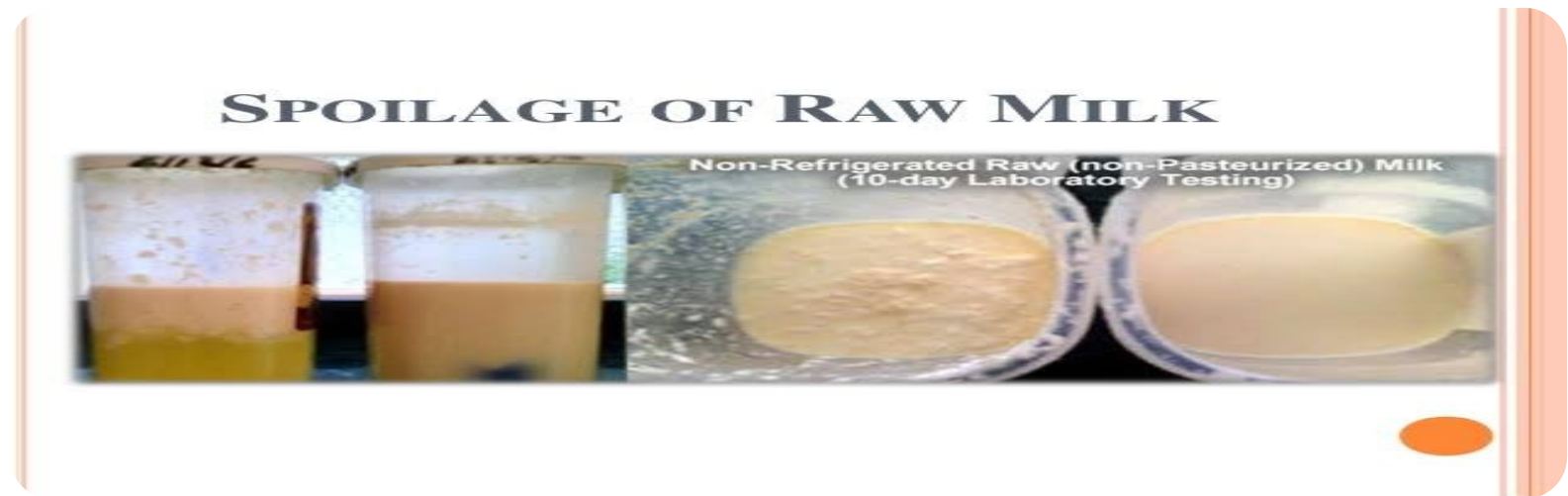




Types of Microbial Spoilage

The microbiological changes in milk and milk products are produced as a result of fermentation of one or more of the milk constituents by the causative microorganisms. These milk fermentations may be of **normal type (curdling of milk)** or **abnormal type (gassiness, ropiness, proteolysis, lipolysis, sweet curdling)**. When two or more fermentation occurs simultaneously in the product, it is called **mixed fermentations (production of both acid and gas by coliforms)**.



Natural souring:

1. Holding raw milk for sometime in ambient condition, the immediate effect is souring followed by curdling.
2. 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 is inhibited by accumulation of acid or whole of the lactose is exhausted.
3. The mechanism of acid coagulation involves interaction of lactic acid produced with the calcium bound to casein thus leading to precipitation of casein which appears as curd. Casein starts precipitating in the pH range of 4.64 to 4.78.
4. Causative organisms: Lactic streptococci, *Lactobacillus*, *Leuconostocs*, *Bacillus coagulans* (lactic acid producing spore former)

The coagulum formed by **lactic streptococci, lactobacilli** and ***S. thermophilus*** is smooth with typical clean sour flavour (preferred for desirable fermentations in dairy industry). Organisms like ***B. coagulans, coliforms*** produce a coagulum along with other undesirable flavours due to the liberation of certain volatile flavouring substances from lactose, proteins and milk



Significance

- The increased acidity is unfit for consumption, so raw milk giving positive clot-on-boiling test is rejected at platform.
- The producers mask the developed acidity by adding caustic soda which is undesirable from public health point of view.

