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S.Y. B.Sc. (Sem. IV) (CBCS)

MICROBIOLOGY

[401]: APPLIED AND ENVIRONMENTAL MICROBIOLOGY

Unit 3
DAIRY MICROBIOLOGY

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Milk as medium

- Milk as food contains component that are essential to humans such as protein, carbohydrates, fat, water, all the vitamin B, A, D calcium and phosphorus. It also provides energy.
- An important protein in milk is casein. This is the base for cheese making and is a very important nutrient for humans and animals.
- In addition to casein milk contains whey protein (20%). The whey protein is in most cases not incorporated in cheese; they remain in whey. It has very high nutritive value.
- Milk contains sugar, fat and important source of minerals and vitamins which is very important for human and animal.

Composition and characteristics of various types of milk

- The composition of mother's milk and milk from cow, buffalo, goat, camel, donkey, and lama is shown in table.
- There are other differences between the various kinds of milk, there is a lot of provitamin A in cow milk, giving its yellow color but not buffalo goat and sheep milk. In the sheep and goat milk these carotenoids are already converted in to the colorless vitamin A.
- Cow milk accounts for 91% of the worlds milk production, buffalo, goat, and sheep milk account for 5.9%, 1.6% and 1.7% respectively.

Main constituent	Range (%)	Mean (%)
Water	85.5 – 89.5	87.0
Total solids	10.5 – 14.5	13.0
Fat	2.5 – 6.0	4.0
Proteins	2.9 – 5.0	3.4
Lactose	3.6 – 5.5	4.8

- Proteins perform a variety of functions in living organisms ranging from providing structure to reproduction. Milk proteins represent one of the greatest contributions of milk to human nutrition. Proteins are polymers of amino acids. Only 20 different amino acids occur, regularly in proteins.
- The content and sequence of amino acids in a protein therefore affect its properties. Some proteins contain substances other than amino acids, e.g. lipoproteins contain fat and protein. Such proteins are called conjugated proteins as phosphoprotein, lipoproteins and chromoproteins.
- The phosphate is linked chemically to phosphoproteins, the casein in milk is an example. A combination of lipid and protein forms the lipoprotein and are excellent emulsifying agents. Chromoproteins are proteins with a colored prosthetic group and include hemoglobin and myoglobin.

Sr. no.	QUESTION	ANSWER

1	What is the component of milk?	Protein,carbohydrates,fat, water,vitamin B,A,D and calcium and phosphorous
2	Which protein found in milk is base of cheese making?	Casein protein
3	What is the reason behind yellow color of cow milk?	Provitamin A
4	How many percentage of cow milk is consumed by world population?	91%
5	Give example of chromoprotein	Hemoglobin & myoglobin

NORMAL FLORA OF MILK

- In India and other developing countries, hand milking, utensils, water in unhygienic conditions are major sources of microorganisms in milk.
- The microorganisms present in milk in the teat canal of milk admit bacteria into the raw milk. First few millimeters of such milk may contain around 15,000 bacteria per millimeter, but milk of later stage contains less microorganisms. Hand milking increases the chance of contamination.
- *Staphylococcus epidermis, Micrococcus, Pseudomonas, Flacobacterium, Erwinia*, and some fungi are present in raw milk. Hand milking allows the entry of *E. coli, Acinetobacter Johnsoni*.
- The organisms present in the storing containers contaminate the milk duringprocessing and transportation.
- Pathogens can enter milk through diseased cattle Some of them are *Mycobacterium bovis, Brucella*, etc. Some microorganisms are able to grow on the udders of the cowlike species of *micrococcaceae; Bacilliaceae, Escherichae. Lactobacillaceae* etc. So, they are most commonly found in the milk.

TYPES OF MICROORGANISMS IN MILK

Microorganisms present in the milk can be discussed on various types (1) Biochemical Types (2) Pathogenic Types (3) Temperature Types

1) **Biochemical Type**: Milk contains some normal flora under favorable conditions of growth. These microorganisms cause raw milk to develop clean, bit sour natural flavor. This change is brought about by: *Streptococcus, S. cremoris* and some *lactobacilli*.They convert lactose to lactic acid, which changes its natural favor, taste etc. This change is called "Normal fermentation of milk". However, proteolytic activity and lipolytic activities are not easily detected in milk. Some other microbes show different activities and changes in the milk. For e.g.,

2) **Pathogenic Types**: Due to better techniques available today for preservation of milk there are less and less diseases prevailing which are caused by milk. Milk and dairy products are considered as model foods from quality, production, and processing and distribution point of view. There are good methods for analysis of milk and milk products which are generally not available for other foods. There are few varieties of diseases caused by milk. The source of milk may be the cow or the milking man. In the following manner disease may be transmitted: Pathogens from cow milk - human beings + cow (T.B. etc.).

3) **Temperature Types:** Milk microorganisms may be classified on the basis of their temperature and heat resistance property. Low temperatures are used to preserve milk and high temperatures are used to destroy microorganisms and to improve the quality of milk. Thus, temperature characteristics are very important for microbes.

- Milk contains *Psychrophiles*, *Mesophiles*, *Thermophiles* and Thermoduric microorganisms. Storage temperature will decide that decide that which bacteria will predominate in milk. Pasteurized milk can be stored in the refrigerator for a week and longer but *Psychrophiles* will eventually bring about deterioration change in favor and odor of the milk. Thermophiles and thermoduric bacteria cause problems during pasteurization. Certain microbes like *Bacillus*, *Stearothermophilus*, etc. resist during pasteurization. Thermoduric and thermo tolerant bacteria cause hurdle during pasteurization as they survive at the pasteurization temperature (60-70°C). Some examples of thermoduric bacteria are; (1) *Microbacterium lacticum* (2) *Micrococcusluteus* (3) *S. thermophilus* (4) *Bacillus subtilis*.

Sr. no.	QUESTION	ANSWER
1	What is the major source of microorganisms in milk ?	Hand milking, utensils, water in unhygienic conditions
2	What are the different types of microorganisms present in milk?	Biochemical type, pathogenic type, temperature type
3	What changes are seen in milk if biochemical type of microorganisms present in it?	They convert lactose to lactic acid, which changes its natural favor, taste etc.
4	Example of temperature type microorganisms present in milk	*Psychrophiles*, *Mesophiles*, *Thermophiles* and Thermoduric microorganisms
5	What changes are done by	They brings

	Psychrophiles ?	deterioration change in favor and odor of the milk

SPOILAGE OF MILK AND MILK PRODUCTS

- Milk is known to be balanced food as well as highly nutritious food as it is Rich in proteins, fats, carbohydrates, vitamins, minerals, essential amino acids, etc.
- Milk and its products consist of numerous nutrient content, it serves as an excellent growth medium for all of the microorganisms (i.e., bacteria, viruses, fungi, and protozoans).
- The microbial content of raw milk is important for the production of hygienic milk foods.
- Milk and its products such as milk, butter, cream, and cheese are all susceptible to microbial spoilage.

