Dairy Microbiology

Key Terms

Mastitis

Somatic Cells

Microorganisms

Bacteria

Psychrotrophic

Thermoduric

Thermophilic

Endospores

Coliform

Indicator Organisms

Pasteurization

Dairy animals, including: cows, sheep, goats, and buffalo, like humans are natural reservoirs of bacteria. Many of these bacteria are not harmful to humans, but some may be harmful to humans even though the dairy animals are not affected and appear healthy.

- Milk may become contaminated with bacteria during or after milking. ***Mastitis*** is an inflammation of the udder, typically caused by a microbiological infection. Mastitis can occur in all mammals, including humans. These infections can be caused by many types of microbes which can be transmitted from both environmental sources (for example, contaminated water, soil, and bedding) and other infected dairy animals). These microbes enter the udder and multiply. The microbes can enter milk as it passes through the udder during the milking process. Mastitis in dairy animals can be a source of disease causing bacteria (pathogens) and spoilage organisms in milk.

- Milk from dairy animals infected with mastitis generally has higher total bacteria counts and somatic cell counts than milk from uninfected animals. Therefore, bacterial counts and somatic cell counts are used by dairy farmers and processors as indicators of milk quality. In general, the higher the counts, the lower the milk quality. Milk from mastitic dairy animals may have off-flavors and may undergo deterioration of the milk fat and protein more quickly than milk from healthy dairy animals.

- ***Somatic cells*** are cells from the dairy animal (predominantly white blood cells, otherwise known as leukocytes) that are normally present in milk. During most mastitis infections, the number of somatic cells present in the udder increases to help the animal fight the infection. There are several types of somatic cells that have different functions in fighting infection. Somatic cells can contain lipolytic and proteolytic enzymes, which degrade fats and proteins, respectively. An increase in somatic cells count during a mastitis infection increases the amount of destructive enzymes present in the milk, which increases the rate of deterioration of the milk fat and protein. This effect can cause reduced yield as well as flavor and/or odor defects.
  - There are specific tests and electronic procedures designed to detect somatic cells.
    - eg. DeLaval Cell Counter (DCC, DeLaval Inc., Kansas City, MO)
      - Some varieties of animals have specific tests (eg. Pyronine Y Methyl-Green for goat and sheep milk.)
      - International standards vary.

Microorganisms in Dairy Products

Microorganisms are small forms of life which cannot be seen with the naked eye. Examples of microorganisms include: **bacteria**, yeast, mold, protozoa, and viruses.

Why are we concerned with microorganisms in dairy products?

- **Public Health Concerns**
  - Organisms that cause illnesses; preventing contamination and/or survival
- **Regulatory Standards**
  - Designed and implemented to protect public health
- **Dairy Product Quality**
  - Keeping consumers happy
  - Preventing spoilage
  - Proper dairy starter culture

Microbial Standards for Raw and Pasteurized Milk

Milk	Test	Maximum Limit
Pre-pasteurized milk for Grade A use	Total bacteria	Individual producer not to exceed 100,000/mL[1]
		Commingled not to exceed 300,000/mL[2]
	Somatic cell count	Individual producer not to exceed 750,000/mL
	Drugs	No positive test on drug residue detection
Grade A pasteurized milk	Total Bacteria	20,000/mL
	Coliforms	Not to exceed 10/mL
Raw milk[3]	Total bacteria	30,000/mL
	Somatic cell count	Not to exceed 750,000/mL
	Drugs	No positive test on drug residue detection

[1]Individual producer milk is milk that is still on the farm.
[2]Commingled milk is milk that has left the farm and has been mixed with other individual producer milk in a tank, either during shipment or at the processing plant.
[3]New York State regulations for raw milk intended to be consumed as raw milk.

Bacteria

Bacteria are single celled organisms that can only be seen with a microscope ("microorganisms"). All processes needed for life occur within a single cell. Bacteria are considered *prokaryotes*. Their basic cell structure differs from cells of plants and animals (*eukaryotes*); for example they lack a true nucleus and have a unique cell wall. Bacteria can be found wherever life exists; some are considered useful, such as those responsible for nutrient conversion (e.g., decomposition) and food fermentations (e.g., cheese), while others are harmful, such as those responsible for food spoilage and disease.

