FE 305FOOD MICROBIOLOGY
Microbial Spoilage in Milk and Dairy Products

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Spoilage in Milk

Raw Milk and Treated Milk

Milk contains nutrients in sufficient and balanced proportions. Milk also constitutes an ideal medium for the development of microorganisms, since it is at pH values (6.4-6.8 pH) where the majority of microorganisms can easily grow.

Raw Milk and Treated Milk Contamination Sources

The sources of contamination listed below can contaminate milk with a wide variety of microorganisms and multiply rapidly, negatively affecting the durability of dairy products.

- The main carbohydrate in milk is the lactose,
  - the microorganisms with lactose-hydrolyzing enzymes (lactase or β-galactosides) have an advantage over those unable to metabolize lactose.
- Milk fat can be hydrolyzed by microbial lipases
  - to produce small molecular volatile fatty acids (butyric, capric and caporic acids).

Sources of Contamination in Raw Milk

- **Contamination from Dairy Animal**: Microorganisms can be transmitted to milk from the skin, udder of the dairy animal or from fecal (fecal) contamination contaminated with them.
- The most common disease causing microorganisms called mastitis, which are seen in the mammary tissue of the animal, are *Streptococcus species, Staphylococci, Streptococci and Eschericia co*li.
- The milk of animals with diseases such as tuberculosis and brucellosis contain bacteria that cause these diseases. These bacteria that cause important diseases in humans are *Mybacterium tuberculosis and Brucella* species.
- **Feed, Dust-Soil and Airborne Contamination**: Most contamination of milk is by soil-based microorganisms (*Bacillus subtilis and B. mesentericus*). In addition, various bacteria that cause infection in humans can be transmitted through air and feed.

Sources of Contamination in Raw Milk

- **Contamination from Barn Material, Containers Used, Milking Machines and Other Tools-Equipment**: Failure to disinfect the tools and equipment used is an important factor that increases the risk of contamination in milk. From tools-equipment that are not cleaned adequately, milk is contaminated with rapidly growing lactic *Streptococci* and coliform bacteria, gram negative bacteria such as *Pseudomonas, Alcaligenes and Flavobacterium*.
- **Contamination from Milking Personnel**: Presence of porters (carriers of disease agents) in barn workers, especially in milking workers, causes secondary contamination in milk. Many milk-borne diseases (typhoid fever, dysentery, salmonellosis, etc.) occur with the consumption of raw milk contaminated by carriers. Especially *Staphylococci, Micrococci and Streptococci* can easily be transmitted to milk during manual milking.

Sources of Contamination in Drinking Milk

- The most important microorganisms contaminating pasteurized milk are *Pseudomonas, Alcaligenes, Aeromonas, Enterobacter, Flavobacterium and Acinetobacter* species. The presence of coliform bacteria in pasteurized milk is the most obvious evidence of contamination. In addition, the presence of *Pseudomonas and Aeromonas* is also a sign of secondary contamination. Secondary contamination in pasteurized milk; It can be realized from tanks, filling machines or packaging material.
- Some strains of *Bacillus* species can survive after UHT application in sterilized milk, especially due to insufficient application of temperature-time norms. There are two reasons for microbiological contamination in sterilized milk:
  - Insufficient temperature-time application and
  - contamination in pipes, tanks and aseptic packaging system after sterilization.
- Microorganisms in raw milk and drinking milk can cause very different changes and spoilage in these products.

Microbial Spoilage in Raw Milk and Drinking Milk

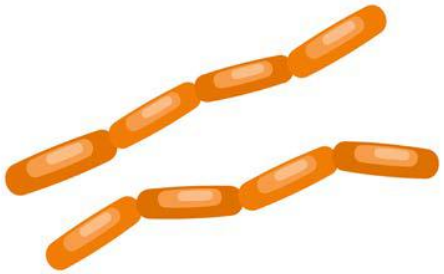
- **Sourness and Acid Formation**: Lactic acid bacteria form an important part of the microorganisms found in milk. lactose in milk; It is broken down into lactic acid and other organic acids by lactic acid bacteria and some other microorganisms. The indicator of acid formation is the sourness in taste and the formation of clot in its structure. The most important factors of this spoilage are bacteria such as *Streptococcus lactis, S. thermophilus, Lactobacillus bulgaricus, L. thermophilus, Bacillus acidolactis*. However, although not as effective as these, members of the *Clostridium, Bacillus, Micrococcus and Mycobacterium* genera also cause such spoilage.