The milk source is the mammals such as cows, buffalo, sheep, and goats. Milk is not sterile when obtained from animals. The sources of milk contamination are:

1. the contamination from milking animals
2. the interior and exterior of the udder
3. the coat of the milking animals
4. the surrounding air of the farm and storage
5. the animal feed
6. the quality of water used
7. the quality of milk handling equipment and storage tanks used biofilms formation on diary plant pipes.

SPOILAGE OF MILK

- The main components of milk are water, fat, protein, and lactose.
- Its high water activity, moderate pH (6.4–6.6), and high nutrient content make milk an excellent medium for microbial growth.
- Both raw milk and pasteurized milk contain many types of microorganisms, they are refrigerated, yet they have limited shelf life.
- During refrigerated storage (at dairy farms and processing plants) before pasteurization, only *psychrotrophs* can grow in refrigerated milk storage such as *Pseudomonas, Flavobacterium, Alcaligenes, Listeria monocytogenes, Yersinia enterocolitica, some coliforms, and Bacillus spp*.
- Those that spoil milk after heating are the thermoduric microorganisms surviving pasteurization such as *Micrococcus*, some *Enterococcus*, *Streptococcus*, some *Lactobacillus*, and spores of *Bacillus* and *Clostridium.*
- Molds and yeasts are usually eliminated during the pasteurization process therefore they cause milk spoilage after the heat treatment such as *Aspergillus, Byssochlamys, Cladosporium, Candida spp*.
- To a lesser extent, the protozoan pathogens such as *Cryptosporidium* and *Giardia* have been found to contaminate milk.

The defects that occur in milk due to microorganism are:

1. Off flavors
2. Discoloration
3. Gas production
4. Lactic acid production/ Souring
5. Proteolysis
6. Lipolysis with development of rancidity
7. Sweet curdling

Kind of defect	Related microorganisms
Shorter shelf life, rancidity, and bitterness	*Bacillus* spp.
Gelation	Psychrotrophic bacteria (Gram-negative and Gram-positive)
Increase of free fatty acids and casein hydrolyses, destabilizing the casein micelles (acid coagulation of milk)	*Bacillus* spp
Undesirable flavor: unclean, fruity, bitter, rancid, yeasty	*Pseudomonas fragi, P. fluorescens Flavobacterium, Acinetobacter, Alcaligens*
Sour (acid, gas)	Lactic acid bacteria
Ropy or slimy	Coliforms, *Pseudomonas* spp, *Alcaligenes, Micrococcus, Bacillus subtilis*
Flavor Sour or acid flavor	*Streptococcus lactis, leuconostoc, clostridium spp*
Burnt or caramelflavor	*Streptococcus lactis*
Color changes -Blue milk	*Pseudomonas syncyanea, Streptococcus Lactis*

Sr. no.	QUESTION	ANSWER
1	List the product made from the milk	Butter, cream, cheese etc.
2	Why milk is excellent medium for growth of microorganisms?	Due to its high water activity, moderate pH and high nutrient content
3	List of psychrotophs present in milk	Pseudomonas, Flavobacterium, Alcaligenes, Listeria monocytogenes, Yersinia enterocolitica, some coliforms, and Bacillus spp.
4	Which kind of diffects are done by	Shorter shelf life,

	Bacillus spp.?	rancidity, and bitterness, Increase of free fatty acids and casein hydrolyses, destabilizing the casein micelles (acid coagulation of milk)
5	What kind of defects occurs in milk due to microorganisms?	Off flavors Discoloration Gas production Lactic acid production/ Souring Proteolysis Lipolysis with development of rancidity Sweet curdling

SPOILAGE OF MILK PRODUCT

- The microbial quality of milk product mainly depends upon:
- The type of milk and milk product used (raw milk, condensed milk, dried milk, cream, butter, etc.)
- The product used for its enhancement such as gelatin, nuts, fruits, sugar, chocolate, coloring agent, etc.,
- Sanitary level of types of equipment, the efficiency of pasteurization, and hygienic level during production and packaging.

SPOILAGE OF MILK CREAM

- The cream is a milk product made from a butterfat layer deposited on the top of milk before homogenization.
- Cooled milk is used for the production of cream so psychrotrophic bacteria are the main causes of spoilage are caused by *Pseudomonas, Alcaligenes, Acinetobacter, Aeromonas,* and *Achromobacter.*
- At room temperature, the main spoilage-causing organisms *are Corynebacterium, Bacillus, Micrococcus, Lactobacillus, and Staphylococcus.*
- The cream is highly susceptible to pathogenic microorganisms like *E. coli, Salmonella Typhimurium,* and *Listeria monocytogenes*.

Kinds of defect	Related Microorganisms
Bitty cream, sweet curdling	*Bacillus cereus*
Bitterness, thinning in sterilized cream	*Bacillus licheniformis, B. coagulans, Bacillus subtilis*
Yeast or fruity flavors, gas formation	*Candida lipolyticum, Geotrichum candidum.*
Surface taints	*Penicillium* spp
Foamy	*Candida, Torulopsis*

SPOILAGE OF BUTTER

- Butter is a milk product made by the separation of milk and subsequent churning of the cream.
- The main source of microorganisms found in butter is cream which is starting material of butter. Therefore, the main spoilage is caused by *Pseudomonas, Alcaligenes, Acinetobacter, Aeromonas,* and *Achromobacter.*
- The primary spoilage organisms in butter are molds such as *Thamnidium, Cladosporium,* and *Aspergillus.*
- The pathogenic microorganisms like *Listeria monocytogenes, Brucella, Mycobacterium, Campylobacter jejuni, Yersinia enterocolitica,* and *Salmonella Typhimurium.*

Kinds of defect	Related Microorganisms
Surface discoloration and taints	Bacteria: *Pseudomonas putrefaciens, Flavobacterium spp, Alteromonas* Molds: *Penicillium, Aspergillus, Mucor, Cladosporium, Rhizopus* spp
Lipolytic spoilage	*Rhodotorula, Cryptococcus, Torulopsis, Candida lipolytica*
Yeasty smell	*Geotrichum candidum*

SPOILAGE OF CHEESE

- Cheese is a fermented milk product that is made by coagulating the casein present in milk by using the enzyme rennet.
- The ripening in cheese is achieved due to the proteolytic and lipolytic activities of various microorganisms.
- The low moisture content of hard and semi-hard ripened cheeses makes them susceptible to fungi compared to bacteria.
- Soft and fresh cheeses are spoiled easily due to their higher pH, moisture content, and lower

salinity.