Control measures

- Immediate chilling of raw milk
- Adequate pasteurization of milk followed by cooling
- Milk production should be practiced under hygienic conditions
- Sanitized utensils to be used

Gassiness:

- 1. Production of carbon dioxide by certain microorganisms is responsible for this defect.**
- 2. Gassiness in some cases is associated with acid production in high fat milk where there will be foaming as the gas escapes the partially coagulated mass and the defect is known as 'frothiness'.**
- 3. Frothiness is due to the associative action of acid producing bacteria and gas producing yeasts.**
- 4. The production of gas can lead to blowing of cans.**
- 5. Causative organisms: Lactose fermenting yeasts, Coliforms, *Clostridium butyricum***

Ropiness/sliminess:

- 1. Ropy fermentation is brought about by the growth of bacteria leading to change in consistency of the product that forms threads of viscous masses, when poured.**
- 2. Ropiness develops only on storage and milk is drawn out as fine threads and may appear gel like consistency. Sometimes the change is so prominent that the milk can be drawn into a long thread.**
- 3. Ropiness has particularly been observed in milk held at low temperature as many causative microbes show active growth in milk at low temperature. At higher temperatures these ropy bacteria are overgrown by LAB.**
- 4. Ropiness is developed before detectable acid development and it decreases as the acidity increases.**

Causative organisms: Gram-negative rods (*Alcaligenes viscolactis*, *Achromobacter lacticum*); Micrococci; Coli-aerogenes group (Consists of ropy strains belonging to *Enterobacter*, *Citrobacter* and related genera: *Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*); Aerobic spore formers (*Bacillus cereus*, *B. subtilis*, *B. circulans*); LAB (develop during successive period of propagation :*Lactococcus lactis* ssp. *lactis* biovar *hollandicus*, *Lactobacillus casei*, *L. bulgaricus*).

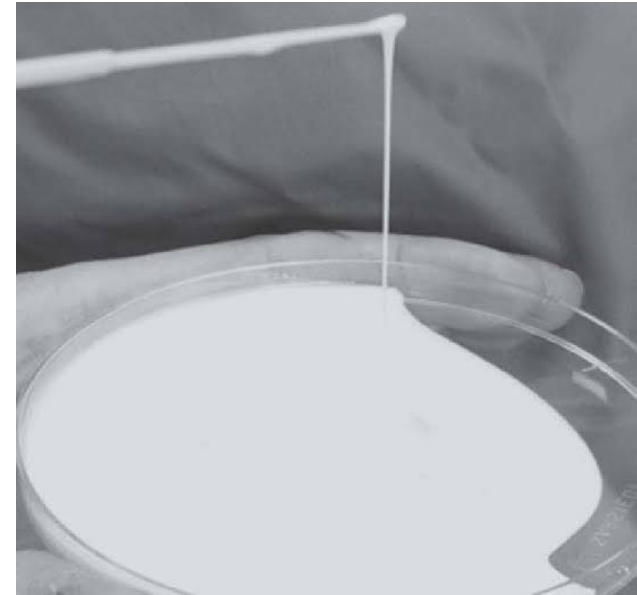
Source: Bacteria causing ropiness are generally not present in the aseptically drawn milk, but come from water, dairy equipment, dust laden air, coat of cows, cow feed etc and enter the milk drawn.

Materials that are capable of producing ropiness are:

True gums or gum like substances: polysaccharides. Gums are derived from lactose that is fermented by Coliforms, *L. lactis* ssp. *lactis* biovar *hollandicus*, Streptococci, *Leuconostoc*, Lactobacilli.

Nitrogenous mucus like substances: Mucins are combination of proteins with a carbohydrate radical may be produced by peptonizing bacteria (Micrococci, Gram-negative bacteria and aerobic spore formers)

Exopolysaccharides: Produced as capsules associated with cell or as slime unattached to the cell. *Alcaligenes viscosus* produces capsular material.



Significance of ropiness: Ropiness causing bacteria are not harmful to consumers. However, this defect affects the acceptability of products that occurs less frequently in pasteurized milk. Ropiness is particularly observed in milk held at low temperature. At higher temperature, lactic acid bacteria dominate ropy bacteria. Ropiness is observed higher in summer, more prevalent in spring and autumn.