Lactobacillus bulgaricus

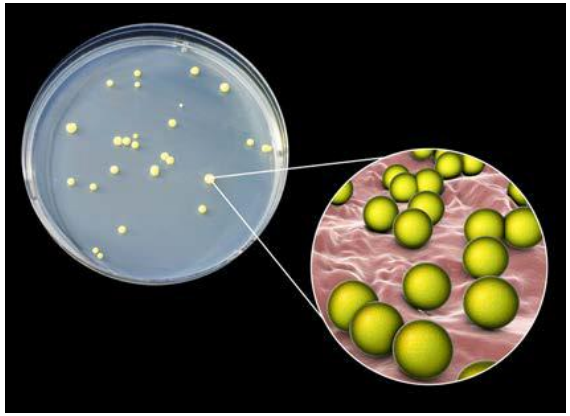
Streptococcus thermophilus

Microbial Spoilage in Raw Milk and Drinking Milk

- **Gas Formation:** Gas accumulation in milk occurs with the formation of acid. Gas formation factor in milk and various products, especially coliform group bacteria and *Clostridium* gas-forming species. *Bacillus* species and heterofermentative lactic acid bacteria cause gas formation by producing hydrogen (H_2) and carbon dioxide (CO_2).
- **Breakdown of Proteins:** As a result of proteolytic bacteria breaking down the proteins in milk (proteolysis), rancidity occurs. Bitterness is caused by certain peptides that are released as a result of proteolysis. Bacteria responsible for proteolysis are mostly capable of multiplying at low temperatures. The most important are *Alcaligenes, Bacillus, Flavobacterium, Micrococcus, Pseudomonas, Clostridium, Serratia and Proteus* species.



B*acillus cereus*



Micrococcus luteus

Microbial Spoilage in Raw Milk and Drinking Milk

- **Lipolytic Degradation**: This degradation occurs as the breakdown of milk fat into glycerine and fatty acids. Milk fat can be broken down by various bacteria, yeasts and molds. As a result of these decompositions, butyric acid odor (sour-acidic) and rancid taste (bitter) formation occur. The most important spoilage factors are *Pseudomonas, Enterobacter, Alcaligenes, Bacillus, Micrococcus, Clostridium* species. *Aspergillus, Mucor, Geotricum, Rhizopus* molds are spoilage factors in milk fat.
- **Creep Event**: Different bacteria cause the creep event in milk and cream. The spoilage factor is *Enterobacter, Alcaligenes viscolactis, Streptococc*us species and lactic acid bacteria. It is especially seen in milk stored at low temperatures. In addition, creep formation is reduced at low pH values.

Bacteria that cause changes in milk color and color changes in milk

Causing Bacteria	**Color formed in milk**
Pseudomonas aeruginosa	Yellow-Green
Pseudomonas syncyaneae	Blue
Serratia marcescens	Red
Micrococcus rubens	Red
Pseudomonas syxantha	Yellow

Microbial Spoilage in Raw Milk and Drinking Milk

- **Change in Color of Milk:** This defect in milk is caused by the growth of microorganisms in pigment or the various reactions in milk with enzymes. The factor of this deterioration is bacteria and the color change they cause is given in Table given in previous slayt.
- **Taste Changes:** Torula amora, a yeast species, causes bitter taste in milk. Streptococcus lactis var. maltigenes can cause a burnt and caramel-like taste, and some other bacteria can produce a nutty and soapy taste.
- Microorganisms that cause deterioration in pasteurized milk are *Pseudomonas, Flavobacterium, Chromobacterium, Alcaligenes* and Coliform bacteria that are transmitted to milk after pasteurization. These deteriorations are taste defects defined as nasty, putrid, unpleasant and fruity.
- The measurement of taste defects depends on the amount of microbial enzyme degradation of milk protein, fat and lactose.
- The active remaining lipolytic and proteolytic enzymes of bacteria in sterilized (UHT) milk cause coagulation with taste and aroma defects.

