CHAPTER 19

Spoilage of Milk and Milk Products

19.1 Introduction

Milk is subjected to a variety of preservation treatments due to its highly perishable and putrid characteristics and also owing to the presence of undesired pathogens in it. Dairy processing involves pasteurization, commercial sterilization, fermentation, dehydration, refrigeration, and freezing. Milk products are marketed as heated milk, yogurt, kefir, kumiss, cream, butter, cheese, condensed and dried milk, and others. Spoilage of milk and milk products results with off-flavor and odor formation and changes in both texture and appearance. In this chapter, contamination and spoilage of milk and milk products and interaction of microorganisms with dairy foods are overviewed.

19.2 Milk Composition and Microbial Contamination

Table 19.1 lists the major nutritional components of milk. Milk has carbohydrates, fats, casein, lactalbumin, and free amino acids that provide a good nutrient source for microorganisms. The pH of cow's milk is about 6.4. The fats in milk are mainly present in globules as an oil-in-water emulsion. They are coated with a surface material of proteins and polar lipids. The milk fat consists of triglycerides (98%), diacetyl glycerol, phospholipids, and fatty acids.

The minimum heat treatment requirement for milk to be sold in fluid form is 72 °C for 15 s, but most processors use slightly higher temperatures and longer holding times. This pasteurization inactivates all pathogens and many spoilage microorganisms, but this treatment may also encourage the survival of thermoduric and thermophilic spoilage microflora by destroying inhibitory chemicals and computing microorganisms, and activating spores. Heat treatments affect microbial growth by increasing both the available nitrogen throughout protein denaturation and sulfhydryl compounds.

Sources of the initial microflora in raw milk include the interior of the udder, udder surfaces, milking equipment, transport lines, storage tank, environment (such as air and water), and workers. Raw milk may also be contaminated from

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Table 19.1 Approximate nutritional compositions of raw milk.

Component	Amount	Component	Amount
	($g\ ml^{-1}$)	Magnesium	0.12
Water	0.873	Citrate	1.78
Lactose	0.0482	Chloride	1.12
Fat	0.0339	Phosphorus	0.79
Casein	0.029	*Nonprotein nitrogen (NPN)*	($mg\ ml^{-1}$)
Vitamins	0.001	Total NPN	0.34
Minerals	0.007	Urea	0.25
Minerals	($mg\ ml^{-1}$)	Peptide	0.04
Sodium	0.56	Amino acid	0.06
Potassium	1.44	Creatine	0.04
Calcium	1.38		

the animal manure and flies. Undesirable bacteria from these sources are lactic *Streptococcus*, coliforms, psychrotrophic Gram-negative thermoduric bacteria (such as *Micrococcus*, *Enterococcus*, and *Bacillus*), and *Brevibacterium*. Soil, water, animals, and plant material constitute the natural psychrotrophic bacterial contaminants for raw milk. Filling equipment are most often the source of psychrotrophs. Contaminated bacteria grow well in milk and reduce its keeping quality. The contamination from these sources can be effectively controlled by proper cleaning and sanitizing methods. The sanitizing agent quaternary ammonium compounds are ineffective on the Gram-negative bacteria on the utensils while reducing Gram-positive bacteria, where hypochlorites are effective on both of them. Milking equipment is generally fabricated from stainless steel, which is easily cleaned and sanitized; however, some parts, rubber or other nonmetal materials, are difficult to sanitize, and hence bacteria attached to microscopic pores or cracks are difficult to inactivate by chemical sanitizers.

Fresh raw milk generally contains different numbers of microorganisms depending on the care employed in milking, cleaning, and handling of milk utensils. Storage of raw milk at refrigerator temperature for several days can lead to growth of psychrotrophic species of several bacterial genera: *Aerococcus*, *Bacillus*, *Lactobacillus*, *Leuconostoc*, *Microbacterium*, *Micrococcus*, *Propionibacterium*, *Proteus*, *Pseudomonas*, *Streptococcus*, coliforms, and others. The pasteurization of milk eliminates a great deal of psychrotrophic bacteria, but thermoduric bacteria (such as *Bacillus*, *Lactobacillus*, and *Streptococcus*) and thermophilic can survive.

Coliforms are present on udder skin because of fecal contamination, so ineffective cleaning of this area before milking will contribute high coliform populations to raw milk. Coliforms can also associate with inadequately cleaned milk equipment. Soil, water, animals, and plant materials are the natural habitat of psychrotrophic bacteria contaminating with milk. Plant materials (such as grass and hay used as animal feed) mostly contain over 10^8 psychrotrophs per gram.

Water is also an important source of psychrotroph and its use in cleaning and rinsing milking equipment causes an indirect contaminant in milk. Psychrotrophic bacteria from water are very active producers of extracellular enzymes. The udder area of the cow can harbor high levels of psychrotrophic bacteria, even after washing and sanitizing. Utensils and storage tanks are other sources of psychrotrophic bacteria contaminating with raw milk. Milk residues on unclean equipment provide a growth environment for psychrotrophic bacteria, which contaminate milking machines, pipelines, and holding tanks during water rinses.

Spore-forming bacteria in raw milk are generally quite low (<5000 bacterial cells per milliliter), but spoilage caused by them does not always correlate with the initial number of spore formers in the raw milk. Spore-forming bacteria in raw milk are predominantly *Bacillus* spp. (such as *B. cereus*, *B. licheniformis*, *B. megaterium*, and *B. subtilis*). *Clostridium* spp. are present in raw milk at low levels. Populations of spore-forming bacteria in raw milk vary seasonally. *Bacillus* and *Clostridium* spp. are at higher levels in the raw milk collected in winters than in summers, because in winters cows lie on spore-contaminated bedding materials and consume spore-containing silage. Psychrotrophic *Bacillus* spp. may be introduced into the raw milk from the processing plant.

The microorganisms most frequently involving mastitis include *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberus*, *Escherichia coli*, *Corynebacterium bovis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Mycoplasma*, and yeasts. Microorganisms causing mastitis are important for a number of reasons: They change the composition of the milk, they may be pathogenic for man (such as *Brucella*, *L. monocytogenes*, and *S. aureus*), they can cause economical loss, and they can contain residues of antibiotics that are used for mastitis treatment.

19.3 Spoilage

The main carbohydrate in milk is lactose, the microorganisms producing lactose-hydrolyzing enzyme (lactase or β-galactosidase) 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 caproic acids). Types of some microbial spoilage of milk are given in Table 19.2.

19.3.1 Raw Milk Spoilage

Raw milk is an excellent medium for microorganisms due to high moisture, nearly neutral pH, and rich in nutrients. Microbial spoilage of raw milk occurs from the metabolism of lactose, nitrogenous compound (such as proteins, amino acids, ammonia, urea, and others), unsaturated fatty acids, triglycerides, and minerals.

Table 19.2 Some types of microbial spoilage of milk.

Spoilage	Spoilage bacteria	Enzyme	Metabolic product
Acid proteolysis	*Lactobacillus*, *Micrococcus*, *B. cereus*	Proteinases, lactase	Peptides, amino acids
Alcoholic flavor	Yeasts	Alcohol dehydrogenase	Ethanol
Bitter flavor	Psychrotrophic bacteria, *Bacillus*	Proteinase, lipases	Bitter peptides
Fishiness	*Aeromonas hydrophila*	Proteinase	Fish flavors
Fruity flavor	*P. fragi*, *Staphylococcus*, *Candida*, *S. cerevisiae*, *P. notatum*, *C. butyricum*	Esterase	Ethyl esters, lactons
Caramel and malty flavor	*L. lactis* var. *maltigenes*	Oxidase	3-Methylbutanol
Putrefaction	*P. putrefaciens*, *Clostridium*	Proteinase	Peptones, amino acids
Rancid flavor	Lipolytic bacteria	Lipase	Free fatty acids
Ropy texture	LAB	Polymerase	Exopolysaccharide
Sour and acid flavor	*Lactococcus lactis*	Lactase	Lactate
Sour flavor at 10–40 °C	LAB, coliforms, *Enterococcus*	Lactase	Lactate, acetate
Sour flavor at 37–50 °C	*L. thermophiles, S. thermophilus*	Lactase	Lactate, acetate

19.3.1.1 Organoleptic and Physical Changes

Microorganisms cause different undesirable organoleptic and physical changes in raw milk. Refrigeration of raw milk prevents growth of most mesophilic microorganisms, while psychrotrophic microorganisms (such as species of *Pseudomonas*, *Flavobacterium*, *Micrococcus*, *Bacillus*, *Enterobacter*, *Aeromonas*, and *Alcaligenes*) are able to grow. Although psychrotrophic microorganisms present initially in low numbers, but extended refrigeration allows their growth to high numbers. Organoleptic changes (such as malty, rancid, yeasty, bitter, fruity, or putrid off-flavors), pigments, and ropiness can associate with growth of psychrotrophic microorganisms.

Different off-flavors are produced by microorganisms: (i) Sour or acid flavor is caused by *Lactococcus lactis*. Volatile fatty acids are produced by coliforms and *Clostridium.* (ii) Bitter flavors result from proteolysis and lipolysis. (iii) Burn or caramel flavor is caused by *Lactococcus lactis* subsp. *lactis* var. *maltigenes*, which resembles the cooked flavor of overheated milk. (iv) Miscellaneous flavors—barny flavor is caused by *Enterobacter oxytocum*, soapiness with ammonia production by *Proteus sapolactica*, malty flavor by *Micrococcus*, fruit flavor by *Pseudomonas fragi*, proteolytic flavor by *Pseudomonas mucidolens*, fishiness by *Aeromonas* *hydrophila*, putrefaction by *Clostridium* and *Pseudomonas putrefaciens*, and fruity and alcoholic flavors by yeasts. Lactones, which can give fruity and coconut-like flavor to dairy products, can be produced by various microbial growth (such as *Candida* and *Saccharomyces cerevisiae*) and molds (such as *Penicillium notatum* and *Cladosporium butyricum*).

