Preservatives in milk

- Mercuric Chloride: 300- 800 ppm
- Formalin: 0.4/100ml
- Hydrogen Peroxide
- Potassium dichromate

LP system

- lactoperoxidase- enzyme (bovine milk - 30 µg/ml)
- Thiocyanate- substrate
- Hydrogen peroxide – Promoter

sodium thiocyanate and H2O2 when added in 14:30 mg/ litre improves keeping quality to 10Hr at 37 °C

bactericidal to Gram-ve and bacteriostatic to Gram +ve

Standardization

- adjusting the fat and solids-not-fat (SNF) content of milk to meet specific standards or requirements.
- By removal of excess fat or addition of skim milk or cream
- standardized milk must have a minimum fat content of 4.5% and SNF content of 8.5%

Pasteurization

- process of heating every particle of milk to at least 63°C for 30 min or 72°C for 15s or to any temperature-time combination which is equally efficient, in a properly operated equipment.
- After pasteurization, the milk is immediately cooled to 5°C or below.
- started by Louis Pasteur in Wine and Dr. Soxhlet in milk

Importance and Drawbacks

- **Importance of Pasteurization**

  - To render milk safe for human consumption by destroying all the pathogenic microorganisms.

  - To improve keeping quality of milk by killing almost all spoilage organisms (85-99%)

- **Drawbacks of Pasteurization**

  • It diminishes the cream line or cream volume (by denaturation of cryoglobulins)

  • Pasteurized milk may increase the renneting time.

  • It fails to destroy bacterial toxins and Accumulation of milk-stone in the heating section

METHODS OF PASTEURIZATION

1. Batch or holding pasteurization (LTLT)	63 ° C for 30 minutes
2. High Temperature Short Time (HTST) pasteurization/ Flash pasteurization	72 ° C for 15 sec
3. Electric pasteurization	Using electricity for 15-20 sec
4. Vacuum pasteurization (vacreation)	under reduced pressure by direct steam
5. Ultra high temperature pasteurization	135 ° C to 150 ° C for no hold
6. In- bottle pasteurization	63-66 ° c for 30 minutes
7. Stassanization	74 ° c for 7 sec
8. Uperization/ultra – pasteurization	150 ° c for a fraction of a second

LTLT

- Batch or Holding pasteurization
- heated to a minimum of 62.7°C and held at this temperature for minimum 30 min and cooled as rapidly as possible to 4°C.
- The LTLT pasteurizer may be of three types

- *Water – jacketed vat*
- *Water–spray type*
- *Coil-vat type*

HTST - Flash pasteurization

- **Modern method** : 72 ° C for 15 sec
- regenerative heating and cooling in plate heat exchanger
- Heating by hot water or steam
- Coolant: Chilled water or glycol

- Pressure in HTST: Pasteurized milk 15 psi; raw milk 14 psi; heating/cooling media 12-13 psi

* Regeneration efficiency: 85-90%

= temp. after regenerative heating – initial temp. / temp. after final heating – initial temp.