Types of bacterial spoilage in milk and milk products

Milk and milk products	Type of spoilage	Bacteria
Raw milk (at 10-37°C) (at 37-50°C) (>50°C)	Souring Souring Bitter and putrit Fruity flavor Malty flavor Ropiness	*Lactobacillus lactis* *Str. thermophilus* *Pseudomonas* spp. *Staphylococcus* spp. *Lac. Lactis* subsp. *lactis* var. *maltigenes*, *Lb. maloaromicus* *Alcaligenes viscolactis*

Types of bacterial spoilage in milk and milk products

Milk and milk products	Type of spoilage	Bacteria
Pasteurized milk	Bitter taste (proteolysis)	*Pseudomonas, Flavobacterium, Bacillus.*
	Souring	*Streptococcus*, *Lactobacillus.*
	Acid proteolysis	*Micrococcus, B. cereus, Lactobacillus, Clostridium.*
	Sour flavor	*B. circulans*
	Sweet proteolysis, slime	*Alcaligenes, Proteus*, *B. cereus*
		Lb. lactis, *Lac. lactis* subsp. *lactis* var. *maltigenes.*
	Malty flavor	*Lactococcus*, *Mic. freudenreichii,*
	Ropiness	*Alcaligenes viscolactis.*
	Blue color	*Pseudomonas syncyanea*, *Lb. lactis.*
	Gases	*Bacillus*, *Clostridium.*

Milk and milk products	**Type of spoilage**	**Bacteria**
Cream and soft cheese	Surface taint Bitter	*Pseudomonas putrefaciens* *B. cereus*
Hard and soft cheese	Slime and off flavor Pink discoloration Holes in curd Rancidity, soapiness	*Pseudomonas, Leuconostoc, Bacillus* spp. *Lactobacillus* and *Leuconostoc* spp. *Bacillus, Pseudomonas* and *Leuconostoc* spp. *Micrococcus, Serratia and Pseudomonas* spp.
Cottage cheese	Slime curd, putrid odor Unclean taste Discoloration	*Pseudomonas* spp. *Escherichia coli* *Flavobacterium* spp.
Concentrated milk	Acid proteolysis	*Bacillus* spp.
	Sweet coagulation	*Bacillus coagulans*, *B.stearothermophilus* *B. cereus*,
	Bitter flavor	*B. subtilis*, *B. licheniformis*, *C. botulinum*
	Swelling	*C. sporogenes*
	flat sour	*B. stearothermophilus*, *B. lieheniformis*, *B. macerans*, *B. subtilis*

Psychrotrophic spoilage

- Pasteurized milk is expected to have a shelf life of 14 to 20 days.
  - contaminated psychrotrophic microorganisms and
  - The enzymes from growth of psychrophic bacteria in raw milk
    - can spoil or cause defects in milk and milk products.

i) Psychrotrophic bacteria in milk

- Raw milk is primarily spoiled by psychrotrophic aerobic Gram-negative rods in the family *Alcaligenes*, *Flavobacterium*, *Neisseriaceae* and *Pseudomonadaceae*.
- About 65 to 70 % of psychrotrophic in raw milk are *Pseudomonas* spp.
- Other genera of psychrotrophic bacteria are *Bacillus*, *Aerococcus*, *Micrococcus*, *Staphylococcus* and the family *Enterobacteriaceae*, and
  - they usually grow together with *Pseudomonas* spp. when milk is stored at 3 to 7°C.

Psychrotrophic spoilage

- The psychrotrophic spoilage of milk is generally lipolytic,
  - with production of extracellular lipases, phospholipases and other hydrolytic enzymes.
- The bacterial species most often associating with flavor defects in refrigerated milk are *P. fluorescens*, *P. fragi, P. lundensis* and *P. putida*.
- Psychrotrophic bacteria commonly present in raw milk are sensitive to pasteurization.
- But their enzymes are resistant heat treatmen.

ii) Sources of psychrotrophic bacteria in milk

- Soil, water, animals and plant material are the natural habitat of psychrotrophic bacteria contaminating with milk.
- Plant materials, such as grass and hay used for animal feed, may contain psychrotrophs.
- Water used on the dairy farm contains psychrotrophic bacteria,
  - its use to clean and rinse milking equipment provides a direct means for their entry into milk.

