development of thermophiles not a problem as holding time is less.
10. The process can be interrupted and quickly restarted.
11. Automatic precision controls ensure positive pasteurization.
12. This imparts less cooked flavour to the milk.

13. It is well suited for regeneration heating and cooling.

Disadvantages

1. The system is not well adapted to handle small quantities of several liquid milk products.

2. Gaskets require constant attention for possible damage and lack of sanitization.

3. Complete drainage is not possible.

4. Margin of safety in the product sanitary control are so narrow that automatic control precision instrument is required in its operations.

5. Pasteurization efficiency of high thermoduric count raw milk is not as great as it is when the holder system is used.

6. Greater accumulation of milk stone in heating section (due to higher temperature of heating).

8.4 UHT Pasteurization

Ultra High Temperature pasteurization (UHT) was developed in 1950's. In this method milk is heated to 135 - 150°C for no hold (a fraction of a second).

UHT process is carried out by two main ways.

1. Indirect heating system

2. Direct heating system.

Indirect heating system: These are self contained continuous sterilizing plants, and are to some extent like the conventional HTST pasteurizing plants, although the operating pressures are higher. The heat exchanger may be of plate type, tubular coil type or sometime scraped surface type.

The heating medium is steam under pressure. Most of the plants employ either plate or double or triple concentric tube heat exchanger. The operating principle is same for all plants. The operating temperature is achieved by regeneration and indirect steam heating.
Fig 8.6 Milk is pumped from the balance tank 1. Through the first regeneration section of the heat exchanger. 2. And is filtered it. It then passes to a section. 3. Heated by low pressure steam (0.35 – 0.45 atm) controlled by hand operating valve. In this section the milk is heated to 85°C and then the milk is homogenized 4. The milk is held to 5-7 mts in a holder tank. 5. To reduce the amount of deposit formed on the heating surfaces from the milk at later stages. The homogenizer provides the pressure to pass the milk through the unit. A spring loaded relief valve. 6. Set at about 5 atm is connected between the homogenizer outlet and the balance tank to prevent excessive pressure developing in the plate assembly. Then the milk passes through a second regenerator section. 7. and through a section. 8. Heated by stream at a pressure of 5-6 atm.

Milk leaves this section at about 135 - 145°C and after a short period of 2-4 sec. holding period, it passes through a flow diversion value. 9. Operated by the control system and the two regenerator sections(7,2) to the milk outlet. The second regenerator is by passed by a line with a hand-operated valve in order to give manual control of the outlet temperature. This will be 70°C for hot filling before an in-bottle sterilizing process. If the milk has to be cooled further more regeneration will be used.

Milk diverted by the flow diversion value must be cooled to below boiling point before it can be returned to the balance tank. This is done in water.
cooling section of the heat exchanger (10) preset values (11) are connected as restrictions.

A temperature sensitive element (x) measures the temperature of milk as it leaves the heater and the controller provides air pressure to a diaphragm valve in the steam line of the final heating section.

**Advantages**

1. Produces the milk of high bacteriological quality.
2. Little effect on colour and flavour of milk.
3. Control system is simple compared to direct system.
4. Water and electricity requirements are less than direct system.
5. Steam consumption is same as in the case of direct system.

**Disadvantages**

It forms deposits on the heating surfaces, which is difficult to clean.

**Direct Heating System**

In this system product is heated by direct contact with steam. This is accomplished either by injecting steam into the product or by admitting the product into a chamber containing an atmosphere of high pressure steam (infusion heaters). The injectors are smaller and less expensive than the infusers, but requires a higher operating temperature. The steam used must be of culinary quality and with some products, it may be necessary to remove the steam, which condenses in the product so that the original composition may be maintained.

Milk is heated by mixing it directly with steam at high pressure, so that the steam is condensed and gives up its latent heat there by heating the milk almost instantaneously to 140 – 150°C. The excess water is removed by evaporation in a vacuum chamber an the milk is at the same time cooled by the extraction of latent heat. Undesirable volatile odours may also be removed during this evaporative cooling.

Milk is supplied from float controlled balance tank by a pump to the preheater, where it is heated to 80°C with hot water. Then it passes through a steam chamber. The steam chamber may be a steam injection head, where steam is injected into the milk, or a steam pressure chamber which is filled with steam and milk is sprayed in this chamber. Here milk temperature is raised to 140°C - 150°C in a fraction of second. Milk then passes through holding tube which it takes 2.5 to 4 sec to traverse. The milk now sterilized. Continues
through a flow diversion valve (some plants do not have FDV) to a constant vacuum vessel. Here the milk temperature falls instantly to about 81 °C to 82 °C. the flashed vapor is condensed in the condenser.

Fig 8.7 UHT Pasteurizer
Milk is drawn from the vacuum vessel by an aseptic pump and passed to an aseptic homogenizer and then to aseptic final cooler, through an aseptic precooler, in which its temperature is lowered to about 20°C. Milk is then aseptically packaged. The diverted milk goes back to balance tank for vacuum chamber.

The vacuum in the expansion vessel is so controlled that the amount of the water evaporated is equal to the amount of steam condensed, so that the total solids percentage of milk remains unaltered. Apparently it may be achieved, if the milk temperature at the outlet of expansion vessel is equal to its temperature immediately before it was mixed with steam.

Advantages

1. The plants will run for long period compared to indirect system without cleaning.
2. Produces good quality product.
3. Off flavours are removed.
4. Able to process variety of products with little modifications.

Disadvantages

1. High initial cost.
2. High operating cost.
3. More complex and so more difficult to operate.

Stassanization: This type of pasteurization is carried out in tubular heat exchanger consisting of three concentric tubes. The principle of its operation is that heating of milk to the desired temperature by passing it between two water heated pipes through the narrow spaces of 0.6 – 0.8 mm. The milk is heated to 74°C (165°F) for seven sec. The rest of the process resembles HTST system.

8.5 Sterilization of milk

Sterilized milk may be defined as milk which has been heated to a temperature of 100°C or above or such lengths of time that it remains fit for human consumption for at least 7 days at room temperature.

Commercially sterilized milk must

(a) Keep without deterioration for a sufficient period to satisfy commercial requirement.
(b) Be free of microorganisms and toxins harmful to health of consumer.

(c) Be free of any microorganisms capable to proliferate, it should not show any signs of bacterial growth.

**Sterilization System and Plants**

There are three methods of milk sterilization as indicated below.

(a) In container sterilization, in which milk is bottled and heated for 20 to 40 mts at temperature between 110 and 120°C.

(b) Ultra high temperature process discussed under UHT pasteurization

(c) Two-stage process, where the milk is first sterilized according to UHT process, then boiled and finally subjected to further heat treatment to destroy any spores which may have entered during bottling.

**Incontainer Sterilization.**

In this process the milk is heated in container at a temperature of 100°C - 120°C, usually by steam. The temperature of milk rises slowly on account of the slow heat penetration especially when the container is not agitated in the sterilizer, because of this and for the fact that the bottles do not withstand sudden and extreme temperature changes, the milk must be sterilized by a time, temperature combination where by the temperature is low and the time correspondingly is long. This tends to give the milk a rather strong flavour and a brownish colour especially when the bottles are not agitated in the sterilizer. In container sterilizers are grouped in two categories i.e.

Batch Sterilizer. Continuous sterilizer.

**Batch Sterilizer:** these sterilizers use steam as heating medium. Batch sterilizers may be either stationary type or rotary type. The simplest type is the stationary autoclave or sterilizer. This is a pressure vessel, either cylindrical or rectangular in cross-section, designed to hold steam under a pressure sufficient to give required sterilizing temperature. The sterilizer is fitted with doors at one or both ends which are provided with gaskets to make them air tight when closed. The autoclaves are equipped with indicating pressure gauge, indicating thermometers and temperature and pressure controller to maintain the inside steam pressure of required level. The sterilizer may also be equipped with a timer so that after the lapse of the desired holding period steam supply will automatically stopped and a bell will ring. An air vent is provided usually at the top of the sterilizer to facilitate the removal of air.
When the sterilizer is fully loaded with crates of bottles, the door is closed and sealed and the vessel is put under steam pressure corresponding to the required treatment temperature. After the desired processing time, the steam is vented out to atmosphere. The crates of bottles are removed either for natural air cooling or for cooling by blown air from fans. Water cooling can also be done in the autoclave before the bottles are removed.

Some sterilizers are built so as to agitate the milk during heating by rotating the load of bottles. The shell of the sterilizer may rotate in a horizontal axis. In this way, the rate of heat transfer to milk is increases. These are called batch type sterilizers. In this sterilizer the heat treatment of milk is more uniform than in non rotating batch type.

![Fig 8.8 Schematic diagram of a rotary batch sterilizer](image)

Batch process is relatively wastes full of steam, and large heat losses are unavoidable. The steam consumption will be 0.2 – 0.5 kg per litre of milk.

**Continuous sterilizer**

In the system the containers of milk are loaded mechanically in to a conveyor which carries them in continuous sequence through the plant, so that the milk automatically subjected to the required sterilizing process. These sterilizers can be divided into two kinds – namely those operating with steam as the sterilizing medium and those using hot air, those operating with steam are either hydrostatic or hydrolock system.
**Hydrostatic Sterilizer**: consists of a number of towers which together form a number of ‘U’ shaped passages. One of the towers filled with steam, is maintained at high temperature.

The other towers are filled with water to seal off the steam tower from the atmosphere to allow a high pressure of steam, and hence a high temperature. The product in the containers is conveyed through the machine in carriers fitted between two endless chains. An automatic feed system introduces the containers into the carrier. At the end of cycle, an automatic discharge mechanism removes the containers from the machine. A bottle normally leaves the outlet seal at the temperature of 85°C. Further cooling is obtained either by passing the bottle through a cooling tank or lower, or by spraying water at controlled temperature on to the bottle. This sterilizer is economical in the use of steam, water and labour. Steam consumption in 0.1 to 0.15 kg per litre of milk.

Hydrolock sterilizers contain a water sealed rotary valve, not commonly used.

Hot air sterilizer have various forms. In one form the bottles are crated and the crates are loaded to a conveyor which move through a tunnel. They are first heated by hot water spray of increasing temperature and then by hot air at temperature up to 145°C circulated by fans. The hot air is normally heated by steam pipes. Cooling is effected with hot water sprays in stages reducing temperatures. In another form the bottles are heated and cooled by passing through water baths instead of sprays.

The hot air sterilizers have the advantage of the temperature is obtained without pressure. But thermal efficiency is poor.

The raw milk on receipt should be strictly examined and only high quality milk should be used for production of sterilized milk. Take only with no developed acidity and with least number of spore forming bacteria. The milk is promptly cooled to 5°C for bulk storage and check bacterial growth. Then milk is preheated to 35 - 40°C for efficient filtration / clarification, so as to remove visible dirt etc., and to increase the aesthetic quality. Then milk is again cooled to 5°C so as to preserve its quality. Then it is standardized to confirm legal requirements and stored at 5°C until further processing.

Then milk is preheated to 60°C for efficient homogenization to prevent any visible cream layer formation. Then milk is homogenized at 60°C with 2500 PSI pressure. The homogenized milk should be clarified to remove the sediment formed during homogenization process. Then milk is filled in bottles and sealed with special caps (of crown seal type). Then bottles are sterilized by any method discussed in this chapter. Usually the temperature applied is 108 -
11 °C (225 - 230 °F) for 25 – 30 mts. The sterilized milk bottles are gradually cooled to room temperature (sudden cooling causes breakage of bottles) and stored at room temperature.

The official checking test for efficiency of sterilized milk is turbidity test.

Flow diagram of sterilization of Milk

**8.6 Homogenization Definition – Advantages and Disadvantages**

Homogenization refers to the processing of forcing the milk through a homogenizer with the object of sub-dividing the fat globules. According to United States, Public Health Service, homogenized milk is milk, which has been treated in such a manner as to ensure breakup of the fat globules to such an extent that after 48 hours of quiescent storage, no visible cream separation occurs on the milk, and the fat percentage of the milk in the top 100 ml of milk in a quart of bottle, or of proportionate volumes in containers of other sizes, does not differ
by more than 10 percent of itself from the fat percentage of the remaining milk, as determined after thorough mixing (in efficiently homogenized milk, the fat globules are sub-divided to 2 microns or less in diameter).

**Advantages**

1. No formation of cream layers/plug.
2. Fat in milk does not churn due to rough handling or excessive agitation.
3. Better adapted to bulk dispensing, mixing not necessary.
4. More palatable due perhaps to brighter appearance, heavier body and richer flavour.
5. Produces soft curd and is better digested, hence recommended for infant feeding.
6. Less susceptible to oxidized flavour development.

**Disadvantages**

1. Increased cost of production.
2. Returned homogenized milk difficult to salvage, fat recovery is a problem.
3. Sediment appearance to a greater degree.
4. Curdling is cookery.
5. More susceptible to production of activated or sunshine flavour defect.
6. Greater tendency for milk seepage through bottle caps.

### 8.7 Packing of milk (Prepack) and storage

Fluid milk for immediate consumption is packed in glass, plastic or laminated container. The bottle are sealed with aluminum caps. Sachets are single service containers. The four sided tetra pack is a very effective package for fluid milk product.