Individual bacteria are named by *Genus* and *species* (e.g., *Bacillus cereus*, *Pseudomonas fluorescens*), as are all living organisms. They are classified according to their appearance and structure and by specific characteristics of their metabolism and growth, including nutrient requirements, growth temperatures, oxygen requirements, their ability to use specific substrates (e.g., certain sugars), and by specific by-products of their metabolism. Currently, genetic profiling techniques have become standard tools in the identification/classification of bacteria, often beyond species level (e.g., sub-species, sub-types, allelic types). There are literally thousands of species of bacteria, but only select groups are of concern to the dairy industry. The following will describe the general characteristics important for characterizing bacteria that are common in milk and dairy products.

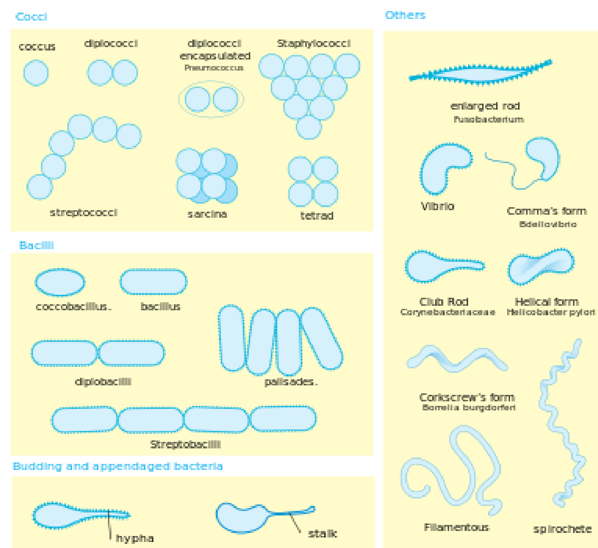
To see bacteria, a microscope is required, generally one with a magnification of 1000X. Bacteria are measured in microns (1 micron = 1/1000 mm = 1/25,000 inch). When a standard light microscope is used, bacterial cells are normally stained to make them easier to see. Bacteria can be observed in milk by staining a dried milk smear on a microscope slide with a specific "milk-stain" (e.g., Levowitz-Weber Stain). Bacteria grown in a petri dish (e.g., on a semi-solid nutrient "agar" media) or in a nutrient broth can be smeared and dried on a slide and stained with a simple stain (e.g., methylene blue) or complex stain (see *gram-stain*, next page) for observation. Bacteria exist in a variety of shapes, sizes and arrangements, which can be defining characteristics.

Typical of what might be seen in milk and dairy products are:

<u>Cocci</u> -Spherical cells, 0.4 - 1.5 microns. Occur as single cells, pairs, chains or clusters.
(e.g., Genera - *Streptococcus*, *Staphylococcus*).

<u>Bacilli</u> -Rod shaped, 0.5 - 30 microns. Occur as single cells, pairs or chains
(e.g., Genera - *Lactobacillus*, *Bacillus*, *Pseudomonas*).

<u>Spirillum</u>- Spiral-shaped rods, 2-30 microns. Occur most often as single cell; rare in dairy.
(e.g., Genus – *Campylobacter* (Spiral).



Ruiz, Mariana. Bacterial Morphology Diagram. Digital image. Wikipedia.N.p., n.d. Web. 8 Aug. 2014.

Gram-Stain Reaction

- Most bacteria are classified as either "**gram-positive**" or "**gram-negative**." This is typically determined by the **gram-stain procedure**, which is used to view and differentiate bacteria under the microscope; it is one of the first steps used when classifying bacteria. The gram-stain is a four step procedure with Crystal Violet (blue) and Safranin (red) as the primary stains. Depending on the characteristics of the bacteria (i.e., different cell wall structures), they will stain either blue (gram-positive) or red (gram-negative). In some cases an organism classified as "gram-positive" may stain red or appear grainy with blue and red shades. These organisms are often referred to as "gram-variable":

- Gram-positive (blue) … e.g., *Bacillus* (rod); *Streptococcus* (cocci); *Staphylococcus* (cocci)
- Gram-negative (red) …. e.g., *Pseudomonas* (rods); *E. coli* & other coliform bacteria (rods)
- Gram-variable … Stain blue or red depending on conditions; most are truly Gram-pos.

- There are a few generalizations based on the gram-stain reaction that can be made of microorganisms common to dairy products. For example, **gram-negative bacteria do not survive pasteurization**; bacteria that do survive are gram-positive (*but not all gram-positive bacteria survive*); certain gram-negative bacteria, if present, will spoil milk faster under refrigeration compared to gram-positive spoilage organisms; specific antibiotics are more effective against gram-positive than gram-negative bacteria.