Prevention and control

- a. Clean milk production and effective heat treatment.
- b. Post-pasteurization contamination should be avoided.
- c. Stables should be kept cleaned and disinfected.
- d. Supply of clean water should be accomplished.
- e. After washing hands prior to milking, workers should not handle straw or fodder.

Sweet curdling:

- 1. Curdling without pronounced 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. This defect is commonly 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. The activity of this enzyme is known to increase at higher temperature.**
- 4. Little or no acid production is observed during sweet curdling.**
- 5. Under Indian conditions, boiled milk if allowed to cool and stand in same container for long time the product will undergo sweet curdling. This defect sometimes, becomes apparent on re-heating the product and also occur in pasteurized milk, if stored for longer times under refrigerated conditions.**
- 6. Under farm conditions, milk contains microbes but the defect does not appear, as the commonly encountered LAB curdle the product by acid production.**

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

Significance: Sweet curdling is prevalent in heat treated products, particularly in summer. However, it is also observed in refrigerated and boiled milk stored for longer durations.



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 mycooides*. It does not grow rapidly, as it may be inhibited by lactic acid bacteria.
3. **Pasteurization:** It kills most of the bacteria, especially lactic acid bacteria. The flora is dominated by heat resistant bacteria which are of proteolytic type and attack proteins causing sweet curdling.