Psychrotrophic spoilage

- Psychrotrophic bacteria from water are often very active producers of extracellular enzymes.
- The teat and udder area of the cow can contain high levels of psychrotrophic bacteria,
  - even after washing and sanitizing.
  - These psychrotrophs probably originate from soil.
- Milking equipment, utensils and storage tanks are another major source of psychrotrophic bacteria.
- Milk residues on unclean equipment provide a growth environment for psychrotrophic bacteria,
  - which contaminate milking machines, pipelines and holding tanks with water rinses.
- Proper cleaning and sanitizing procedures can effectively reduce contamination from these sources.

Psychrotrophic spoilage

iii) Growth and defects of psychrotrophic bacteria

- Generation times of psychrotrophic *Pseudomonas* spp. are 8 to 12 h at 3°C and 5 to 10 h at 5°C respectively.
  - These growth rates are sufficient to cause spoilage within 5 days
    - if the milk initially contains only one cell/ml.
- The growth of psychrotrophic bacteria can cause defect with the production of extracellular enzymes.
- Enzymes can cause rancid and fruity flavors in milk
  - products appear when population of psychrotrophs reaches $l0^{6-7}$ cfu/ml.
- Bitter and putrid flavors, and coagulation result from proteolysis.
- *P. fluorescens*, *P. aeruginosa* and *P. fragi* produce lipase and protease.

Prevention of Spoilage in Raw and Drinking Milk

- The milking process should be carried out under hygienic conditions.
- After milking, the milk should be cooled to <8 °C if it is to be collected daily, and to <6 °C if it is not to be collected daily.
- Tools and equipment such as containers, carriers, tankers that will come into contact with milk must be made of materials that can be cleaned properly and easily, resistant to corrosion, and will not pose a danger to human health, and will not adversely affect the physical and chemical properties of milk.
- These used materials should be cleaned and disinfected at least once a day.
- Here are some rules to be followed by people performing raw milk milking and related operations:
  - Milkers should wear suitable and clean milking clothes, wash their hands before milking and pay attention to cleanliness during milking.
  - Persons who may contaminate milk during milking should be removed from milking.
  - All persons working in milking should document that they do not have diseases that would prevent them from working in such a job, by having porter examination every 3 months and lung checks every 6 months.

Prevention of Spoilage in Raw and Drinking Milk

- The points to be considered in order to prevent the deterioration of drinking milk are as follows:
  - The pasteurization process (time-temperature) must be safely controlled.
  - All surfaces that come into contact with milk should be cleaned and disinfected before use.
  - Contamination from the operating air must be prevented.
  - Transport and storage should be done in the cold.
  - Raw cow milk to be used in the production of heat-treated drinking milk, dairy products and milk-based products must comply with the following standards in the Turkish Food Codex on Raw Milk and Heat-Treated Drinking Milk 2000/6:
  - Somatic cell count is an important criterion in raw milk as it is used to detect the presence of mammary disease (mastitis) of the animal.
    - Somatic cell count (per ml) should be ≤ 500,000.
    - If the somatic cell count is > 1,000,000, it means sick breast, and 250,000 – 500,000 means normal breast.

Prevention of Spoilage in Raw and Drinking Milk

- In the controls made by random sampling of raw cow's milk;
- The total number of viable bacteria should be ≤ 100,000 at 30 °C (per ml),
- *Salmonella* should not be present in 25 ml,
- Pathogenic microorganisms should not be present in 25 gr.
- According to the Turkish Food Codex, in the final product controls carried out by random sampling method in the production facility where heat treatment is applied;
- 25 gr should not contain pathogenic microorganisms,
- Coliform (in ml) should be 5 cfu/ml,
- In the sowing made after 5 days of incubation, the total number of live bacteria should be within the limits of 50,000-500,000 cfu/ml at 21 °C.
- Sowing in sterilized UHT milk after 15 days of incubation at 30 °C [total viable bacteria count ≤ 10 (at 30 °C, 0.1 ml)].