**Packing in Cans:** Milk is filled in cans made of stainless steel or aluminum and having the capacity of 20 or 40 litres. Before filling milk into the cans, it should be ensured that the cans have been properly cleaned and sterilized. Milk can be filled into the cans directly from the outlet point of the pasteurizer or by providing a holding tank in between.

**Packing in Bottles:** Inspite of the weight of the bottles and disadvantages attached to their return and sterilization of empty bottles, bottles
had been still the most widely used container. However the dairies have shifted to sachet packing. The bottle should be 500 ml or litre of good shape with adequate resistance.

There are two types of bottle fillers in use i.e. gravity fillers and vacuum fillers. In both types, filling nozzles are arranged in a circle. The unit comprising of the float chamber. Filling nozzles and supports assembly revolves the bottles are automatically fed in to the bottle supports, each support raises its bottle so that its mouth is pressed against the soft rubber gasket of the filling nozzles. The milk flows in to the bottle, while it is travelling with this assembly and just before it has made one complete revolution, the filled bottle is automatically brought down and removed and its place is taken by an empty bottle.

In vacuum filler, the supply tank is under moderate vacuum. A high speed centrifugal blower supplies the vacuum by connecting its section in let to the top of the supply tank. Vacuum pipe is connected to the supply tank, vacuum through the filling valve. The lower end of the tube may be opened or closed with ports on the side. As the bottles rises towards the outer tube upward against the spring, this connects the bottle to the vacuum chamber through the vacuum pipe and the airport. And then allows the milk to flow down the annular passage into the bottles.

The height of filling is determined by the distance the vacuum tube (or the position of air port on the tube) extends into the bottle. In case of fillers, which have ports on the vacuum tube, no air flows on to the filler except that contained in the bottle. Another form of vacuum filler is “valveless” type. Vacuum fillers are exact and will not fill a broken bottle. Milk does not drip from the filler valve when a bottle is not under the valve.

Gravity fillers are similar in construction as vacuum fillers with filling valves. However the supply tank is under atmospheric pressure in place of the vacuum. Therefore they will fill even broken bottles and milk will drip from the filling valves when a bottle is not in a position.

In both the type of fillers, the force is gravity and the speed of filling is governed by the head of the milk over the bottle. Vacuum fillers are mostly used because of the advantages mentioned earlier. The adjustment for bottles of different sizes ad capacities differ in various makes. It involves proper centering of the bottles below the nozzles, and adjusting the clearance between the raised bottle support and the nozzles.
The filled bottles leave the filler assembly and are transferred to the revolving capper has three or more magazines each with a movable support beneath it. The support raises the bottle on to the conveyor for inspection and crafting. The rising bottle actuates and mechanism, which slides a cap over the bottle and then brings the cap and bottle mouth against a plunger to force the cap on to place. The plunger is backed up by a spring so that a cap is seated under regulated pressure. The two most commonly used material for sealing of bottles are aluminum caps and crown corks.

**Packing in Single Service - Containers**

Single service containers have the advantages of resistance to tampering, less weight, less bulk, no return of empties and no cleaning problem. On the other hand they are costly and opaque. There are two types i.e. prefabricated packs and the other in form and fill cartons.

Prefabricated cartons are made of card board and coated inside with paraffin or plastic. The fillers for perfabricated cartons operate in a manner very similar to a gravity filler in glass bottles. A pre measured volume of milk dispensed into the carton. High speed fillers upto 260 cartons per ml are available. This is not popular in India.

Form and fill carton system is mostly used now a days. Carton is received as a scored and sized plastic coated blank with bonded side seam. As three components, bottle sidewalls and top or in the form of a roll of heat scalable material. The packing material used is laminated paper, consisting of duplex
craft paper coated inside with polyethylene and outside with wax. Another type of laminated paper consists of a thin aluminum foil sandwiched between an outer craft paper and inner polyethylene coating.

Of different form – fill and seal machines, one is tetra pack for packing of sterilized and pasteurized milk. The machine forms, fills and automatically packs tetrahedron shaped containers (which have least surface to volume ratio) in one continuous operation. In operation, the packing material supplied in rolls, is fed from a reel through an enclosed sanitary chute to the top of vertically designed machine. The paper strip then travels downwards and is formed into a tube by guide rods and forming rings.

The polyethylene coating on the paper acts on the sealing medium. The vertical tube is filled with the product through a filling pipe extending into the tube. Electrically heated jaws, positioned at right angles to each other, alternatively pinch the product – filled tube, forming a chain of individual tetra pack containers. Then a cutter automatically divides the chain into individual units. The individual packages are conveyed to an automatic packer, which positions 18 cartons into a plastic case.

As the strip of packing material unwinds from the cell, a device punches holes in the material and also heat seals paper ‘pull tabs’ over the holes. The pull tab opening is provided near the apex of one side of each finished carton. Removing the tabs uncover a hole through which a straw may be inserted or the milk may be drained.

Special equipment is available for adopting tetra pack machines to aseptic packing of sterilized milk.

Prepack

Generally two types of sachet filling machines are available in dairy industry. They are Prepack and fill pack. The machines available in a capacity of 2500 and 5000 packs per hour with a packing size of one litre, half litre, and 200 ml.

The components of machines are enclosed in a stainless steel cabinet. The major items are either of stainless steel or treated aluminium covered by a weather proof paint coating. Heat scalable film rolls are mounted inside the compartment located in the rear bottom. They are supported on idler rollers or guide rollers. The film is exposed to UV lamp in order to sterilize it; just, before wrapping. The film is overlapped and sealed in to a tube by impulse heated element known as vertical electrode. The downward movement of the film is controlled by a set of nip rollers made of rubber.
Below the nip rollers the film tube enters the horizontal sealer. Here simultaneous seals, across the bottom of each pocket and across the top of preceding sachet, are made with the same horizontal electrode. This also separates one sachet from the other. Injection of product takes place between the strokes of horizontal element, controlled by pneumatic solenoid value. The heat of both the sealing elements are controlled by the circulation of soft water and the movement is controlled by the compressed air (6 kg/cm²).

Fig 8.10 Pneumatic circuit of Machine (Prepac)
Pneumatic system is one of the important systems of the machine which controls the entire mechanical working. The compressed air is passed through an FRL unit, which filters the air, regulates the air pressure to 6 kg / cm² and lubricates the air. A flow of 3-4 drops of oil per mt, is ideal. Pneumatic circuit is given in the fig 8.10.

When solenoid valve ‘A’ closes, i.e. no supply is given to it.

(a) 1 and 3 ports are connected i.e. no supply is given to it.

(b) 2 and 4 ports are connected i.e. return air is going to exhaust when solenoid valve ‘A’ is given supply.

(c) 1 and gets connected

d) 3 and 4 gets connected and air gets exhausted through 4 to exhaust.

When solenoid ‘B’ is not energized, 1 and 2 gets connected. In sachet filling machine an electronic programmer with printed circuit cards have been incorporated. These get the power supply of 24 V DC through a power cord. This supply is required for clutch and beak coils, solenoid coils and D.V.P. coils. Printed circuit cards controls the operation of clutch and brake, scaling heat and injection of the product.

Starting of Machine

1. Put on the main switch
2. Preset the pack length
3. Turn ‘on’ the vertical seal switch and adjust the temperature.
4. Check the film for desired overlapping, beneath the vertical seal electrodes.
5. Turn ‘on’ the auto switches and check the film movement.
6. Check the strength of vertical seal.
7. Turn ‘on’ the horizontal seal switch and check for the seal strength and pack separation.
8. Put the machine on manual operation.
9. Check the filling of the product by inching the machine by turning ‘on’ and ‘off’ the injection switch in quick succession for accuracy.
10. Put the machine on ‘Auto ‘ and turn on the injection switch and make the final adjustment.
11. When the film roll is to be changed for continuous operation, the machine is to be put on. New roll is to be placed and to be joined with the end part of the previous roll and check for the overlapping and sealing. Then switch over the machine to ‘Auto’.

**Aseptic Packing of UHT Milk**

A UHT sterilization system demands reliable aseptic filling avoiding bacterial contamination, because a good sterilization process can be completely nullified by it during and after filling. An aseptic packing system has three main requirements. The container material and any closure system must be adequately sterilized product in a sterile atmosphere and the filled container must be sealed in the similar environment. All the parts must be connected together in such a way as to prevent contamination between the stages.

In a simple aseptic packing system, the UHT plant is connected directly to aseptic straight-line slit filler, supplying milk at a constant flow rate. It is possible to connect the UHT plant to two or more aseptic fillers. Often fillers are intermittent in operation but the packing machine must be capable of dealing with the continuous flow from the UHT plant. Therefore an aseptic tank is incorporated between the UHT plant and filler(s). A by-pass line drawn from the line connecting the UHT plant and the filler connects the aseptic tank. This tank serves as a buffer for a sterile product that is fed to the filling unit(s) in a continuous flow. The aseptic tank will permit a constant flow of milk to the fillers even during the cleaning of UHT plant.

Before starting production, the tank and UHT plant are sterilized at 130°C for 20 to 30 mts with steam. After sterilization, the steam is cutoff and the tank is cooled and air is supplied from a compressor via two pasteurized bacteriological fillers. By regulating the air flow to and from the tank by venting correct pressure is kept on milk to suit the filling machines. The container sterilization and aseptic filling and sealing system would depend on the type of container. Commonest types packages are cans and form and fill cartons.

**Aseptic Canning** : In this system, the filler and closing machine are enclosed in inter connected rectangular boxes. Cans from air cleaner enter the can sterilizer through a narrow passage along a conveyor. The sterilizer box is filled with super heated steam at 230°C - 290°C and the can temperature is raised to 200°C. An atmosphere of superheated steam is maintained in filler, sealing machine and inter connectivity conveyor system to maintain sterility. As the cool sterilized milk flows continuously from the UHT plant, it is filled into the sterilized cans coming from the can sterilizer. The filled cans are then conveyed to the sealing machine. The can covers, sterilized with super heated steam in a vertical chamber, are placed on the cans are sealed by the machine.
Aseptic Cartooning: This system of aseptic packing using tetra pack machine is gaining more attention. The paper strip passes through a bath of dilute hydrogen peroxide at 80°C to sanitize it. Chlorine spray also has been used. After the paper has been formed and heat seeded in to a vertical tube, it passes on to electric heating element which is totally enclosed by the paper tube. The heating element raises the temperature of the inside surface of the paper tube to 200 - 250°C, which renders the sterilizing affect and disintegrates the existing hydrogen peroxide.

The hot zone also prevents atmospheric contamination from passing down the tube and reaching the milk surface. The milk supply tube is thermally insulated and passes down through the heating element. The paper tube containing the milk is heat scaled transversely as has been described earlier.

Fig 8.11 Schematic diagram of Tetra Pack System
Summary

Pasteurization definition, objectives, objection, formulation of standards and various types of pasteurization methods discussed. The batch method, HTST, UHT, Vacuum pasteurization methods were described with the help of sketch flow diagram. Sterilization of milk, types of sterilizers were discussed. The packing of milk, types of packing including prepak and aseptic packing of milk were briefly explained. The various effects of different heat treatment on milk quality and on milk constituents were discussed.

Short Answer Type Questions

1. Define pasteurization.
2. Mention three standards applied for pasteurization.
3. What are the different methods of pasteurization?
5. What is the function of FDV?
6. What is the benefit of regeneration section?
7. Classify different methods of UHT pasteurization.
8. What are the requirements for a commercial sterilized milk?
10. Name the official checking tests for checking efficiency or pasteurization and sterilization.
11. What is prepak?
12. Define aseptic packing.
13. What is browsing?

Long Answer Type Questions

1. What are the objectives, objection and standards for pasteurization process?
2. Briefly explain different types of batch pasteurization.
3. Write detail HTST system with the help of sketch diagram.
4. What are the advantages and disadvantages of HTST system?
5. Explain indirect UHT system.
6. Briefly write about direct UHT treatment with the help of flow diagram.
7. Explain vacuum pasteurization process.
8. Briefly write about the preparation of sterilized milk.
10. Describe in detail about aseptic packing of milk.
11. What are the various effects of heat on milk and milk constituents?
Learning Objectives

After studying this unit, the student will be able to

- Understand about Detergents and Sanitizers
- Different methods and cans of Cleaning and Sanitization.
- Cleaning and Sanitization of HTST Pasteurizers.

9.1 Detergents and Sanitizers – Desirable Characteristics

Cleaning or washing of dairy equipment implies the removal of soil from the surface of each machine. Detergents or cleaning/washing compounds are the substances capable of assisting cleaning.

The soil consists primarily of milk and milk product residues which may be more or less modified processing treatment or interacts on with water or cleaning materials previously used or by dust, dirt or other foreign matter.
Milk stone is an accumulation of dried milk solids and salts from hard water and washing solutions. All dairy equipment should be properly cleaned as milk provides an excellent medium for the growth of microorganisms. At the same time, detergents used for cleaning should be so selected as not to affect the material of the equipment.

**Desirable Characteristics of a good detergent**

1. Good alkalinity
2. Should be freely, easily, quickly and completely soluble.
3. Should not have corrosive action on metal surface
4. Good wetting power or ability to make a contact with the surface to be cleaned.
5. Should make emulsion with fat and remove the same from the surface (emulsifying power.)
6. Good dissolving power or ability to dissolve protein.
7. Good deflocculating power or the ability to break up dirt particles.
8. Germicidal power or effectiveness in killing microorganisms.
9. Penetrating power or the ability to penetrate the milk films on equipment surfaces.
10. Sequestering and chelating power
11. Free rinsing
12. Economical

Dairy detergents are broadly classified into alkalies, acids, polyphosphates and chelating agent and surface active/wetting agents.