- The bacterial cheese spoilage is caused by *Clostridium* spp., (especially *C. pasteurianum, C. butyricum, C. sporogenes,* and *C. tyrobutyricum), Bacillus polymyxa, Flavobacterium, Pseudomonas spp., Alcaligenes,* and *Achromobacter*.
- Yeasts are common cheese spoilage organisms e.g., *Candida spp., Debaryomyces hansenii, Geotrichum candidum,* and *Pichia spp.*
- The mold spoilage in cheese is caused mainly by *Penicillium spp.* and *Cladosporium spp*.
- Major pathogenic bacteria found in cheese are *Listeria monocytogenes, Salmonella spp.,* and *Escherichia coli.*

Kinds of defect	Related Microorganisms
1. Cheese	
Gassy, Butyric acid	*Clostridium tyrobutyricum*
Gassy, floating, or split curd	*Leuconostoc, S. lactis subsp. Diacetylactis*
Moldy	*Penicillium, Scopulariopsis, Mucor, other molds*
Bitterness, putrefaction and rancid odor, liquefaction, gelatinization of curd, and slime and mucous formation	*Pseudomonas fluorescens, P. fragi*
Undesirables flavor: rancid taste in hard cheeses	*Bacillus spp*
off-white, tan, or yellow surface discolorations	*Geotrichum*
2. Soft cheese	
Black mold	*Mucor*
3. Cottage cheese	
Slimy curd, putrid odor	*Pseudomonas*
Discoloration	*Flavobacterium,* yeasts, molds
Slimy, gelatinous	*Pseudomonas, Alcaligenes, Flavobacterium, coliforms*
Fruity	Yeasts
4. Cheddar cheese	
Sweet, yeasty, fruity	Yeasts
5. Swiss cheese	
Gassy, sweet Off odor	Yeasts (Torulopsis)

Off odor	*C. sporogenes*

SPOILAGE OF ICE-CREAM

- Ice cream is a frozen milk product produced by freezing a pasteurized mixture of milk, cream, and milk solids other than fat, sugars, emulsifier, and stabilizers.
- It includes various flavor-enhancing ingredients like nuts, fruit pulp, confectionary products, eggs, and egg products.
- Being a nutritious food, ice-cream serves as a good medium for microbial growth due to the high nutritive value, almost neutral pH, and long storage duration.
- It is a frozen milk product hence ice cream spoilage is mainly caused by psychrotrophs such as *Pseudomonas, Flavobacterium, Alcaligenes, Listeria monocytogenes*.
- Certain molds such as *Aspergillus*, *Fusarium*, *Geotrichum*, *Mucor*, *Penicillium*, and yeasts such as Zygosaccharomyces, Saccharomyces, and Cryptococcus also cause ice-cream spoilage.
- Major pathogenic bacteria found in ice-cream are Listeria monocytogenes, *Salmonella* spp., and *Escherichia coli*.
- The defect found in ice cream due to this spoilage causing microorganisms are:
    1. bitterness and off-flavor
    2. rancidity
    3. greenish pigments
    4. discoloration
    5. surface taints

Sr. no.	QUESTION	ANSWER
1	How cream is made from the milk?	It is made from a butterfat layer deposited on the top of milk before homogenization
2	Give example of Bactria that spoiled the milkcrème	Pseudomonas, Alcaligenes, Acinetobacter, Aeromonas, and Achromobacter.
3	Who is primary spoilage of butter?	Thamnidium, Cladosporium, and Aspergillus.
4	Reason behind the putrid odor in cottage cheese	*Pseudomonas*

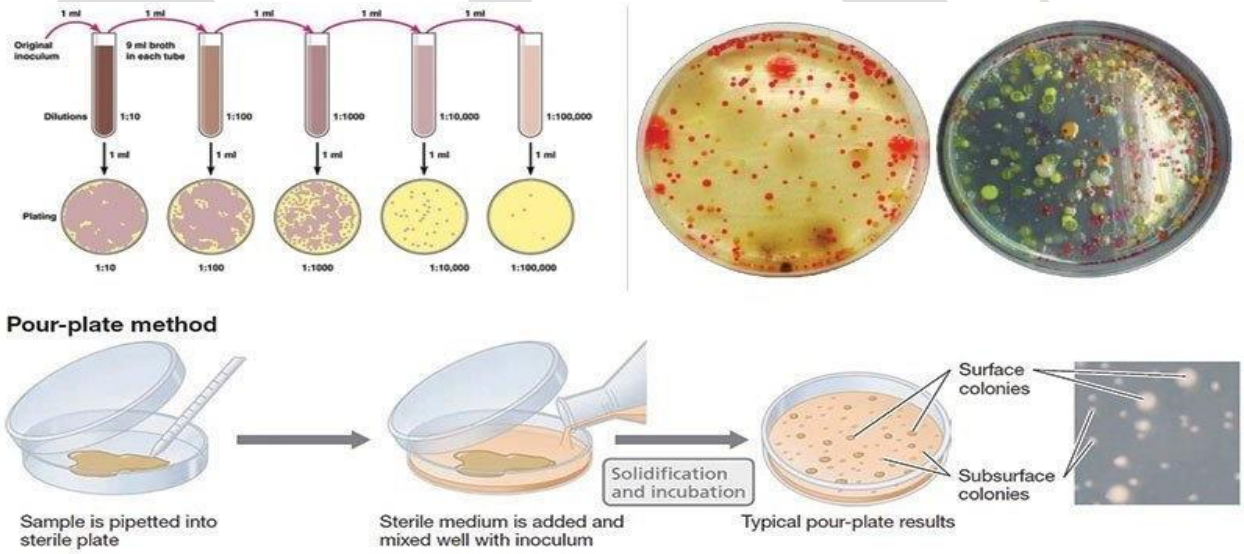
5	Give name of flavor enhancing ingredients present in ice-crème	Nuts, fruit pulp, confectionary products, eggs and eggs products.

MICROBIAL ANALYSIS OF MILK

Several tests are available now a day for the microbial analysis of the milk. some of test include standard plate count (SPC), direct count, methylene blue, resaszurin test.

1) Standard plate count

- In nature, microbial populations do not segregate themselves by species but exist with a mixture of many other cell types.
- In the laboratory, these populations can be separated into pure cultures.
- These cultures contain only one type of organism and are suitable for the study of their cultural, morphological, and biochemical properties.
- At times also the determination of viable cells is very crucial in many microbiological procedures.
- To accomplish this, the serial dilution–agar plate technique is used.
- Briefly, this method involves serial dilution of a bacterial suspension in sterile water blanks, which serve as a diluentof known volume.
- Once diluted, the suspensions are placed on suitable nutrient media.
- It should be allowed to incubate at 32-35 degree Celsius for 24-48 hours.



Advantages of SPC

- Easy to undertake.
- Will detect lower concentrations than surface spread method because of the larger sample volume.
- It requires no pre-drying of the agar surface.
- The most common method for determining the total viable count is the pour-plate method.

- The pour plate technique can be used to determine the number of microbes/ mL in a specimen.
- It has the advantage of not requiring previously prepared plates and is often used to assay bacterial contamination of foodstuffs.

Disadvantages of SPC

- The use of relatively hot agar carries the risk of killing some sensitive contaminants, so giving a low result.
- Small colonies may be overlooked.
- In the case of solid sample dissolving in water, some species may suffer a degree of viability loss if diluted quickly in cold water; consequently, isotonic buffer (phosphate-buffered saline for example) or peptone water are used as solvents or diluents.
- Colonies of different species within the agar appear similar — so it is difficult to detect contaminants.
- The reduced growth rate of obligate aerobes in the depth of the agar.
- Preparation for the pour plate method is time consuming compared with streak plate/and or spread plate technique.