Psychrotrophic (Psychrotolerant) Bacteria

Psychrotrophic or psychrotolerant bacteria are capable of growth at 44.6°F (7°C) or less. Psychrotrophs are of primary concern to the dairy industry since they grow and cause spoilage in raw or processed dairy products commonly held under refrigeration.

- The most commonly occurring psychrotrophs in milk are gram-negative rods, many belonging to the genus *Pseudomonas*. Gram-negative psychrotrophs generally do not survive pasteurization, thus they occur in processed milk and dairy products as post-pasteurization contaminants (PPC). Some strains of thermoduric bacteria are capable of growth under refrigeration storage (*see below*).

- Psychrotrophs are common in the dairy environment. Milk soils (e.g., on dirty equipment) can support the growth of psychrotrophs and other contaminants that can contaminate subsequent milk. Marginal cooling can result in relatively large numbers of these organisms in milk. Psychrotrophs might also be present in low numbers in untreated water supplies used for rinsing dairy equipment.

- Psychrotrophic bacteria produce a variety of enzymes that cause chemical deterioration of milk resulting in off-flavors. Some of these enzymes are not inactivated by pasteurization or by other heat treatments and may continue to degrade milk products, even when the bacterium is destroyed. This has been shown to be a concern with shelf-stable (Ultra-High Temperature) milk, but there is limited information relative to conventionally pasteurized milk.

Thermoduric and Thermophilic Bacteria

Thermoduric bacteria are a miscellaneous group of bacteria that are capable of surviving pasteurization or other heat treatments. ***Thermophilic*** bacteria thrive at high temperatures. As such, they not only survive heat treatments, but they can also grow. As a general rule, all thermoduric and thermophilic bacteria are gram-positive. Spore-forming bacteria (e.g., *Bacillus, Paenibacillus*) comprise some of the most heat-resistant bacteria.

- Chief sources of thermoduric/thermophilic bacteria in milk are poorly cleaned equipment including old rubber parts, areas of milkstone build-up, separators and other difficult to clean or neglected areas (soil build-up). They may contaminate milk at the farm or at the plant. Poor pre-milking hygiene procedures (e.g., dirty dairy animals) may also influence levels of heat-resistant bacteria in raw milk, especially of spore-formers.

- Very high thermoduric/thermophilic counts in raw milk could result in counts in the pasteurized milk made from that milk that exceed the 20,000 cfu/ml legal limit. This is rare as counts are normally < 500 cfu/ml.

- Although most heat-resistant bacteria are not psychotrophs, some are. In the absence of gram-negative psychrotrophs, certain thermoduric bacteria can grow and cause spoilage in pasteurized milk. Heat Resistant Spore-Forming Psychrotrophs (HRSP), such as strains of *Bacillus* and *Paenibacillus*.

- ***Endospores***, or bacterial "spores," are protective, dormant structures that allow an organism to survive under adverse conditions.
    - When conditions become unfavorable (e.g., lack of nutrients), vegetative growth ("multiplication") stops and "spores" begin to form within the cell.
    - During sporulation a thick coating develops and encases the cell's genetic material. Spores forming inside a cell may be seen as swollen, possibly clear, areas or may not be apparent at all. Special spore stains facilitate seeing spores under the microscope.
    - Bacterial spores released from the cell have increased resistance to heat, drying, nutrient deprivation, chemicals, sanitizers, and other conditions that would normally kill the vegetative, actively growing cell.
    - Spores can remain dormant for extended periods of time (e.g., for years). When conditions become favorable, a spore can "germinate" and return to an actively growing state. Spores may be "activated" into growth by heat or some other "trigger."

    Spores are produced by only a few select groups of bacteria. Bacteria in the genera *Bacillus*, *Paenibacillus*, and *Clostridium* are common gram-positive, spore-forming rods, which have some importance to dairy. Some strains of spore-formers stain gram-variable. Spores are commonly found in soil, manure and other environmental sources

Bacteria of Concern

The bacteria present in dairy products may cause disease or spoilage. Human illness from milk-borne pathogens is usually associated with consumption of raw milk or products made from raw milk such as fresh cheeses.

Because there is a risk of pathogen contamination in milk produced from healthy dairy animals under sanitary milk conditions, pasteurization of milk prior to consumption will destroy pathogens and provide protection for illness associated with consumption of dangerous microbes. Human illness has been linked to pasteurized milk products but these cases usually have been a result of contamination of the product after pasteurization or improper pasteurization.