**(a) Alkalies**

1. **Soap powder:** Soap powders are understood to be alkaline salts of fatty acids. They have excellent emulsifying properties and therefore are used for removing fat films. They are harmless to metals and to the hands of the operator. Solutions of soap compounds rinse poorly and tend to leave a film on the cleaned surface that readily harbours bacteria and so are not desirable as cleaners on the milk side of equipment. These are suitable for washing wood work, scrubbing floors etc. Their functions can be improved when used with stronger alkalies.
2. **Phosphates**: These are available as phosphate, pyrophosphate, and hexametaphosphates. They are most effectively in combination with soda ash and soda bicarbonate. Trisodium phosphate is effective for cleaning and heating surfaces, such as pasteurizers, but corrodes aluminium and tin. It dissolves protein, is an excellent emulsifier of fats, holds dirt particles in suspension and precipitates the hardness of water as floccules. Other polyphosphates that are being used as an ingredient in compound cleaning agents prevent the formation of stone by forming soluble compounds with calcium and magnesium salts and usually have no harmful effects on metals or human skin.

3. **Caustic Soda (Sodium Hydroxide)**: Caustic soda is the most alkaline cleaner. This is used when vigorous action is desired. It breaks up and dissolves protein particles. Saponifies fats and precipitates the hardness of the water as floccules. It has good bactericidal action and good solvent properties but causes skin irritation and is harmful to painted surfaces. It should not be used on tinned surfaces as it destroys the tin coating and aluminium rapidly. This is more suitable for mechanical bottle washers and for vacuum pan and stainless steel heat exchangers in which heavy protein films are encountered.

4. **Soda ash (sodium carbonate)**: It is an effective remover of film of fats and protein materials, is better for general cleaning purpose. It is an excellent water softener. When mixed with more active chemicals, it acts as a buffering agent and assists in cleaning. It corrodes both aluminium and tin, and is irritating to the human skin.

5. **Modified Sodas (sesquicarbonate)**: These products are a mixture of soda ash and sodium bicarbonates. They are useful for hand washing operations, as they do not cause skin irritation.

6. **Sodium metasilicate** ($\text{Na}_2 \text{SiO}_3 \cdot \text{SH}_2 \text{O}$): It has good wetting emulsifying and deflocculating power. It is effective in preventing lime scale in medium hard water and is an efficient water softener. It is a good solvent of protein material. It has pronounced buffer action, and has proved suitable for use in every type of creamery equipment. It is readily soluble in hot and cold water and rinses easily.

   **(b) Acids**: For milk stone removal, mild acids have been found most satisfactory. Among acids used are nitric, phosphoric, tartaric, citric, gluconic and hydroxyacetic acids in strength of approximately 0.1%. Most acid cleaners are combined with wetting agents to provide the greatest possible penetration of soil.

   Milk protein are coagulated in weak acid solutions which, in turn, are readily dissolved in weak alkaline solutions. For scale removal, the solution
should have high dissolving power and high neutralizing value on the deposits, low corrosiveness on equipment or other surfaces with which it comes in contact. It should be relatively safe to handle and shall be harmless, it residues should get in to food products per chance.

1. Nitric Acid: It is a strong inorganic acid that can easily dissolve milk stone and hard water scale, it attacks tinned metals very strongly but not aluminium or stainless steel. The strongly oxidizing acid has a stabilizing effect upon stainless steel and has also a good disinfecting effect. It burns the skin. It is widely used in cleaning in place (CIP) of plant employed for the heat treatment of milk. For this purpose, acid with strength of 60% is normally used.

2. Phosphoric acid ($\text{H}_3\text{PO}_4$): It is moderately strong inorganic acid which is used to some extent instead of nitric acid.

3. Organic Acids: Such as acetic, oxyacetic, gluconic, tartaric and citric acids have, even in stronger concentrations, a relatively high pH, so that their corrosive effect upon metals is very much less than even weak solutions of nitric acid. They have a good buffering ability, so that they can be used to remove milk stone and hard water scales. They are only slightly irritating to human skin.

(c) Wetting Agents: Water and most aqueous solutions wet the surface with difficulty unless such surfaces are absolutely free of fats or oils. Surfaces active or wetting agents in solution improve the wetting of particles and penetration of the solution in to capillary pores and minute spaces between and under soil particles and equipment surfaces. They assist in forming stable dispersions and emulsions of soil, which is there by easier to remove from the surfaces to be cleaned. Too high a concentration, however would tend to insulate these particles from chemical attack by the solutions.

There are three groups of surfactants, anionic, cationic, non ionic and depending upon how they dissociate in aqueous solution. The most common group is the anionic one. These compounds ionize with negative anion being the active species. They are excellent detergents but poor sanitizers. Examples are sulphosoaps, sulphated alcohols and alkyl aryl sulphonates.

Non ionic wetting agents are also in use ad many of these are liquids. Foaming characteristics vary from low to high; examples are condensation products between ethylene oxide and an alkyl phenol. The cationic group includes the quaternary ammonium compounds. They possess poor detergent properties but are very good sanitizers.
(d) Sequestering Agents: Prevention of water hardness precipitation may be achieved by using sequestering / chelating agents. There are three main classes of chelating agents. The first Ethylene diamine tetra acetic acid (EDTA) and its sodium salts. They are heat stable and are compatible with quarternary ammonium compounds (QAC). It greatly increases the anti-redeposition power of a blend of detergents and also has a bacteriostatic property. The second class consists of sodium of gluconic and heptonic acids which are rather stronger chelating agents for calcium and magnesium than EDTA, but requires high concentration of caustic soda solution (2-5%) for effectiveness. The third class comprises of the poly phosphates. They are not heat stable.

(e) Inhibitors and antifoaming agents: Inhibitors are used to minimize the corrosive attack of acids and alkalies on metals eg. Sodium Sulphite is used to protect tinned surfaces from attack by alkalies and Sodium silicate protects aluminium and its alloys from attack by mild alkalies. Anti foaming agents may be incorporated for special applications such as bottle washing. Where foam may be generated by pumping and jetting action during detergent recirculation.

Sanitizers

Sanitization implies the destruction of all pathogenic and all most all non pathogenic microorganism from equipment surface. Sanitizers are the substances capable of destroying all pathogenic and all most all non pathogenic microorganisms.

Desirable Characteristics of a good Sanitizer

1. Non toxic
2. Quick acting
3. Relatively non corrosive to hands and equipment
4. Easily and quickly applied
5. Relatively inexpensive

The commonly used dairy sanitizers are hot water, steam, chemical sanitizers (chlorine compounds, iodophors and Quarternary ammonium compounds)

Hot Water: It is one of the most effective germicidal agents as it can contact all clean surfaces of the equipment. It is used in sufficient quantities and it kills a large percentage of the bacteria. To be effective as germicidal agent, water should have temperature of less than 80°C and be circulated for 15 mts. This temperature is taken at the outlet of the processing equipments.
Steam: It is very effective for sterilizing vats, pipe lines and equipments, which can be at least partially closed during the process. The equipment should attain a temperature of 78°C for at least 15 mts or 93°C for 5 mts.

Chemical Sanitizers

Chemical sanitizers or sterilants are very effective germicidal agents.

1. Chlorine compounds: Chlorine sanitizers generally corrosive to aluminium, copper tinned surfaces and stainless steel. Corrosion by chlorine is increased by higher temperatures and concentrations. Inorganic compounds include sodium hypochlorited and chlorinated trisodium phosphate, and organic compounds are dichloroisocyanurate and chloramines – T, the inorganic agents may be used as sanitizing agents alone. Organic agents may be used with detergents.

Sodium hypochlorite containing 10-15% active chlorine with a pH 7-8 will do a good job. The lower pH though increases its effectiveness, but solutions below 7 is highly corrosive to all metals. Chlorinated Trisodium phosphate contains about 3 – 3.5 % available chorine. It is used in combination with appropriate salts. The solution pH used is 8 -8.5. Chlorine – T contains 25% active chorine and rather a slow sanitizer. The working solution should be of pH 7 for satisfactory effectiveness. Halane has 68% available chlorine and behaves much like chloramine – T, Di and Trichloroisocyanuric acid and their salts have 60 – 90% available chlorine and are least corrosive for all chlorine compounds. They can be used at solution pH at as high as 9.5 – 10.0.

The methods of application can include circulation with 200 ppm (0.02%) for 5 mts through pumps, pipelines and coolers immersion in a 200 ppm solution for 5 mts; spraying large open holding vats with 300 ppm solutions with 5 mts contact time. Fogging is carried out closed vats and tankers with 500 ppm solution with special automizing equipment and brushing cheese vat surfaces and agitators weighting vats and similar open vessels with a 400 ppm solution.

Quaternary Ammonium Compounds (QAC) are non irritant to skin and cationic. They possess both antibacterial and surface active properties. They should not be used with anionic wetting agents in which case their effectiveness is greatly reduced. Hard water salts also reduce their bacterial effectiveness. They form deposits on glass surfaces and their last traces are difficult to remove from equipment by rising. Even small residues of QAC may be harmful to starter culture organisms. Their use therefore is limited.

Iodophores: Iodophores are prepared from iodine and suitable nonionic wetting agent which serves as “carrier” of Iodine. Acidified conditions enhance their bacterial activity and those approved for use in the dairying industry are
invariably acidified, usually with phosphoric acid. The presence of surface active agents and acids confers detergent properties on these Iodophors and all are classified as detergent sanitizers. However they show only poor affectivity against fat residues.

They will if used regularly, help to prevent accumulation of milk stone, but they should not be expected to remove existing milk stone. They cannot be used at higher temperatures, say higher than 50°C, as Iodine vapors will be released which are highly corrosive for all metals. Unless the acid content is fairly low, they can corrode all non stainless steel metals to some extent. Some plastic materials and rubber gaskets absorb Iodine and are stained brown.

Acids like nitric acid and phosphoric acids are now being used as sanitizers or detergent sanitizers eg. 0.5 litres of nitric acid (60%) per 100 litres of water is considered adequate. Sodium hydroxide at 1.5 – 2 % solution at 45°C for 2 mts is effective against non-spore forming bacteria.

9.2 Cleaning and Sanitation Methods – Hand, Machine and CIP Systems

**Principle:** In the selection of any particular detergent, consideration should be given to type of soil, quality of water supply, material of surface and the equipment to be cleaned and method of cleaning viz., soaking, brushing, spraying and/or recirculation. Detergents are invariably used as an aqueous solution. In the selection of dairy sanitizers there are two types.

(a) **High temperature Sanitizing:** Advantages are penetrating ability and quick drying of equipment.

(b) **Low temperature Sanitizing:** Advantages are, permits sanitizing immediately before equipment is used (when hot equipment will be injurious to the quality of milk / milk products) avoids excessive strain on the equipment and permits flushing out of equipment immediately before use. Generally, chlorine at 15 - 20°C containing 150 – 200 ppm available chlorine for 1-2 minutes contact time is used.

The usual procedure for cleaning and sanitization of major items of dairy equipment should consist of ---

**I. Draining:** To remove any residual loose milk and any other matter.

**II. Pre-rinsing:** With cold or tap water to remove as much milk residue and other matter as possible.

**III. Warm to hot detergent washing with detergent solutions of 0.15 to 0.60 % alkalinity, to remove the remaining milk solids.**
IV. **Hot water rinsing**: To remove traces of detergents.

V. Sanitizing to destroy all pathogens and almost all non pathogens.

VI. **Draining and drying**: To help prevent bacterial growth and corrosion.

The selection of detergents and sanitizers for different surface materials of equipment are shown below.

<table>
<thead>
<tr>
<th>Material</th>
<th>Cleaning</th>
<th>Sanitization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Stainless steel</td>
<td>All alkalies may be used. Care should be taken with acids.</td>
<td>All sanitizers may be used.</td>
</tr>
<tr>
<td>2. Mild steel</td>
<td>All alkalies may be used. Acids should be used together with inhibitors.</td>
<td>All sanitizers may be used.</td>
</tr>
<tr>
<td>3. Tinned steel / copper</td>
<td>Weak alkalies together with sodium sulphite as inhibitors should be used.</td>
<td>All sanitizers may be used.</td>
</tr>
<tr>
<td>4. Bronze</td>
<td>Weak alkalies together with sodium sulphite as inhibitors should be used.</td>
<td>All sanitizers may be used.</td>
</tr>
<tr>
<td>5. Galvanised</td>
<td>Weak alkalies together with sodium sulphite as inhibitors should be used.</td>
<td>All sanitizers may be used.</td>
</tr>
<tr>
<td>6. Aluminium alloy</td>
<td>Weak alkalies, together with sodium silicate as inhibitors should be used.</td>
<td>All sanitizers may be used.</td>
</tr>
<tr>
<td>7. Glass</td>
<td>All alkalies and acids may be used.</td>
<td>All sanitizers may be used.</td>
</tr>
<tr>
<td>8. Vitreous enamel</td>
<td>Weak alkalies, together with sodium silicate as inhibitors should be used.</td>
<td>All sanitizers may be used.</td>
</tr>
<tr>
<td>9. Plastics</td>
<td>Cleaning temperature should not be above the softening point of plastic.</td>
<td>Only chemical sanitizers should be used.</td>
</tr>
<tr>
<td>10. Rubber</td>
<td>Strong alkalies should be used to remove any fatty material stuck to the surface.</td>
<td>Only chemical sanitizers should be used.</td>
</tr>
</tbody>
</table>
Note

Chlorine sanitizers, if left in contact with metal surface, cause corrosion. So it should be preferably used just before processing.