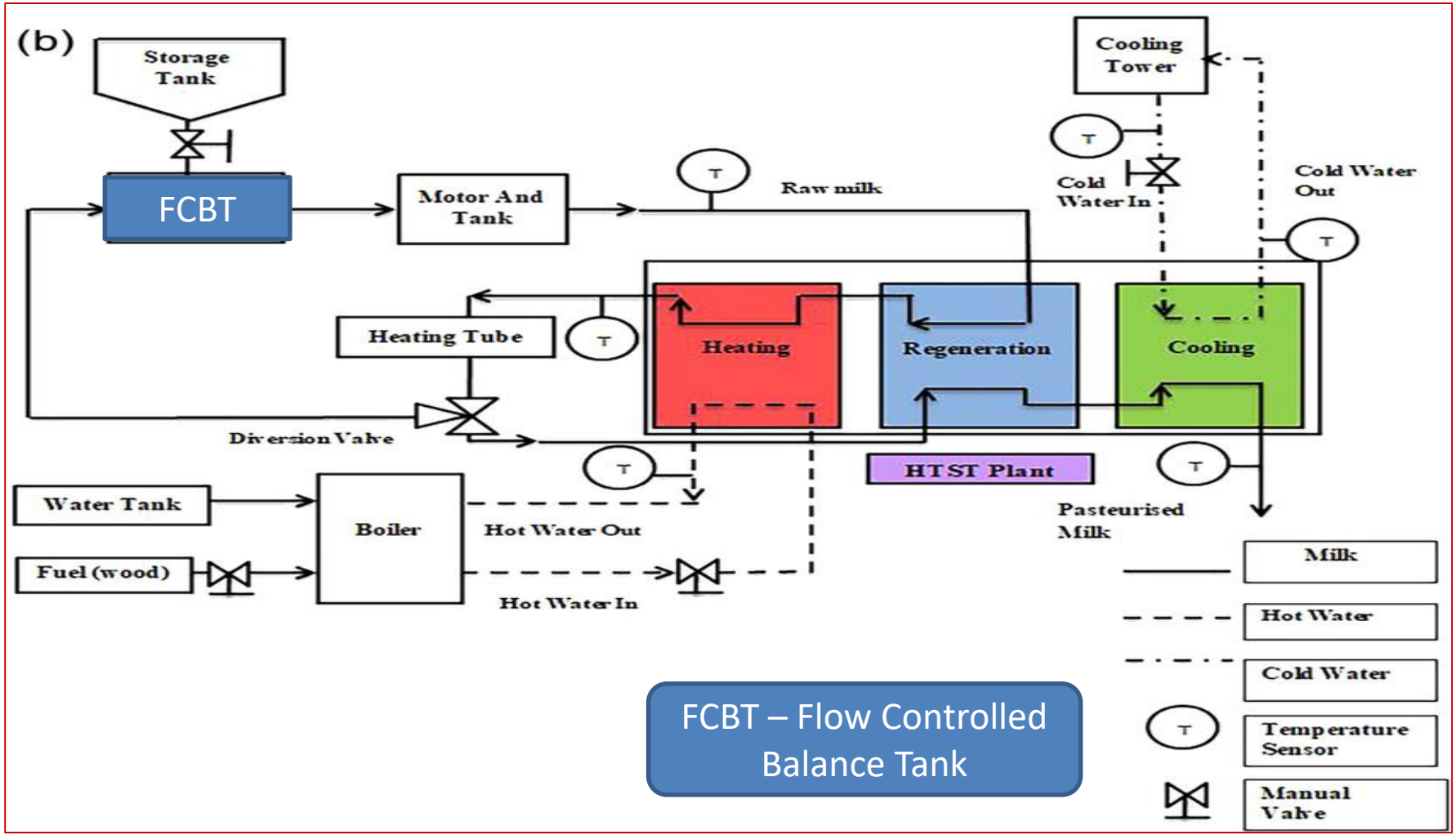
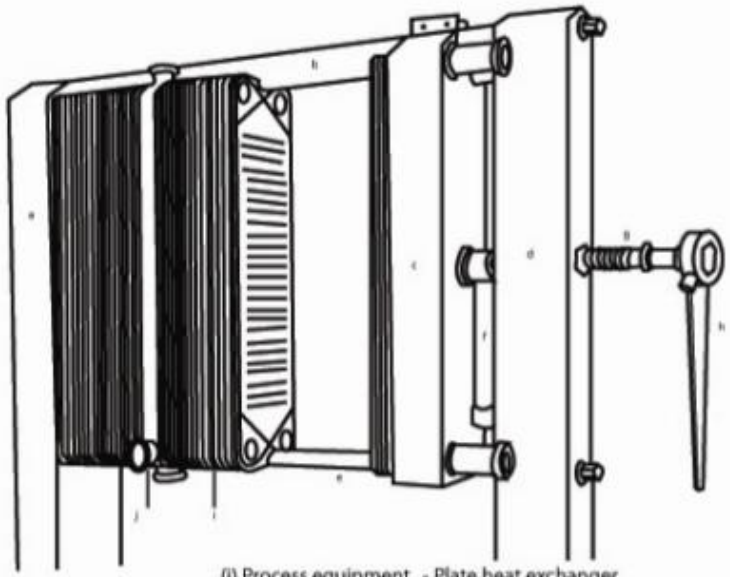

(b)
Storage Tank
FCBT
Motor And Tank
Raw milk
Cooling Tower
Cold Water In
Cold Water Out
Heating Tube
Heating
Regeneration
Cooling
Diversion Valve
HTST Plant
Water Tank
Boiler
Hot Water Out
Fuel (wood)
Hot Water In
Pasteurised Milk
Milk
Hot Water
Cold Water
Temperature Sensor
Manual Valve
FCBT – Flow Controlled Balance Tank