2) Direct Microscopic Counts

- Studies involving the analysis of materials including food, water, milk, and, in some cases, air require quantitative enumeration of microorganisms in the substances.
- Many methods have been devised to accomplish this, including direct microscopic counts, use of an electronic cell counter such as the Coulter Counter, chemical methods for estimating cell mass or cellular constituents, turbidometric measurements for increases in cell mass, and the serial dilution– agar plate method.
- Direct microscopic counts require the use of a specialized slide called the Petroff-Hausser counting chamber, in which an aliquot of a eukaryotic cell suspension is counted and the total number of cells is determined mathematically.
- The Petroff-Hausser counting chamber is a thick glass microscope slide with a chamber 0.02 mm (1/50 mm) deep in the center.
  The chamber contains an etched grid and has improved Neubauer rulings.
- (1/400 square mm).
  The rulings cover 9 mm^2. The boundary lines (Neubauer rulings) are the center lines of the groups of three.
- The center square millimeter is ruled into groups of 16 small squares, and each group is separated by triple lines, the middle one of which is the boundary.
- The ruled surface is 0.02 mm below the cover glass, which makes the volume over a square millimeter 0.02 mm3 (cubic mm). All cells are counted in this square millimeter.
- The number of cells counted is calculated as follows:

Number of cells per mm = number of cells counted * dilution * 50,000

[The factor of 50,000 is used in order to determine the cell count for 1 ml: 1 ml = 1000 mm3 = (50 times the chamber depth of 0.02 mm) * 1000.]

A variation of the direct microscopic count has been used to observe and measure growth of

bacteria in natural environments. In order to detect and prove that thermophilic bacteria were growing in boiling hot springs, T.D. Brock immersed microscope slides in the springs and withdrew them periodically for microscopic observation. The bacteria in the boiling water attached to the glass slides naturally and grew as microcolonies on the surface.

Advantages of direct microscopic count

- Rapid, Simple and easy method requiring minimum equipment.
- Morphology of the bacteria can be observed as they counted.
- Very dense suspensions can be counted if they are diluted appropriately.

Disadvantages of direct microscopic count

- Although rapid, a direct count has the disadvantages that both living and dead cells are counted.
- Only dense suspensions can be counted (>107 cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity.
- It is not sensitive to populations of fewer than 1 million cells.
- Small cells are difficult to see under the microscope, and some cells are probably missed.
- Precision is difficult to achieve
- A phase contrast microscope is required when the sample is not stained.

Methylene Blue Dye Reduction Test

- Methylene Blue Dye Reduction Test, commonly known as MBRT test is used as a quick method to assess the microbiological quality of raw and pasteurized milk. This test is based on the fact that the blue color of the dye solution added to the milk get decolorized when the oxygen present in the milk get exhausted due to microbial activity. The sooner the decolorization, more inferior is the bacteriological quality of milk assumed to be. This test is widely used at the dairy reception dock, processing units and milk chilling centers where it is followed as acceptance/rejection criteria for the raw and processed milk.

Grading of raw milk based on MBRT:

- MBRT test may be utilized for grading of milk which may be useful for the milk processor to take a decision on further processing of milk. As per BIS 1479 (Part 3): 1977 criterion for grading of raw milk based on MBRT is as below:

5 hrs and above	Very good
3 to 4 hrs	Good
1 to 2 hrs	Fair
Less than ½ hrs	Poor

Procedure: The test has to be done under sterile conditions. Take 10 ml milk sample in sterile MBRT test tube. Add 1 ml MBRT dye solution (dye concentration 0.005%). Stopper the tubes with sterilized rubber stopper and carefully place them in a test tube stand dipped in a serological water bath maintained at 37 ± 1^0C. Record this time as the beginning of the incubation period. Decolorization is considered complete when only a faint blue ring (about 5mm) persists at the top.

Recording of Results - During incubation, observe color changes as follows:

- If any sample is decolorized on incubation for 30 minutes, record the reduction time as MBRT - 30 minutes.
- Record such readings as, reduction times in whole hours. For example, if the color disappears between 0.5- and 1.5-hour readings, record the result as MBRT - 1 hour; similarly, if between l.5 and 2.5 hours as MBRT - 2 hour and so on.
- Immediately after each, reading, remove and record all the decolorized samples and then gently invert the remaining tubes if the decolorization has not yet begun.

RESASZURIN TEST

The Resazurin test is designed for assessing the quality of raw bulked milk. This is essentially a rapid bacteria estimation and can be carried out either as a 10-minute pass or fail, a 3-hour triple reading test or as the standard and generally accepted 1 hour test.

BLUE ⟶ PINK ⟶ COLOURLESS

Resazurin gives milk a characteristic blue color and the test is based on the ability of bacteria in the milk to reduce the blue dye. The quality of the milk is judged by noting the degree of color change - from blue through mauve and purple and pink and finally colorless - after a stated period of incubation, or the time required to reduce the dye to a predetermined color.

Sr. no.	QUESTION	ANSWER
1	Give the name of the test for microbial analysis?	SPC, direct count, MBRT, resaszurin test
2	Use of petroff-hausser counting chamberf	It is used for direct microscopic counts
3	If the decolorization of milk occurs within ½ hrs what is the quality of milk	poor
4	What is the rapid test for microbial analysis ?	Reasazurin test
5	Full form of MBRT	Methylene blue reduction test

GRADING OF MILK

- The importance of milk grading lies in the fact that dairy products are only the raw materials from which they were made. It is important that dairy panel have knowledge of

sensory perception and evaluation techniques. The identification of off - flavors and desirable flavors, as well knowledge of their and quality dairy products understanding of the principles of sensory evaluation is necessary for grading.

- A foes and smell. At taste, smell, touch and sound. The greatest emphasis, however, is placed.
- The actual no of the bacteria or microorganisms permissible in the milk varies undoing to the milk standard ordinances. Ending to the cities, but there are certain standards which are widely accepted Radically there are two grades,
    - grade-A
    - grade-B
- **Grade A: Raw Milk for Pasteurization**: It is the raw milk from properly premises, and equipments as prescribed in the ordinances. supervised dairies and having standards of sanitation of workers, cattle, Individual dealing with cattle and cattle itself should be free from infection. The bacterial count should not be exceeding 2,00,000 organisms / ml. It must not have MBRT of less than 5.5 hrs. and RRT of less than 2.75 hrs.
- **Grade A: Pasteurized Milk**: In all cases grade A pasteurized milk should show effective pasteurization as an evidence by satisfactorily performed phosphate test. After pasteurization and before delivery, the milk shall not have a bacterial count exceeding 20,000 / ml. Also, there should not be coli forms count exceeding 10 / ml
- **Grade B: Pasteurized Milk**: This milk does not contain the bacterial count standard for Grade - A pasteurized milk and certain other sanitary requirements. Such milk may be used in some commercial processes. Many communities now permit the sale of only Grade A Milk.
- **Certified Milk**: If the milk is to be offered for the sale, unpasteurized, it is then required that it must be carefully supervised under certain conditions. The American Association of Medical Milk Commission (AAMMC) has established rules and regulation like:
    - Veterinary inspection of the cows especially, for tuberculosis infectious mastitis.
    - Inspection of the sanitation of barns and utensils.
- Milk produced under such rules and regulations is called as "certified milk "baby milk". The coliforms standard for certified raw milk is 10 While the coliforms standard certified milk is 1/ml. Certified milk is said to contain larger amounts of vitamins, essential for infants than milk which has been heated. All the persons occupied in preparation of the certified mille must be examined periodically, for the typhoid and dysentery types of infection. Examination for the presence of the organisms that cause diphtheria, tuberculosis, scarlet fever and other transmissible diseases and are also required for certified milk handlers.