The spoilage bacteria in raw milk are mostly aerobic Gram-negative psychrotrophic rods, such as *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, and some coliforms. About 65–70% of psychrotrophic microorganisms in raw milk is *Pseudomonas* spp. Psychrotrophic spoilage microflora of milk is generally proteolytic, lipolytic, and phospholipolytic, but not lactose hydrolyzing. *Pseudomonas* spp. (such as *P. fluorescens*, *P. fragi*, *P. putida*, *P. lundensis*, and *P. aeruginosa*) outgrow other bacteria when milk is stored at 3–7 °C. They can produce extracellular heat-stable lipases, proteinases, and phospholipases. Milk products that may be affected by residual heat-stable enzymes include ultrahigh temperature (UHT) milk, butter, cheese, and fluid milk products.

Pseudomonas spp. by their proteolytic enzyme activities can hydrolyze proteins and peptides to bitter, putrid, and unclean flavor and coagulate milk. Rancid and fruity flavors result from lipolysis of fats.

Raw milk is rapidly cooled after collection and is kept cold until pasteurization. But there is often sufficient time between milk collection and consumption for psychrotrophic bacteria to grow. Generation time of psychrotrophic *Pseudomonas* spp. in milk is 8–12 h at 3 °C and 5–10 h at 5 °C. These growth rates are sufficient to cause spoilage within 5 days even if the milk initially contains only one *Pseudomonas* per milliliter.

19.3.1.2 Souring

Spoilage of milk and milk products results from growth of fermentative bacteria when storage temperatures are sufficiently high for psychrotrophs. Genera of bacteria producing acids in milk and milk products are *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. Alkaline reaction occurs by *P. fluorescens* and *Alcaligenes viscolactis* at chilling and refrigeration temperatures. Little acid formation takes place in milk held at temperatures near freezing, but proteolysis may take place at this temperature. If raw milk is not refrigerated and kept at room temperature, growth of mesophiles predominates. These include species of *Bacillus*, *Clostridium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Proteus*, *Pseudomonas*, coliforms, and others.

The unpleasant sour odor and taste of spoiled milk result from the production of small amounts of acetic and propionic acids by lactic acid bacteria (LAB). Lactose hydrolyzing *Lactococcus lactis* subsp. *lactis* will generally predominate to cause souring with enough acid production to lower the pH and inhibit growth of other microorganisms. A malty flavor can also result from growth of *Lactococcus lactis* subsp. *lactis* var. *maltigenes*. When coliforms, *Enterococcus*, *Lactobacillus*, and *Micrococcus* grow in milk, they cause curdling, gas formation, proteolysis, and lipolysis. At higher temperatures, from 37 to 50 °C, *Streptococcus thermophilus* and *Enterococcus faecalis* grow to produce acid and then growth can be followed by *Lactobacillus* (such as *Lactobacillus bulgaricus*). Some of *Lactobacillus* can grow at temperatures above 50 °C, but produce less acid. The pasteurization of milk kills some acid-forming bacteria, but heat-resistant thermoduric LAB (such as *S. thermophilus*, *Enterococcus*, and *Lactobacillus*) can survive.

19.3.1.3 Proteolysis

Psychrotrophic species of *Alcaligenes*, *Flavobacterium*, *Bacillus*, *Micrococcus*, and *Pseudomonas* can grow at low temperatures and produce extracellular proteinases. Most of proteinases in raw milk are produced by *P. fragi* and *P. fluorescens*. Proteinases hydrolyze proteins (proteolysis). The hydrolysis of proteins at low temperatures produces bitter, fruity, and putrid flavors due to release of peptides. Types of proteolysis are (i) acid proteolysis due to proteolysis and acid production at the same time, (ii) proteolysis with little acidity or alkalinity, (iii) sweet curdling, which is caused by rennin-like enzymes of bacteria, (iv) slow proteolysis by intracellular enzymes of bacteria after lyses of their cells, and (v) residual proteolytic activity of heat-stable proteinases. Acid proteolysis is caused by *Enterococcus faecalis* var. *liquefaciens*, *Macrococcus caseolyticus*, and *Bacillus cereus*.

Factors Affecting Proteinase Production and Activity

Psychrotrophic *Pseudomonas* spp. can produce extracellular proteinases in raw milk during the late exponential and stationary phases of growth. Proteinase activity increases during stationary and death phases of microbial growth (because of the release of preformed enzymes from lysed cells besides extracellular enzymes). The effect of temperature on proteinase production is not parallel to its effect on growth. The temperature for optimum production of proteinases by psychrotrophic *Pseudomonas* spp. is lower than the temperature for optimum growth. Relatively higher amounts of proteinases are produced at temperatures as low as 5 °C, while optimum growth temperature is 20 °C for *Pseudomonas*. Proteinase production by *Pseudomonas* spp. is inhibited in milk at 2 °C. *Pseudomonas* spp. are obligate aerobes and oxygen is necessary for proteinase synthesis.

Decimal reduction time (*D* value) of proteinase ranges from 50 to 200 s at 140 °C, this is sufficient to retain significant residual active proteinases after milk processing at UHT treatment.

Proteinase-Inducing Spoilage

Heat-resistant proteinases of psychrotrophic bacteria cause spoilage in processed milk because of enzyme-retaining activity after the heat treatment. Proteinases hydrolyze casein in milk to liberate bitter peptides (putrid off-flavors). Proteolytic putrid off-flavors associates with lower molecular weight products such as ammonia, amines, and sulfides. Bitterness in UHT processing (commercially sterile) milk develops when psychrotrophic bacteria grow in raw milk (up to

10^7 viable cells per milliliter) to produce proteinases and residual active enzymes retained in product after heat treatment. Low level of proteinase activity in UHT milk can result in coagulation of milk proteins during storage. UHT milk is more sensitive to proteinase defects than raw milk due to either heat-inducing change in casein micelle structure or heat inactivation of proteinase inhibitors in milk.

19.3.1.4 Lipolysis

Psychrotrophic bacteria (such as *Alcaligenes*, *Bacillus*, *Clostridium*, *Micrococcus*, *Pseudomonas*, *Proteus*, and *Staphylococcus*) produce extracellular lipases in milk and hydrolyze milk fat. Lipase-producing bacteria are *Pseudomonas* spp. *P. fragi* and *S. aureus,* and these enzymes are fairly heat resistant. Lipases hydrolyze fat to fatty acids and glycerol. Oxidation of the unsaturated fatty acid results in the formation of aldehydes, acids, and ketones. They can cause odors and unwanted tastes in milk. Combined effects of oxidation and hydrolysis of fat cause rancidity.

Factors Affecting Lipase Production and Activity

Milk is an excellent medium for lipase production by psychrotrophic *Pseudomonas* spp. and is produced in the late exponential and stationary phases of growth. Optimum synthesis of lipase generally occurs below the optimum growth temperature of bacteria. For example, optimum temperature for production of lipase is 8 °C for *P. fluorescens,* which has optimum growth at 20 °C. Temperatures for activity of lipases range from 22 to 70 °C with optimum between 30 and 40 °C. Optimum pH activity ranges from 7.0 to 9.0. In milk, lipases show extreme heat resistance and retain high residual activity at UHT processing.

Lipase-Inducing Spoilage

The triglycerides in raw milk occur in globules that are protected from enzymatic degradation by a membrane. Lipolysis of triglycerides occurs in milk if the membranes of triglycerides are disrupted by excessive shear force (from pumping, agitation, homogenization, freezing, etc.). Raw milk contains a mammalian lipase (milk lipase) that rapidly acts on the fat if the globule membrane is disrupted. Most cases of rancidity in raw and pasteurized milk result from lipases activity, rather than from the bacterial lipases. Phospholipase from psychrotrophic bacteria can degrade the fat globule membrane and this enhances milk lipase activity. Milk lipase is heat labile, so most heat-treated milk products will not have residual milk lipase activity.

When psychrotrophic bacteria grow in raw milk, they produce lipases and retain activities during heat treatments. They can hydrolyze lipids during processing and storage of milk. The rancid flavor (soapy) and odor formation due to action of lipase result from the liberation of fatty acids. *P. fragi* produces a fruity off-flavor in milk by esterifying free fatty acids with ethanol. Ethyl butyrate and ethyl hexanoate are short-chain acids of ethyl esters of acetate, propionate, and isovalerate. Short-chain unsaturated fatty acids may be oxidized to ketones and aldehydes to produce off-flavor. However, lipase-inducing spoilage are not as common as those resulting from microbial proteinases. Lipolytic off-flavors production in UHT milk generally takes several weeks to months.

19.3.1.5 Ropiness

Ropiness (sliminess) results with the formation of polymers from microbial and nonmicrobial activities on polysaccharides. Most LAB (such as *Lactobacillus* spp.), *Alcaligenes* spp. (such as *A. viscolactis*), and coliforms produce extracellular polymers that increase the viscosity of milk and cause ropiness. The polymers produced from polysaccharides contain glucose and galactose with small amounts of mannose, rhamnose, and pentose. The main sources of bacteria causing ropiness are water, manure, utensils, and feed. Nonbacterial ropiness can also occur in milk. This may be due to (i) the presence of fibrin and leukocytes (from mastitis) in milk, (ii) the thickness of cream (at the top), and (iii) the films of casein or lactalbumin.

Bacterial ropiness is caused by slime capsular polymers that are located on the surface of cells. Bacteria causing ropiness are *A. viscolactis* and *Micrococcus freudenreichii* (surface ropiness); *Enterobacter aerogenes* (ropiness near the top); *Enterobacter cloacae*, *E. coli*, *Klebsiella oxytoca*, and *Bacillus* (ropiness throughout the milk); and certain species of LAB (such as *S. thermophilus*, *L. bulgaricus*, *Lactobacillus casei*, *Lactobacillus. plantarum*, *Lactococcus lactis* subsp. *hollandicus*, *Lactococcus lactis* subsp. *lactis*, and *Lactococcus lactis* subsp. *cremoris*).

19.3.1.6 Color Changes and Gas Formation

Pigmented bacteria and molds can change the color of milk. Blue milk is caused by *Pseudomonas syncyanea* and *Geotrichum*, deep-blue color by *P. syncyanea* growing together with *L. lactis*; red milk by *P. synxantha*, *Serratia marcescens*, *Brevibacterium erythrogenes*, *Micrococcus roseus*, and *Flavobacterium* spp.; and brown milk by *P. putrefaciens* and *P. fluorescens*. The chief gas formers are coliforms in raw milk. *Clostridium*, *Bacillus*, and thermoduric heterofermentative *Lactobacillus* can produce gas in heat-treated milk.