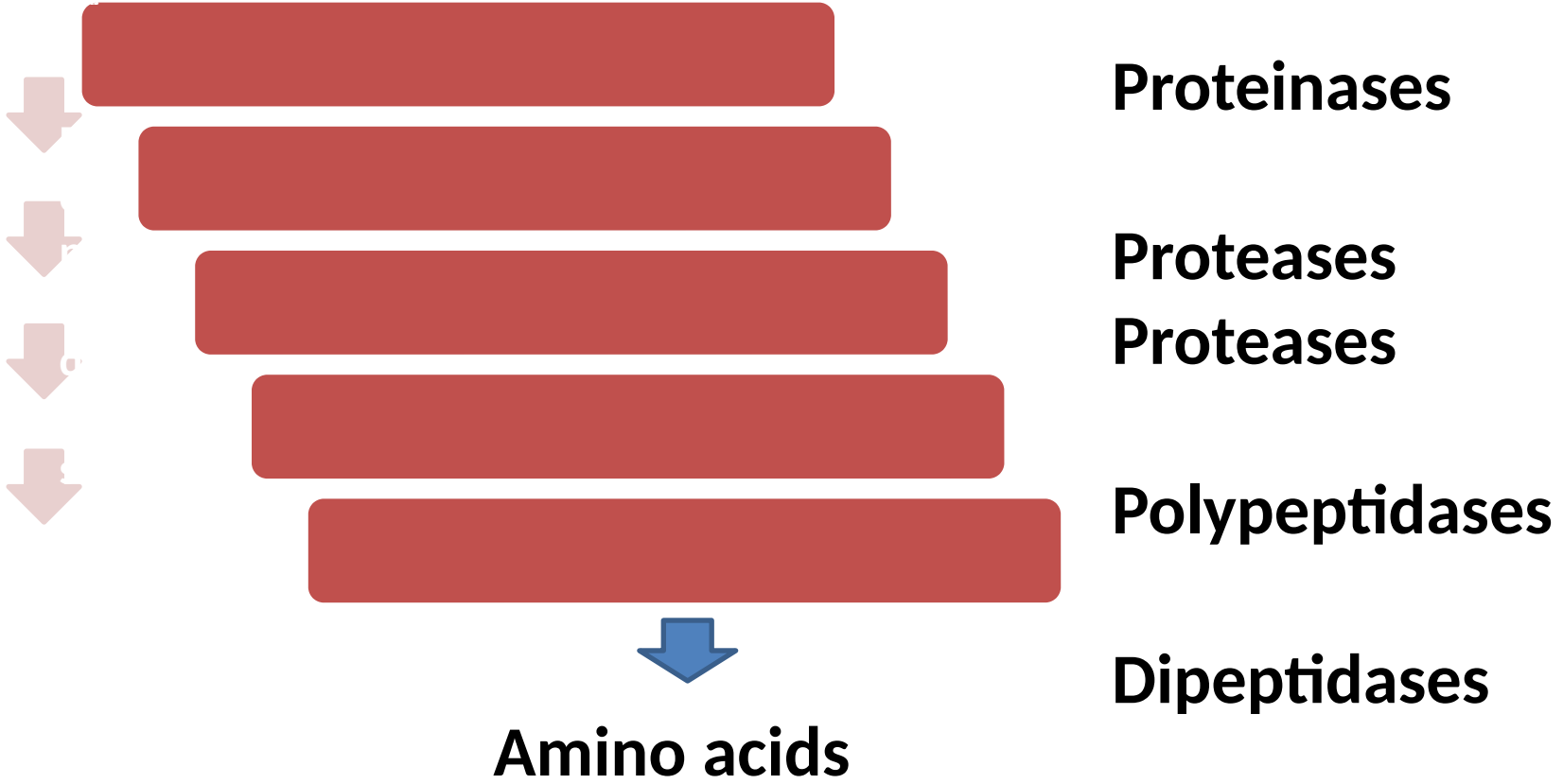
Precaution and Control

- **Avoid contamination**
- **Control storage temperature (milk cooled at farm level)**
- **Pasteurization at a temperature higher than normal**

Proteolysis:

- 1. It is a process in which casein or some insoluble casein derivatives are broken down to water soluble compounds through the action of microorganisms or 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. Since, milk contains only small amounts of non-protein nitrogenous substances to sustain prolonged growth, microorganisms depend on enzymes that hydrolyze milk proteins.**
- 3. Changes in milk proteins, as a result of psychrotrophs growth or enzymatic action, are important in keeping quality of milk and milk products.**
- 4. Raw milk supplies contain heat stable proteases that can attack caseins and whey proteins leading to coagulation of milk and development of bitter taste.**

- 5. Proteolytic flavours are attributed to the breakdown of proteins and amino acids and thus proteolysis is accompanied by development of bitterness due to the presence of peptides that are bitter in taste.
- 6. Psychrotrophic proteinases primarily act during refrigerated storage of raw milk before heat treatment.



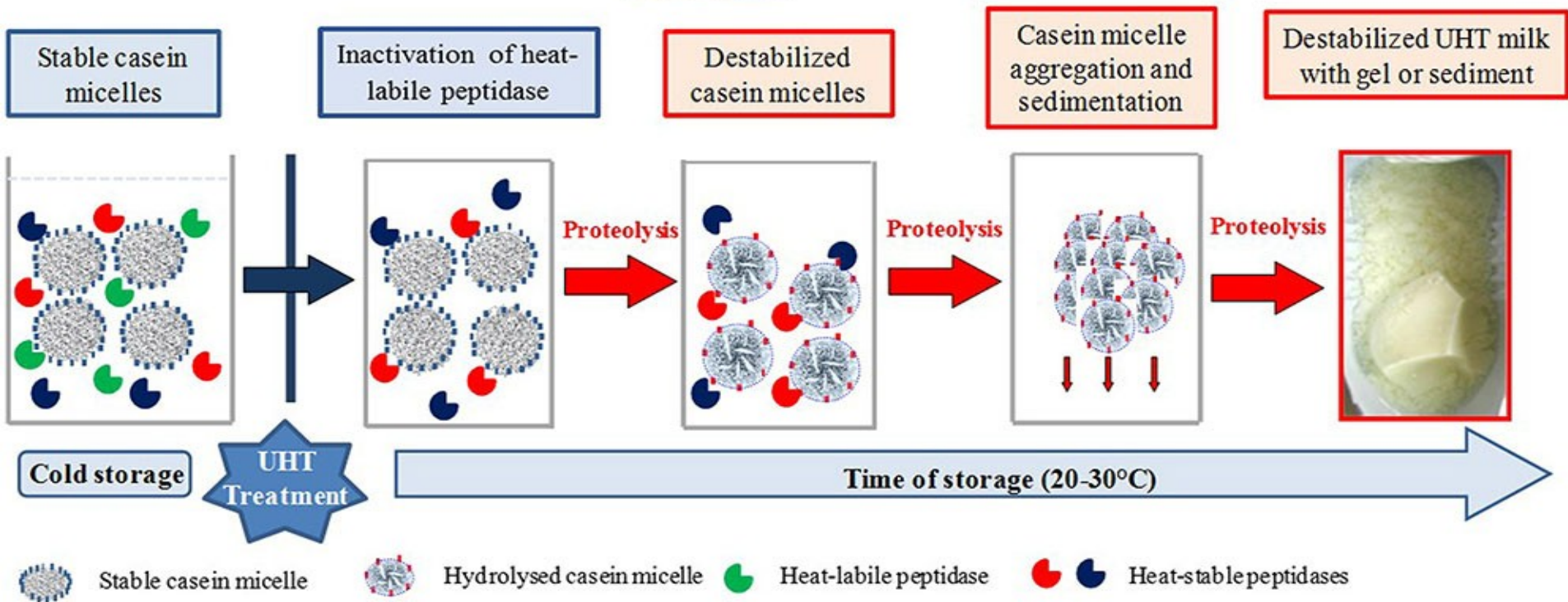
Causative organisms:

Psychrotrophs are active proteolytic and grow at 7°C or less, especially *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Achromobacter*, *Acinetobacter*, *Aeromonas* and *Enterobacter liquefaciens*. Some thermotolerant bacteria includes *B. subtilis*, *B. cereus* etc.

Significance of Proteolysis:

1. With the extended period of refrigerated storage, psychrotrophs become important to the quality of fluid milk.
2. The proteolytic action of psychrotrophs in raw milk is important to the sterilization of milk at ultra high temperature (UHT).
3. Psychrotrophic proteinases act during the refrigerated handling of raw milk before heat treatment.
4. UHT sterilized milk coagulated in the presence of heat resistant *Pseudomonas* protease and ensuing κ -casein degradation can be detected by the release of sialic acid into the milk during storage.
5. Proteolysis may also predispose the caseins and whey proteins to damage by heat treatment.

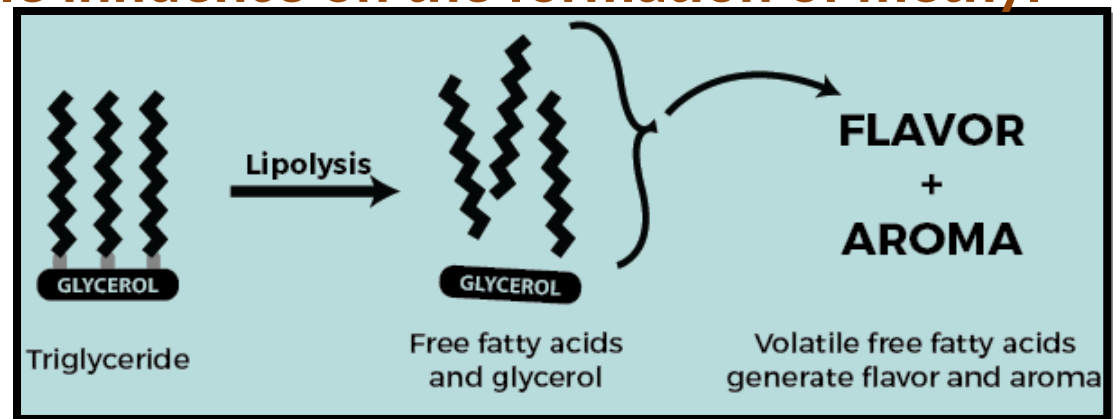
UHT milk



Lipolysis:

- 1. Hydrolysis of milk fat and subsequent production of off-flavours in dairy products.**
- 2. Lipolysis is carried out by lipase resulting into the accumulation of free fatty acids. The lower chain fatty acids particularly butyric and caproic are responsible for lipolytic off-flavours.**
- 3. The onset of lipolysis in milk at any stage from milking, storing and processing should be avoided, since rancid milk or cream will not yield products of high flavour quality.**
- 4. The intrinsic milk lipase is present in sufficient quantities to cause hydrolysis of milk fat. However, fat globule membrane protects the milk triglycerides from attack by lipolytic enzymes, whose activity is further impaired by their association with casein and possibly by the presence of lipolytic inhibitors.**

5. Lipolytic microorganisms and their enzymes produced in bulk cooled milk/ cream by psychrotrophs that survives HTST pasteurization and UHT sterilization, causes lipolysis during subsequent storage of heat processed milk even at low temperature.
6. Lipolysis is favorable for flavor of cheese. The hydrolysis of cheese constituents is essential for the development of mold ripened cheese. Not only the lipolytic activity of molds but also further metabolism of fatty acids especially their oxidation to methyl ketones.
7. Addition of lipase of *Candida lipolytica* improve the quality of blue veined cheese made from raw or pasteurized milk and yeasts of *Torulopsis* had a desirable influence on the formation of methyl ketones.