Methods of Cleaning and Sanitization

The methods of cleaning and sanitization of dairy equipment are hand washing and mechanical or machine washing and cleaning-in-place (Inplace-cleaning).

I. Hand Washing: The following points, in general should be remembered while cleaning the equipment.

(a) The equipment should be rinsed with water at 43°C – 50°C.
(b) The equipment is washed with warm water containing suitable washing powder. All parts are brushed to loosen dirt particles.
(c) Equipment is rinsed first with warm water and then with hot water.
(d) All pumps, valves fittings and sanitary pipelines are completely dismantled for washing.
(e) All equipment are completely sanitized with steam, hot water at 82°C or by chemical sanitizer.
(f) All parts are permitted to dry and are left exposed to circulating air, Rack is provided for storage of sanitary pipes and parts.
(g) Pumps and sanitary pipelines, fittings or valves are not assembled until ready for use.
(h) After an equipment is assembled, it is sanitized with hot water, steam or chlorine solution.
(i) Pump parts, valves, sanitary fittings and freezer dashers should be handled carefully during washing and sanitizing operations in order to protect the machined surfaces.
(j) Convenient dry place should be provided for storage of washing powder and chemical sterilizers.

The normal cleaning and sanitization of hand washed dairy equipment in organized dairies should be done as follows.

1. Prepare 0.8 to 1.0% of detergent mixture in tap water, so as to give a minimum alkalinity of 0.5 percent (pH over 11.) in a wash up tank and maintain the temperature at about 50°C.
2. Thoroughly rinse the utensils with clean cold water.

3. Introduce the detergent solution in to the equipment (quantity of solution to be determined by requirement and experience). Thoroughly brush the equipment surface, inside and outside, with a clean can-brush.

4. Wash the utensil with enough fresh cold water using a clean brush again, if needed, to remove all traces of detergent.

5. Allow the equipment to drain thoroughly and let it dry (for at least 1-2 hours).

6. Sanitize the equipment surface by steam / hot water after cleaning and / or by rinsing with chlorine solution (200 ppm available chlorine) just before using.

**Bottles may be hand washed as follows.**

A three compartment tank (with drainage outlet for each) is selected. Two thirds of first compartment is filled with water at 50 - 55°C containing alkali detergent. The second compartment is filled with water only at 50 - 55°C and the third with cold water with 150 – 200ppm available chlorine. The drained bottles are put in the first compartment and allowed to soak for few minutes, then brushed with a clean bottle brush both inside and outside. Then the bottles are placed in the second compartment for sufficient time and after careful emptying are placed in the third compartment. Then bottles are left up side down to drain and dry.

**II. Mechanical / Machine Washing:** This consist mainly using machine which will perform work by fully automatic or semiautomatic systems. Examples are can washers and bottle washers.

The can washers may be either rotary or straight through / tunnel type. The capacity may be 4 -12 cans and lids per minute. The mechanical bottle washer may either be soaker type (soaking) or hydro (jetting) or soaker – hydro (part soaking and part jetting). Further it may be of the come back or straight-through type; in the former, loading and unloading take place at the same end, while in the latter, they are done at opposite ends. Generally, soaker-hydro-comeback types are popular for smaller capacities and straight-through-hydrotypes used are larger capacities. The stages of treatment in a mechanical bottle washers are given below.

(a) Prerinse, using water at 32 – 38°C.
(b) Detergent wash, usually 1-3 percent caustic soda, together with chelating and wetting agents given preferably in two stages at different temperatures within 60 - 75°C. Sanitizes the bottles as well.
(c) Water rinse, to remove all traces of detergent. Reduces bottle temperature for next stage. Water temperature varies from 25 - 45°C and is usually recirculated.

(d) Cold water rinse, normally recirculated chlorine water (containing 35 – 50 ppm. Available chlorine) is used to prevent recontamination of bottle.

(e) Draining after the bottles come out of the machines.

**Soaker Type Washer**: Is used in large dairy plants, soaking of completely full bottles in caustic solutions is the main action of cleaning bottles. The cleaning efficiency depends on the action of the solution in which bottles are immersed.

![Fig 9.1 Schematic drawing of a straight-through soaker type bottle washer](image)

Before the bottles are transported from one soaker tank to the next, they must be emptied by being approximately tilted. They are refilled in the next tank. The advantage of various zones is temperature adoption (to prevent bottle breakage due to thermal shock) and the division into zones in which the amount of soiling matter is different.

**Jet Type Washers**: The jet water sprays the solution inside and outside of bottles, through a series of jets in order to wash and sanitize them. The inverted bottles pass over different zones having a number of nozzles through which the solution and water is sprayed at a pressure about 2 kg / cm².
Division of zones offers the already mentioned advantages in this case as well. With the aid of jetting, strongly adhering soil components can be removed, even those which are soluble only with difficulty or are insoluble.

**Soaker Jet Type Washer**: It combines the advantages of soaking and jetting and is therefore suitable for washing the bottles used for sterilized milk filling.

In all bottles washers it is used to load manually or semi-automatically onto one or more conveyers travelling to the filling machines. In all washers two endless chains, one either side of the washer, run in vertical plane over suitable sprockets and guides. Two chains are synchronized and are generally moved mechanically with an intermittent motion, one pitch at a time. Connecting the two chains are a large number of carriers. Which provide a series of baskets (receptables) in which bottles are taken through the process.
Where soaking tanks are used, the bottles travel down into the tank in a horizontal position, filling as they become immersed; after traveling through the tank, they are carried upwards, emptying as they rise above the liquid. Where jets are used, it is most important that the inverted bottles should centre exactly over a jet during the pause between movements.

### III. CIP Cleaning

It is also called in-place-cleaning (IPC). This refers to the system of cleaning also sanitization which does not require the daily dismantling of dairy equipment. Inplace cleaning is based on taking the detergent to the equipment rather than taking the equipment to the detergent.

**Types of CIP system are.**

1. **Manual Control**: In this, the completion and setting up of the product and CIP circuits is done manually; the valves are hand operated and the entire process is controlled by the operator.

2. **Automation**: Three levels of automation is possible i.e. low level in which setting of CIP and other product circuits is done automatically. Medium level in which setting up of CIP and product circuits as well as different types of treatments are all controlled automatically. High level in which computer is used for complete control of entire product manufacture and CIP system in large plants.

Two different techniques are used in CIP cleaning i.e. single use system and reuse system.

1. **Single use System**: In this system the cleaning solution are used once at the lowest possible strength, and discharging it to the sewer at the end of each cycle. It is therefore limited to very soiled equipment in which the detergents are completely used up in the one passage. Solutions which are not completely used up can to be stored in an additional recovery tank and reused as a prerinser for the next cleaning run.
2. **Reuse System**: In this system, the same solutions are used for a large number of cleaning operations. They are fitted with separate tanks for each type of detergent as shown in Fig: 9.4.

![Diagram of Re-use System of CIP Cleaning](image)

**Fig 9.5 Re-use System of CIP Cleaning** 1. Newtalization 2. Return water 3. Acid 4. lye I 5. lye II 6. Fresh water

The cleaning reagents to the solutions is added as required to maintain its strength and cleaning ability. It requires more space and utilize more parts in the form of tanks, valves, controls etc. Tanks and pipes are cleaned with 0.5 – 1.0% caustic soda solution and milk heating equipment with a 1-2 % solution.

Some recent installation have incorporated system which combine the advantages of single use systems (flexibility and reliability) with water and solution recovery procedures which aid in reducing the total amount of water required for a given cleaning cycle. These systems were designed to recover the ‘spent’ cleaning solution and post rinse water from one cleaning cycle, store it temporarily and then release this detergent rinse water mixture as re-rinse for the subsequent cleaning cycle. This reduces water requirement by 25 – 30% steam requirement by 12 – 15% and chemical consumption by 10 -12%.

**Merits of CIP system**

1. Ensures that all equipment receives uniform treatment day after day, by eliminating the human factor.
2. Less damage to equipment (no dismantling and assembling)
3. Saving of (25% or more) total clean up costs and man hours.
4. Reduces possibility of contamination through human error.
5. Improved plant utilization and appearance.
The closed equipment such as pipelines, plate and tubular heat exchangers. HTST pasteurizers, pumps, homogenizers and evaporators are cleaned by pumping the solution. Open equipment such as tanks, silos, churns and spray driers requires spraying, devices that will give complete coverage of the surface being cleaned.

The success of the CIP system depends upon

1. Proper temperature of cleaning solutions.
2. Adequate velocity of cleaning solutions.
3. Proper selection of pipes and fittings, installation and development of circuits.
4. Use of detergents designed specially for re-circulation cleaning.
5. Proper concentration of detergent solution.

9.3 Cleaning and Sanitization of Cans — Types of Can Washers

A large quantity of milk in dairy is received in cans. The sanitary conditions of cans used in dairy affects the quality of raw milk and finished products produced from the raw milk. Milk cans are constructed of aluminium, stainless steel or tinned steel. The aluminium cans are sturdy and light, but pit easily and are difficult to clean. The problems of rusting, pitting and wearing off a tin coating are eliminated by using stainless steel. The most commonly used milk can is made of steel and is coated both inside and outside with tin.

All cans, after dumping of milk, should be washed thoroughly dried and sterilized. Drying of cans is more important than complete sterilization, as it can is dry, the remaining bacteria will not multiply. If the cans remain wet, bacteria will multiply in the presence of moisture.

Manual Washing: Small dairy plants and milk collecting centres employ manual washing for cans. In hand washing, cans should be prerinsed with water at 32°C to 38°C. three tanks are used for cleaning operations. The first tank contains 200 litres of warm water (52°C) and good balanced alkaline cleaner (115 g to 230 g per litres of water). The detergents used are either soda ash or washing soda. Cans are immersed in the solution and brushed thoroughly inside and out with a going type brush.

They should then be washed in the second tank containing warm water at about 60°C. Finally it should be rinsed in the third tank containing clean and
hot water at about 65°C. If the steam is available, they should be subjected to a treatment, if no steam is available, they should be subjected to a bacterial rinse containing 200 ppm of QAC for 2 mts. (Chlorine should never be used). These cans and covers are then inverted and stored to dry.

**Mechanical Can Washing:** Medium ad large size dairy plants use mechanical washers. Mechanical washers may be either manually operated type or power operated type.

**Advantages**

(a) Occupies little space.

(b) Machine can be operated by a single worker.

(c) Time is reduced.

The cleaning and sanitization procedure for mechanical can washing consists of the following stages.

1. Drainage stage for liquid milk residues.

2. Pump-fed prerinsing with cold or Luke warm water. This is done by passing ordinary water through a jet to clean the milk film remaining in the can. The temperature of water used for rinsing is about 25°C. The water is passed through the jet at a pressure of about 1-2 kg/cm² for 3-6 seconds, so that it can rinse properly.

3. Drainage stage to remove water.

4. Pump fed jetting with detergent at not less than 70°C, this is done by passing the washing solution through jets at a sufficient high pressure to remove all dried milk and cream film inside and outside of the cans. When using an alkaline it should be less than 0.40% for farmers can and 0.15% for dairy cans. Caustic soda must not be used as the detergent, but sodium carbonate and a corrosion inhibitor (sodium sulphite for tin or sodium silicate for aluminium) are suitable.

5. Drainage stage to remove detergent.

6. Hot water rinsing at 85-88°C. the temperature of the can should increase at successive stage, as the sterilization and drying stage of steam and hot air temperature will be higher than 100°C.

7. Final fresh water rinsing with steam and water ejector at 88 – 93°C.

8. Live steam injection (sterilization)
9. Drying with hot air at 95 – 115°C to prevent the corrosion of metal due to moisture and to check bacterial growth. The air used for drying should be filtered.

![Diagram of a rotary can washer]

**Fig 9.6 Operation diagram of a rotary can washer**

1. **Rotary Can Washers**

   Rotary can washer carries the inverted cans on a large rotating table. The table is mounted on a vertical shaft, and is rotated by means of an electric motor through a warm gear drive. The movement may be continuous or intermittent. The cans are loaded manually on the table and it passes through the various sections viz. Pre-rinsing, washing, hot water rinsing, sterilizing and drying. The rinsing water and washing solution is circulated through jets installed blower. After drying the cleaned can is taken out and another can is loaded for washing on the rotating table.

   The capacity of rotary can washers varies from 3 to 6 cans per minute depending on its size, make and the number of treatments given in the washer. The rotary can washer is simple in construction, occupies less floor space, and is used in smaller dairy plants. They can wash the cans of any size and shape, but recontamination of cans by the washer or by the operator is difficult to avoid. These require 1.0 kg steam per can, water 8.7 litre per can and energy 0.86 Kwh per 100 cans.

2. **Straight – Through Can Washer.**

   This type carries the cans through the washer in a straight line by means of a continuously moving conveyor or slide along rail as they move intermittently
from one jetting position to the next. The driving unit at regular intervals show the can forward from one position to the next.