Cheese Microbiology

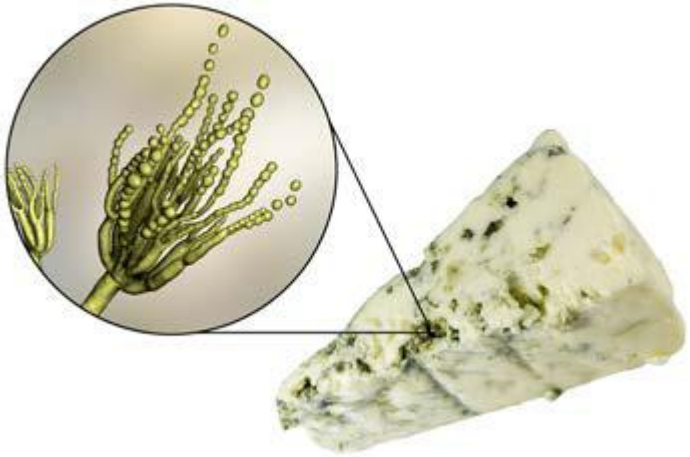
- Cheese is a dairy product obtained by coagulating milk in various ways and removing the clot from the whey and shaping it. The main steps in the transformation of milk into cheese; coagulation, filtration and maturation.
- Since the amount of microbiological load carried by raw milk differs significantly, consumption of cheese produced from raw milk brings along various infection risks. In order to obtain safe products in terms of food safety and consumer health, pasteurized milk is used in most of the industrially produced cheeses.
- As a result of pasteurization of the milk to be processed into cheese (30 minutes at 63-65 °C and 15-40 seconds at 65-74 °C), harmful and pathogenic microorganisms in the milk that will adversely affect the quality of the product become inactive. However, after the pasteurization process, most of the lactic acid bacteria, which provide natural acidification and maturation of the cheese, become inactive during cheese production.
  - In this case, the production of desired (standard) quality cheese becomes difficult.

Cheese Microbiology

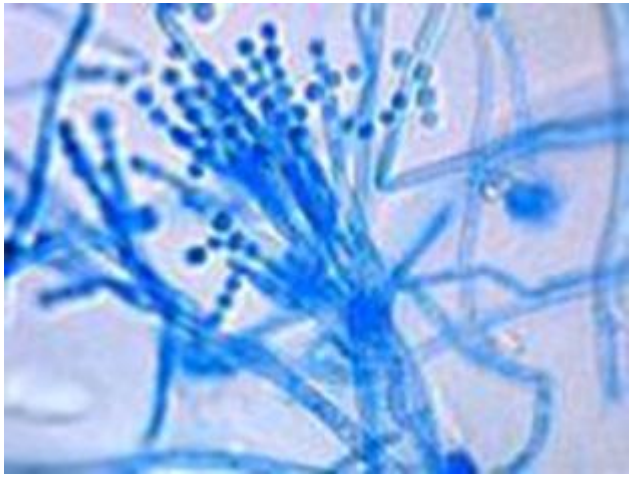
- Microorganism spores resistant to heat treatment that cannot be inactivated by pasteurization or microorganisms that can be transmitted during cheese production stages easily develop in milk and cause various defects in cheese.
- In order to obtain a standard quality product, it is a technological necessity to add lactic acid bacteria lost through pasteurization to milk as a starter culture. lactic acid bacteria; It represents an important microflora for cheese production due to its ability to metabolize lactose to lactic acid during cheese production, to contribute to the development of cheese flavor during ripening, and to inhibit unwanted microorganisms with the metabolites they form. In addition, some of these bacteria also show probiotic properties.
- Starter cultivars used in cheese production are *S. thermophilus, Lactobacillus bulgarius, L. acidophilus, S. lactis, S. cremoris and Leuconostoc* species. In addition, propionic acid bacteria are used in some cheese varieties.
- In addition to lactic acid bacteria, mold starter cultures containing *Penicillium roqueforti and Penicillium camemberti* molds are used in roquefort and camber cheese ripened with molds.

Microbial Spoilage in Cheese

- Although cheeses seem suitable for microorganism activity with the nutrients they contain, the added salt content limits the growth of many bacteria. However, the salt added to cheese and the acid formed by *Streptococcus cremoris, S. lactis, L. lactis and L. helveticus* suppress the growth of many microorganisms.