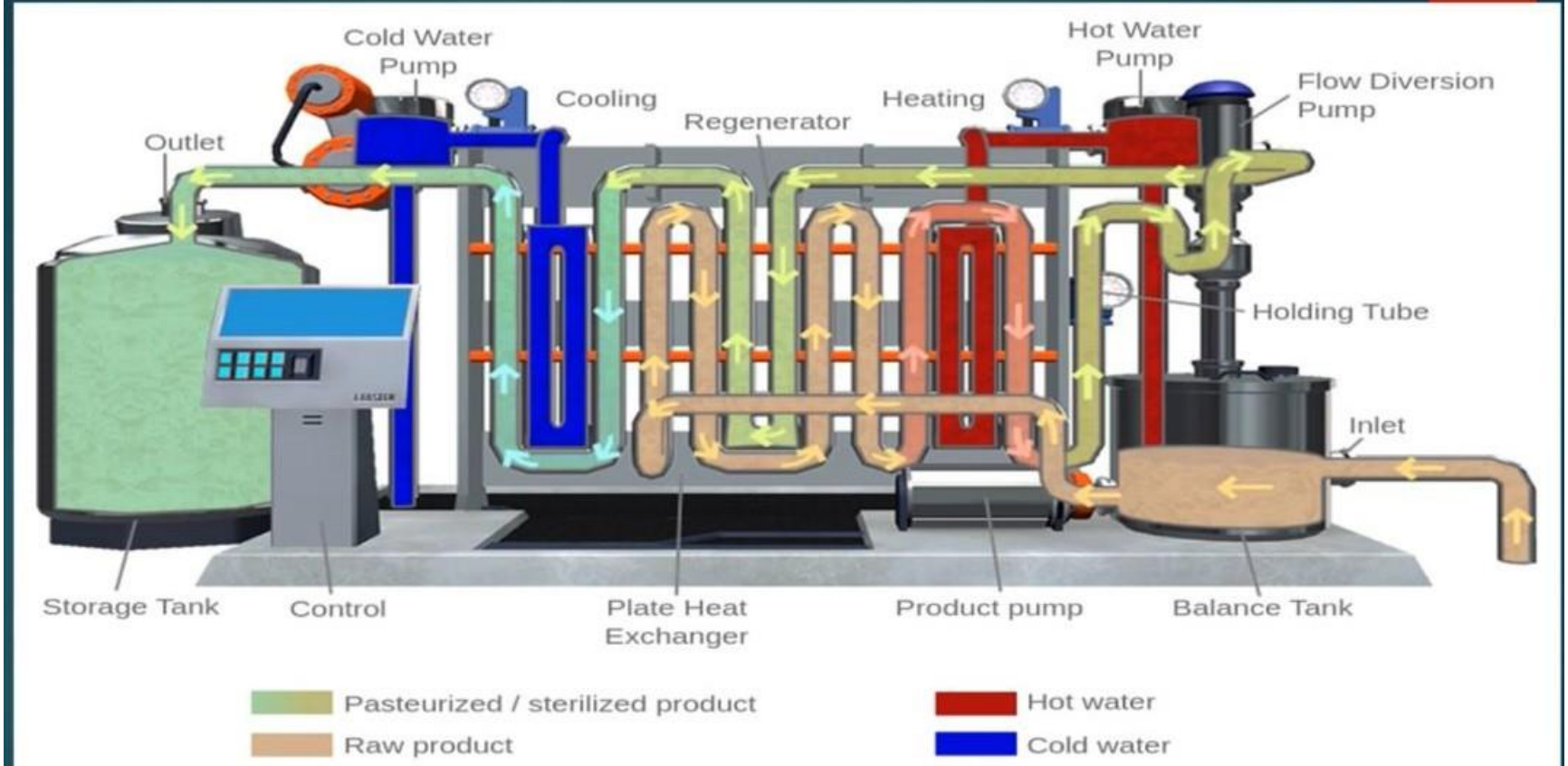
Plate Heat Exchanger



(i) Process equipment - Plate heat exchanger

a. Head Frame, b. Guide bar, c. Follower, d. End support, e. Carrying bar, f. Hinged distance piece, g. Tightening screw device
h. Detachable retchet spanner, i. Bank of plates, j. Connector grid with inlet and outlet bosses

HTST


Cold Water Pump
Cooling
Regenerator
Heating
Hot Water Pump
Flow Diversion Pump
Outlet
Holding Tube
Inlet
Storage Tank
Control
Plate Heat Exchanger
Product pump
Balance Tank
Pasteurized / sterilized product
Raw product
Hot water
Cold water

ADVANTAGES

- complete destruction of Phosphatase enzyme
- Phosphatase Test : Detect adequacy of pasteurization
- Pasteurization --- carried out at a heat treatment temperature above that for phosphatase inactivation and yet below that for cream line reduction.
- Pasteurization ensures **complete destruction of pathogens**, **negative alkaline phosphatase test** and least damage to the cream line

- Vacuum pasteurization (vacreation) : pasteurization of cream under reduced pressure by direct steam