19.3.1.7 Pathogens in Raw Milk

Mycobacterium bovis, *Mycobacterium tuberculosis*, *Brucella abortus*, and *Brucella melitensis* can cause milk-borne diseases. Raw milk is an important vehicle for *Salmonella enterica* subsp. *enterica* serovars (such as *S.* Aanatum, *S.* Thompson, *S.* Heidelberg, *S.* Enteritidis, and *S.* Newport) that cause human infection. *Salmonella enterica* subsp. *enterica* ser. Dublin and *Salmonella enterica* subsp. *enterica* ser. Typhimurium most frequently associate with cattle. These *Salmonella* serovars may show multiple resistances to antibiotics. Campylobacteriosis with *Campylobacter* spp. can also associate with raw milk. Raw milk contaminates with pathogenic *E. coli* through exposure to fecal material and can cause foodborne outbreaks (such as *E. coli* O157:H7). The most probable source of *S. aureus* in raw milk is the infected bovine udder. *S. aureus* can multiply and produce enterotoxin in raw milk. Raw milk may be contaminated with *L. monocytogenes* from poor silage conditions, fecal contamination, and improperly cleaned equipment. Raw milk may be contaminated with *Yersinia enterocolitica*, *Streptococcus agalactiae*, other hemolytic *Streptococcus* and *Coxiella burnetii*. Drinking milk containing bovine tubercle bacilli causes alimentary tuberculosis, especially in infants. In regions where consumption of raw milk continues, multiple outbreaks of brucellosis, salmonellosis, campylobacteriosis, and enterohemorrhagic colitis (by *E. coli* O157: H7) can occur. Raw milk can transmit viruses causing hepatitis A, poliomyelitis, encephalitis, and gastroenteritis. Raw milk may also contaminate with mycotoxins (such as aflatoxin B1, G1, sterigmatocystin, ochratoxin, and others), particularly aflatoxin M1 (AFM1). The presence of AFM1 is the result of hydroxylation of aflatoxin B1 by mammals with subsequent excretion in the milk. Many countries have established limits for AFM1 in raw milk, ranging between "zero" and 1.0 μg per liter of milk.

19.3.2 Fluid Milk Products Spoilage

Types of bacterial spoilage in fluid milk products are given in Table 19.3.

19.3.2.1 Pasteurized Milk Spoilage

Pasteurization of milk kills most of the acid-forming bacteria, but not heat-resistant thermoduric bacteria (such as *Bacillus*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Lactobacillus*, *Micrococcus*, and *Streptococcus*). These thermoduric microorganisms can grow at low temperatures, since some species are also psychrotrophic. Coliforms, *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, and others can enter into milk as postpasteurization contaminants. Pasteurized milk storage in refrigerator has a limited shelf life, mainly due to growth of psychrotrophic contaminants. The most common fermentative spoilage of fluid milk products is souring caused by thermoduric LAB. Lactic acid by itself has a clean pleasant acid flavor and no odor. The unpleasant "sour" odor and taste of spoiled milk result from formation of small amounts of acetic and propionic acid. Sour odor can appear before an acid flavor when the microbial population reaches $\geq 10^6$ cells per milliliter of fluid milk products. Pasteurized milk spoils by the growth of heat-resistant *Streptococcus* utilizing lactose to reduce pH (curdling at 4.59). *Lactobacillus* continues fermentation and may reduce the pH to 4.0 or below. If molds present in milk, they begin to grow on the surface of soured milk and raise the pH toward neutrality, thus allowing the growth of proteolytic bacteria (such as *Pseudomonas*) and causing liquefaction of the milk curd.

Pasteurization temperature activates spore germination and spoilage by spore-forming *Bacillus*, and *Clostridium* chiefly occurs in heated packed fluid milk with gas production. Milk can be coagulated by spore-forming bacteria without significant acid or off-flavor formation. Microbial growth in milk may become visible as bacterial colonies (buttons) at the bottom of the carton. Degradation of

Table 19.3 Types of spoilage in fluid milk products.

Milk products	Type of spoilage	Bacteria
Pasteurized milk	Proteolysis	*Pseudomonas, Flavobacterium, Bacillus*
	Souring	*Streptococcus*, *Lactobacillus*
	Acid proteolysis	*Micrococcus, B. cereus, Clostridium*
	Sweet proteolysis	*Alcaligenes, Proteus*, *B. cereus*
	Malty flavor	*L. lactis*, *L. lactis* subsp. *lactis* var. *maltigenes*,
	Sour flavor	*Micrococcus*
	Repines	*Bacillus circulans*
	Blue color	LAB, *A. viscolactis*
	Gases, coagulation	*P. syncyanea*, *L. lactis*
	Buttons	*Bacillus*, *Clostridium*
		Microbial growth
UHT milk	Off-tastes and off-odors, physical changes	*G. stearothermophilus*, *G. thermosaccharolyticum*
	Pink color, off-flavor, coagulation	*B. sporothermodurans*
	Age gelation, increasing viscosity	Milk and bacterial proteinases
	Rancid flavors, odors	Phospholipases, lipases
	Maillard products	Heat treatment
	Acidity	Free fatty acids, other organic acids
Concentrated milk	Acid proteolysis	*Bacillus*
	Bitter flavor	*B. licheniformis*, *C. botulinum*
	Swelling, off-flavors	*C. sporogenes*, chemical reactions, overfilling, xerophilic mold
	Late sour	*B. licheniformis, B. macerans*, *B. subtilis*, *G. stearothermophilus*
	Buttons, coagulation	*Wallemia sebi*
	Sweet coagulation	*B. coagulans*, *B. cereus*, *T. stearothermophilus*
	Flat sour spoilage	*G. stearothermophilus*. *B. licheniformis*, *B. macerans*

casein with microbial enzymes can cause bitter flavor. Spores of psychrotrophic *Bacillus* spp. surviving at pasteurization can germinate and multiply at low-temperature storage to cause a spoilage known as "bitty." They produce the enzyme lectinase. Lectinase hydrolyzes phospholipids of the fat and causes aggregation of the fat globules that adhere to the container surfaces. Production of rennin-like enzymes by the psychrotrophs can cause sweet curdling of milk at high pH. Nonaseptic packaged refrigerated fluid milk may be spoiled by growth of psychrotrophic *B. cereus* and *Bacillus polymyxa* in the absence of more rapidly growing Gram-positive psychrotrophs (such as *Pseudomonas* spp.). Psychrotrophic *Bacillus circulans* is the predominant spoilage bacterium in aseptically packaged heat-treated milk. This bacterium produces acid from lactose, giving sour flavor to milk. *Bacillus mycoides* is another spoilage psychrotrophic spore former in milk.

19.3.2.2 UHT Milk Spoilage

UHT processing is a method of milk preservation by which both the microorganisms and enzymes are reduced to a commercially acceptable level (commercial sterility; not more than one package (1 l) spoiled per 10,000 or less), which ensures consumer safety and extended shelf life of milk. However, during heat treatment and storage period, a number of changes can occur in chemical, physical, and microbiological characteristics of the product. These changes may make the product unacceptable because of the development of off-flavor, color, and gelatin.

Contamination of spore-forming bacteria (such as *Geobacillus stearothermophilus*, *Bacillus subtilis*, and *Clostridium botulinum*) is possible with milk during UHT process. A large number of spores can occur at the dairy farm. Feed and milking equipment can act as reservoirs or entry points for potentially high-heat-resistant spores into raw milk. Lowering the spore numbers by good hygienic measures can probably further reduce the contamination level of raw milk. All microbial vegetative cells and most bacterial spores in milk are destroyed by UHT treatments. However, some heat-resistant endospores (such as *Bacillus sporothermodurans*, *G. stearothermophilus*, and *Thermoanaerobacterium thermosaccharolyticum*) survive and heat-resistant extracellular enzymes (proteinases, lipases, and phospholipases) retain activities at the UHT processing of milk. These bacteria are not pathogenic and cannot grow at the temperature below 35 °C. They can cause off-tastes and off-odors, and physical changes (curdling or coagulation) in milk. Bacterial heat-resistant extracellular proteinases can cause spoilage of UHT milk by producing bitter peptides. Occasionally, microbial spoilage can occur in UHT-treated milk, usually as a result of contamination during filling operations. Members of *Bacillus* spp. (such as *Bacillus badius*, *B. cereus*, *Bacillus licheniformis*, *B. polymyxa*, *Bacillus subtilis*, and *G. stearothermophilus*) are common spoilage bacteria in such situations. *B. sporothermodurans* spoilage in milk can occur with a pink color change, off-flavors, and coagulation, especially in containers with a low-oxygen barrier (such as plastic bottles). Despite its rather poor growth characteristics in UHT milk, UHT milk can be regarded as a new ecological source for *B. sporothermodurans* because of the lack of competition from other microorganisms.

Heating of milk accounts for two main problems: age gelation and off-flavor development, which limits shelf life of milk. UHT milk is bacteriologically stable for months at ambient temperatures; its shelf life is often affected by age gelation. UHT treatment leads to a much larger production of small-sized casein micelles compared to raw or pasteurized milk. The gel is three-dimensional protein network formation by the whey proteins, particularly β-lactoglobulin, interacting with k-casein of the casein micelle. Age gelation is initiated by proteolytic activity of heat-resistant native milk proteinases (plasmin) and bacterial proteinases. Hydrolysis of milk caseins by proteinases results in a destabilization of the casein micelle. After heat treatment and effect of proteinases, interaction between k-casein and β-lactoglobulin occur, resulting in the formation of β-lactoglobulin–k-casein complex (βk complex). The complex subsequently aggregates and forms the cross-linked proteins, which causes the milk to gel. This phenomenon increases milk viscosity during storage and results in a loss of fluidity.

