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Class- Milk Hygiene/Fifth class

Microbiological spoilage of milk and milk products

It is primarily caused by psychrotrophic bacteria (e.g., *Pseudomonas*) breaking down proteins and fats, resulting in souring, curdling, off-flavors, and discoloration. Common spoilage includes souring from lactic acid bacteria, bitterness from heat-resistant enzymes, and gas production (curd explosion) from coliforms, often occurring due to improper cooling or contaminated equipment.

Microorganisms and spoilage mechanisms:

- Psychrotrophic Bacteria: *Pseudomonas* spp. account for 65–70% of psychrotrophic microbes in raw milk, causing fruity, bitter, or rancid flavors.
- Acidification/Souring: *Lactobacillus* spp. and *Streptococcus* spp. are major contributors to sour milk and curd formation.
- Proteolysis & Lipolysis: *Bacillus* spp. and *Pseudomonas* produce extracellular enzymes that degrade milk proteins and lipids, causing putrid odors and rancidity.
- Gas Production: *Clostridium* spp. and coliforms cause gas, leading to "blown" or bubbly textures in fermented products.



Factors affecting spoilage:

- Temperature: Improper refrigeration allows psychrotrophs to multiply.
- Raw Milk Contamination: Improper sanitation of milking equipment and udder surfaces.
- Post-Pasteurization Contamination: packaging or handling errors allowing, for instance, heat-resistant bacteria to survive.

Common defects in dairy products:

- Milk: Sour smell, coagulation (curdling), and sweet curdling (by *Bacillus*).
- Butter: Rancidity caused by lipolytic bacteria (*Pseudomonas*).
- Cheese: Surface mold growth, surface softening, and gas production.
- Cream: Yeasty or fermented flavors.

Prevention Methods:

- Temperature Control: Rapid cooling to below 4°C to inhibit bacteria.
- Pasteurization: Eliminates vegetative cells, though some heat-resistant (thermoduric) microbes may remain.
- Hygiene: Thorough cleaning of equipment and udders.

Mechanism of microbial spoilage or changes

This can be occurred through fermentation of one or more of the milk constituents by the causative microorganisms. The changes like gassiness, ropiness, proteolysis, sweet curdle, and lipolysis are abnormal fermentations. Presence of mixed microflora in dairy products called associative of action. The associative actions of microorganisms in milk include **synergism, metabiosis, and antibiosis**, which refers to the different ways bacteria, yeasts, and moulds interact with each other in the dairy environment, ultimately determining the rate of spoilage or the success of fermentation.

1. Synergism

Synergism occurs when two or more microorganisms growing together produce changes that neither could achieve alone, or when they enhance each other's growth.

- **Example in Milk:** The production of a "bright blue color" in milk caused by the combination of *Pseudomonas syncyanea* and *Streptococcus lactis*. *P. syncyanea* alone only produces a light brown tint, but in association with *S. lactis*, the pH change allows the intense blue pigment to develop.
- **Fermentation:** In yogurt production, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* demonstrate synergism, where one produces amino acids that stimulate the other, resulting in faster acid production.

2. Metabiosis

Metabiosis is a form of interaction where one microorganism creates favorable conditions for the growth of a second, succeeding organism.

- **Example in Milk:** Natural milk souring follows a metabiotic sequence. Initial microbes lower the pH, which kills off competitors but allows acid-tolerant lactic acid bacteria to flourish. These bacteria then produce lactic acid, which eventually inhibits their own growth, allowing yeasts or molds to grow, which in turn use up the lactic acid and raise the pH.
- **Spoilage:** Early bacteria may degrade complex proteins or fats into simpler substances, which are then used by secondary bacteria to cause further spoilage.

3. Antibiosis (Competitive Inhibition)

Antibiosis involves the production of inhibitory substances by one microorganism that suppress or destroy other organisms.

- **Example in Milk:** Lactic acid bacteria (LAB) are well-known for their antibiosis effect against spoilage microorganisms and pathogens.

- **Mechanism:** Lactic acid bacteria produce organic acids (like lactic acid), hydrogen peroxide, and bacteriocins, which lower the pH and create an environment unsuitable for harmful bacteria like *E. coli* or *Salmonella*.

Milk fermentations may be of normal type (curdling of milk) or abnormal type (gassiness, proteolysis, lipolysis, sweet curdling). When two or more fermentation occurs simultaneously in product, it is called mixed fermentations (production of both acid and gas by coliforms).

“Lactose fermentation is a microbial metabolic process where microorganisms (e.g., *E. coli*, lactic acid bacteria) use the enzyme β -galactosidase to break down lactose into glucose and galactose, producing acids (like lactic acid) and sometimes gas. It is essential for food production (yogurt, cheese) and crucial in clinical microbiology for identifying bacteria, specifically differentiating Enterobacteriaceae on media like MacConkey agar”.

However, fermentation of milk may be normal or abnormal:

A-Natural souring

Fresh milk has normal acidity ranging from 0.14-0.19%. The acidity at which milk sours is usually in the range of 0.20-0.25%, the milk usually curdles when acidity further reaches to 0.50-0.65%. Generally, acidity continues to increase even after coagulation of casein till the LAB (lactic Acid Bacteria) is inhibited by accumulation of acid or whole of lactose is exhausted.



Mechanism of acid coagulation:

Interaction of lactic acid produced with calcium bound to casein thus leading to precipitation of casein which appears as curd. Casein starts precipitating in the pH range of 4.64- 4.78.