-equipment used is called vacreator

Thermization: 62° C - 65° C for 15-20 seconds

- Sterilization : 115 ° C for 15 min. or 145 ° C for 3 sec to ensure preservation of milk at room temperature for a period of not less than 15 days.
- Sterilized milk shall show a negative turbidity test

Index organism for pasteurization : Coxiella burnetti

Shelf life of milk

- At room temp. for 3 hour immediately after milking
- Cooling at 5°C : 24 hours
- Pasteurization: 4-7 days
- UHT : few months

Loss of nutrients

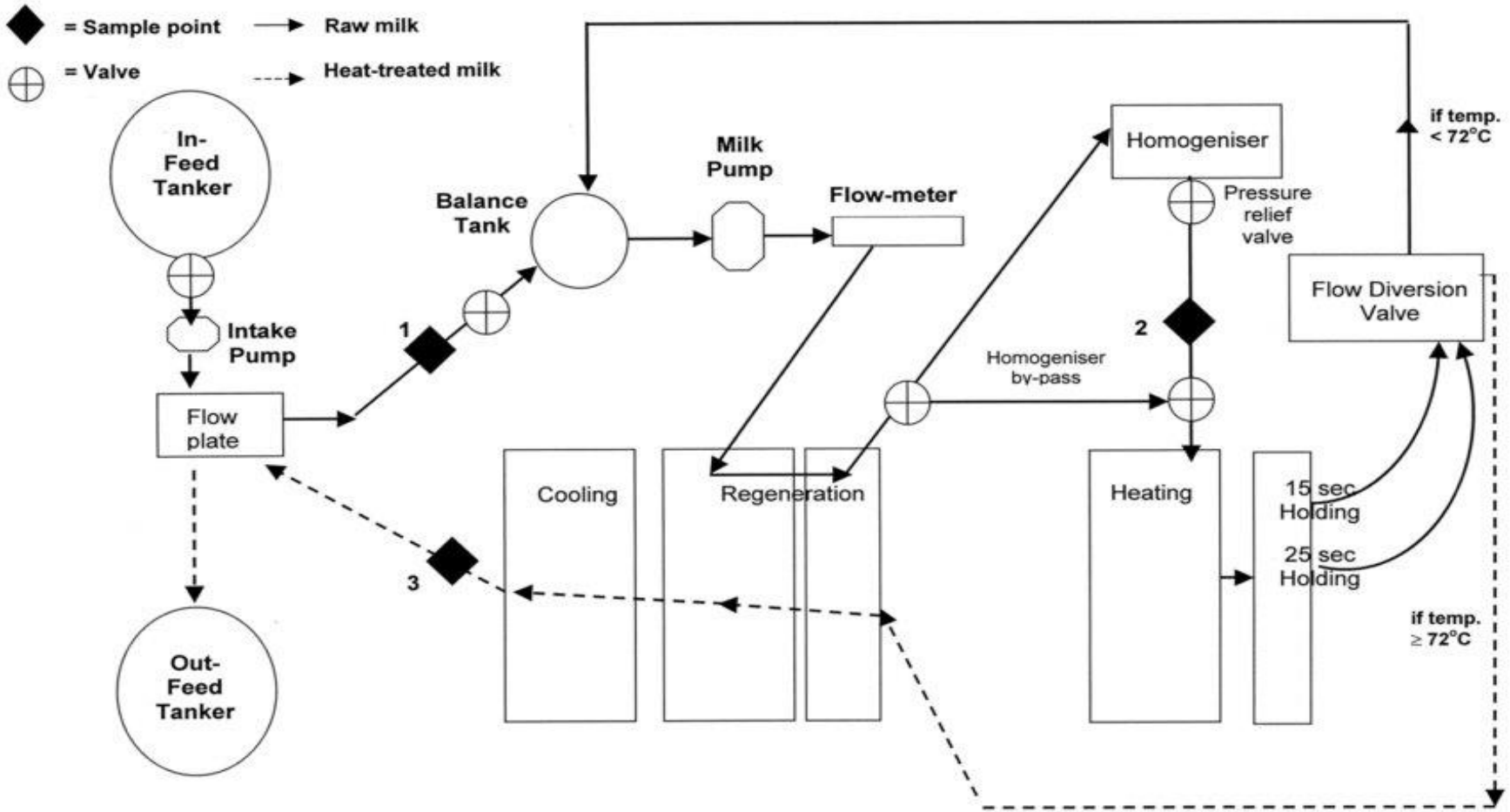
- **Pasteurization:** 10% Vitamin B1 and 20% of Vitamin C. Lactose - not much influenced by the normal pasteurization conditions
- **Sterilization:** 30-50% Vitamin B1 and 50% of Vitamin C
- Lactose- browning and isomerisation reactions