Sr. no.	QUESTION	ANSWER
1	How many types of grade are there? Give name	2 types 1)grade –A 2) grade – B
2	What is the bacterial count for grade A in row milk for pasteurization?	2,00,000 organisms/ml.

3	What is the MBRT result for grade-A?	Less than 5.5 hrs.
4	Full form of AAMMC	American association of medical milk commison
5	What is coliform standerd for certifird row milk ?	10

FERMENTED MILK BEVERAGES AND MANUFACTURESS DAIRY PRODUCT

Starter culture:

- Starter cultures are an essential component of nearly all commercially produced fermented foods. Simply defined, starter cultures consist of microorganisms that are inoculated directly into food materials in order to bring about desired and predictable changes in the finished product. These changes may include enhanced preservation, improved nutritional value, modified sensory qualities, and increased economic value.
- Although many fermented foods can be made without a starter culture, the addition of concentrated microorganisms, in the form of a starter culture, provides a basis for ensuring that products are manufactured on a consistent schedule, with consistent product qualities.
- Fermented foods and beverages have long been manufactured without the use of commercial starter cultures.
- Traditional methods of production include back slopping, or using a small amount of the finished specifically preserved product to inoculate a new batch, the use of microorganisms found naturally on the product, and the use of special containers that allow for the survival of the starter culture microorganisms within cracks and pores.
- These traditional methods allow for the development of individual varieties of fermented foods and beverages, and they are still practiced today for small- to mid-scale production facilities, as well as in less developed countries and in homemade-type products.
- Traditional methods, however, are prone to slow or failed fermentations, contamination, and inconsistent quality. In contrast, modern large-scale industrial production of fermented foods and beverages demands consistent product quality and predictable production schedules, as well as stringent quality control to ensure food safety.
- Given that pure culture techniques in microbiology were not developed until Pasteur in the 1860s and that lactic acid bacteria (LAB), in particular, were identified by Lister in the 1870s, it is noteworthy that an industry devoted to producing pure cultures had its beginnings only a short time later.
- In the late 1880s, Storch in Denmark, Weigman in Germany, and Conn in the USA showed that pure cultures could be used to ripen cream, and soon the role of flavor-producing bacteria (i.e., citrate-fermenting diacetyl-producers) was established.
- By 1878 Christian Hansen began a culture business that continues even today to be a major

supplier of starter cultures for the dairy, meat, brewing, baking, and wine industries.

- Initially, starter strains were prepared by the manufacturer by growing pure strains in heat-sterilized milk. Calcium carbonate was often added as a buffer in order to maintain a neutral pH.
- These liquid cultures remained popular until relatively recently, even though they had a relatively short shelf-life due to the loss of cell viability and fermentative activity.
- Eventually, rather crude dry culture preparations were produced which required several transfers in milk to revive the culture to an active state. Freeze-dried cultures also became available, but the early product also required growth in intermediate or mother cultures.
- Frozen cultures, now the most common form for dairy cultures, were not introduced until the 1960s.
- Significant improvements in freezing and freeze-drying technologies have led these types of cultures to dominate the starter culture market.
- The modern starter culture industry provides cultures for nearly every type of fermented food and beverage.
- Most culture houses also produce and sell the media used to propagate starter cultures, the enzymes used to coagulate milk for cheese production, and other ancillary products.
- In addition, the starter culture companies maintain a staff of highly trained microbiologists who provide expert technical service and support when issues or problems related to culture performance arise.
- And although there are many small culture manufacturers throughout the world, specializing in cultures for specific products or applications, the industry is dominated by a small number of large companies.

Sr. no.	QUESTION	ANSWER
1	Define starter culture	starter cultures consist of microorganisms that are inoculated directly into food materials in order to bring about desired and predictable changes in the finished product
2	What is the benefit of starter culture?	It ensures that the products are manufactured on a consistent schedule, with consistent product quality
3	Lactic acid bacteria identified by ……..	Lister
4	Who began culture business?	Christina Hansen
5	For manufacturing of starter culture	Calcium

	…… used as a buffer	carbonate

Cheese:

Cheese is one of the oldest foods, probably developed roughly 8,000 years ago. About 2,000 distinct varieties of cheese are produced throughout the world, representing approximately 20 general types and an annual economic value of about 75 billion U.S. dollars.

Often cheeses are classified based on texture or hardness as

- soft cheeses (cottage, cream, Brie),
- semisoft cheeses (Muenster, Limburger, blue),
- hard cheeses (cheddar, Colby, Swiss)
- very hard cheeses (Parmesan).

• All cheese results from a lactic acid fermentation of milk, which results in coagulation of milk proteins and formation of a curd. Rennin, an enzyme from calf stomachs but now produced by genetically engineered microorganisms, can also be used to promote curd formation. After the curd is formed, it is heated and pressed to remove the watery part of the milk (called the whey), salted, and then usually ripened. The cheese curd can be packaged for ripening with or without additional microorganisms.

• *Lactococcus lactis* is used as a starter culture for a number of cheeses. Starter culture density is often over 10^9 colony-forming units (CFU) per gram of cheese curd before ripening. However, the high salt, low pH, and the temperatures that characterize the cheese microenvironment quickly reduce these numbers. This enables other bacteria, sometimes called nonstarter lactic acid bacteria (NSLAB}, to grow; their numbers can reach 10^7 to 10^9 CFU/g after several months of aging. Thus, both starter and nonstarter LAB contribute to the final taste, texture, aroma, and appearance of the cheese. In some cases, molds are used to further enhance the cheese.

- Obvious examples are Roquefort and blue cheese. For these cheeses, *Penicillium roqueforti* spores are added to the curds just before the final cheese processing. Sometimes the surface of an already formed cheese is inoculated at the start of ripening; for example, Camembert cheese is inoculated with spores of *Penicillium camembert*. The final hardness of the cheese is partially a function of the length of ripening.
- Soft cheeses are ripened for only about 1 to 5 months, whereas hard cheeses need 3 to 12 months, and very hard cheeses such as Parmesan require 12 to 16 months of ripening.

- **The ripening process** also is critical for Swiss cheese; gas production by *Propionibacterium* contribute to final flavor development and hole or eye formation in this cheese. Some cheeses, such as Limburger, are soaked in brine to stimulate the development of specific fungi and bacteria.