Causative organisms: *Lactic streptococci*, *Lactobacillus*.

B-Sweet curdling

1. Curdling without pronounced acid (little or no acid) production is sweet curdling. This defect is due to the production of an extracellular enzyme similar to rennin by bacteria that precipitate casein before the development of sufficient acidity.
2. Observed in milk and cream particularly that are moderately heat treated particularly when held at higher temperatures during summer months.
3. Rennin like is produced generally by aerobic spore formers (*Bacillus*). Activity of this enzyme is known to increase at higher temperature.
4. Boiled milk if allowed to cool and stand in same container for long time, it will undergo sweet curdling.



Causative microorganisms: Cocci - *Streptococcus liquefaciens*; Aerobic sporeformers – *B. cereus*; Psychrotrophic spore formers- *B. cereus*, *B. licheniformis*; Non-spore forming rods: *Proteus* spp., Yeasts and molds

Significance: Sweet curdling is prevalent in heat treated products, particularly in summer.

Factors affecting sweet curdling

1. High temperature: More prevalent in summer
2. Age of milk: Milk held at ambient temperature for more than 24 h due to production of more rennin by *Bacillus mycoides*. It does not grow rapidly, as it may be inhibited by lactic acid bacteria.
2. Pasteurization: It kills most of the bacteria, especially lactic acid bacteria.

C-Proteolysis

1. Casein or some insoluble casein derivatives are broken down to water soluble compounds through action of microorganisms/enzymes.
2. Milk contains different proteolytic enzymes that may bring about alterations of milk constituents. Some of these are naturally present in milk, while others are elaborated by microorganisms.
3. Changes in milk proteins, as a result of psychrotrophic growth or enzymatic action, are important in keeping quality of milk and milk products.
4. Heat labile proteases that attack caseins and whey proteins leads to coagulation of milk and development of bitter taste.

Causative organisms: Psychrotrophs are active proteolytic and grow at 7°C or less (*Pseudomonas fluorescens*, *Acinetobacter* etc.).

Significance of Proteolysis:

1. With the extended period of refrigerated storage, psychrotrophs become important to the quality of fluid milk.
2. Psychrotrophic proteinases act during the refrigerated handling of raw milk before heat treatment.

D-Lipolysis:

1. Hydrolysis of milk fat and subsequent production of off- flavours in dairy products.
2. Lipolysis is carried out by lipase resulting into accumulation of free fatty acids. Lower chain fatty acids (butyric and caproic) are responsible for lipolytic off- flavours.
3. Lipolysis in milk at any stage from milking, storing and processing should be avoided, since rancid milk/cream will not yield high flavour quality products.

4. Lipolytic microorganisms and their enzymes produced in bulk cooled milk/cream survives HTST pasteurization and UHT sterilization, causing lipolysis during subsequent storage of heat processed milk at low temperature.

Causes of lipolysis:

1. Intrinsic milk lipase: Present in sufficient quantities to cause hydrolysis of milk fat. However, fat globule membrane protects milk triglycerides from attack by lipase and hence, little or no lipolysis occurs.
2. Lipolytic microbes or enzymes: Psychrotrophs (*Pseudomonas fragi*), Other types (*B. subtilis*), Yeast and molds (*Geotrichum candidum*)

Precaution and control

1. Clean milk production practices
2. Cooling of milk as per recommendation

E-Bitty cream (Broken cream):

1. Bitty cream is due to effect of spore formers on milk.
2. Occurs in pasteurized and raw cream.
3. Characterized by appearance of flakes in the cream layer that do not mix even when milk is shaken. If such milk is used in tea, flakes float on surface making it unacceptable.
4. Flakes are of two types: Mechanical/physical origin and their flakes occur when fat globule membrane is partly disrupted and globule stick together occurring as cream plug. Second type is of bacterial origin and is produced partly by lecithinase enzyme of *B. cereus* var *mycoides* that attacks phospholipid part of fat globule membrane and partly from the coagulation of casein associated with membrane.

Reasons: failure of refrigeration, seasonal variation, prolonged storage etc.

Abnormal colours (discolouration): Colour of milk is largely controlled by feed but the milk of certain breeds is always more coloured due to the fact that milk fat contains more carotene and less vitamin A.

Colours	Organism responsible
Blue	<i>Pseudomonas syncyanea</i>
Yellow	<i>Pseudomonas synxantha</i>
Red	<i>Serratia marcescens</i>
Greenish	<i>Pseudomonas fluorescens</i>

Abnormal flavours (off-flavours):

- 1- Fruity Flavour: It is due to ethyl ester formation usually catalysed by esterase produced by psychrotrophs or lactic acid bacteria.
- 2- Malty flavour: Caused by malty strains of *Lactococcus lactis* ssp. *lactis* var *maltigenes*.
- 3- Bitter flavour: Caused by proteolytic microorganisms especially *Pseudomonas* spp
- 4- Fishy flavour: Caused by *Pseudomonas ichthyosmius*, due to conversion of lecithin to trimethylamine.
- 5- Musty potato flavour: Caused by *Pseudomonas mucidolens*.
- 6- Phenolic flavour: Caused by *Bacillus circulans*. Cresols are responsible for this defect.
- 7- Unclean flavour: Increase in the production of dimethyl sulphide caused by rod shaped Gram-negative psychrotrophic.
- 8- Medicinal flavour: Caused by *Aerobacter aerogenes* in pasteurized market milk.