BACTOFUGATION

- process of removal of microorganisms from milk using centrifugal force.
- Most of the microorganisms are inactivated by pasteurization. However, the highly heat resistant spores survive pasteurization
- special form of separation of microorganisms (99%), mainly spore formers (Bacilli/Clostridia)

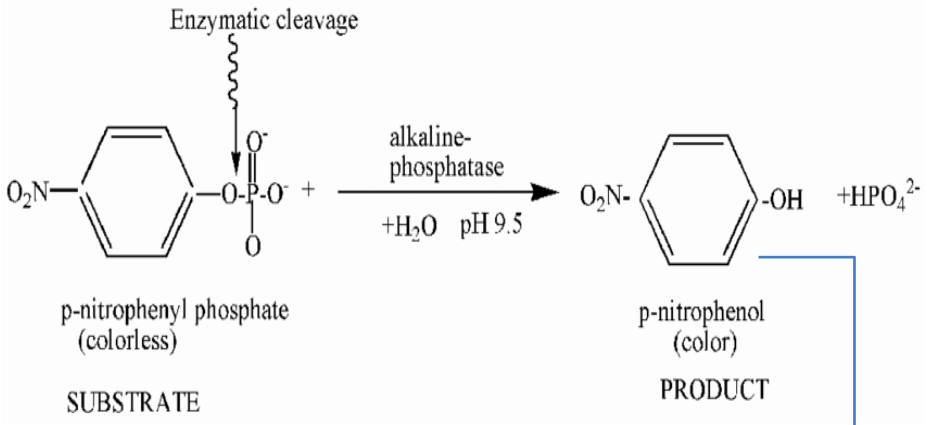
Homogenization

- Process in which fat globules in milk are broken down in to smaller size (<2 μm) and distributed evenly into milk serum by applying high pressure.
- surface area increases by a four- to six- fold
- No cream can be separated from homogenized milk
- Principle: milk is forced at high pressure through a narrow valve with velocity 100 - 200 m/s. This can cause high shearing stresses, cavitations and micro-turbulence. The globules becomes deformed, wavy and then breakup.
- Temperature of 65-70°C (to inactivate lipase enzyme) and a pressure of 150 - 200 bar (15-20 MPa) and additional 5-10 Mpa in two stage homogenization


= Sample point
Raw milk
= Valve
Heat-treated milk
In-Feed Tanker
Balance Tank
Milk Pump
Flow-meter
Homogeniser
Pressure relief valve
if temp. < 72°C
Flow Diversion Valve
Intake Pump
1
2
Homogeniser by-pass
Flow plate
Cooling
Regeneration
Heating
15 sec Holding
25 sec Holding
3
Out-Feed Tanker
if temp. ≥ 72°C

Efficiency of pasteurizarion

- Scharer Rapid Phosphatase Test.



Blue

- The shelf life of pasteurized milk kept at less than 8°C is: (PPSC 2016)

a) 1-2 days b) 3-5 days c) 6-8 days d) 10-15 days

- An indicator organism for efficient pasteurization is: (PPSC 2022)

a) Mycobacterium tuberculosis

b) Coxiella burnetti

c) Mycobacterium bovis

d) Bacillus anthracis

- Due to homogenization, area of milk fat increases (UPPSC)

(a)2 times (b) 3 times (c) 4 times (d) 5 times

- Efficacy of pasteurization is judged by: (PPSC 2016), (OPSC, MPPSC)

a) Dye reduction test

b) Alkaline phosphatase test

c) Amylase test

d) Malachite test

- Which of the following is not a part of lactoperoxidase system in milk (HPSC 2018)

a. Phosphatase
b. Hydrogen peroxide
c. Thiocyanate
d. Lactoperoxidase

- In a positive phosphatase test, a para-nitrophenol is liberated that gives……. colour under alkaline condition. (MPPSC)

[A] pink [B] violet [C] blue [D] yellow

- In the HTST type of pasteurisation of milk the temperature and time is (RPSC 2013)

(1) 73 °C to 76 °C for 60 seconds

(2) 75 °C to 78 °C for 45 seconds

(3) 72.2 °C to 72.8 °C for 15 seconds

(4) 74.2 °C to 75.2 °C for 30 seconds

COLOSTRUM V/S MILK

- colostrum – 1st milk or beestings (fed 1/10th of the body weight for 3-5 days)
- less water and lactose compared to milk
- More protein, fat, immunoglobulins, total solids
- contains trypsin inhibitor to protect immunoglobulins from digestion
- antibodies transfer doesn't occur through placenta in ruminants
- IgG1 > IgM > IgA > IgG2 (IgG1 most abundant immunoglobulin in bovine milk while IgA in human mik)
- Contains Bifidus factor (Human milk)