Proteolysis and lipolysis of UHT milk components during storage at room temperature is a major factor limiting the shelf life. The changes reduce the quality of UHT milk, developing off-flavors, fat separation, sedimentation, and defects in texture. Indigenous proteinases or those from bacteria can retain activity in UHT milk during storage and cause bitterness. However, bacterial proteinases are more important than plasmin in quality deterioration of UHT milk. Residual or reactivated heat-stable proteolytic enzymes cause instability of casein micelles and appearance of bitter off-flavors in UHT milk. Peptides and amino acids are known to cause a variety of undesirable taste sensations. Hydrophobicity of amino acids yields bitter peptides. Changes in nitrogenous compounds can also occur in UHT milk during storage. These changes would be due to proteolysis of proteins. Fat hydrolyses results with liberation of volatile fatty acids (such as butyric acid) and oxidation of free or unsaturated fatty acids. Heat-stable bacterial phospholipases and lipases hydrolyze lipids in UHT milk, and the release of short-chain fatty acids (C4 through C8) results in the rancid flavors and odors, whereas the release of long-chain fatty acids results in a soapy flavor. Oxidation of free unsaturated fatty acids to aldehydes and ketones results in an oxidized flavor. Fruity off-flavor results from esterification of short-chain fatty acids with alcohols. Phospholipases are heat-stable enzymes and can cause bitter off-flavors due to the release of fatty acids by native lipase in raw milk. Therefore, undesirable changes in organoleptic characteristics of UHT milk would be due to decomposition of milk fat and protein as a result of lipolysis and proteolysis processes during storage.

Minor changes in macro and micronutrients occur during UHT processing of milk. Besides losses in nutrients, new components are also formed due to degradation of lactose during heat process. HMF and other browning reaction precursors appear in milk because of Maillard reaction between lactose and lysine on heating milk. The increase in acidity during storage of UHT milk would be due to increase in the concentration of free fatty acids, lactic acid, and other organic acids, which results from degradation of milk components—mainly fat and lactose. Changes in calcium phosphate equilibrium might also be responsible for increased acidity. UHT treatment can also cause a slightly burn taste.

Storing raw milk for more than 72 h at refrigeration temperatures might result in the formation of thermoresistant bacterial enzymes, mainly by psychrotrophic bacteria that significantly limit the shelf life of the milk. A psychrotrophic population, as low as 10^4 cells per milliliter, may be sufficient to produce heat-stable proteinases, lipases, and phospholipases in raw milk. Heat-stable enzymes of bacterial origin retain activity, and they can cause lipolysis and proteolysis in milk during storage of the UHT milk. Proteolysis in UHT milk can cause the development of bitter flavor leading to an increase in viscosity with initiation of gelation during storage, which is a major factor limiting its shelf life and market potential. Other enzymes responsible for the proteolysis are the native milk alkaline proteinase (heat-sensitive) and plasmin (heat-stable). Proteolysis of casein by plasmin is also responsible for gelation and bitter flavor of UHT milk during storage.

19.3.2.3 Spoilage of Concentrated Milk Products

Concentrated milk products can be divided into three groups: (i) evaporated milk, (ii) sweetened or unsweetened condensed milk, and (iii) concentrated milk. These products are subjected to sufficient heat treatments to kill microorganisms. Evaporated milk is prepared by heating milk at 93–100 °C and then evaporating it under partial vacuum (at 50 °C). Evaporated milk results with 7.5% milk fat and 25% total solids. It is packed in hermetically sealed cans and they are commercially sterilized at 115–118 °C for 14–18 min. Unsweetened condensed milk is prepared by heating milk at 100–120 °C for 1–3 min followed by chilling to 70 °C to stabilize the proteins. It is then concentrated at 45–70 °C under partial vacuum. After concentration, milk is homogenized, supplemented with stabilizers, cooled to 14 °C and packed in hermetically sealed cans. Unsweetened condensed milk results with 10–12% fat and 36% total solids. Sweetened condensed milk is prepared by heating milk at a high temperatures (from 71 to 100 °C for 10–30 min) and then condensed at 50–57 °C under vacuum. Condensed milk results with 8.5% fat, 28% total solids, and 42–64% sugar (sucrose).

Only thermophilic bacterial spores can survive high-temperature treatment of concentrated products. Exposure of milk to heat treatment over 43 °C or higher can activate germination of spores that subsequently grow. Under such conditions, *Bacillus* spp. (such as *B. coagulans*) can cause coagulation of products. Swelling of can containing concentrated milk is caused by gas-forming anaerobic spore formers (such as *Clostridium* spp.), overfilling of the can, and action of acid constituents of concentrated product on the iron of the can. Bitterness usually results from proteolysis by *Bacillus* and *Clostridium* spp. Thermophilic bacteria are a problem only if the product is stored at elevated temperatures (over 35 °C). Halophilic and xerophilic microorganisms (such as *Micrococcus* and fungi) are able to grow due to their low water activity. Growth of xerophilic fungi may cause blowing of cans and development of off-flavors. *Wallemia sebi* forms small brownish “buttons” of mycelium and coagulates casein without gas production.

Poor hygienic conditions allow contamination of spoilage microorganisms. Due to low a_w (≤0.85), these products are susceptible to spoilage with gas production by postprocess contaminant yeasts (such as *Kluyveromyces marxianus*, *Debaryomyces hansenii*, *Candida famata*, *Candida kefyr*, *Rhodotorula mucilaginosa*, *Yarrowia lipolytica*, and *Pichia*). Yeasts are able to produce proteolytic or lipolytic enzymes in condensed milk. If the containers have enough headspace, postprocess contaminated molds (such as *Aspergillus* and *Penicillium*) can grow on the surface of product. *Bacillus* spp. may produce acid and cause acid-proteolytic spoilage. In canned-condensed product, spoilage associates with the growth of spore-forming bacteria. "Sweet coagulation" is caused by *Bacillus coagulans*, *B. cereus*, and *G. stearothermophilus*. Protein destruction, in addition to curdling, can also be caused by *B. licheniformis* and *B. subtilis.* Can swelling or bursting may be caused by the growth of *Clostridium sporogenes* with the production of gases. "Flat sour" spoilage (acidification without gas production) can result from the growth of *B. licheniformis*, *Bacillus macerans*, *B. subtilis*, and *G. stearothermophilus*.

Water activity of concentrated milk products is about 0.85, and this is only borderline for *S. aureus*. However, anaerobic conditions in the condensed milk prevent growth of *S. aureus* and enterotoxin formation. Sweetened condensed milk may be produced with low-sugar content and high a_w and used, for example, for pastry and confectionery. In such products, growth of *S. aureus* is possible if the temperature is abused. Spores of *Clostridium* and *Bacillus* spp. may be present in these products, but their growth is prevented by low a_w. Growth and survival of pathogens in ultrafiltered milk is similar to that in milk, except that *Listeria monocytogenes* can grow faster and achieve higher populations.

19.3.2.4 Dried Milk Spoilage

In the case of low-heat milk powders, the heat treatments correspond to pasteurization; in the case of medium-heat milk powders, temperatures of 85–95 °C for 20–30 s are applied and temperatures above 120 °C for up to 30 s are used to obtain high-heat milk powders. Due to the extremely low a_w (0.30–0.40) of dried milk products, growth of spoilage microorganisms is not possible. Condensation of water on packaging material may allow growth of microorganisms and must be avoided. Extent of microbial destruction during drying depends on the types of microorganisms, drying temperature, and retention time of process. Thermophilic and thermoduric bacteria may survive the thermal treatments of drying process. The flora of dried milk includes (i) thermoduric *Micrococcus* usually associated with dairy equipment, (ii) thermoduric *Streptococcus* (mainly *S. thermophilus*) that grows at temperatures between 3 and 15 °C, (iii) thermoduric species of *Enterococcus* (such as *E. bovis*, *E. durans*, and *E. faecalis*), (iv) thermoduric species of *Corynebacterium* coming from milk, (v) bacterial spores of mostly *B. subtilis,* and (vi) miscellaneous contaminants, such as *E. coli* that indicates poor sanitation and postprocess contamination from the human reservoir.

Whole milk powder contains bacterial lipases and they can cause rancidity as a potential problem in dried milk. Low-fat milk powders (such as nonfat dried milk, whey, and whey protein concentrate) may contain residual lipases, which become active when these products are used in fat-containing food formulations.

Dried milk may be rehydrated and consumed directly, but it is more commonly used as ingredients in a number of products (such as bakery, yogurt, chocolate, confectionery, baby foods, ice cream, and animal feeds). *L. monocytogenes* and *Salmonella* (such as *S. enterica* subsp. *enterica* var. Typhimurium and *S. enterica* subsp. *enterica* var. Agona) may survive insufficient heat treatment of dried milk or contaminate during processing of milk powder. Outbreak of *S. aureus* can occur due to preformed staphylococcal enterotoxin when the milk powder is used as an ingredient. *Enterobacter sakazakii* may present in dried milk and can cause neonatal meningitis with the consumption of contaminated infant formula. The product must be protected against microbial contamination, from pasteurization to the packaging operations.

19.3.2.5 Ice Cream Spoilage

Ice cream mix is preheated and homogenized to improve texture in the frozen product and then pasteurized. Ice cream mix is pasteurized at 68 °C for 30 min or at 80 °C for 25 s. Flavors and other ingredients (such as color, fruits, candies, flavors, chocolate, and nuts) are generally added after pasteurization. Pasteurized mix is cooled and transferred to an aging tank, where the mix is stored for at least 4 h at temperatures between 2 and 5 °C. This phase is necessary to allow stabilization of the mix, crystallization of the fat, and adsorption of protein onto fat globules. After aging, ice cream mix is frozen at −5 to −8 °C and while mixing, air is introduced into the mix. Volume of the ice cream increases due to incorporation of air from 30 to 100% of the mix volume. Partially frozen mix is packaged and immediately placed in a hardening room or freezing tunnel to freeze at −25 to −30 °C. The freezing prevents microbial growth.

Microbial destruction is extensive during heat treatments of ice cream mix and survivors are primarily spores. Adding ingredients after pasteurization may contribute significant microbial contamination. Another important reservoir of microorganisms is the workers. If ice cream mix is frozen, microbial spoilage does not occur. If there is an uncontrolled storage between pasteurization and freezing, ice cream readily supports microbial growth. Both contamination and temperature abuse of the mix may easily occur during processing. Types of microorganisms contaminating with ice cream are *Streptococcus*, *Micrococcus*, *Pseudomonas*, spore formers, and coliforms. Ice cream may contain spoilage yeasts (such as *Pichia anomala* and *C. versatilis*). Xerotolerant yeasts (such as *Z. rouxii*, *Z. bailii*, *Z. bisporus*, *D. bansenii*, and *S. cerevisiae*) may also cause spoilage on ice cream.