![Fig 9.7 Schematic drawing of a straight-through can washer](image)

The prerinsing (position 2) is done by spraying sufficient amount of wash through jets inside the can. The position 4 employs an acid cleaning by circulating acid solution inside the can instead of usual alkaline solution. In the washing (position 4 and 5) the washing solution at 65 - 70°C is circulated for two times. At position 6 and 7 hot water at a temperature between 80°C and 90°C is used for rinsing. The high temperature of water saves the amount of steam consumed in the next treatment. The sterile rinsing (position 8 and 9) is done by passing steam through jets at about 107°C temperature. After sterilizing, hot air drying (position 10) is done by passing air at 124°C temperature, the temperature of the hot water and washing solution is controlled by means of thermostats.

The capacity of straight through can washers varies from 6 – 16 cans per minute. This type is used in large size dairy plants. It is easily accessible for maintenance and cleaning of inner parts. There is no recontamination of cans either by the washer or by the operator. It occupies more floor space in dairy and requires space and close inspection while in operation.

The requirements are 0.85 – 1.8 kg of steam per can, water 4.0 to 4.5 litres per can, energy between 1.0 and 2.4 Kwh.

**Selection of Can washers**: In selection of can washers the following points should be taken into consideration.

1. Number of cans to be washed per day in a given period of time
2. Quality of drying is necessary.
3. Available space for can washer and can storage before and after washing.
4. Steam requirements and capacity of boiler available to meet the steam requirements of the washer.

5. Relative cost of manual washing and mechanical washing.

6. Facility requirements for maintenance.

**Maintenance of Can washers.**

1. The water valves should be opened, cleaned and water pressure 2 to 4 kg/cm² should be checked. If pressure is less, it is due to clogged jets and defective pump. Which should be rectified. The pump strainer inlet and outlet lines should be checked for the accumulation of lime and scale.

2. The can washers should be cleaned as and when necessary. All the wash sprays, nozzles and strainers should be checked and cleaned.

3. The mechanical difficulties are caused by lack of regular and sufficient lubrication failure to make adjustment for wear and failure to replace worn out parts. Most of the mechanical difficulties can be avoided by proper lubrication of the moving parts, making adjustments for wear and replacing the worn out parts.

4. The temperature and pressure of washing solution, hot water steam and drying air should be maintained at optimum level. The proper strength of solution is also important.

5. Heavy scale deposits in can washers are usually the result of inadequate daily cleaning, use of hard water and use of solution at inadequate temperature and strength. The scales can be removed by using inhabited acid at a temperature of about 50°C.

**9.4 Cleaning and Sanitization of HTST Pasteurizers and other equipment**

**HTST Pasteurizer:** The CIP method is followed.

After the day’s operation, cold water followed by warm water at a temperature not exceeding 38°C is passed through the equipment to rinse out the remaining milk. Rinsing is completed when the water, leaving the plate sections becomes clear. The outlet pipe from the plate heating section is then connected with the down steam side of the milk regenerate and the outlet of the final cooling section is extended to the top of the float control tank. By this arrangement, the timing pump and flow diversion valves are by passed by the cleaning solution. These are washed separately. A centrifugal pump is connected in the line between...
the float controlled tank and the up stream side of the milk regenerator to circulate the acid leaning solution.

An acid cleaning solution of 0.5% is prepared. This solution is pumped through the plate and circulated for zero minutes at a temperature between 60°C - 65°C. After the acid solution treatment is completed, hot water is added to the solution container. After the solid materials have been flushed away, the outlet pipe from the unit is removed so that warm rinse water is pumped from the container through the plates and on to the floor. This rinsing should continue for 10-15 minutes. The hot water is then turned off. And the outlet pipe to the solution container is replaced so that a mild alkaline solution should be circulated through the plates following the acid solution treatment. A detergent (soda, ash, tri sodium phosphate or sodium silicate) solution of strength 0.25 – 0.5 is prepared. The alkali solution should be circulated at 60-75°C for 30 minutes.

After alkali solution treatment, the cold water is pumped through until the plates are cooled to at least room temperature; Sterilization is continued by circulating hot water (80°C - 90°C) for 15 minutes through the system or by chlorine solution (Containing 100 ppm chlorine) at 20°C for 10 minutes or by a combination method.

In an alternative method alkali treatment precedes acid treatment. In this method a 0.7 – 1.5% solution of caustic soda 70°C - 75°C is circulated for 30 minutes after rinsing. Then cold water is pumped through the plant for 5 -10 minutes to rinse away any residual detergent. This is followed by the circulation of 0.5 – 1% acid solution at 70°C for 30 minutes. The pasteurizer is then flushed with water for about 5-10 minutes. Sterilization is done as before.

**Milk Storage Tanks / Milk Tankers**

All interior surfaces of storage cans and tank trucks should be rinsed with water at 50°C. The agitator should be removed, if it is removable. Large vats and tanks may be washed either with a mechanical spray mechanism or by hand. When washing by hand, the cleaning solution should be made up in a bucket and carried in to the vat or tank. By using a long handled brush, all surfaces can be scrubbed thoroughly. Particular attention must be paid to cleaning of sight glasses, vents pipe opening, manhole. Gasket and agitator. The final step is through rinsing with hot water, followed by air drying with all valves, lids, vents and manhole open for maximum aeration. The sterilization may be done either by live steam or by using 200 ppm chlorine solution.

The introduction of mechanical methods put an end to the inadequacies or the manual cleaning method. There are high pressure jetting methods, where a small amount is sprayed on the walls under high pressure and low pressure
jetting methods in which large amounts of liquid coming from jet nozzles or rotating turbine nozzles are jetted over the tank walls. For spraying tanks and vessels, spray cleaning devices of various types are used.

![Spray Cleaning devices](image)

**Fig 9.8 Spray Cleaning devices**

The CIP method of cleaning of tanks using above mechanical devices as follows.

(a) Pre rinse with tap water.

(b) Drain for 3 – 5 minutes.

(c) Hot detergent wash with sodium hydroxide solution (sodium hydroxide 90 parts, sodium thiosulphate 9 parts and washing agent 1 part.) of 0.35 – 0.5 % strength at 71 °C for 15 – 20 minutes. Once or twice a week, an acid – alkali program may be used. The acid may be phosphoric or nitric. This should be followed by alkali as above.

(a) Drain for 3-5 minutes

(b) Post rinse with hot water at 65 - 75°C

(c) Drain for 3 -5 minutes

(d) Sanitize with hot water at 90°C for 2 - 3 minutes or chlorine solution at 15 – 20°C containing 150-200 ppm available chlorine for a contact time of 1 -2 minutes.

(e) Drain for 1 – 2 minutes.

(f) Hot air blow for 1-2 minutes.
Glass Enamel Milk Vats: These are cleaned like other equipment’s except for alkali preparations, which should not be used. Alkali attacks the glass coating, etches the enamel and injures it.

Batch Pasteurizer: The equipment is pre-rinsed using a brush and bucketful of solution of general purpose detergent at 43°C, it is scrubbed. Finally it is rinsed with hot water. The agitators should be cleaned separately. Thermometer holes in the lid, and the air space heaters, if present, should not be forgotten. These should be scrubbed thoroughly. The entire outside surface of the pasteurizer and the lid should be washed each time the interior is cleaned. Batch pasteurizers and also uninsulated tanks and vats equipped with covers may be sterilized with steam.

A short hose or pipe may extend from the steam line into the vat through the thermometer opening or manhole. The outlet valve should be left open to permit the condensed steam to drain from the vat. After the steam has been applied for 20 – 30 minutes, the steam valve should be closed and cover of the vat raised to permit the steam to escape. After the steam has escaped and hot surface dried, the cover should be replaced to prevent contamination. Large insulated vats and tanks are best sterilized with a spray of chlorine solution.

Surface Milk Coolers: The pre rinse is immediately followed with cleaning solution and while the solution is flowing over the cooler surface, all the parts of the cooler are brushed vigorously. Particular attention is paid to the joints on the under side and ends of each section. The washing by cleaning solution is followed by the final rinse during which the brush should not be used. Surface coolers may be sterilized before re-using by allowing hot water at 88°C to flow over the surface for 10 – 15 minutes. When a milk cooler is enclosed with metal covers, steam from a hose may be passed in to the cooler compartment for 15 – 20 minutes and the tubes heated to a sterilizing temperature. Alternatively 200 ppm chlorine solution may be circulated for 10 minutes. After sterilization, the covers should be opened to permit the steam to escape and the tubes to dry.

Milk Pumps: After the flow of milk has ceased, head of pump is removed and it is cleaned thoroughly with water at 50°C. Impellers are removed and placed in the cleaning solution. Intake and discharge parts of pumps and chamber are washed thoroughly with a pipe brush. The impeller is brushed, placed in a wire basket, rinsed with hot water, and permitted to dry. The pump and pipelines after reassembly may then be sterilized by pumping hot water (88°C) through them. Hot water or chemical sterilization is preferable for milk pump as steam will not easily pass through certain types of pumps.

Milk Pipes: These are rinsed free of milk by passing warm water through them. They are then taken apart and soaked in general purpose detergent
solution for 20 minutes. They are then brushed in a special in trough used for washing sanitary pipes and fittings. Next they are rinsed thoroughly with hot water and permitted to drain and dry. Sterilization is accomplished just before next use by passing small flow of steam through the pipeline for 10 – 15 minutes or by using a 200 ppm chlorine solution of hot water.

**Separators and Clarifiers**: Immediately after the day’s run, the equipment is rinsed with 50°C water until the discharge is clear. It is dismantled, the bowl and disc removed and each piece rinsed with warm water before placing it in the wash vat. The wash vat should contain an alkaline cleaning solution and each disc should be washed separately. All discs and other parts should then be thoroughly rinsed with hot water and racked to drain and dry. Next day, when the machine has been reassembled, it is sanitized with hot water or chlorine solution of 200 ppm strength.

**Homogenizers**: After each days use, cold water is pumped through the machine without pressure until water at the discharge is clear. Then a 0.5% solution of detergent at 50°C- 60°C is circulated. Thereafter all parts coming in contact with the milk are dismantled and by using a brush, internal parts of the block and all removable parts are washed in the washing powder solution. Removable parts are placed on draining rack and rinsed thoroughly with hot water at 77°C. Block inside is rinsed in the same way.

Piston packing is washed as in the second step, rinsed with hot water and placed in glass jar containing chlorine solution 200 ppm. With the compressed filtered air, the inside of block and parts, that have been removed to drain rack are dried. Just before use, the machine is assembled and 200 ppm chlorine solution is circulated for 5 mts. Rinse water is pumped for 3 mts. To take care of water left in the machine and to prevent the bottling of unhomogenized milk, enough of the first milk is set aside through the machine, equal to 2% of the rated hourly capacity of the machine.

**Evaporator**: The CIP system consists of

(a) Warm water rinse (45°C)

(b) After the water has run clear, cleaning with a 1 – 4% alkaline solution (80°C) by spraying and circulation for 45 – 60 m.

(c) Discharge of caustic solution and a worm water rinse.

(d) Circulation of a 0.3 – 0.5% acid solution (70°C) for 20 – 3- minutes to remove mineral deposits.

(e) Discharge of the solution and a worm water rinse (60°C)
(f) Cleaning of external surfaces with a cleaning solution if necessary with brushing. Final rinsing with slightly chlorinated water.

(g) Immediately before use, another sterilization is necessary. A rinse with 75 ppm available chlorine for 5 minute is good. Because of danger of corrosion, this solution must immediately rinsed off again.

**Dryers**: The CIP system followed for evaporator is followed, except that before start up, of the dryer must be well dried. For sterilization, hot air to the walls of dryer for 10 minutes at 90°C.

### 9.5 Dairy Effluents – Treatment Measures

Dairy wastes (effluents) may have its origin from disposal of spoiled products or by products, Spillage, overflow, leakage, rinsing from washing of equipment etc. The volume of effluent from a dairy processing plant is related to the particular product being produced, normally processing of one litre of milk yield 8 – 10 litres of waste water depending upon types of products manufactured, quality of water utilized, type of process in operations and control of management. The dairy wastes are particular as compared to other industrial wastes, because of relative high concentrated efficient, danger of shock, load of waste water, particularly whey and butter washings water in to drains.

**The strength of dairy waste is expressed in different terms**

1. **B.O.D.**: Biological or bio chemical oxygen demand is the quantity of oxygen in mg consumed by biological agents in a sample of waste incubated over a period of five days at 20°C. Expressed as mg BOD per litre or ppm BOD. The value indicates the organic matter content in the waste.

2. **C.O.D**: Chemical oxygen demand is the quantity of oxygen in mg O₂ or ppm which is necessary for the chemical oxidation of organic substances in the presence of potassium dichromate and silver sulphate as catalysts. It includes oxygen utilized for oxidation of compounds that is not utilized by micro organisms.

3. **T.O.C**: Total Organic Carbon content i.e. amount of organic carbon content per litre of water expressed as mg per litre.

The strength of dairy waste i.e. B.O.D varies from 300 – 2000 mg / litre depending upon type of product made and quantity of milk processed.