Constituents	colostrum	milk
Water	70-74	87
Total solids	28	13
Fat	1-12(6)	4
Protein	**21.3**	**3.3**
Globulin	15.1	0
Casein	4.7	2.8
lactalbumin	1.5	0.5
lactose	**2.5**	**4.9**

Dairy Microbiology

- Milk is sterile at secretion in the udder
- contaminated by bacteria even before it leaves the udder
- Bacterial count of milk – 500-1000/ml (10000/ml when drawn in to pail)
- Freshly drawn milk has a temperature of approximately 38^0C which is highly suitable for bacterial growth.

SOURCES OF CONTAMINATION

a) Interior of the udder: bacterial count of milk varies between 500 and 1000/ml
b) Environmental: bacteria accumulated on the surface of body get dislodged during the milking process and enter the pail contributing a load of 10,000 bacteria or more per ml. of milk
c) Milker or Handler: typhoid fever, diphtheria, scarlet fever, septic sore throat
d) Utensils
e) Wholesaler, retailer and the vendor
f) During transportation

Type of bacteria	Temp. range	Optimum growth temp.	Example
Mesophilic	20 & 40°C	37°C	S. aureus, E. coli
Thermophilic (Heat loving)	55-70° C	55° C	Bacillus stearothermophilus
Thermoduric (spore forming)	60-63° C	35-37°C	Micrococcus varians
Psychotropic (Cold loving)	Can survive refrigerated temp.	15 - 20°C	Pseudomonas sp. Alkaligenes sp.
coliforms		37° C	E.coli

Spores can survive UHT

Thermophilic – can survive and grow above pasteurization temp. while Thermoduric Can survive but not grow

TYPE OF MICROBES

Lactic acid bacteria (LAB): GRAS bacteria

- Homofermentive: able to ferment lactose to lactic acid e.g. *Lactobacillus acidophilus, L. delbrueckii, L. Helveticus*

-Heterofermentative: which produces end Products other than lactic acid e.g. *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus reuteri*

Milk fermentation

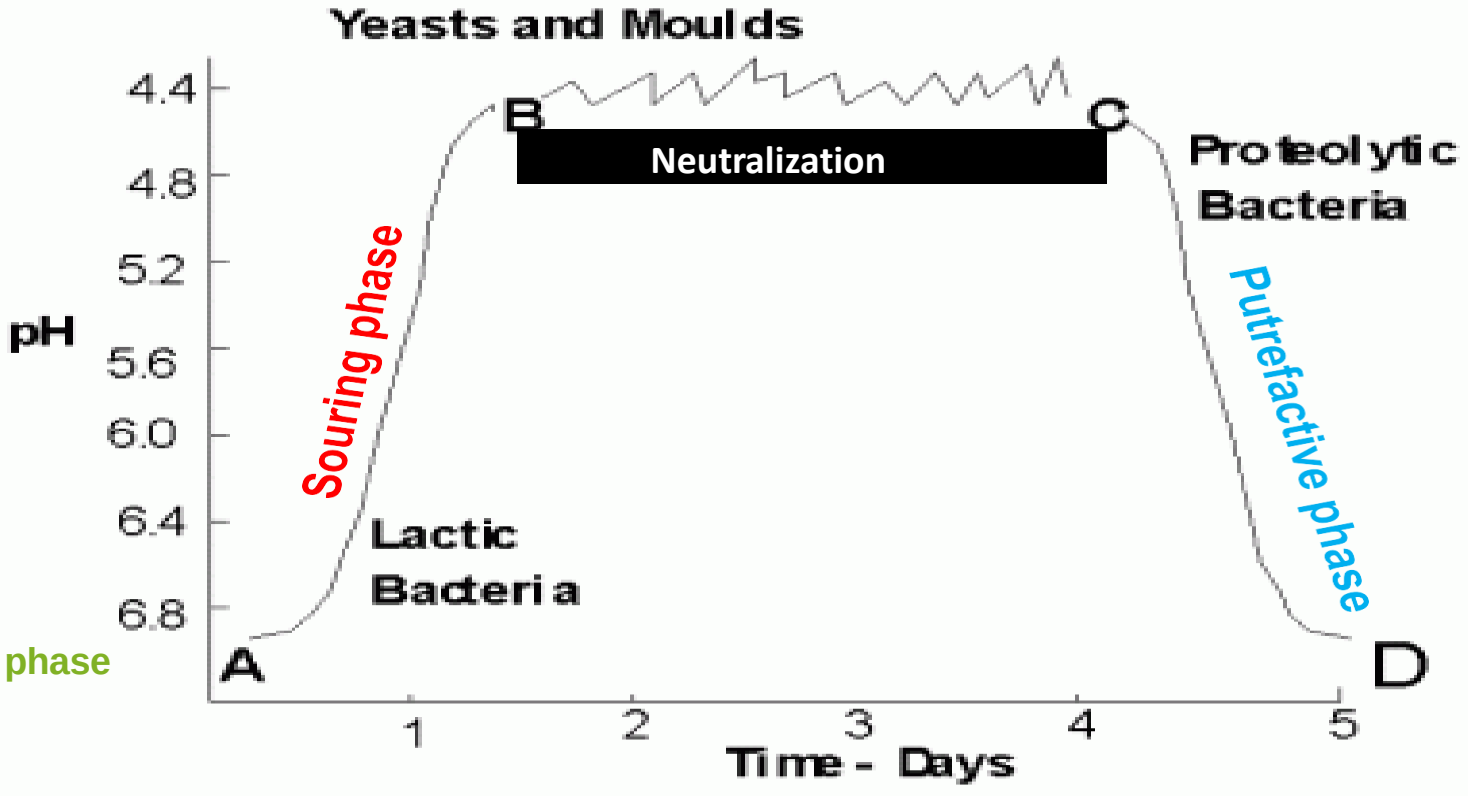
The process by which a change is produced in milk through microbial activity