When ice cream is made from raw milk, pathogenic microorganisms may associate with products. Most foodborne outbreaks occur with homemade ice cream due to use of raw milk, cream, eggs, inadequate heat treatment, and contamination from infected handlers. Similar problems also sometimes result from consumption of commercially manufactured ice cream. *Salmonella*, *Listeria*, *Staphylococcus*, *Mycobacterium*, *Campylobacter*, and *Brucella* may associate with ice cream. *Salmonella* are the hazard pathogen of most concern in ice cream. Raw or improperly pasteurized eggs may contribute *Salmonella* (such as *S.* Enteritidis) to ice cream. Subsequent temperature abuse permits growth of *S. aureus* and enterotoxin production. Postpasteurization contamination of microorganisms can occur with product. Contamination of pathogens is probably possible during transport of pasteurized ice cream mix in tanks that are also used to transport unpasteurized raw egg. The ice cream mix is not subsequently repasteurized and pathogens, if present, may survive in ice cream for months. Control measures to prevent microbial contamination and growth include the use of high-quality supplies, excellent sanitation during preparation of the ice cream, protection from recontamination, and use of adequate processing and storage conditions.

19.3.3 Fermented Milk Products Spoilage

Buttermilk, yogurt, and cheese are a few of the fermented milk products that are generally produced by inoculating milk with specific starter culture. They differ in acidity, water activity, susceptibility to spoilage, and storage stability.

19.3.3.1 Cheese Spoilage

Cheese is a ready-to-eat food and easily contaminates with microorganisms from processing and storage environments. Cheese undergoes spoilage with bacteria, yeasts, and molds (Table 19.4). Factors determining the type of spoilage of cheese are processing conditions, water activity, pH, salt, moisture ratio, temperature, characteristics of the lactic starter culture, types and viability of microorganisms, and characteristics and quantities of residual enzymes. The types of spoilage microorganisms and their effects depend on the characteristics of cheese, survival during processing step, contamination during processing, and microorganisms either on the surface or in the interior of cheese. Many bacteria, especially psychrotroph, can contaminate with cheese during processing. Psychrotrophic Gram-negative bacteria can readily spoil the cheese, and cause visual and physical defects, such as green or yellow slime, fruity odors, and putrid off-flavors. Bacterial spoilage may occur in fresh cheese having a sufficiently high pH (such as cottage cheese). Microorganisms can contaminate curd from wash water. Microorganisms can produce gases during processing, at the beginning of ripening, and during ripening periods.

Spoilage by LAB

Some species of LAB produce unwanted flavor in cheese. The use of high ripening temperatures (such as 15 °C rather than 8 °C) encourages growth of heterofermentative *Lactobacillus*. *Lactobacillus brevis* and *Lactobacillus casei* subsp. *pseudoplantarum* can produce gas in cheese. *Lactobacillus casei* subsp. *casei* produces a soft defect in cheese. Some unripened soft cheese with vacuum packaging and storage at refrigerated temperature are spoiled by heterofermentative *Leuconostoc* spp. This is characterized by gas and liquid accumulation in the package. Another common spoilage of ripened cheese is the appearance of white crystalline deposits on the surface. These deposits do not affect flavor, but reduce consumer acceptability. *Lactobacillus* spp. produce higher amount of lactic acid during cheese ripening and

Table 19.4 Types of microbial spoilage in cheese.

Spoilage	Microorganisms	Products
Early gas	Coliforms, yeasts, *Lactobacillus*	CO_2, H_2
Fruity	*Lactococcus*, *Pseudomonas*, yeasts	Ethanol, esters
Late gas	*Clostridium* spp.	CO_2, H_2
Phenolic flavor	*Lb. casei* subsp. *alactosus*, *Lb. casei* subsp. *rhamnosus*	Phenolic compounds
Pink discoloration	*Lb. bulgaricus*, *Leuconostoc*, *Propionibacterium*, *Fusarium culmorum*, yeasts	High redox potential
Rancid	*Pseudomonas*, *Micrococcus*, *Serratia*	Free fatty acids
Slime and off-flavor	*Acinetobacter*, *Enterococcus*, *Bacillus*, *Alcaligenes*, *Pseudomonas*, *Leuconostoc*	Polymeric products
Soft spoilage	*Lb. casei* subsp. *casei*	Organic acids
White crystals	Heterofermentative *Lactobacillus* spp.	Excessive D-lactate
Ropiness	*Micrococcus, Pseudomonas, Serratia, Leuconostoc*	Polymers
Holes in curd	*Bacillus, Pseudomonas, Leuconostoc*	CO_2, H_2

this results in the formation of insoluble calcium lactate crystals. *Lactobacillus casei* subsp. *alactosus* and *Lactobacillus casei* subsp. *rhamnosus* associate with the development of phenolic flavor, similar to that of horse urine after 2–6 months of aging. Fruity off-flavor in cheese is usually caused by LAB (usually *Lactococcus* spp.) with production of esterase. The major esters contributing fruity flavor to cheese are ethyl hexanate and ethyl butyrate.

Early Blowing (Coliforms Spoilage)

If cheese milk contains high number of coliforms (heterofermenters of lactose) surviving during processing or it is contaminated during processing with coliforms, excessive gas formation (CO_2 and H_2) can occur during cheese processing or at the beginning of ripening or storage due to the growth of coliforms. Therefore, this gas is called early gas defect (or early blowing). This defect on cheese or container appears during pressing, salting, or at the beginning of ripening, when lactic acid fermentation fails to rapid lowering of pH. Mold-ripened cheese with increased pH are also susceptible to coliform growth. Approximately 10^7 coliforms per gram are required to cause a gassy defect. Gas formation results with small, spherical, and shiny appearance on the cheese. This defect associates with unclean flavor and aroma. The excessive gas formation can cause blowing of can or container. Besides coliforms, some yeasts, *Bacillus subtilis*, and *Lactobacillus* spp. can also cause early gas defects on cheese. Use of milk with low number of coliforms, sufficient acid development during cheese processing, strict sanitation and hygiene, and adequate heat treatment of milk will control early gas blowing defect.

Late Blowing (Spore Forming Bacterial Spoilage)

Butyric acid bacteria can break down the lactate in cheese during ripening. Butyric fermentation in cheese is most commonly caused by *Clostridium tyrobutyricum*. This defect is also caused by *Clostridium perfringens*, *Clostridium butyricum*, and *C. sporogene*. Butyric fermentation is a type of spoilage in cheese. This fermentation produces excessive H_2 and CO_2 gases, and causes abnormal cheese flavor due to butyric acid. Gas pressure builds up within the cheese, disrupting the structure of cheese with holes and cracks and destroying the texture and eventually rupturing the can or container. This gas defect occurs during ripening period of cheese, mostly after 10 days (therefore called late blowing). *C. tyrobutyricum* is more frequent in winters and is present in milk from cows fed silage, while *C. butyricum* is more frequent in summers. Even 10 spores of *C. tyrobutyricum* per liter of milk result in late blowing. This type of spoilage occurs in cheese due to relatively high pH, high moisture, low interior salt level, and survival of spores during processing conditions. The contamination of *C. tyrobutyricum* with milk occurs from silage, but also frequently during the cheese processing. The use of wooden cheese vats and utensils may harbor bacterial spores. Anaerobic bacterium does not grow well in milk, but may grow in the anaerobic environment within large cheese blocks. This spore-forming bacterium is resistant to standard pasteurization temperatures and heat treatment activates spore germination.

Spoilage by Psychrotrophic Bacteria

Psychrotrophic bacteria (such as *Pseudomonas*, *Alcaligenes*, *Achromobacter*, and *Flavobacterium*) have primary importance in cheese spoilage since these bacteria produce very active proteolytic and lipolytic enzymes. The presence of psychrotrophic bacteria in cheese indicates postprocess contamination since these bacteria are sensitive to pasteurization. These bacteria grow rapidly at normal refrigeration temperature (7 °C) and produce lipases. Lipases can lipolyze fats in cheese. Lipolysis produces short-chain fatty acids that combine with ethanol to form fruity esters. *P. fluorescens*, *P. fragi*, and *P. putida* cause bitterness, putrefaction, rancid odor, liquefaction, gelatinization, and slime formation on cheese. The most common bacterial spoilage is "slimy curd" caused by *Alcaligenes* spp. (such as *A. viscolactis*). *Alcaligenes metacaligenes* causes flat and poor flavor spoilage in cheese. *Flavobacterium* spp. produce yellow flavin pigments that discolor cheese. Psychrotrophic *Bacillus* species cause bitterness and proteolytic changes, and the product is dark pigmented. *P. fluorescens* causes discoloration because of the formation of water-soluble fluorescent pigments. Other *Pseudomonas* spp. also causes darkening or yellowing of the cheese surface. Yellow discoloration of cheese surface may be attributed to production of flavin pigment by *Flavobacterium* spp.

Spoilage by Fungi

Molds, especially from the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Monilia*, *Mucor*, *Penicillium*, and others, lead to undesirable changes in cheese; *Penicillium* most frequently occurs on cheese. *Penicillium*, *Mucor*, and other molds grow well on cheese and produce stale, yeasty, and musty flavors. During the first days of ripening, yeasts and/or molds (such as *Debaryomyces hansenii*, *Geotrichum candidum*, and *Penicillium camemberti*) colonize on the surface of cheese and utilize lactate. This leads to deacidification of the cheese surface, enabling the growth of less acid-tolerant bacteria (such as *Arthrobacter arilaitensis*, *Brevibacterium aurantiacum*, *Brevibacterium linens*, and *Corynebacterium casei*). Development of visible molds on the surface of cheese is often the first sign of spoilage, followed by the appearance of musty off-taints and odors. Molds from the raw materials can play a major role in spoilage. Some molds can grow under low oxygen tension. Molds commonly present in vacuum-packaged cheese include *Penicillium* spp. and *Cladosporium* spp. Cream cheese are susceptible to spoilage by heat-resistant molds (such as *Byssochlamys nivea*). *B. nivea* grows in reduced oxygen atmospheres, as well as in atmospheres containing 20, 40, and 60% CO_2 with less than 0.5% oxygen. Once this mold presents in the milk supply, it can be difficult to eliminate during normal processing of cheese. The *D* value of *B. nivea* ascospores in milk and cheese is 1.3–2.4 s at 92 °C, respectively.