**The B.O.D of some of dairy products are**

- **Milk**: 1, 10,000 mg B.O.D
- **Skim Milk**: 72,000 mg B.O.D
Whey: 44,000 mg B.O.D

As per the Bureau of Indian Standards (B.I.S), for disposal of industrial effluents as application of dairy wastes are

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Tolerance into inland surface water</th>
<th>Limits for into public sewer</th>
<th>Industrial onland irrigation</th>
<th>Effluents into marine coastal area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. B.O.D. mg/lit max.</td>
<td>30</td>
<td>350</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2. C.O.D mg/lit max</td>
<td>250</td>
<td>--</td>
<td>-</td>
<td>250</td>
</tr>
<tr>
<td>3. PH valves</td>
<td>5.5 to 0.90</td>
<td>5.5 to 0.90</td>
<td>5.5 - 9.0</td>
<td>5.5 to 9.0</td>
</tr>
<tr>
<td>4. Dissolved solids (inorganic/mg</td>
<td>2100</td>
<td>2100</td>
<td>2100</td>
<td>-</td>
</tr>
<tr>
<td>5. Suspended solids</td>
<td>100</td>
<td>600</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>6. Temperature °C max</td>
<td>40</td>
<td>45</td>
<td>-</td>
<td>45</td>
</tr>
</tbody>
</table>

**Effluent Treatment Methods**

The selection of waste treatment method for a specific application is done by considering the above effluent standards, location of sites, waste water characteristics, waste water variation, and cost of treatment etc. The methods are classified as mechanical methods, chemical methods and biological methods. Mechanical and chemical methods are used to separate solid wastes from waste water which are further disposed.

**Mechanical Methods**: These are 1. Screening using siever. 2. Filtration- using filter. 3. Sedimentation: Allowing for gravity sedimentation. 4. Flotation: Aided by gas bubbles to which the particles of impurities becomes
attached and removed. 5. Reverse osmosis would also remove dissolved constituents, but it is not suitable in large scale.

**Chemical Methods**: Consists of precipitation of dissolved substances by means of suitable precipitating agents such as iron sulphate, iron chloride, aluminium sulphate. A sedimentable coagulum is formed which also contains suspended solids. The formed coagulum may be removed by using any mechanical methods.

**Biological Methods**: These methods are most suitable methods. Dissolved or colloidally suspended organic compounds are decomposed by oxidation with the aid of aerobic or anaerobic microorganisms. Dairy wastes are normally treated by

i. **Conventional Methods**: Eg: Activated Sludge method, trickling filters

ii. **Low cost treatment Methods**: Eg: Aerated Lagoons Stabilization pond, oxidation Ditch.

iii. **Anaerobic Methods**: Eg: Septic Tank method.

**Activated Sludge Method**: This is one of the most popular methods used in the treatment of dairy waste water, this is given in the fig. 9.9.

![Activated Sludge Method Diagram](image)

The degree of purification is high in this method. The first step is to allow sedimentable impurities to sediment in a settling tank. Then the waste water is passed in to aeration tank where actual biological purification takes place. The influent waste water and recycled sludge enter the tank at the head and are aerated for period of about 6 – 8 hours. The air is incorporated by pressure jet or surface aerators and amount of air incorporated should be sufficient to raise the oxygen content in aeration tank at least 1-4 mg/litre. The optimum
temperature to aerobic digestion is 30°C. The water is also agitated to prevent the flocculated material from settling. The sludge contains desirable microorganisms which decompose organic matter. Then the waste water and sludge mixer passes into sedimentation tank where the flocculated material settles within 2-4 hours forming a layer of sludge at the bottom.

The sludge is returned at a rate of approximately 25 – 50% of influent flow rate. This process provides aerobic biological treatment using suspended growth of bacterial floc. High concentration of organisms, maintained by sludge return reduces the size of reactor. The sludge should not remain for too long time (more than 6) as it can start to decompose by purification and gases evolved will float to the surface. Part of organic matter, then increasing the sludge amount. The solids/sediments obtained in primary settling tank and excess sludge from final settling tank are decomposed by digestion method.

The sludge floc is generally produced by the growth of zoogale bacteria and other organisms in the presence of oxygen.

**Advantages**

1. Occupies least space for comparable loading rates.
2. BOD and suspended solids removal is good to excellent
3. Operator can exercise control over the process
4. No odour or fly problems.
5. Wasted sludge is fairly stable.

**Disadvantages**

1. High capital cost
2. High operating cost
3. Requires skilled operator

**Trickling Filters (BIO FILTERS)**

Trickling filters or percolating filter contains broken pieces of plastic or any other material which serve as support for the growth of bacteria, yeast, fungi, protozoa and nematodes. The depth of rock varies with a particular design but ranges from 0.9 to 2.5 meters. The media grows into slimy skin on the filtering medium. Aerobic heterotrophic bacteria play role in form of jelly like matrix which enmeshes microorganisms and suspected particles forming a strong floc. The important species is zoolea ramigera which produce extra cellular mucopoly saccharide responsible for floc formation.
Liquid waste water is distributed over the top of the bed by rotary distributor. A stream of air is often passed through the filter medium. By aeration the cells are destroyed by their own metabolism (endogenous oxidation) thus greatly reducing sludge volume. Sloughing of sludge is a natural process and must occur in order to maintain an active biological film on the media. Trickling filters are followed by clarification in order to intercept and remove sloughed solids from the filter.

Single passage of efficient through the filter will not give satisfactory results. The effluent may be passed several times through the same filter.

![Schematic diagram of a percolating filter](image)

**Fig 9.10 Schematic diagram of a percolating filter**

**Advantages**

1. Initial cost is less than activated sludge method.
2. BOD and suspected solid removal efficiency good.
3. Greater space requirements than activated sludge method but less than lagoon system.
4. Low to moderate maintenance cost.
5. Can be arranged in series or with other biological oxidation system.

**Disadvantages**

1. Prohibitive cost for smaller industries.
2. No operator control on the process.
3. Start up time is 3 – 4 weeks to build up functional system.
**Aerated Lagoons** : This is extension of activated sludge process. It is a single unit system in which there is no sludge return. It consists of simple earthen basin 2.5 to 3.5 meter deep in which mechanical surface aerators are installed on floats or on a permanent base to aerate the liquid contents.

In this method single tank acts as biological oxidation and sedimentation tanks. Other principle and operation is just like activated sludge process. This process uses low concentration of activated sludge solids in the range of 50 – 500 mg / litre. This is developed by Central Public Health Engineering Research Institute, Nagpur.

**Oxidation Ditch**

Oxidation ditch is an oval shaped closed circuit channel of simple construction.

Raw waste enters into the channel, where mechanical rotors vigorously aerate the mixer liquor containing a high concentration of activated sludge solids in the channel and keep the mixed liquor in circulation. The channel depth is 1 – 1.5 m and can be constructed in simple masonry on earth work as long as water tightness is ensured. Oxidation ditch is essentially an extended aeration system, in which low organic loading high biological solids concentration in the range of 4000 mg / litre and extended period of aeration is normally used. This system requires sludge return involving separation of solids from the effluent and returning the same in to the system. National Environment Engineering Research Institute (NEERI), Nagpur has developed this system.

**Advantages**

1. Effluent removal of BOD and suspected solids.
2. No odour and fly problem.
3. Lower operating cost.

**Disadvantages**

1. Higher initial cost
2. Energy is required for aerators, pumps etc.
3. Problem in winter season.

**Rotating Biological Contractors (RBC)**

This process is a modification of the trickling filter process, in which fixed biological film is rotated through the waste water. The apparatus consists of a number of large diameter (12 ft) light weight plastic discs mounted on a
horizontal shaft to form rotating biological contractors or discs. The discs are about half immersed and slowly rotates as waste passes through a horizontal pen tank. The tank usually has a semi circular bottom to fit the centres of the disc. Microorganisms attach to the surface of the discs and grow by assimilating nutrients from the waste water.

The discs are rotated at 2 r.p.m, while submerged to about 40% of their area. Organic matter is absorbed by the biomass on the discs and is subsequently oxidized in the presence of oxygen. A positive means of excess film sloughing is provided by the shearing action caused by the rotation of discs. A clarification facility is needed to remove the sloughed biological solids from the discs.

**Composition of Different Treatment Methods.**

<table>
<thead>
<tr>
<th>Type</th>
<th><strong>Effluent</strong></th>
<th><strong>Reliability</strong></th>
<th><strong>Cost Operating cost</strong></th>
<th><strong>Land Required</strong></th>
<th><strong>Response to shock</strong></th>
<th><strong>Economical life (year)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bod</td>
<td>Ammonia</td>
<td>Phos</td>
<td>Capital</td>
<td></td>
<td></td>
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<tr>
<td>Activated Sludge</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++ H</td>
<td>H</td>
<td>L</td>
</tr>
<tr>
<td>Oxidation Ditch</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++ H</td>
<td>H</td>
<td>A</td>
</tr>
<tr>
<td>Aerated Lagoon</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++ A</td>
<td>A</td>
<td>H</td>
</tr>
<tr>
<td>Trickling filter</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>++ H</td>
<td>H</td>
<td>A</td>
</tr>
<tr>
<td>Biological Disc</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++ H</td>
<td>L</td>
<td>L</td>
</tr>
</tbody>
</table>

**Legend:**

- **Excellent** +++
- **Good** ++
- **Fair** +
- **Poor** o
- **H** – High
- **A** – Average
- **L** – Low
Summary

The desirable properties of detergents and sanitizers and various detergents and sanitizers agents were discussed. The importance of quality of water for cleaning operations was explained and remedies discussed to remove hardness from water. Hand, machine and CIP system of cleaning and sterilization methods explained in detail. Cleaning and sanitization of various equipment like cans, HTST pasteurizers, tanks Coolers, Pumps, Pipelines, homogenizer, evaporator, were discussed. The various factors affecting methods of dairy waste disposal were described in detail to control the pollution.

Short Answer Type Questions

1. Define detergents and sanitizers.
2. What are sequestering agents?
3. Give some examples for acid detergents.
4. Classify different types of surfactants.
5. What is CIP system?
6. What are the inhibitors used in cleaning solution for aluminium and tinned surface vessels?
7. How rotary can washer differs from straight-through can washer.
8. Define B.O.D.
9. Define C.O.D.
10. What are different mechanical methods used for disposal of dairy effluents?
12. What do you mean by RBCS?
13. Define TOC.

Long Answer Type Questions

1. Mention the desirable characteristics of a good detergent and sanitizers.
2. Briefly write about various alkali detergents.
3. Write in detail about chemical sanitizers.
4. Briefly write about treatment of water for preparing cleaning solutions with appropriate reactions.

5. How do you select the detergents and sanitizers for different surface materials.

6. Briefly write about hand washing methods.

7. Explain CIP method of cleaning and sanitization.

8. Give the steps of hand washing of cans.

9. Describe in detail about various types of can washers.

10. Write the CIP procedure for HTST pasteurizer.

11. What are the different factors affecting efficiency of cleaning and sanitization?

12. Briefly write about Trickling filter.


14. Discuss in detail septic tank method.
UNIT 10
Steam and Refrigeration

Structure

10.1 Properties of steam
10.2 Steam Boilers – Types of Water tube and Fire tube
10.3 Steam Requirements in Dairy
10.4 Direct and Indirect Refrigeration Systems
10.5 Vapour Compression Cycle, Compressor types and construction details
10.6 Bulk Coolers – Plate Chillers – Shell and Tube Chillers
10.7 Common Problems in Refrigeration System and Remedies

Learning Objectives

After studying this unit, the student will be able to

- Understand about Steam and Refrigeration.
- Steam boilers and their different types of water tube and fire tube.
- Steam requirements in Dairy
- Understand direct and Indirect refrigeration
- Different types of Chillers in Steam.
- Problems and Remedies in Refrigeration.
10.1 Properties of Steam

Steam is vapourized water, which is formed from water by adding sufficient heat or reduced by the pressure. When water is heated, vaporization occurs because the vapour pressure of the liquid exceeds the vapour pressure of the surrounding atmosphere.

Steam is colourless, however, when dispersed with liquid drops, it is whitish in colour. At low pressure steam is lighter than air, thus it tends the rise in air and condenses on ceilings. If 1 kg of water at 0°C is heated to 100°C, approximately 100 kcal the heat added is known as sensible heat. When heat is added to ice, then melting starts, and when the solid has been completely transformed into liquid there will be rise in temperature. When the liquid is transformed into vapour, it absorbs 539 k cal per kg of heat without change in temperature.

Sensible heat is the quantity of heat (h) in kcal required to raise the temperature of 1 kg of water from 0°C to the saturation temperature at which water begins to boil at a given pressure. Sensible heat of water may be found approximately by multiplying its specific heat by the temperature rise.

The latent heat of vapourization is defined as the quantity of heat in kcal, required to convert 1 kg of water at saturation temperature for given pressure to 1 kg of dry saturated steam, at the pressure. It decreases as the pressure increases and it becomes zero when the critical pressure is reached.

The total heat steam \( H_s \) is the sum of sensible heat and latent heat and it increase with pressure.

\[
H_s = h + L
\]

\[
H_w = h + x L
\]

Where \( H_w \) is the total heat of wet steam and \( L \) is the latent heat.