Penicillium roqueforti



Penicillium camemberti

Microbial Spoilage in Cheese

- However, some bacteria, especially yeast and molds, develop in cheese and cause undesirable changes. Undesirable changes seen in cheeses are:
- **Color Errors**: Bacteria such as *Serratia marcescens and Proteus vulgaris* develop on the cheese surface and cause red and brown colored formations. *Penicillium casei and Cladosporium herbarum* molds cause black and dark brown spots; some other molds cause white and green appearances. *Torula* yeast strains cause the crust to take on a white oily appearance.






Microbial Spoilage in Cheese

- **Formation of Faulty Pores and Clefts:** It is usually caused by gas-forming microorganisms. With this defect, soft cheeses take a spongy shape; hard cheeses also swell and change shape. In advanced cases, cracks and disintegration are seen in hard cheeses.
- The reason for the small pores seen in the first days of maturation is coliform group microorganisms. Especially *Escherichia coli and Enterobacter aerogenes* cause this defect; it can reproduce in some yeasts and form this porous structure.
- Propionic acid bacteria and *Clostridium* species are responsible for the pores formed in the later days of maturation. Clostridiums form few but large pores.
- **Taste Change**s: *Streptococcus* species break down proteins to form peptides that produce bitter taste. *Clostridium* species cause deterioration by breaking down proteins into malodorous products such as hydrogen sulfide (H_2S), indole, skatol.
- **Creep Event**: Such formations occur with the proliferation of *Bacillus mesentericus and members of the genus Proteus, Alcaligenes and Pseudomonas*.

Yoghurt Microbiology

- *Streptococcus thermophilus and Lactobacillus bulgaricus* are used as starter cultures in yogurt. The high temperature applied to the milk during yoghurt production and the low pH that occurs during production make yoghurt a safe food.
- Because yogurt has a low pH, most bacteria cannot thrive. However, some lactic acid bacteria that can grow at low acidity can cause aroma disorders.
- The main microorganisms that cause deterioration in yogurt are yeast and molds that multiply easily in acidic environments. These are the causes of deterioration, especially with gas formation. The spoilage factor is the genus of yeasts *Candida, Saccharomyces, Rhodotorula, Kluyveromyces* species.
- Molds causing spoilage in yoghurt are *Mucor, Penicillium, Rhizopus and Aspergillus*. As a result of their growth in yogurt, they can create a musty odor and taste.
- One of the most important reasons for the sour taste in yogurt is the overgrowth of the starter culture, *L. bulgaricus*.

Butter Microbiology

- Butter contains a significant amount of milk fat (82%), varying amounts of water (15%) and other nutrients (0.5% carbohydrate and protein). The higher the water content in butter, the greater the risk of microorganism proliferation.
- In the production of butter, *Streptococcus lactis, Streptococcus cremoris, Leuconostoc cremoris* and some other *Leuconostoc* species are used as starter cultures. Microorganisms (some bacteria and molds) in the milk that remain alive after the applied heat treatment and the microorganisms that are subsequently contaminated create a breeding environment in butter.
- Most of the microbial spoilage seen in butter comes from the cream from which the butter is made. In unsalted butter, coliform bacteria such as *Pseudomonas* and *Enterococcus* thrive and spoil the flavor. As a result of the breakdown of protein and fat by *Pseudomonas* species, a fruity odor, putrite spoilage, and ester-like flavor are formed in butter. Also, black spots occur on butter.
- Yeasts (*Candida* species) that can ferment lactose develop and produce gas. *Bacillus cereus* bacteria cause metallic taste and food poisoning; *Mucor stoloniferous* mold to lipolytic and proteolytic degradation; *Rhodotorula, Candida, Torula* yeast strains cause stains and yeast taste on the surface.

Ice-Cream Microbiology

- Ice cream is a dairy product obtained by freezing the mixture formed by the combination of milk, sugar, stabilizer substance, flavoring and coloring substances after applying heat treatment (pasteurized).
- In the heat treatment applied to milk in ice cream production, some microorganisms can survive and freeze; It can be exposed to secondary contamination from machinery-tools, utility water, air, employees and packaging material.
- Microbial spoilage usually occurs during the preparation or thawing of the product before freezing. Common deterioration is rancidity caused by the growth of acid-forming bacteria.
- Microorganisms that can be found in ice cream and cause undesirable taste changes are *Clostridium, Micrococcus, Bacillus, Enterococcus and Corynebacterium* species. In addition, *Salmonella* and molds transmitted from various additives can also be seen in ice creams.