Coliform bacteria are defined as "aerobic or facultatively anaerobic, gram-negative rods, that ferment lactose with the production of acid and gas." These characteristics allow selective detection and counting of these types of bacteria in milk and dairy products. They are considered "**indicator organisms**" because they are easy to detect and their presence in food & water indicate some form of contamination; e.g., the presence of "fecal" coliforms (*E. coli*) suggests the possibility of fecal contamination. Coliforms ***do not*** survive pasteurization. When detected in processed milk or dairy products, they indicate recontamination after pasteurization.

Sources of Bacteria in Raw Milk Products:

Bacteria from a healthy teats
Flora of mastitic animals
Exterior of animal
Dairy barn environment
Milk contact surfaces: dirty equipment
Inappropriate milk storage time/temperature

Raw milk may also harbor other organisms associated with foodborne illness, including *Salmonella*, *Listeria*, *Campylobacter*, *Yersinia* and certain strains of *E. coli*. These organisms are also killed by pasteurization. However, cross-contamination of processed dairy products with raw milk and/or the direct consumption of raw milk have resulted in relatively recent outbreaks of foodborne illnesses involving these organisms.

Sources of Bacteria in Pasteurized Milk Products:

Recontamination
Personnel: poor hygiene and/or procedures
Milk contact surfaces: dirty equipment
Environment: plant, water, air, etc.
Thermoduric bacteria that survive pasteurization

Pasteurized milk products can also be contaminated by poor processing and handling conditions and/or poor worker hygiene.

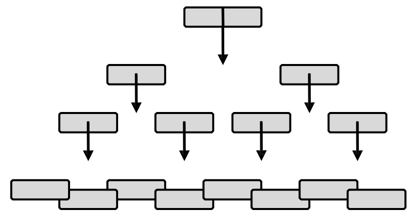
Organisms Common to Milk and Dairy Products

Grouping/Organisms	General Characteristics and Importance to Milk or Milk Products
Gram-Positive Cocci:	
Enterococcus spp.	Short chains or pairs of cells. "Fecal" streptococci (but are not coliform); common in fecal matter, but also in the dairy farm environment. Used as indicator organisms in some foods. Acid producers. Some strains have some heat resistance.
Lactococcus lactis	Short chains or pairs. "*Lactic*" streptococci; produce lactic acid. Some strains are used as "mesophilic" dairy starter cultures. High numbers may occur in poor cooling of raw milk. Some strains produce a "malty" defect in milk as well as acid defect.
Micrococcus spp.	Irregular clusters or tetrads, cells tend to be larger. Some are associated with udder skin. Some strains are thermoduric and are associated with milkstone on equipment.
Staphylococcus aureus	Single, pairs or irregular clusters. A cause of contagious mastitis. May cause food poisoning due to toxin development if present in high numbers in foods.
Streptococcus agalactiae	Chains, often very long. May appear as chains of pairs or with oval cocci stretched in the direction of the chain. Cause of contagious mastitis.
Streptococcus uberis	Pairs and chains of moderate length. Considered a cause of environmental mastitis, although some evidence suggests that it may be spread cow to cow.
Streptococcus thermophilus	Chains, moderate to long. Dairy "thermophilic" starter culture (incubation ~110°F) used for making yogurt and certain cheeses.
Gram-Positive Rods:	
Corynbacterium bovis	Irregular shaped rods, some "club" shaped. Cause of bovine mastitis, although some strains may be natural inhabitants of the skin and mucosal membranes.
Lactobacillus delbrueckii sub-sp. *bulgaricus*	Long rods, some chains. Dairy "thermophilic" starter culture (incubation ~110°F) used for making yogurt and certain cheese.
Microbacterium lacticum	Irregular rods, some "V-Forms." Thermoduric bacterium, some strains with relatively high heat resistance for a non-spore former.
Gram-Positive Rods, Spore-Forming:	
Bacillus cereus	Relatively large, thick rods. Some strains are psychrotrophic. Some strains cause foodborne illness if allowed to grow to sufficient levels (toxin mediated).
Bacillus spp. (other spore-formers)	Many different spore-forming *Bacillus spp.* in milk. Rods vary in size. Some are psychrotrophic, some are not. Some are gram-variable. Most are thermoduric in the spore state, but not as vegetative cells. Common in soil & dairy environment.
Clostridium tyrobutyricum	Anaerobic spore-former that causes "late gas blowing" defect in certain Swiss and Dutch style cheeses. Associated with poor silage and dirty cows.
Paenibacillus spp.	Spore-forming group with psychrotrophic strains important in their potential to survive pasteurization and limit milk shelf-life. Most were previously classified as *Bacillus spp*. Some strains may stain as "gram-variable."
Gram-Negative Rods:	
Pseudomonas fluorescens (also *P. putida*, *P. fragi*)	Rods, often in pairs, end-to-end. Psychrotrophic strains of bacteria that are a main cause of reduced shelf-life due to post-pasteurization contamination.
Escherichia coli (*E. coli*)	"Fecal Coliform" associated with manure/environmental contamination. Used as an indicator organism. Some pathogenic strains (e.g., O157:H7). May cause mastitis.
Coliform Bacteria	*Enterococcus*, *Citrobacter*, *Klebsiella, E. coli*. Associated with fecal/environmental contamination. Some strains are psychrotrophic. Some may cause mastitis. Coliforms are used as indicators of post-pasteurization contamination in milk and other products.