Yeasts most frequently associated with cheese spoilage include *Candida*, *Pichia*, *Yarrowia lipolytica*, *G. candidum*, *K. marxianus*, and *D. hansenii*. The low pH and the nutritional profile of most cheese are favorable for the growth of yeasts. The characteristic yeast spoilage is gassiness, odors, off-flavors, and slime formation. Surface moisture, lactic acid, peptides, and amino acids favor rapid growth of yeasts on cheese. Many yeasts produce alcohol and CO_2, resulting in yeasty taste. Yeasts can produce large amounts of gases (such as CO_2) in vacuum- and modified atmosphere-packed cheese. This can cause swelling of packages. Some proteolytic yeast produces sulfides, resulting in an egg odor. Yeasts cause spoilage, especially in the fresh or soft cheese during storage. Yeasts also grow on the surface of ripened cheese. If the surface becomes wet, yeasts can cause slime formation. A "fermented yeasty" flavor in cheese results from growth of *Candida* spp.

Yeasts and molds can contaminate with cheese during processing from air, brine, equipment, the environment (floors, walls, ventilation ducts, etc.), ingredients (such as fruit concentrates, cereals, honey, chocolate and cocoa, nuts, or spices), and stabilizing agents (such as thickeners). Molds and yeasts contamination can be prevented during ripening by rigorous cleaning procedures, supplying sterilized air through filtration or ultraviolet treatment of room. In countries where there is legal allowance, natamycin or sorbic acid may be incorporated into the packaging to prevent cheese spoilage.

Other Spoilage

Hard-ripened cheese (such as cheddar) can have a bitter taste due to rapid production of bitter peptides during ripening. Amines (such as histamine and tyramine) can be produced from the decarboxylation of the respective amino acids tyrosine and histamine. Decarboxylase enzymes can be produced by amine-positive mesophilic LAB or by secondary microflora of the cheese (such as *Enterococcus* and coliforms). Lyses of the microbial cells release the enzymes during the ripening process and can cause decarboxylation of amino acids.

Cheese is more susceptible to spoilage by bacterial lipases than proteinases, because lipases are concentrated along with the fat in the curd. Proteinases are removed with the whey fraction during cheese manufacture. The acidic environment of most cheese limits but may not eliminate proteinase and lipase activities. Some cheese (such as camembert and brie) increase in pH to near neutrality during ripening. Psychrotrophic lipolytic and proteolytic bacteria cannot grow in cheese because of the low pH, salt content, and low storage temperature, but they can grow in high-moisture fresh cheese (such as cottage cheese). Psychrotrophic bacteria have strong diacetyl reductase enzyme, which reduces diacetyl to acetoin and other reducing products. This reduction causes loss of desirable flavor of cheese.

Pathogens and Toxins

Several factors influence the presence and survival of pathogens in cheese, namely, characteristics of the pathogens (such as heat, acid, and salt tolerance), the initial number of pathogens, physiological condition of cheese, and the steps of cheese processing. However, cheese is the vehicle for several foodborne pathogens. The principal high-risk pathogenic microorganisms with cheese are *Salmonella*, *S. aureus*, *L. monocytogenes*, and *E. coli* O157:H7. Medium-risk species include *Y. enterocolitica*, *B. abortus*, *M. bovis*, *C. burnetii*, and *A. hydrophila*. *B. melitensis* can present in fresh cheese made from unpasteurized milk, most commonly in Mediterranean countries. Several outbreaks of salmonellosis due to the consumption of contaminated cheese can be attributed to faulty control of the cheese-making process or use of contaminated raw milk with serovars of *Salmonella* (such as *S.* Heidelberg, *S.* Muenster, *S.* Typhimurium, *S.* Newport, and *S.* Enteritidis). Milk from cows suffering mastitis is a significant source of enterotoxigenic *S. aureus*. High *S. aureus* populations ($>10^6$ cfu per gram of cheese) associate with the production of enterotoxin. *C. perfringens* and *C. botulinum* spores may be present in milk and survive pasteurization, but conditions in the cheese prevent germination of spores. Improper pasteurization of cheese milk allows survival of *L. monocytogenes*. The higher incidence of *L. monocytogenes* associates with soft and mold-ripened cheese. Mold-ripened cheese have high moisture levels, high pH due to lactate metabolism by molds, and susceptibility to surface contamination during the ripening process. *L. monocytogenes* is able to multiply during the manufacturing processes of cheese. Slicing and repacking of cheese can contribute cross-contamination of microorganisms. *E. coli* O157:H7 is relatively acid tolerant and this is confirmed with the cheese-making process.

Common mycotoxin-producing molds in cheese include Penicillium, Aspergillus, and Fusarium. Mycotoxin productions are affected by different mold growth conditions, including substrate composition, physiological factors (such as temperature, water activity, pH, and oxygen concentration), and biotic factors (such as strain-dependent mycotoxin production). Many types of cheese are excellent substrates for the growth of molds. Different mycotoxins occur in cheese at highly variable concentrations depending on cheese origin. Contamination of mycotoxins with cheese can occur indirectly with milk contamination or directly by mycotoxin-producing spoilage molds. The main indirect contaminant is AFM1 in cheese. AFM1 in the final product is due to the addition of this mycotoxin into milk by dairy animals that have fed on moldy AFB1 contaminated feedstuffs. AFB1 is converted into AFM1 by enzymes mainly present in the animal liver and then passed into their urine and milk. Low storage temperature of cheese can decrease AFM1 concentration. The indirect contamination of cheese with other mycotoxins can also occur depending on the process conditions of cheese. Direct mycotoxin contamination can occur exogenously from the growth of molds during cheese processing and endogenously from the presence of mycotoxin-producing commercial mold cultures. However, mold growth on the cheese surface does not indicate presence of mycotoxins in cheese. Mycotoxin production depends on types of mycotoxicogenic molds and processing conditions. Mold optimum growth temperature cannot correlate with mycotoxin production temperature. Contamination of cheese by molds is detrimental to cheese quality, appearance defects, off-flavors, and formation of toxic secondary metabolite. The most common mycotoxins stable in cheese are aflatoxins, citrinin, cyclopiazonic acid, roquefortine, sterigmatocystin, and mycophenolic acid. Others, including patulin, penicillic acid, and penitrem toxin, are not produced by molds in cheese due to the microaerophilic conditions. The efficient ripening cultures, selection of nonmycotoxigenic strains, cold storage, and packaging conditions can prevent the growth of molds and contamination of mycotoxins.

Cheese can contain proteins, enzymes, cofactors, water, salt, and bacteria, thus representing an ideal environment for biogenic amine production from free amino acids by decarboxylase enzymes of microorganisms during cheese ripening. Amino acid decarboxylases are not widely distributed among bacteria. Some species of bacterial genera can produce decarboxylase enzymes, such as *Bacillus*, *Citrobacter*, *Clostridium*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Shigella*, *Photobacterium*, and LAB. *Lactobacillus*, *Pediococcus*, and *Streptococcus* can decarboxylate one or more amino acids. The following conditions are necessary for the decarboxylation of amino acids:

- Availability of free amino acid, but not always leading to amine formation
- Presence of decarboxylase-producing microorganisms.
- Conditions allowing bacterial growth, decarboxylase synthesis, and decarboxylase activity

Biogenic amines are organic substances in food, which induce toxicological and health risks. Histamine, tyramine, cadaverine, putrescine, and 2-phenylethylamine can be formed in many kinds of cheese. They induce toxicological risks and serious health hazards when they present in cheese in significant amounts or ingested in the presence of potential factors, such as amine oxidase-inhibiting drugs, alcohol, and gastrointestinal diseases. Not all amines are equally toxic; histamine, tyramine, and 2- phenylethylamine are more major toxic biogenic amines. Many factors affect the formation of biogenic amine in cheese, such as availability of free amino acids, pH, water activity, salt and moisture levels, temperature, bacterial number, synergistic effects between microorganisms, and the presence of decarboxylase-producing microorganisms. Large amounts of biogenic amine in cheese indicate a failure in a hygienic condition, quality of milk, and processing. The enzymes monoamino oxidase and diamino oxidase play an important role in the detoxification of biogenic amines.

19.3.3.2 Butter Spoilage

Butter contains around 15% water, 81% fat, 0.4% carbohydrates, 0.6% proteins, and 2.5% ash, and can be salted or unsalted. Cream for butter production is heated at 71 °C for 30 min or at 93 °C for a few seconds. The microbiological quality of butter depends on the quality of cream and the sanitary conditions used in the processing. The main source of microorganisms for butter is cream, milk, equipment, water, and processing conditions.

Buttermilk, cream, and butter can be spoiled by psychrotrophs, coliforms, yeasts, and LAB. Bacteria can cause five types of spoilage in butter: First, the surface taint (putrid), which is caused by *Shewanella putrefaciens*, *P. putrefaciens*, and *Flavobacterium* spp. at chilling and refrigerating temperatures within 7–10 days. Second, the rancidity of butter, which is caused by hydrolysis of butterfat with liberation of free fatty acids. Rancidity and fruity odors are caused by the growth of *P. fragi* and *P. fluorescens*. Third, flavor formations—Malty flavor (due to formation of 3-methylbutanol) is caused by *Lac. lactis* subsp. *lactis* var. multigenes. Diacetyl can be reduced by diacetyl reductase enzymes produced by *Pseudomonas*, *Lactococcus*, and coliforms in these products, leading to a "green" or yogurt-like flavor from an imbalance of the diacetyl to acetaldehyde ratio. Coliforms and *Enterococcus* can grow in water phase of butter and produce flavor defects. Cheesiness is caused by *Lactobacillus*, rancidity by lipolytic bacteria and molds, barny flavor by *Enterobacter*, yeasty flavor by dissolving metals in high-acid cream, unclean flavor by coliforms, fishiness by *A. hydrophila*, and ester-like flavor by *P. fragi*. Chemical reactions (oxidation of unsaturated fats, rancidity, and trimethylamine formation from lecithin) can also produce flavors. Fourth, discoloration caused by growth of molds, yeasts, and bacteria on the surface of butter. Dark, smoky, or greenish color on butter are caused by *Alternaria* and *Cladosporium*, small black color by *Stemphylium* and *P. nigrificans*, green color by *Penicillium*, bright reddish-pink color by *Fusarium culmorum*, and black color by *Torula* spp. Fifth, the formation of excessive viscosity in buttermilk and sour cream with the growth of encapsulated, slime-forming *Lactococcus*.