Forms of Steam

(a) Saturated Steam: It is the vapour at the temperature corresponding to the boiling point of the liquid at the imposed pressure. For each liquid, a certain definite boiling point exists for each pressure, when heat is applied to saturated vapour and to the liquid with which it is in contact, more liquid evaporates, but the temperature remains constant. Similarly if heat is removed, more of the vapour condenses but the temperature will remain constant.

(b) Dry saturated and wet steam: If saturated steam does not contain any water it is known as dry saturated steam. It contains just sufficient heat
energy to maintain all of the water in gaseous state. If saturated steam contains liquid particles, it is known as wet steam. Wet steam does not contain sufficient heat to maintain all water in gaseous state. If some of the heat energy is absorbed from the dry saturated steam, the steam becomes wet.

(c) **Supersaturated Steam**: If the temperature of the steam is greater than that of boiling point corresponding to the pressure of steam generation, the steam is known as super heated steam. If super heated steam is brought in contact with water, it will give up parts of its heat to the water.

**Dryness Fraction**: Saturated steam consists of dry saturated steam and water particles in suspension. The dryness fraction of steam in the ratio of the weight of dry steam in a certain quantity of steam to the weight of wet steam. It is denoted by ‘x’.

\[ X = \frac{W}{W_s} \]

Where W is the weight of dry steam in a certain quantity of wet steam \( W_s \).

**Thermal Properties of steam**

The specific heat of the dry saturated steam increase with an increase in pressure. At pressure below 7 kg / cm\(^2\) there is an increase in specific heat with an increase in temperature, while above this pressure, there is a decrease in specific heat with an increase in temperature. The specific heat of steam at normal atmospheric pressure is 0.4 \( \text{kcal} \) per kg (c).

The latent heat decreases with an increase in temperature of evaporation, the total heat, however, increases with an increase in temperature of evaporation and with an increase in degree of super heat. The viscosity of steam increase with an increase in pressure and with an increase in temperature of super heated steam (at constant pressure). The heat content of steam is given in steam tables; typical values for the heat content of dry saturated steam under conditions found in dairy practice are given in the table.

<table>
<thead>
<tr>
<th>Pressure kg/cm(^2)abs</th>
<th>Saturation temp °C</th>
<th>Sensible heat (H) kcal/kg</th>
<th>Latent heat (L)</th>
<th>Total heat (H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>99.12</td>
<td>99.15</td>
<td>539.5</td>
<td>638.7</td>
</tr>
<tr>
<td>1.033</td>
<td>100.00</td>
<td>100.00</td>
<td>539.0</td>
<td>639.0</td>
</tr>
<tr>
<td>2.0</td>
<td>119.6</td>
<td>119.85</td>
<td>525.9</td>
<td>645.8</td>
</tr>
<tr>
<td>3.0</td>
<td>132.84</td>
<td>133.3</td>
<td>516.7</td>
<td>650.0</td>
</tr>
<tr>
<td>4.0</td>
<td>142.89</td>
<td>143.5</td>
<td>509.6</td>
<td>653.1</td>
</tr>
</tbody>
</table>
A steam boiler is closed vessel in which steam or other vapour is generated by direct application of heat resulting from the combustion of fuel or by use of electricity or nuclear energy. The function of a steam boiler is to transfer the heat produced by burning of fuel of water ad thus to produce steam. The boiler output is denoted by boiler horse power (bhp) which is defined as the evaporation into dry saturated steam of 15.64 of water per hour at a temperature of 100 °C.

1 BHP = 8.43.56 kcal / hour

**Classification of Boilers**

(a) **Fire Tube and Water tube**: On the basis of heat transfer, the boilers are classified as fire tube and water tube. A tube boiler is one in which the product of combustion of fuel gases, pass on inside the tubes. A combination of water and fire tube is one in which part of tube arrangement is of the fire tube type and part of water tube type.

In addition to water and gas passages the principle difference between the water tube and fire tube boilers are

1. The tubes in fire tube boilers are contained within the shell or drum where as in water tube boilers they are located outside the shell or drum.

2. As the fire tube boiler become larger, the capacity becomes limited because of the larger size shell required. The water tube boiler has a distinctive

<table>
<thead>
<tr>
<th>Pressure kg / cm²abs</th>
<th>Saturation temp ºC</th>
<th>Sensible heat (H) kcal/kg</th>
<th>Latent heat (L)</th>
<th>Total heat (H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>151.11</td>
<td>152.1</td>
<td>503.7</td>
<td>655.8</td>
</tr>
<tr>
<td>6.0</td>
<td>158.08</td>
<td>159.3</td>
<td>498.5</td>
<td>657.8</td>
</tr>
<tr>
<td>7.0</td>
<td>164.17</td>
<td>165.6</td>
<td>493.8</td>
<td>659.4</td>
</tr>
<tr>
<td>8.0</td>
<td>169.61</td>
<td>171.3</td>
<td>489.5</td>
<td>660.8</td>
</tr>
<tr>
<td>10.0</td>
<td>179.04</td>
<td>181.2</td>
<td>481.8</td>
<td>663.0</td>
</tr>
</tbody>
</table>
advantage, as tube arrangements can take many different forms to obtain more heating surfaces.

3. The water tube units are capable of greater capacity and pressure, which would be impossible in the fire tube unit. The largest modern steam generators are of water tube design.

The fire tube boilers are of simple and rugged construction and of relatively low first cost. The larger hot water capacity makes it possible to meet steam and load changes quickly. High pressures and large diameter shell requires thick plates. Hence there is a definite economical limit on pressure and capacity that can be reached with the fire tube type.

Water tube units, having higher capacity larger heating surfaces are exposed to the radiant heat of the fire they are not subjected to overheating and can be constructed of heavier plate for higher pressures. Most parts of water tube boiler are accessible for cleaning, repair and inspection. The general design permits higher operating efficiencies, and the furnace designs are such that various fuels can be used within making major alterations.

(b) Forced circulation and natural circulation: This classification is based on natural or forced circulation.

c) Externally fired or internally fired: Based on place of firing.

d) Horizontally or Vertically: based on shape.

Water Tube Boiler

The water tube boiler has a steam drum at the top and a mud drum at the bottom. Baffles are provided to deflect the hot gases back and forth between the tubes a number of times to enable greater heat absorption by the boiler tubes. They also permit designing for better temperature difference between tubes and gases throughout the boiler. Baffles help to maintain gas velocity, eliminate dead pockets, deposit fly ash and for proper removal and prevent high draft losses.

The firing door is either inward opening type or with self locking door latches which omit spring or friction contact. The reason is that the door should not be blown upon the pressure inside the furnace in case of tube rupture or furnace explosion. The main causes of tube failures in water tube boilers are solid deposits, corrosion, low water condition and slagging of gas passages.
Bent tubes are used in water tube boilers as they are more flexible than straight tubes. Boilers can be made wide and low, where head room is limited or narrow and high where floor space is at a premium. Bent tube boilers allow more heating surface to be exposed to the radiant heat of the flame. They allow for free expansion and contraction of the assembly. They enter the drum radially to allow many ends of tubes to enter the drum and allow greater flexibility in boiler tube arrangement than is possible in straight tube boilers. The steam drum serves as convenient collecting points in the steam water circuit and for separation of steam and water.

The above figure shows two typical curves, the upper curve indicates the fuel gas temperature from furnace to the exit portion and the lower curve shows feed water enter the boiler. The feed water temperature is slowly raised by the hot gas to its final steam, temperature. The various tubes provide the necessary heat transfer to accomplish just this.

The water tube boiler is safer, largely because most of the water at the hottest art of the furnace is in small tube, thus if a tube ruptures; only a comparatively small volume of water is instantly released to flash into the steam. All parts are more assessable for cleaning inspection and repairs. Large boilers can carry much greater loads and respond more readily to sudden changes and fluctuations in demand. The drum in water tube boilers is not exposed to radiant heat of the fire. The capacity and pressure can be increased which is impossible with the fire tube boilers. For the same diameter and thickness of tube a water tube boiler has more heating surface than a fire tube type.
10.3 Steam Requirements in Dairy

Considerable quantity of steam is required for processing, cleaning and sterilization in dairy. A plant using batch pasteurizer requires 0.28 to 0.35 kg of steam per kg milk processed. HTST pasteurizer using regenerator requires about 0.2 to 0.3 kg of steam per litre of milk. Approximate steam requirements for different processing operations:

<table>
<thead>
<tr>
<th>Process</th>
<th>Steam KG / 1 litre of Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reception</td>
<td>0.05</td>
</tr>
<tr>
<td>Separation</td>
<td>0.05</td>
</tr>
<tr>
<td>Pasteurization (HTST)</td>
<td>0.30</td>
</tr>
<tr>
<td>Bottle Washing / 100 bottles</td>
<td>1.3</td>
</tr>
<tr>
<td>Sterilization Batch</td>
<td>0.4</td>
</tr>
<tr>
<td>UHT direct</td>
<td>0.12</td>
</tr>
<tr>
<td>Ghee making</td>
<td>0.036</td>
</tr>
<tr>
<td>Rotary can washer / can</td>
<td>0.51</td>
</tr>
<tr>
<td>Straight through / can</td>
<td>0.43</td>
</tr>
<tr>
<td>Ice cream</td>
<td>0.25</td>
</tr>
<tr>
<td>Cheese making</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evaporator</th>
<th>Steam kg / kg of water evaporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Single effect</td>
<td>1.1</td>
</tr>
<tr>
<td>(b) Double effect</td>
<td>0.6</td>
</tr>
<tr>
<td>(c) Triple effect</td>
<td>0.46</td>
</tr>
<tr>
<td>(d) Roller drier</td>
<td>1.1</td>
</tr>
<tr>
<td>(e) Spray drier</td>
<td>2.1</td>
</tr>
</tbody>
</table>

The total plant requirements are approximately 30-40 kg of steam per hour per 1000 litres of milk processed per day. Low pressure steam below (2.0 kg / cm² abs) can be used where high temperature above 115°C are not required. Low pressure steam is less expensive because of reduced labour costs, lower fuel costs, and less equipment maintenance. Low pressure steam requires large pipe size.
The first step in calculating the size of boiler needed for a given dairy plant is to find the steam requirements of each equipment, second, determine the steam needed for heating water. Third make a processing chart which will show the time and duration of use of each equipment to use steam. Fourth, sum up the total requirement for given period and maximum requirement at a time to determine the capacity of the boiler.

10.4 Direct and Indirect Refrigeration Systems

The refrigerants are classified as primary refrigerants and secondary refrigerants. The primary refrigerants directly take part in the refrigeration system and so it is known as direct refrigeration system. The working of direct refrigeration system is well explained in chapter 8.3.

The secondary refrigerants are first cooled with the help of the primary refrigerants and are further used for cooling purpose. So this is known as indirect refrigeration system. Under many circumstances it is not desirable to carry the heat from the heat generating source directly by refrigerant, hence it is carried by using secondary refrigerant as water or brine. The heat carried by the secondary refrigerant is given to the refrigerant in the evaporator and recirculated continuously. The indirect refrigeration system, in which the evaporator cools a circulating medium, has the following advantages over the direct cooling system.

1. The indirect system is easy to control and easy to handle compared with primary refrigerant.

2. The pipeline used for carrying the heat by secondary refrigerant from the source is considerably smaller compared with the pipeline used with direct expansion refrigeration system and this is because, the specific volume of the chilled water or brine is considerably low compared with the specific volume of the refrigerant vapour. Therefore the pipeline diameter required for secondary refrigerant is considered lower than the refrigerant pipeline diameter.

3. The indirect system keeps coils and pipes containing a toxic refrigerant away from the load placed. The secondary refrigerant also eliminates long refrigerant also eliminates long refrigerant lines with their possibilities of leakage and their penalizing pressure drips. The commonly used secondary refrigerants are water, sodium chloride brine, calcium chloride brine and propylene glycol. When the required temperature to be achieved is above the freezing point of water, then water is universally used as secondary refrigerant.
**Brine**: Brine is a solution containing the salt in dissolved condition in water. When the temperature required to be maintained are below the freezing point of water, then brine solutions are used.

The freezing temperature of the brine is lower than the freezing temperature of water and it decreases with the increase in salt concentration. If, the concentration is increased beyond a certain point, the freezing temperature of brine increases instead of decreasing. The solution at this concentration is known as “Eutectic Solution” and the lowest freezing temperature is achieved at Eutectic point. The Eutectic temperature of calcium chloride brine is -55°C at corresponding salt concentration of 30 % by weight.

The Eutectic temperature of sodium chloride brine is - 21 °C at corresponding salt concentration of 23 % by weight.

Calcium chloride brine is more preferable over sodium chloride brine, when required temperature is below -20°C. It is commonly used for product freezing, cooling and ice making plants. The major disadvantage of this brine is dehydration effect on food with which it may come in contact. The brine solution should not come in contact with the refrigerated foods. At concentration of 5% and 10 % the crystallization starts at -2.4 °C and – 5.4°C respectively.

Sodium chloride brine is used where the use of calcium chloride brine is objectionable. This brine is commonly used for freezing the meat and fish with the help of brine spray. At 5% and 10 % concentration the crystallization starts at -2.8 °C and -6.44 respectively.

**Brines to be suitable as a simple refrigeration carrying medium should.**

- (a) Remain liquid under all temperatures to which they are subjected.
- (b) Be essentially non corrosive when in contact with metals.
- (c) Have a sufficiently high specific heat.
- (d) Undergo no changes when in contact with refrigerants.