Causes of lipolysis:

1. **Intrinsic milk lipase:** Present in sufficient quantities to cause hydrolysis of milk fat. However, fat globule membrane protects the milk triglycerides from attack by lipase and hence, little or no lipolysis occurs.
2. **Lipolytic microbes or enzymes:** **Psychrotrophs** (*Pseudomonas* spp. mainly *P. fragi*, *P. fluorescens* and *Achromobacter lipolyticum*), **Other types** (*Micrococcus freudenreichii*, *B. subtilis* and *B. coagulans*), **Yeast and molds** (*Candida lipolytica*, *Geotrichum candidum*, *Penicillium* spp. and *Aspergillus* spp.)

Precaution and control

3. **Clean milk production practices**
4. **Cooling of milk as per recommendation**
5. **Processing of milk prior to microbial growth reaching log phase.**

Bitty cream:

1. Bitty cream or broken cream is a well known example of the effect of spore formers on milk. This defect occurring in pasteurized and raw cream, is characterized by the appearance of flakes in the cream layer that do not mix even when milk is shaken. If such milk is used in tea, the flakes float on the surface making it unacceptable to consumers.
2. The flakes are of two types: **Mechanical/ physical origin** and there flakes occur when fat globule membrane is partly disrupted and globule stick together, and when this condition is pronounced, it occurs as cream plug. The second type is of **bacterial origin** and is produced partly by lecithinase enzyme of *Bacillus cereus*, *B. cereus* var *mycoides* that attacks phospholipid part of fat globule membrane and partly from the coagulation of casein associated with membrane.

- 3. Bacterial flakes do not change materially, when heated to 100°C, whereas mechanical flakes change at 40–45°C.**
- 4. Bitty cream is a major spoilage problem of pasteurized milk. Reasons: failure of refrigeration, seasonal variation, prolonged storage etc.**
- 5. *Bacillus* occur naturally in soil and get into skin and hair of animals. First, all the spores present in milk collect in the cream layer where they develop into microcolonies. Flocks form around these and at the beginning of the appearance of bitty cream, each micro colony has formed one flock. Milk is not a favorable sporulation medium for many sporeformers but, if diluted 1 to 50 with water, conditions may become conducive for rapid and complete sporulation of some strains.**
- 6. Clean milk production methods is the precaution for this defect.**

Abnormal colours (discolouration):

The 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. A number of different abnormal colours are developed depending on the growth of microorganism:

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):

Microbial off-flavours are invariably encountered in raw milk through occasional neglect or failure in refrigeration. Improvement in processing operations has resulted in lower bacterial count and hence, associated off-flavours can be traced to post-pasteurization contamination with psychrotrophs.

Fruity Flavour: It is due to ethyl ester formation usually catalyzed by esterases produced by psychrotrophs or lactic acid bacteria. Ester formation by *Pseudomonas fragi* involves liberation of butyric and caproic acids from one and three positions of milk triglycerides and are esterified with ethanol. Predominating esters are Ethyl butyrate, Ethyl hexanoate.

Malty flavour: Caused by malty strains of *Lactococcus lactis* ssp. *lactis* var *maltigenes*

Bitter flavour: Caused by proteolytic microorganisms especially *Pseudomonas* spp.

Fishy flavour: Caused by *Pseudomonas ichthyosmius*, due to conversion of lecithin to trimethylamine.

Musty potato flavour: Caused by *Pseudomonas mucidolens* and *Pseudomonas graveolens*

Phenolic flavour: Caused by *Bacillus circulans*. Cresols are responsible for this defect.

Unclean flavour: Increase in the production of dimethyl sulphide caused by the contamination of milk with rod shaped Gram-negative psychrotrophic bacteria.

Medicinal flavour: Caused by *Aerobacter aerogenes* in pasteurized market milk.