Requirements and Conditions for Microbial Growth

If One Bacterial Cell Reproduced Every Hour, in 24 Hours There Would Be ~17,000,000 Cells

Hour	Count	Hour	Count
0	1	9	512
1	2	10	1,024
2	4	11	2,048
3	8	12	4,096
4	16	:	:
5	32	18	262,144
6	64	:	:
7	128	:	:
8	256	24	~17,000,000



and so on .....

- **Nutrient Requirements**
  - Some microbes have very specific needs, others more general.
    - Energy Source- carbohydrates, proteins, lipids
    - Carbon Source- carbohydrates, proteins, lipids, carbon dioxide
    - Nitrogen Source- proteins, peptides, amino acids, ammonia, nitrates
    - Vitamins- primarily water soluble B-vitamins
    - Minerals, Metal Ions & Salts- potassium, phosphorus, calcium, magnesium, iron

- **Available Water**
  - Reducing available in water in food restricts or prevents microbial growth
  - Standing fluid (water, milk, condensate) supports environment contaminants
  - Most bacteria require a water activity of greater than 0.91 and generally will not grow in or spoil foods that are lacking available water.
    - Some cheeses have water activities less than 0.90 due to concentration and water binding by salts and solutes.
- **Acidity or pH**

0 . . . . 7 14
Acidic Neutral Alkaline

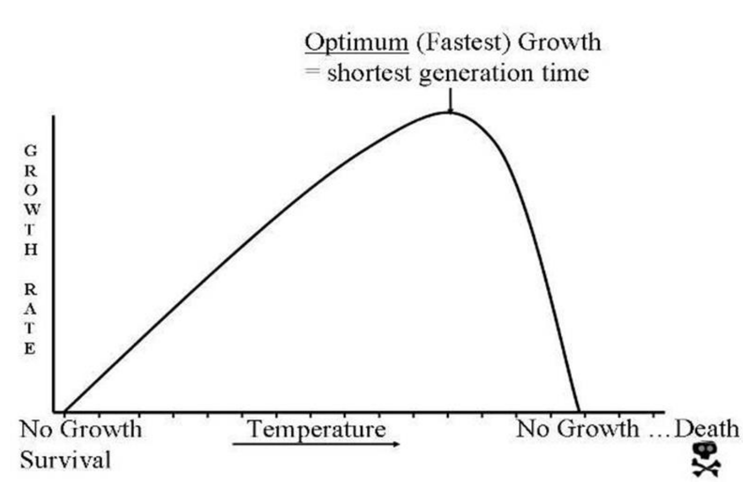
  - Most bacteria grow best at a neutral (7.0) or slightly higher pH, although this varies with different organisms.