General fermentation

1. Germicidal (Destruction phase)
2. Souring phase
3. Neutralization
4. Putrefactive phase

Specific fermentation

1. Souring
2. Souring & gassiness
3. Proteolysis
4. Alkaline fermentation
5. Ropiness



Germicidal phase

F Natural Fermentation of Raw Milk

Souring/ curdling

due to the production of acidity (lactic acid from lactose) by lactic acid bacteria

Sour flavor is because of volatile acids, diacetyl and acetaldehyde

normal acidity of fresh milk	0.13 to 0.15%
Milk sours	0.20 to 0.25%
milk curdles	0.50 to 0.65%

- e.g. Lactococcus, Lactobacillus, Leuconostoc, Streptococcus and Enterococcus.

ROPINESS OR SLIMINESS

- growth of bacteria leading to change in consistency of the product that forms threads or viscous masses when poured.

-Ropiness because of Polysaccharides and Mucins

E.g. **Alcaligenes viscolactis** – More common, B.cereus, B.subtlis, Coli aerogenus group

PROTEOLYSIS

- casein or some insoluble casein derivatives are broken down to water soluble compounds through the action of microbes or their enzymes
- E.g. Pseudomonas, Bacillus
- Important for development of body and texture in Cheese

SWEET CURDLING

- curdling without pronounced acid production
- Due to production of rennin like enzymes by bacteria which causes precipitation of casein without production of acid
- E.g. Bacillus cereus , B. subtilis, E.coli

LIPOLYSIS

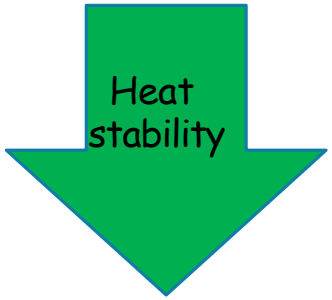
- hydrolysis of milk fat by lipase resulting in to the accumulation of free fatty acids

- butyric & caproic responsible for off flavors

- E.g. Pseudomonas

- Gas forming bacterias: Coliaerogenus, Clostridium
- Coliaerogenus group – E.coli, Klebsiella, Enterobacter – possess the enzyme β-galactosidase, which is critical for lactose fermentation
- Stormy Fermentation: *Clostridium perfringens*

PLATFORM TESTS



Organoleptic tests – Color, Flavor & taste

Acidity/pH

Alcohol alizarin, Clot on boiling, Alcohol test

2 min resazurin test

Lactometer

Organoleptic tests

Also called Rapid Platform tests

Flavor:

- Rancid - Pseudomonas fragi,
- phenolic flavors - Bacillus circulans
- Fishy flavor - Pseudomonas icthyosmius, due to conversion of lecithin to trimethylamine.
- Cooked flavor is due to the sulfhydryl group

- Taste: Bitter taste in milk may be due to Serratia liquefacines

- Sediment test

Tests for acidity

- Clot on Boiling (COB) Test: give indication about the susceptibility of milk to heat processing and its keeping quality.
- rapid method to determine the acidity in milk
- Milk with high acidity (More than 0.17% LA) gets coagulated on boiling.
- Other test to determine acidity : Alcohol test, pH test, Alcohol alizarin test