Sr. no.	QUESTION	ANSWER
1	How many varieties of cheese are produced throughout the world?	Around 2000
2	Which enzyme is used to promote	Rennin

	curd formation?	
3	Example of very hard cheese	parmesan
4	Watery part of milk called…..	Whey
5	Full form of NSLAB	Nonstarter lactic acid bacteria

YOGURT

- Yogurt is most popular of fermented milks and known in several countries with realtering names
- TA Jugurt or Eran in Turkey.
- Lehnan in Lebanon and Arabian countries.
- Dahi in India.
- The tis cheese a high Zabady in Egypt and Sudan - Filmjolk in Scandinavia.
- However, it is a traditional food in Balkans and Middle East. According to FAO / WHO (1997). "YOGHURT is a coagulated milk product obtained by lactic acid fermentation through the action of *S. thermophilus* and Lactobacillus bulgaricus from milk and milk products with or without optional additions milk powder, whey powder etc. and the final product must contain viable organisms in abundance.

- The microflora of yoghurt can be categorized as; + essential - constituting starters - non - essential occasionally added for special benefits + contaminants - unwanted group Special yoghurts: With a view to increase the shelf life, the yoghurt may be heat treated at 60-70°C for 1-5 mins.
- Such yoghurt is called pasteurized JUHT / Heated / long life yoghurt. The treatment destroys the live starter and hon - starter bacteria and enzymes.
- It also reduces the viscosity, increases whey separation, decreases flavor and creates legal complications as it does not obey FAO / WHO definitions, where presence of viable starter organisms in abundance is a requirement hydrolyzed yoghurt with specially prepared for people who glucose and galactose.
- This increases the sweetness in the improves the starter growth.
- Several diabetic and therapeutic you also prepared, which makes use of selected strains of *L. acidophies, B. bifidum* alongwith normal yoghurts starters.

BUTTERMILK

- Butter milk is popularly known as Chhash or La True buttermilk remaining after the cream is churned into the butter.
- However, more the cultured butter milk is produced by souring skim milk with me generally around 0.8 to 0.9% and typical diacetyl flavor.
- The *Streptococci and Leuconostock*. They produce mild sour taste with w temperature of 21C is most suitable, as it gives proper balance of a pro and aroma producers in the product.
- Sometimes milk may be supplemented with 0.1-0.2 % citrate to have high flavor intensity in

buttermilk. Agitations cooling also affect the flavour of the product.

- **Bulgarian Butter Milk:** The variety of the cultured milk often at Bulgarian butter milk was formerly used for therapeutic reasons,but a been replacedlargely for the use of acidophilus milk
- It differs from the ordinary cultured milk because of its greater acidity. It lacks the aroma and flavors are much more viscous and may be gelatinous. Bulgarian buttermilk is mixed with cultured butter milk to make a product having sharp acidity and thick consistency.
- **Preparation:** Whole or skimmed milk is heated to 190 ° F for 30 minutes and cooled to 105-110 ° F and incubated the 2-3 % starter. The milk is incubated until a titration reading 1.2-1,5 % is reached. The milk is cooled at BOT stirred and packed culture which is used for this type of milk is *L. bulgaricus*.

Sr. no.	QUESTION	ANSWER
1	Yogurt is known as ……. In india	Dahi
2	How bulgarian buttermilk is differ form ordinary?	Because of its high acidity
3	Which culture is used to prepare Bulgarian buttermilk?	l. *bulgaricus*
4	Incubation time for bulgaruan butter milk is…….	30 minutes
5	Butter milk is known as …… in india	chhash

ACIDOPHILUS MILK:

- ACIDOPHILUS MILK Consumption of the acidophilus milk has resulted mainly because of therapeutic and health promoting preparation claimed for it. In Soviet Union, the milk acidophilus with the strain L. acidophilus has been widely used.
- **Starter Propagation:** Strains of L. acidophilus are difficult to maintain and propagate because of their requirements of fastidious nutrients. It is fa this purpose that along with the milk for acidophilus culture, addition sugar, addition of nutrients like tomato juice etc. is done. Sterile culture should be propagated in sterile milk containing 5 % tomato juice or 1 extract and glucose. Mother cultures are stored at 4°F and are transferred times weekly to maintain activities.
- **Preparation of Acidophilus Milk:** Skimmed or whole milk may be used Fresh milk is heated for 15 minutes. After this, milk is cooled and is inoculated with 3-5 % inoculum. Inoculum is mixed properly and is incubated ad milk coagulates. Then it is slowly broken and cooled 5-10% lactose or dextrin may be added. The milk is then stored, packed and stored at 45 ° F. Acidophilus yeast is another product made *from L. acidophilus*.

KEFIR

- Kefir like yoghurt,is a milk product first discoveredby the nomadic tribes living the cold areas of the Caucasus. When making kefir, acid, gas and some alcohol s produced. Like sour milk and buttermilk, it has a special aroma which is Brent from that of yoghurt. Kefir is made by using a ' yoghurt plant ', which is actually a misleadingname as it is not a plant and it has nothing to do with yoghurt.
- Kefir is a rather foamy and effervescent fermented milk that contains about 1% Iactic acid and 0.5 1.0 % alcohol, and is popular in eastern Europe and Mongolin. The starter culture consists of small, white ' kefir grains ', about 2. 10 mm in diameter. These grains contain a complex and quite variable microbial community, but little is known about how they develop. The grains usually esotain LAB such as *Lactobacillus spp. (L. delbreuckii subsp. bulgaricus) plus Lactococcus spp. (L. lactis subsp. lactis) , Leuconostoc spp.* , and *S. thermophilus ,* acetic acid bacteria *(Acetobacter aceti* and *Acetobacter rasens) ,* contaminants such mold *(Geotrichium spp.)* , and a number of yeast species such as *Saccharomyces* and *Kluyveromyces,* but the principal yeast species present is *Candida kefir (* synonym: *Candida kefyr ,* telemorph*: Kluyveromyces marxianus*).
- The yoghurt plant ' is in fact a cauliflower - like lump of chalky crystals and microorganisms consisting of yeasts and bacteria. The yeasts produce alcohol and gas, while the bacteria convert the milk sugar into lactic acid . You may be able to obtain a piece of this from someone who regularly makes kefir. If not, get some Kat tried kefir granules at the local market. You will need : a fresh raw milk, a saucepan , a heat source , a thermometer , a Pan , a glass bottle with wide neck, a bottle which can be closed tightly. dan kefir granules or a ' yoghurt plant, a sieve , cooling facilities, clean water, a teaspoon, and a cool place to store the product.
- Boil the milk and put it into a bottle, which has been thoroughly cleaned and sed with hot water. Do not fill the bottle completely,let the milk cool down to 20 ° C (use a thermometer).
- Add one tablespoon of kefir granules soaked in water to each half litre of milk in the bottle and cover it loosely so that the gas produced can escape. You can use a fresh yoghurt plant instead of the soaked granules Keep the bottle at a temperature of 16 to 18 ° C.
- After 24 hours the milk will have become a little thicker, forming some froth : this is kefir.
- Sieve the kefir and use the (washed with clean water) granules, which remain in the sieve to make fresh kefir again The kefir is now ready for consumption or can ripen for some days to get more flavour.