### 10.5 Vapour Compression Cycle, Compressor types and construction details

The vapour refrigeration system now – a- days are universally used for all purpose of refrigeration. The principle advantages are.

- Smaller size unit for given capacity of refrigeration.
• Less running costs.

The major disadvantages, is that it requires greater safety and prevention of leaks.

In this system, the vapour alternately under goes a change of phase from vapour to liquid and liquid to vapour during completion of cycle. The latent heat of vaporization is utilized for carrying heat from refrigerator.

At atmospheric pressure, liquid ammonia evaporates at – 33.3 °C (saturation temperature corresponding to 1.033 kg/cm² abs) and under these conditions 1 kg liquid is changing to vapour absorbs 327.4 k cal (latent heat of evaporation). Thus the simplest form of vapour refrigeration system consists of an open vessel containing a liquid refrigerant such as ammonia. The ammonia evaporates at temperatures below those surrounding the container and in doing so absorbs heat. Such systems are not used as they are uneconomical.

With properly auxiliary equipment, however, the refrigerant can be recovered and reused in a cycle process; moreover, the temperature of evaporation of the refrigerant can be controlled by controlling the pressure. Thus if the liquid ammonia is maintained at a pressure of 2.138 kg/cm² abs, saturation or evaporation temperature is -18 °C, and the latent heat of vapourization is 316 k cal per kg, if the absolute pressure is 4.20 kg/cm², the evaporating temperature is -1 °C and the latent heat of vapourization is 302.67 kcal per kg.

The refrigerant changes from liquid to vapour or from vapour to liquid may be controlled by controlling the pressure, of refrigerant. If the ambient temperature surrounding the refrigerant and its container is above the saturation temperature corresponding to the refrigerant pressure, than evaporation, and consequently absorption of heat, takes place, if the refrigerant is already in the vapour state and if the temperature is surrounding the refrigerant and its container is below the saturation temperature corresponding to the refrigerant pressure, condensation occurs.

A complete vapour compression refrigeration system requires an evaporator to contain the boiling liquid refrigerant, a compressor to pump the refrigerant vapour from low pressure to high pressure side of the system and to control the pressure with in the evaporator; and a condenser for removing the heat from the refrigerant gas, so that it may be returned to liquid from. In addition, a receiver for storing the liquid refrigerant under high pressure and an expansion
valve for controlling the rate of flow of liquid refrigerant between the high and low pressure sides of the system are needed as shown in the figure.

![Diagram of Ammonia Refrigeration System](image)

**Fig 10.2 Vapour Compressor Ammonia refrigeration system**

The high pressure (10.82 kg/cm² ga) liquid ammonia is held in the receiver. The liquid passes to the entrance of the expansion valve. The temperature of liquid ammonia is 30°C, the saturation temperature at 10.82 kg/cm² ga. The liquid refrigerant is throttled through the expansion valve into the evaporator. Here, low pressure of 1.33 kg/cm² ga maintained by operation of the compressor. The liquid ammonia is evaporated at a temperature of -15°C corresponding to the surrounding evaporator pressure. The refrigerant is no longer in a stable state, since the objects surrounding the evaporator are at a temperature higher than -15°C and thus supply the latent heat absorbed through the coil walls.

The ammonia vapour from the low pressure side of the system is drawn into the compressor and discharged into the high-pressure side by the compressor. The high-pressure ammonia gas discharged into the condenser. Water passing over the condenser coils removes first the heat of super heat and then condenses the vapour by removing the latent heat. The heat removed by the condenser is equal to that absorbed in the evaporator plus the equivalent or energy supplied.
to the vapour through the compressor. All the processes occur simultaneously, only the action of the reciprocating compressor being intermittent in operation.

10.6 Bulk Coolers – Plate Chillers – Shell and Tube Chillers.

Bulk Milk Coolers

There are many makes and manufacturers of the bulk milk coolers. The construction of one design is shown in the figure 8.3.

![Fig 10.3 Bulk Milk Cooler](image)

The unit consists of milk measuring gauge, agitator, dial thermometer and refrigeration channels. Milk is stored in the tank and the cooling is done either by circulating chilled water or refrigerant through the coils surrounding the tank. When cooling starts the agitator is rotated for efficient heat transfer. Chilled water is circulated by means of a pump.

The Ice bank system requires a compressor working 80 - 90% of the time. With the direct expansion, larger compressor is needed, but works for only 25 – 30% of the time. Milk comes into the tank at 30 - 37°C and cools to 2°C in 1.5 to 2 hours. The maximum temperature of blend (when the warm milk is added into the tank) should be about 10°C. This type of cooler is expensive to install and its full advantages cannot be utilized unless milk is collected every other day. This type of cooler is used in collecting centres.

Plate Chillers

A popular heat exchanges for fluid of low viscosity such as milk is the plate heat exchanger, where heating or cooling fluids flow through alternate tortuous passages between vertical plates as illustrated in figure.
The advantages are

- High efficient
- Occupy less space
- Compact and simple
- Easily cleaned
- Low in cost
- Versatile, sanitary
- Easily inspected

The places are supported in a press between a terminal block in each heating and cooling section. The heat transfer takes place through stainless steel plates. The plates are stamped from 18:8 stainless steel sheet of 20 gauge thicknesses and are found in various shapes, sizes and designs. An approximate of 3 mm space is maintained between the plates by a non-absorbent rubber gasket or seal, which is vulcanized to the stainless steel. Gaskets along the edges of the plates and around the ports separate the various flow streams.

The plates are arranged to form streams and passes with each stream alternating with a passage carrying the heating or cooling medium. Streams may be parallel or in series, and the heat exchange medium may flow counter or parallel to the product flow, as desired. The plates are numbered and the total number depends upon the capacity and the amount of heat to be transferred. The plates are tightened in to place with a jack or screw device on the frame.
and are normally mounted vertically in banks. To save space, the recent trend is to have the long edge of the plate in a vertical rather than in a horizontal position.

The plates are designed to provide uniform but not excessive turbulent flow of products with a high heat transfer rate. Raised sections on the plate in the form of knobs, diamonds and the channels help to provide the turbulent action required. Greater capacity is secured by adding more plates. Normally the operating pressure of plate heat exchanges will be 2 kg / cm$^2$, the plate thickness will vary from 1.25 to 3.00 mm and the spacing between plates ranges from 1.25 to 7.75 mm. The channeled corrugated or dimpled surface provide turbulent flow for higher heat transfer rate and adds strength to the plates permitting the use of thin material.

Approximately 2.5 to 4 times the quantity of the product is circulated for cooling a product from 27$^\circ$C to 4$^\circ$C using a coolant at 1$^\circ$C.

**For proper operation of the plates it should.**

1. Be sealed tightly, so there is no dripping.
2. Be designed so that all the plates are utilized for heat transfer.
3. Allow product to be drained from the heat exchange plates without opening the plates.
4. Provide venting so that air is eliminated during start up and operation.

**Shell and Tube Chillers**

When the required heat transfer surface is large, the recommended type of exchanger is the shell and tube type. In this type of cooler, large heat transfer surface can be achieved economically and practically be placing tubes in a bundle; the ends of the tubes are mounted in a tube street. This is very commonly accomplished by expanding the end of the tube into a close fitting hole in the tube sheet by a process called rolling. The resultant tube bundle is then enclosed by a cylindrical casing (the shell), through which the second fluid flows around.

The form of the shell and tube exchanges is just shown in shell and tube condenser. The fluid flowing through the tubes enters a header or channel where it is distributed through the tubes in parallel flow and leaves the unit through another header. Either hot or cold fluid may flow in the shell of the exchanger surrounding the tubes.

Parallel flow through all tubes at a low velocity gives a low heat transfer coefficient and low pressure drop. Because of structural considerations it is rarely possible to space the tubes in the tube sheet so closely that the area of the
path outside the tubes will be as small as that inside the tubes, and therefore the velocity of the fluid outside the tubes will be low in such constructions. To remedy this condition, baffles are placed outside the tubes to strengthen the path and decrease the cross section of the path of the second fluid.

The liquid passes back and forth at high velocity which gives good heat transfer coefficients. The baffle consists of circular discs of sheet metal with one side cut away. These sheets are perforated to receive the tubes. The baffles are held in place by means of one or more fluid rods. Thus, the baffling increases the velocity of the liquid outside the tubes and causes it to flow more or less at right angles to the tubes. This causes an added turbulence which aids in reducing the resistance to heat transfer outside the tubes, two film coefficients an be improved, and therefore overall coefficient $V$ is correspondingly increased.

Cleaning of both shell and tube bundles is difficult. At best to allow provisions for easy removal of the tube bundle for cleaning and to allow for thermal expansion, a floating- head exchange is used, but add to cost of fabrication. Shell side cleaning is very difficult without removing the tube bundle. The nature of the shell side fluid is also important and will influence the selection of the type of exchanger. Since the shell side of the exchanger is difficult to clean, the least corrosive and cleanest require the use of expensive alloys and to the corrosive fluid should not be passed through the tubes to save the cost of an expensive alloy shell.

A fluid that would normally be flowing in laminar (straight layer) flow in the tubes should be placed in the shell to improve the heat transfer characteristics. High – pressure fluids should flow through the tube to avoid expensive high – pressure shells.

### 10.7 Common Problems in Refrigeration System and Remedies

#### 1. Causes for high head pressure.

(a) Insufficient condenser water

(b) Air in the system

(c) Scales on the condenser

(d) Too much refrigerant

#### 2. Causes for low head pressure.

(a) Too much condenser water or too cold condenser water
(b) Not enough refrigerant
(c) Leaky compressor valves

3. Causes for Low suction Pressure
   (a) Clogged strainer
   (b) Not enough refrigerant
   (c) oversized compressor

4. Causes for higher suction pressure.
   (a) Too much load on the evaporator
   (b) Oversized evaporator
   (c) Faulty suction valve.

5. Causes for low refrigeration capacity
   (a) Clogged strainer or expansion valve.
   (b) Frosted cooling coil
   (c) Too large evaporator pressure drop

6. Unit runs with poor refrigeration
   (a) Wrong thermometer setting
   (b) Moisture in the system which freezes intermittently

7. Compressor becomes too hot during operation
   (a) Not enough refrigerant
   (b) Air in the cycle
   (c) Discharge valve not in working order.
   (d) Condenser contaminated
   (e) Safety valve in the compressor is not seal, sealed is damaged.

The remedies for the above problems is only to correct the causes.

Summary

The properties of steam, forms of steam and thermal properties of steam were detailed. The quality of water is important to avoid damage to the boiler surfaces and water softening was fully explained. The various types of boilers
were mentioned. The boiler accessories, controls and safety devices controls were explained. The requirement of stem for various dairy operations were discussed.

A steam boiler is closed vessel in which steam or other vapour is generated by direct application of heat resulting from the combustion of fuel or by use of electricity or nuclear energy. The function of a steam boiler is to transfer the heat produced by burning of fuel of water ad thus to produce steam. The boiler output is denoted by boiler horse power (bhp) which is defined as the evaporation into dry saturated steam of 15.64 of water per hour at a temperature of 100°C.

The basic heat transfer and thermodynamics were discussed in detail. The direct and indirect refrigeration systems were explained. The vapour compression cycle is explained with the help of sketch diagram. The types of constructional details is condensor evaporator compressor and expansion valves were discussed. Chillers and cooler were discussed. The various refrigerants and desirable properties of a good refrigerants were covered. The construction of cold storages were discussed. The common problems in refrigeration system and remedies mentioned.

**Short Answer Type Questions**

1. Define Latent heat.

2. What is sensible heat?

3. Define Saturated steam.

4. What is dryness fraction of steam?

5. What is importance of quality of water in steam production?

6. Mention different types of boilers.

7. What is the function of pressure gauge in boiler?

8. What are the functions of blow off valve?

9. How much steam is required to evaporate 1 kg of water from different types of evaporators.

10. How much steam is required to pasteurize 1 kg of milk by HTST, UHT and sterilization methods?
11. Define Conduction.

12. Define Convection.

13. What is Direct refrigeration?

14. What is indirect refrigeration system?

15. What are the important parts in vapour compressed refrigeration system?

16. What are the functions of Evaporator?

17. Give the function of Compressor.

18. Mention various types of Condensers.

19. Define refrigerant.

20. Give the B.P freezing point and refrigeration effect of ammonia.

21. What are the materials used for insulation of cold storage?

22. What are the causes for high heat pressure in refrigeration system?

**Long Answer Type Questions**

1. Write briefly about various types of steam.

2. Explain the thermal properties of steam.

3. How water is softened for using in a steam boiler?

4. Classify various types of boilers.

5. With the help of diagram explain water tube boiler.

6. Write briefly about boiler accessories.

7. Briefly write about conduction of steam of heat transfer.

8. Briefly write about conduction process of heat transfer.

9. With the help of sketch diagram explain vapour compressed refrigeration cycle.
10. Briefly discuss about different types of compressors.

11. Write about various types of condensers.

12. Discuss in detail about types of evaporators.

13. Briefly write about expansion devices in refrigeration system.

14. Explain about the bulk milk cooler.

15. Explain characteristics of ammonia and Freon.

16. What are the desirable characteristics of an ideal refrigerant?

17. Write about constructional designs of cold storage.

18. Briefly explain about insulation of cold storage.

19. Mention the problems and remedies in refrigeration system.