LABORATORY TESTS

Test	Interpretation	Remarks
Dye reduction test	extent of bacterial contamination	MB reduction test, Resazurin test
Direct microscopic count	type of microorganism	Both live and dead bacteria
Standard plate count	extent of bacterial contamination	Only live bacterias
Freezing point	adulteration of milk with water	Most sensitive test for detecting adulteration with water
Coliform count	faecal contamination	Should be less than 100cfu/ml in raw milk

DYE REDUCTION TESTS

1. Methylene blue reduction test: to find relative number of bacteria in a milk sample

- Very good: not decolorized in 5 hours
- Good: decolorized in less than 3-4 hours
- Fair: decolorized in less than1-2 hours
- Poor: decolorized in less than $\frac{1}{2}$ hour

2. Resazurin reduction test : procedure similar to Mbreduction test. Result in 10minutes

STANDARD PLATE COUNT

- basis for grading milk
- gives rough estimate of viable microbial growth in the sample
- All plate counts are expressed as the number of cfu /ml.
- SPC doesn't indicate the quality of microbial populations in terms of pathogens and non-pathogens.
- generally accepted as the most accurate and informative method of testing bacteriological quality of milk

STANDARD PLATE COUNT

Bacteria CFU/ml	Grade
Up to 2 lakhs	Very good
2-10 lakh	Good
10-50 lakh	Fair
More than 50 lakh	Poor

SPC for pasteurized milk – not more than 30,000 cfu /ml

Bacteriological standards of raw milk (IS-1479 PART III-1997)

Grades	Direct microscopic count per ml (lakhs)	Standard plate count per ml (lakhs)	Methylene blue reduction time (hr)	One hour resuzurin disc. (No.)	Presumptive coliform test (in 0.01 ml) i.e. 1 in 100
Very good	NS	< 2	> 5	NS	absent
Good	< 5	2-10	3-4	4 or higher	absent
Fair	5-40	10-50	1-2	3.5 to 1.0	absent
Poor	40-200	> 50	< 1/2	0.5 to 0	present
Very poor	> 200	NS	NS	NS	NS

NS : Not specified

Bacteriological standards of pasteurised milk (IS-6397-1971)

Test	Requirement
Standard plate count	Maximum 30000 cfu/ml
Coliform count	absent in 1:10 dilution
MBRT	more than 4 hr
Alkaline phosphatase	test negative

ADULTERANTS

- Adulteration --- addition of cheaper & resembling substances to milk or removal of one or more valuable constituents (like fat).

Common adulterants in milk:

1. Water – most common adulterant in milk
2. Starch, cane sugar
3. condensed milk or milk powder
4. urea, detergents, sodium bicarbonate
5. mixing of cow & buffalo milk

Iodine solution Test	Starch adulteration in milk
Nitric acid	Skim milk powder
Bromocresol purple solution	Detergent in milk
p - dimethyl amino benzaldehyde	Urea adulteration in milk
Resorcinol	Cane sugar detection
Rosallic acid test	Sodium Carbonate
Storch's peroxidase test	Heated milk in fresh milk
Hansa Serum (Hansa Test)	Mixing of cow & buffalo milk
Picric acid solution/ Mercuric Nitrate	Gelatin in milk
Delvo kit test	Detect antibiotic and sulpha residues
Lactometer reading, freezing point, nitrate detection	Water in milk
Baudin test	Vegetable oil adulteration in ghee

Table 3. Important tests to detect adulterants in milk

Adulterant	Test	Interpretation
Thickening agents		
Starch	Boil 10 ml milk --> cool and add 1 ml of 5% iodine	Blue colour
Gelatin	10 ml milk + 10 ml mercuric nitrate, shake + 20 ml water and shake well. Filter. Filtrate + equal volume of saturated aqueous picric acid.	Yellow ppt.
Cane sugar	2 ml milk + 1 ml HCl + 0.1 g resorcinol, boil for few minutes	Red colour
Sucrose	10 ml milk + resorcinol	Red colour
Milk powder	10 ml milk + 1 drop formalin place at 60°C for 10 minutes	Peculiar odour
Skim milk powder	10 ml milk + few drops of nitric acid	Yellow colour
Calcium carbonate	10 ml milk + 1 ml conc. HCl	Effervescence
Sodium bicarbonate	5 ml milk + 10 ml alcohol + rosalic acid solution (1:10)	Rose red colour
Urea	i) 0.2 ml urease + 0.1ml 0.5% bromothymol blue + 5 ml milk	Faint blue colour within 10 min for urea and dark Blue colour for synthetic milk.

FAT ESTIMATION

- Gerber test (Fucoma Test)
- Babcock test
- Rose Gottileb and Adam's test