➢ **Ripening of the Kefir:**

- The kefir can be left to ripen', during which time further fermentation takes place.
- This is essential to create kefir's characteristic qualities. Pour the kefir into a well - cleaned bottle , which can be closed , or a bottle with a clip fastening and do not fill more than 3/4 because gas forms during ripening.
- Leave the bottle at about 15 ° C , but do not store it for more than 3 days . Towards the end of the ripening process the whey separates and can be incorporated again by stirring or turning the bottle.
- The end product is a thick, creamy , frothy drink with a sour taste and smell and the produced carbon dioxide is perceivable . If the kefir is left to ripen for more than 3 days, the milk may curdle and the drink becomes too sour.
- The temperature and the time are important as they determine the flavour. The kefir can be kept in a refrigerator or a cellar for a few days .

- The nun Storage of the Kefir Granules : If kefir production is stopped for some Jepends time , the kefir granules can be dried as follows : Put them into a sieve and rinse well with clean water until all remaining milk is removed.
- Place them wa on a clean cloth and leave to dry in a clean place , but not in the sun , until the granules have shriveled up . The granules can be kept in a closed bottle in a cool place for 12 to 18 months .
- The rinsed granules can also be kept in a freezer . Kefir granules can also be kept in a pot with water at 4 ° C , but they become inactive after 8 to 10 days.

Sr. no.	QUESTION	ANSWER
1	What is the starter culture foe acidophilus milk?	l.acidophilus
2	Kefir was discovered by……..	Nomadic tribes
3	…… is another product made from l. acidophilus	Acidophilus yeast
4	Kafir is made from which?	Yoghurt plant
5	Define kafie	Kefir is a rather foamy and effervescent fermented milk that contains about 1% Iactic acid and 0.5 1.0 % alcohol , and is popular in eastern Europe and Mongolin

PRESERVATION OF MILK:

- Milk and its products consist of numerous nutrient content, it serves as an excellent growth medium for all of the microorganisms.
- Thus, various preservation methods are used to eliminate the growth of spoilage-causing microorganisms and maintaining the nutritional properties of milk.
- Several techniques have been used to limit the growth of organisms in milk and milk products.

Thermisation

It is the most commonly used method used for milk preservation by heating the milk at a mild temperature at 57-68°C for 15-20 seconds and rapidly cooling at <6°C. This method is effective against spoilage-causing bacteria however it doesn't eliminate the pathogens such as *L. monocytogenes*. The main objective of thermisation is to reduce the growth of psychrotrophic bacteria and extend the shelf-life of milk.

Pasteurization

Pasteurization is a method of food preservation that involves the application of heat, usually below 100° at a certain time. It aims to reduce the number of viable pathogens and spoilage-causing microorganisms (e.g., *Coxiella burnettii, Brucella abortis, Mycobacterium tuberculosis)* to extend the shelf-life of milk without harming the milk quality. In milk, thermoduric species such as *Micrococcus spp., Enterococcus faecium* and *Enterococcus faecalis, Bacillus subtilis, Bacillus cereus*, and *certain lactobacilli*. are killed by the process of pasteurization.

Four common types of milk pasteurization vary with temperature and time the milk is held at that temperature.

- **Vat Pasteurization/Low temperature**, long-time pasteurization in which the milk is heated at 63°C for 30 min.
- **High Temperature/Short Time (HTST)** in which, the milk is heated at 72°C for 15 s.
- **Ultra-pasteurization (UP)** in which the milk is heated at 138°to 150° C for one or two seconds
- **Ultra-High-Temperature (UHT)** in which the milk is heated at 280° F for only two seconds.

The objective of pasteurization are:

- To destroy pathogenic organisms, present in milk.
- To ensure the quality of milk and milk products.
- To destroy the unwanted organisms, present in milk and milk products.

Ultra-high temperature or sterilization:

Sterilization is a method of food preservation that involves the application of heat, usually

more than 100° at a certain time to kill almost all bacteria followed by packaging in air-tight containers either before or after heat treatment. The sterilized milk can be stored at room temperature for a longer period.

There are two methods of sterilization.

- **Conventional method / In-bottle sterilization** method in which the product is packed before heat treatment and the packed product is heated at 105-110°C for 30-45 min.
- **UHT method / Aseptic method** in which the product is heated at 135-150°C for 1-20 seconds followed by instant aseptic filling into sterile containers.

The objective of sterilization are:

- To ensure the quality of milk and milk products at room temperature without refrigerated storage making it safe for human consumption.
- To ensure the destruction of microorganisms (pathogenic and spoilage-causing microorganisms, vegetative and spore forms, viruses).

Dehydration

It is defined as the process of removal of water normally present in milk by the application of heat under controlled conditions by evaporation. In this method, the water activity of milk is reduced to prevent the growth of spoilage- causing microorganisms.
The objective of this methods are:

- To reduce the growth of spoilage-causing and pathogenic organisms and extend the shelf-life of milk.
- To reduce the volume and weight of milk without compromising the nutritive value.

The methods of dehydration used in milk preservation are:

- **Spray-drying**: in this process, the pre concentrated liquid food is dispersed into a stem of hot gas that results in evaporation of water content resulting in instantaneous drying.
- **Drum drying**: In this process, the pre-concentrated product is applied as a thin film on the outer surface of an internally heated rotating metal drum.
- **Fluid bed drying** processing involves drying, cooling, agglomeration, granulation, and coating of particulate materials. The gas (usually air) is passed through a product layer under controlled velocity conditions to create a fluidized state.

Low-temperature treatment

- The foods are stored at temperatures 0–5 °C.
- It causes minimal changes to the nutritional properties of food.
- The main objective of chilling is to reduce the rate of microbial growth and its enzymatic activities which extends the shelf life of milk and milk products.
- It has been used in combination with other methods of food preservations such as irradiation, pasteurization to extend the shelf life of milk and milk products.
- Milk, cheese, yogurt, butter, etc. are stored in chilled storage.

Sr. no.	QUESTION	ANSWER
1	In thermistation milk is presereved at which temperature?	57-68˚c

2	What is objective of thermisation?	to reduce the growth of psychrotrophic bacteria and extend the shelf-life of milk.
3	Full form of HTST	High temperature/Short time
4	Give name of method used in dehydration	Spray-drying, drum drying, fluid bed drying
5	In low temperature food are stored at …….˚C	0-5

OTHER METHODS OF TREATING MILK

1. Microfiltration

- It is used to remove significant numbers of bacteria from milk and extend its shelf life.
- It can be used in a combination with HTST pasteurization.

2. Bactofugation

- It is a centrifugation process that removes the bacteria present in milk.
- It has been used in the cheese industry to minimize the milk spoilage by clostridia that cause 'late blowing'.

3. Ohmic heating

- Ohmic heating is the process of heating the product by passing it through an alternating electric current.
- It is a direct heating method in which the food is in direct contact with the electrode and food itself is a part of an electric circuit.

4. Microwave heating

- Microwave heating is a method in which electromagnetic waves are used to generate heat in food.
- Microwaves used in the food industry for heating are of frequencies 2450 or 900 MHz.
- It has been in commercial practice for milk pasteurization for quite a long time.

5. Pulse Electric Field

- The pulsed electric field is one of the non-thermal food preservation technologies in which food is subjected to short pulses (1-100 µs) of high electric fields with a duration of nano to milliseconds and intensity of 10 – 80 kV/cm to foods placed between two electrodes.
- It has a lethal effect on vegetative bacteria, mold, and yeasts.