Yeasts grow in butter with low a_w and low pH. They can produce off-flavors (such as fermented or yeasty). They can metabolize diacetyl in buttermilk and sour cream, thereby leading to a yogurt-like flavor. Contamination of *G. candidum* often results in a decrease of diacetyl content in low-fat cheese at 4–7 °C. Lipolytic yeasts (such as *Rhodotorula*) may grow on the surface at chill temperatures and may tolerate high salt concentrations. Other yeasts associated with spoilage of butter include *Candida lipolytica*, *Torulopsis*, and *Cryptococcus*.

Rancid spoilage in butter may result from lipolysis of fat by residual heat-stable microbial lipases originating from the raw milk or cream. Rancidity can also occur in butter due to growth of lipolytic psychrotrophic bacteria in butter. Butter is a water-in-fat emulsion. If salt and moisture are not evenly distributed in the product during processing, lipolytic psychrotroph will grow in pockets of water and produce lipases in butter. When processing conditions disrupt the fat globule membranes, it increases the probability of enzyme–substrate interactions. In the production of butter, lipolysis of fats in cream can cause excessive foaming during churning of cream, hence increasing the time of churning. On the other hand, when butter is manufactured from rancid cream, low molecular weight free fatty acids will be removed with the watery portion of the cream (buttermilk), so the resulting butter will not have a typical rancid flavor. Growth of psychrotrophic bacteria in butter occurs only if the product is made from sweet rather than ripened (sour) cream. Sweet cream butter is preserved by salt and refrigeration.

The desired flavor in butter depends on a combination growth of starter cultures *Lactococcus* (lactic acid producers) and *Leuconostoc* (diacetyl producers). Imbalance of the culture growth, improper temperature, extended ripening time, infection of the culture with bacteriophage, presence of inhibitors, and a microbial contamination can lead to an unsatisfactory product. Buttery flavor mainly depends on *Leuconostoc mesenteroides* subsp. *cremoris*. This bacterium converts acetaldehyde to diacetyl, thus reducing the "green" or yogurt-like flavor. A diacetyl to acetaldehyde ratio of 4:1 is desirable for a good butter flavor, whereas the green flavor appears when the ratio is 3:1 or less. Proteolysis by the *Lactococcus* is necessary for the growth of *L. mesenteroides* subsp. *cremoris* and citrate is needed as substrate for diacetyl production.

Pathogenic bacteria can survive and grow in butter and cream. The presence of staphylococcal enterotoxin A is possible in butter since enterotoxin may be formed in the cream used to make the butter and is carried over into the finished product. The growth and survival of *S. aureus*, *L. monocytogenes*, and *C. jejuni* in butter and cream is possible due to the use of contaminated ingredients, poor hygiene, low salt concentration (or inadequate salt dispersal), insufficient heat treatment of cream, and temperature abuse. *L. monocytogenes* may grow slowly from 4 to 13 °C in the butter. AFM1 can contaminate with butter. Most of the mycotoxins present in the cream would be removed with the buttermilk and very little will remain in the butter.

19.3.3.3 Yogurt Spoilage

Yogurt generally has a low pH (with about 1% lactic acid) and is not spoiled by most bacteria. The acid also restricts the growth of food-poisoning bacteria. So, whereas milk is a potential source of food poisoning and has a shelf life of only a few days, yogurt is safer and can be kept for longer time under proper storage conditions. During storage of yogurt, the yogurt bacteria can continue to produce acids and cause an objectionable sharp acid taste. *Bacillus* is the predominant spoilage bacterium in yogurt, followed by *Lactobacillus*, *Streptococcus*, and *Actinomyces*. In general, coliforms cannot survive at high acidity. The presence of coliforms indicates the poor sanitary conditions during production of yogurt.

Yeasts (especially in fruit yogurt) may grow in the acid environment and produce CO_2 as well as yeasty and fruity off-flavors. Yeasts are major spoilage organisms of yogurt in which the low pH provides a selective environment for their growth. Yogurt produced under hygienic conditions with good manufacturing practices should contain no more than 10 cells per gram. However, yogurt having initial yeast counts of >100 cells per gram tend to spoil quickly. Yeasty and fermented off-flavors and gassy appearance are detectable when yeasts grow up to 10^5–10^6 cells per gram. Galactose results from lactose hydrolysis by the lactic starter cultures that are fermented by galactose-positive species of yeasts (such as *S. cerevisiae* and *Hansenula anomala*). The most common yeast spoilage is caused by *S. cerevisiae*. The other yeasts causing spoilage are *Trichosporon brassicae*, *Cryptococcus curvatus*, *K. marxianus*, *Trichosporon cutaneum*, *D. hansenii*, *Pichia farinosa*, *Candida blankie*, and *G. candidum*. In a higher temperature, the growth of yeasts is stimulated, leading to the gas production and aroma and flavor formation in the yogurt. Especially, galactose-positive yeasts can cause blowing in the fruit yogurt packages. The most commonly associated yeasts in yogurt are *K. marxianus* and *S. cerevisiae*. In addition, *Saccharomyces exiguous*, *Rhodotorula glutinis*, *Yarrowia lipolytica*, and *Debaryomyces hansenii* likely cause quality problems in yogurt stored at relatively higher temperatures (such as 15–20 °C). Some metabolites (such as acetic acid) produced by yogurt starter bacteria can show inhibitory effect against yeasts. The major sources of yeasts in yogurt are ingredients that are added into the product after heat treatment. Additionally, the equipment and the surface of packaging machines are potential sources of yeast contamination. The yeasts can grow more easily at high acidic and low-temperature conditions than mesophilic and psychrotrophic bacteria. An average generation time of yeasts at all storage temperatures is 1 h. Traditional concentrated yogurt is more prone to yeast contamination from the air as this product is produced by removing free water from the plain yogurt through gravity drainage at ambient temperature. To overcome this, addition of potassium sorbate (>300 mg kg^{-1}) or sodium benzoate (up to 400 mg kg^{-1}) inhibits yeasts.

Yogurt provides two significant barriers to spoilage and pathogenic bacteria: (a) heat and (b) acidity (low pH). Both are necessary to ensure a safe product. *L. monocytogenes* and E. coli O157:H7 are easily destroyed by pasteurization. Acidity alone does not provide protection against E. coli O157:H7 that is acid tolerant. Some strains of *L. monocytogenes* are able to adapt to acidity and remain in yogurt during storage period. Therefore, milk must always be pasteurized to make yogurt. The survival of these bacteria in yogurt results from underprocessing.

Molds are aerobic microorganisms and require oxygen. Therefore, the molds grow on the surface of the yogurt stored for longer time. The most common molds growing on yogurt are species of *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium*, and *Alternaria*. The level of mold contamination should not exceed 10 cfu per gram of the yogurt. Aflatoxin produced by molds pose risk to human health. Aflatoxin can remain in yogurt throughout the storage period. AFM1 present in milk can be recovered in yogurt because it tends to associate with the casein fraction. AFM1 may adversely affect the growth and acid-producing capacity of yogurt bacteria. Also, the cell morphology of the yogurt bacteria may be deformed by AFM1. In contrast, Aflatoxin B1 has limited effect on the metabolic activities of yogurt bacteria and is largely degraded during fermentation. Other aflatoxins (B2, G1, and G2) can remain in yogurt throughout the storage period without being degraded. The spores of *Aspergillus flavus* and *Aspergillus parasiticus* show great resistance against storage conditions of yogurt.

L. bulgaricus is principally responsible for the production of carbonyl compounds, and *S. thermophilus* is mainly responsible for the development of acidity during yogurt fermentation. Any factor disturbing the growth of these bacteria adversely affects the establishment of aroma and flavor balance in the yogurt. To obtain yogurt with a well-balanced aroma and flavor characteristics, the incubation temperature and inoculation level of yogurt bacteria should be selected properly. As the yogurt bacteria are thermoduric, the incubation temperature of fermenting milk should be incubated at 41–43 °C. Too high or too low starter inoculation levels cause aroma and flavor defects in yogurt, and therefore the level of inoculation should be designed as 2.5–3.0% of milk. Too fast cooling after fermentation also results in aroma and flavor defect in yogurt. Therefore, in order to avoid this problem, two-phase cooling is recommended. Excessive sourness is another major flavor problem in yogurt. Improper storage conditions (such as high storage temperature), high starter inoculation level, and too slow cooling are the principal reasons of defects in yogurt. Cooked flavor develops in yogurt as a result of the heat treatment of milk at high temperatures. In this respect, the heating temperature of yogurt milk should not be higher than 90–95 °C for 5–10 min.

The most widely appearing problems are dry surface and heterogeneous color distribution on the surface of the yogurt. The first problem results from the evaporation of water from the yogurt during cold storage. This problem can be prevented by using a proper packaging material. Growth of yeasts and molds in yogurt causes heterogeneous color distribution on the surface of yogurt stored at higher temperatures. To avoid this problem, proper hygienic measures should be taken. Yogurt milk should be clarified before being produced to yogurt so that any solid materials coming from raw milk are removed completely. Otherwise, unclean appearance may develop in the end product. Bubbles in the coagulum may indicate the coliform contamination, the products with bubbles should be discarded and the sources of contamination should be eliminated effectively. Less likely, a crystal-like structure may be seen on yogurt surface resulting from keeping the product at subzero temperature (at 2–4 °C). Adding milk-based powders into yogurt milk to increase the total solid content is a common practice in yogurt making. Poor-quality powders may not be dissolved properly at the bottom of the yogurt. Mixing the powder with milk at 39–40 °C and passing the milk through a stainless steel mesh can eliminate particulates at the bottom. Major body and texture defects that can occur in yogurt are weak body and whey separation. The most important technological parameter determining the physical stability of yogurt gel network is total solid standardization. In some countries, the use of stabilizer to improve the textural quality of set-type yogurt is prohibited. Increasing the total solid level of yogurt milk to 15.5–16.0% prior to fermentation is a common practice. Insufficient total solid leads to weak body and wheying-off. On the contrary, addition of too much skimmed milk powder or caseinate powder into yogurt base results in a too firm body with increasing whey separation. Similarly, heat treatment contributes desired textural structure to yogurt. Heat treatment of yogurt base at lower temperatures than the usual heating at high temperatures (80–85 °C for 20–30 min or 90–95 °C for 5–15 min) causes textural defects due to incomplete denaturation of whey proteins.