Dairy Food	pH Range	Supports Growth of:
Milk	6.6 - 6.7	Many Microorganisms
Cheese	4.8 - 6.0	Some; May Be Selective
Yogurt	3.8 - 4.2	Few; More Selective
Sanitizers	< 2.5	Kills, Limited Survival

- **Presence or absence of oxygen**
  - *<u>Aerobic</u>* - Requires the *presence* of oxygen for growth
    - certain spoilage bacteria (*Pseudomonas, Bacillus*)
  - *<u>Anaerobic</u>* - Requires the *absence* of oxygen for growth
    - spoilage / pathogenic (*Clostridium*)
  - *<u>Facultative</u> <u>Anaerobe</u>* - *Presence* or *absence* of Oxygen
    - certain coliform bacteria (*E. coli, Enterobacter*)
  - *<u>Microaerophilic</u>* - prefer reduced Oxygen levels
    - certain dairy starter cultures, *Campylobacter*

- **Influence of Inhibitors**
  - *Chemical Sanitizers*
    - chlorine, iodine, acid-based, peroxyacetic acid, quats
  - *Drug Residues*
    - inhibition from antibiotics or drugs; may influence dairy cultures
  - *Preservatives*
    - potassium sorbate, nisin, bacteriocins, organic acids (vinegar), carbon dioxide
  - *Competitive Microorganisms*
    - outgrow other bacteria and/or produce inhibitory compounds

- **Temperature**
  - The optimum growth temperature for a bacterium is the temperature at which its generation time is shortest or it grows the fastest. Each bacterium has a minimum and maximum temperature for growth, which will vary between species and strains and with other environmental conditions. Outside of this range, growth does not occur.
  - Bacteria are often grouped based on their optimum, minimum and maximum temperatures for growth. These are not rigid ranges, as some bacterial species may overlap into adjacent groups.



Thermophilic: “Heat Loving”	Min:	104°F (40°C)
	Max:	140°F (60°C) 122-
	Opt:	131°F (50-55°C)
Mesophilic : Medium Temps	Min:	41°F (5°C)
	Max:	122°F (50°C)
	Opt:	86-98oF (30-37oC)
Psychrophilic: “Cold Loving”	Min:	32°F (0°C) or less,
	Max:	77°F (25°C)
	Opt:	<69°F (<20°C)

Destruction and Elimination of Microorganisms

- ***Sanitation Procedures***
  - *Chemical sanitizers* are routinely used to reduce the load of microbial contaminants that may be present on milk/food contact surfaces. Most dairy sanitizers, when used correctly, kill off a broad spectrum of microorganisms. They generally do not "sterilize" equipment. Sanitization procedures should be performed after washing and immediately before processing, although an additional sanitizing step after equipment washing procedures can be helpful (e.g., an acid sanitizer rinse). Most chemical sanitizers are inactivated by organic matter and are ineffective on poorly cleaned surfaces. Sanitizers commonly used in the dairy industry include chlorine, iodine, quaternary ammonium, acid anionics and peroxyacetic acid.

  - *Hot water sanitization* is commonly used in many dairy plants. Hot water sanitization involves circulating water of at least 170°F (77°C) as determined at the outlet, for at least 5 minutes. Higher temperatures (>185°F) for longer times (10-20 minutes) are recommended to allow heat penetration into areas that are hard to reach. Hot water treatments should be followed by a cooling chemical sanitizer rinse or with cooled pasteurized water. Hot water sanitization often provides greater microbial kill and longer milk shelf-life than achieved with chemical sanitizers alone, but must be used with caution for personnel and equipment.

- **Pasteurization**
  Pasteurization is heat treatment that ensures destruction of the vegetative forms of microorganisms.
  - Pasteurization procedures generally kill a large percentage of the bacteria commonly found in raw milk, including pathogenic organisms and those that rapidly cause spoilage. The higher the temperature used, the less time is required for equivalent kill. The most commonly used defined minimum temperature/time combinations are:

	Temperature	**Time**
Vat/Batch Pasteurization	145°F/63°C	30 minutes
HTST	161°F/72°C	15 seconds

[4]See full table of minimum time/temperature combinations in appendices

- These procedures stand as legal definitions of pasteurization and are outlined in the "*Pasteurized Milk Ordinance*" (PMO), the guidance document specifying requirements and standards for Grade "A" milk products.
    - General objectives in the pasteurization of milk are:
        - Ensuring product safety: destroy pathogenic and potentially pathogenic bacteria
        - Keeping product quality: destroy spoilage bacteria and enzymes
    - The majority of fluid milk plants use High-Temperature/Short-Time pasteurization, with temperature/time combinations often exceeding the stated minimum requirement (e.g., 175°F for 20 seconds).
    - Most bacteria that survive pasteurization generally do not grow or else grow slowly at refrigeration temperatures, causing problems later in shelf-life. ***Contamination after pasteurization with psychrotrophic spoilage bacteria is not uncommon***. When post-pasteurization contamination of a product occurs, both the quality and the safety of the product are jeopardized.