6. High-pressure process (HPP)

- It is a non – thermal pasteurization process in which food is subjected to high pressure in the region of 3300 - 600 Mpa for about 10 minutes.
- The components of microorganisms are destroyed by high pressure that can inactivate pathogenic and saprophytic microorganisms.

7. Ultrasound

- Ultrasonic is a high-power sound wave at frequencies between 16 kHz and 100 MHz.
- In this method, the sonic wave is passed through the milk and the changes occur in the pressure which leads to cavitation, which causes gas bubbles in the liquid causing a bactericidal effect.
- An ultrasound application has been conducted on the *B. subtilis* spores in milk at a temperature range of 70- 95 °C.

8. UV Radiation and Irradiation

- Ultraviolet (UV) radiation is electromagnetic radiation having a wavelength of about 10-400 nm.
- Gamma rays, X-rays, and accelerated electron beams are the sources of ionizing radiation used for the preservation of foods.
- It is applied in combination with the pasteurization of brine during cheese production.
- Irradiation can be used in dairy products to destroy pathogens or all microorganisms.

Sr. no.	QUESTION	ANSWER
1	What is bactofugation?	It is centrifugation process that removes the bacteria present in milk
2	Frequencies of microwave heating are……..	2450 or 900 MHz
3	Full form of HPP	High pressure process
4	What is frequency of ultra sound?	Between 16kHz and 100 MHz
5	Use of microfiltration	To remove significant numbers of bacteria from mik.

USE OF PRESERVATIVE AGENTS

Preservatives are substances that are capable of inhibiting or retarding the growth of microorganisms. Such preservatives used in food can be divided into three types:

1. **Natural preservatives**
2. **Bio preservatives**
3. **Chemical preservatives**

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Preservatives that are used in milk and its products are:

Product	Types of preservatives	Preservatives used
Milk	Natural preservatives	Honey, lecithin
	Bio preservatives	LAB, bacteriocin, hydrogen peroxide
	Chemical preservatives	Benzoic acid, Sorbic acid, nisin, sodium diacetate, boric acid, formaldehyde,
Cheese	Natural preservatives	Salt, essential oils (Thyme, Ginger, Cayenne, Clove, Cinnamon, Garlic, Lemongrass, Oregano, Basil), Lime juice
	Chemical preservatives	Sorbic acid, Potassium sorbate, propionic acid, Natamycin
	Bio preservatives	Lysozyme, Nisin, LAB,
Ice cream	Chemical preservatives	Butyraldehyde, Diethyl glycol, Polysorbate 80, Potassium sorbate,
	Natural preservatives	Amyl acetate(banana oil), Piperonal (vanilla bean), corn starch, Soy lecithin,
Butter	Natural preservatives	Salt, thymine, cumin
	Chemical preservatives	BHA(butylated hydroxyl anisole, BHT(butylated hydroxyl toluene), rosmaricin acid, gallic acid
Yogurt	Chemical preservatives	Sodium benzoate, Potassium sorbate, and Natamycin.

HOMOGENIZATION OF MILK

Homogenization of milk Homogenized milk is that which has been treated in such a manner as to ensure break up of the fat globules to such an extent that after 48 hours of storage, no visible cream separation occurs on the milk in a quart bottle, or, proportionate volumes in containers of other sizes does not differ by more than 10% from the fat percentage of the remaining milk as determined after through mixing. To achieve this, we should have the fat globules in small and uniform sizes. The process of breaking up the fat globules to very small sizes in order to prevent cream formation is known as homogenization. The equipment used for the same is known as homogeniser. The fat globules present in normal milk vary from 0.1 to 3 or 4 microns depending upon the breed of cows and various other factors. By homogenization, we break up the fat globules to below 2 micron sizes.

Homogenization of milk also serves the following purposes.

- Prevents cream formation.
- Increases milk viscosity, it gives richer appearance to tea or coffee.

• Fat globules do not rise readily and there is no necessity for agitating the milk before serving. • Prevents churning of fat during rough handling or excessive agitation.

• Reduces curd tension, i.e. forms a soft curd when homogenized milk is coagulated, i.e. milk becomes more palatable due to brighter appearance, heavier body and richer flavor.

• Milk becomes more digestible partly because of the smaller fat globules and partly because of the lower curd tension. The homogenized milk can be recommended for infants.

• Reduces the chances of separation of fat during the manufacture of evaporated milk and ice-cream, it gives a smoother texture of the product.

• Homogenizer can be used to prepare reconstituted milk by mixing butter oil or butter with skim milk.

• The milk becomes less susceptible to oxidized flavor development. 2 Dairy and Food Engineering-Prof. S K Dash However, if we are interested in recovery of fat, then homogenized milk should not be taken. Fat recovery from homogenized milk is difficult. Four different forms of fat globules in milk

• Single globules unattached.

• Clusters, consisting of two or more globules loosely attached.

• Clumps, consisting of two or more globules tightly clumped together so that the individual appearance of the globules is almost lost.

• Churned or butter particles in which the individual globules have lost their identity. A homogenizer should break up all the clusters, clumps and butter particles present in unhomogenized milk.

Sr. no.	QUESTION	ANSWER
1	How many types of preservatives are there?	3
2	Which natural preservative is used in milk?	Honey and lecithin
3	Give name of natural preservatives for ice-crème	Salt, thymin, cumin
4	Give name of bio preservative used for cheese	Lysozyme, nisin, LAB
5	What is use of homogenizer?	to prepare reconstituted milk by mixing butter oil or butter with skim milk.

THE HOMOGENIZER

- The homogeniser consists of a high pressure pump fitted with a minute orifice having an adjustable opening through which fluids are forced at a very high pressure.
- Thus the fat globules are reduced in size. Working principle of a homogenizer In actual practice

most valves employ a combination of three principles. The homogenization can occur due to shear or due to disruption. The shearing action occurs among the fact globules and in the narrow opening through which the milk is forced to pass.

- The disruption occurs at the breaker ring and also due to sudden pressure drop as the fluid leaves the valve.
- Proper dispersion is equally important.
- Some homogenizers may give excellent break up, but the dispersion is very poor, resulting in excessive clustering and clumping and even churning.
- This cause the development of excessive viscosity, which sometimes makes the product very difficult to pump or cool. In addition to other factors, the size and shape of the orifices are also affected by the volume of milk to be handled per unit time and by the viscosity of product.
- the main components of a homogenizer are: pump, homogenizing valve, breaker ring, tension spring and the valve sheet.
- Temperature during homogenization the following points are important in this context. • The melting point of fat is 33°C. • For inactivation of lipase, we require 55°C.

Sr. no.	QUESTION	ANSWER
1	How homogenizer made up from?	It consists of a high pressure pump fitted with a minute orifice having an adjustable opening through which fluids are forced at a very high pressure.
2	How homogenizer is occurred?	It occurred due to shear or due to disruption.
3	Give name of the main components of homogenizer	Pump, homogenizing valve, breaker ring, tension spring and the valve sheet.
4	The melting point of fat is …….	33˚C
5	Temperature for inactivation of lipase is…….	55˚C