Yogurt spoilage can also be due to unclean equipment, contaminated milk, or poor hygiene of the production staff. All equipment must be thoroughly scrubbed, sterilized with diluted bleach, and thoroughly rinsed in clean water before production starts to prevent microbial contamination. Producers must wash their hands before starting work and anyone with stomach complaints, cough, or skin infections should not work in the yogurt production.

19.4 Preservation of Milk and Milk Products

19.4.1 Asepsis

Spoilage caused by growth of psychrotrophic bacteria on fluid milk depends on contamination levels of raw milk, cooling rate of raw milk after milking, and maintenance of raw milk at cold temperatures. Bacteria can be limited by cleaning udders before milking and using cleaned and sanitized equipment. Removal of residual milk solids from milk contact surfaces is critical to psychrotroph control, since these residues protect cells from the action of chemical sanitizers and provide nutrients for growth. The growth over a period of a day results in a biofilm in addition to containing high numbers of microorganisms.

Keeping quality is improved when low numbers of microorganisms are present. The bacterial content of milk is used to measure its sanitary quality and historical basis of milk. Packaging prevents contamination of microorganisms. Microorganisms probably enter the filler through the vacuum system. Tanks used for holding pasteurized products before packaging can also be a source of psychrotrophic microorganisms. Tank walls may have microscopic fissures or pits that protect microorganisms from cleaning and sanitizing procedures. Contamination of psychrotrophic microorganisms from the environment with milk can be prevented by aseptic techniques. In this technique, equipment and air that contact with pasteurized product must be free from microorganisms.

19.4.2 Removal of Microorganisms

Centrifugation, clarification, or separation will remove some microorganisms from milk. High-speed centrifugation (at 10,000 g) removes about 99% of spores and vegetative cells plus some proteins. The centrifugal removal of bacteria is known as bactofugation and it is not used extensively on a commercial basis.

19.4.3 Use of Heat

The widely used pasteurization process for milk is first heating at 60 °C for 20 min, then at 61.7 °C for 30 min, and finally at 62.8 °C for 30 min in large tanks or vats. This is not a continuous process and is referred to as vat pasteurization. The use of plate heat exchangers and a continuous operation involves high-temperature short-time (HTST) pasteurization process, at least 72 °C for 15 s. This heat treatment was adjusted to inactivate *Coxiella burnetii*, a rickettsia responsible for Q-fever. The HTST system is the most widely used continuous commercial pasteurization process. Sometimes, it is used at 79 °C for 20–25 s to decrease total microbial load and increase the shelf life of the product. The HTST process is called flash pasteurization.

The objectives of milk pasteurization are to (i) kill all pathogens, (ii) reduce spoilage microorganisms, (iii) inactivate some enzymes, and (iv) improve the keeping quality of milk. Pasteurization kills all yeasts and molds, and most vegetative cells of bacteria in milk. Thermoduric non-spore-forming bacteria surviving at pasteurization are *Enterococcus*, *S. thermophilus*, *L. bulgaricus*, *Microbacterium*, and *Micrococcus*. The surviving spore-forming bacteria are (i) species of *Bacillus*, such as *B. cereus*, *B. licheniformis*, and *B. subtilis* (proteolytic); *Bacillus coagulans* (thermophilic); *B. polymyxa* (gas forming), and (ii) species of *Clostridium*, such as proteolytic (*Clostridium sporogenes*) and saccharolytic (*C. butyricum*) bacteria.

Milk treatment processes can involve UHT system. UHT process is a continuous flow for a holding time 1–3 s over 140 °C, which is labeled as ultrapasteurized. The combination of this heat treatment with aseptic packaging results in "sterilized milk." This is commercial sterility, not absolute sterility. These foods may have a shelf life in excess of 90 days at room temperature.

UHT treatments make products microbiologically stable at room temperature. Adding naturally occurring germinant hippuric acid to raw milk allows germination of sufficient spores before heat treatment and this increases shelf life of heated milk. The use of lysozyme in milk inhibits *Bacillus* spp. Spoilage caused by coliforms and LAB in milk is controlled by good sanitation practices during milking, holding at temperatures below 7 °C, and pasteurization.

Control of coliform growth in cheese is achieved by using pasteurized milk, encouraging rapid fermentation of lactose, and application of good sanitation during manufacture.

19.4.4 Low Temperature

19.4.4.1 Refrigeration

Raw milk spoilage prevention with psychrotrophic bacteria involves limiting contamination levels, rapid cooling after milking and maintaining at cold storage temperatures, limiting by cleaning, sanitizing, and drying cows' udders before milking, and using cleaned and sanitized equipment. Removal of residual milk solids from milk contact surfaces is critical to control of psychrotrophic bacteria, since these residues protect cells from the effects of chemical sanitizers and provide nutrients for growth. Fresh milk enters the farm storage tank at 30–37 °C. Farm storage systems should rapidly cool milk to as low temperature as possible while avoiding ice formation. Sanitary standards require cooling of raw milk to 7 °C within 2 h after milking. Milk is often picked up from the farm every 48 h and only three additional milking will be added to the previously milk-collected tank. The second milking should not warm the previously collected milk over 10 °C. Growth of psychrotrophic bacteria in milk is inhibited at 2 °C. But very low temperature causes disruption of the milk fat globule membrane and making it highly susceptible to lipolysis.

19.4.4.2 Freezing

Ice cream and other frozen dairy products are frozen as part of the manufacturing process and stored at low temperatures in the frozen state. Pasteurization reduces numbers of microorganisms, but freezing kills few microorganisms and storage in the frozen state also permits survival of most of the microorganisms for long periods. Butter in storage is held at −18 °C or lower. Frozen milk can be concentrated by freeze-drying methods. Pasteurized whole milk is frozen at about −29 °C and stored in the frozen state.

19.4.5 Drying

Various milk products are made by removing different percentages of water from whole milk. The reduction in moisture and increased concentration of dissolved substances in milk products inhibit the growth of some kinds of microorganisms. Dry products prepared from whole milk are skimmed milk, cream, whey, buttermilk, and ice cream. Usually the milk is preheated before drying (at 65–85 °C for the roller process and at 69–93 °C for the spray process). This heating can pasteurize the milk and kill the vegetative microorganisms.

19.4.6 Use of Preservatives

The use of preservatives in dairy products is permitted to only a limited extent. The use of sorbic or propionic acid or one of their salts is permitted for some hard and processed cheese and yogurt to prevent the surface growth of molds. Sugar in condensed milk can act as a preservative by reducing a_w. High concentration of sugar is inhibitory to the growth of bacteria. NaCl is added in the manufacture of various kinds of cheese. Most fermented products are microbiologically more stable due to low acidity and production of bacteriocins by LAB.

The major natural microbial inhibitors in raw milk are lactoferrin and the lactoperoxidase system. Natural inhibitors of lesser importance include lysozyme, specific immunoglobulins, and vitamin B_{12} binding systems. Human milk contains over 2 mg of lactoferrin per milliliter and cow milk contains 20–200 mg per milliliter. Lactoferrin, a glycoprotein, acts as an antimicrobial agent by binding iron. Common psychrotrophic aerobic spoilage bacteria in refrigerated milk are inhibited by lactoferrin, but the presence of citrate in cow's milk limits its effect, since the citrate competes with iron for binding to lactoferrin.

Lactoperoxidase enzyme catalyzes the oxidation of thiocyanate (SCN^-) with formation of hydrogen peroxide (H_2O_2). This results in the production of hypothiocyanite ($OSCN^-$). Hypothiocyanite oxidizes sulfhydryl groups of proteins, resulting in enzyme inactivation and structural damage on the microbial cytoplasmic membrane. Lactoperoxidase and thiocyanate are naturally present in milk, whereas H_2O_2 is formed in milk when oxygen is metabolized by LAB or added into milk. H_2O_2 is the limiting substrate of the lactoperoxidase reaction. LAB, coliforms, and various pathogens are inhibited by this system.

The effective antimicrobial agents on *Clostridium* spp. are sodium or potassium nitrate, lysozyme, and H_2O_2. Nitrates are not permitted as additive for cheese in some countries. Lysozyme binds to the casein prior to the clotting of the milk, remains active in the curd throughout the entire ripening process, and can disrupt the Gram-positive cell walls once the spores germinate. Lysozyme by itself does not provide complete protection. Lysozyme retains its activity for more than 2 years in cheese. The use of lysozyme will improve the organoleptic qualities of the cheese through (i) inhibiting production of butyric acid (no off-flavors), (ii) greatly reducing production of gas (less cracks and less openings), (iii) preventing longer ripening time (better flavor and better texture), and (iv) overall better grading of the cheese.

Microbial growth would be inhibited by packaging to reduce oxygen (and/or increase CO_2), cold storage, and the use of antimicrobial chemicals (such as sorbate, propionate, and natamycin). Some molds are resistant to antimycotic additives. Sorbate-resistant molds develop in sorbate-treated cheese. Some *Penicillium* spp. may develop resistant to sorbate. *Penicillium* spp. will degrade cheese by decarboxylation producing 1,3-pentadiene. Some *Mucor* spp. degrade sorbate to 4-hexenol, and some *Geotrichum* spp. degrade it to 4-hexenoic acid. Sorbate can also be used as a carbon source or oxidized to CO_2 and water.

19.4.7 Mechanical Reduction of Microorganisms

In practice, microorganisms and spores would be reduced in the processing of milk products. They can be reduced in milk by a centrifugation process known as bactofugation or microfiltration of cheese milk, particularly in countries where the use of chemical inhibitors is banned.