Microbiology of Processed Liquid Milk

Milk is a good source of nutrients and edible energy for both mammals and wide range of microorganisms. Depending on milking practices (cleaning, hand or machine milking) and the temperature and period of milk storage on the farm, the numbers and types of microorganisms in raw milk differ. Milk drawn aseptically contains fairly low level of microorganisms (i.e., less than 10^3 cfu ml–1), and soon after it leaves the udder, it becomes contaminated by spoilage and/or pathogenic microorganisms (often reaching $>10^6$ cfu ml–1). This eventually leads to losses in quality of milk and milk products, as well as threatening public health. A total colony count of more than 10^5 cfu ml–1 of milk indicates poor sanitation of milk production.

Thermization

Thermization of milk is typically achieved by heat treatment at relatively low temperatures (i.e., at 63–65°C for 15–20 s or at 57–68°C for 15 s). The main purpose of thermization is (a) to kill psychrotrophic bacteria, which may release heat-stable proteases and lipases into milk, (b) to extent storage time of raw milk prior to processing, and (c) to enhance keeping quality of the end product. Thermized milk can be stored at maximum 8°C for up to three days. Low temperature inactivation of microorganisms offers advantages for processing UHT milk as well as cheese-making, i.e., preventing blowing in cheese containers. Despite its positive effects on extended storage time of raw milk prior to processing into milk products,

thermization is not a fully reliable tool for ensuring food safety as it cannot eliminate milk-borne pathogens completely. Listeria monocytogenes , for example, can withstand thermization and may well grow in chilled-stored thermized milk Similarly, the effect of thermization on the viability of *Mycobacterium bovis* and *Coxiella burnetii* is far limited.Therefore, thermization should not be considered as an alternative treatment to pasteurization. In case of applying thermization prior to pasteurization, the germination of *Bacillus cereus* spores is stimulated, simplifying their inactivation by further pasteurization. This process is termed “tyndallization”

Pasteurization

Pasteurization is aimed to make milk and milk products safe by destroying all the vegetative pathogenic microorganisms. The time and temperature conditions of pasteurization are designed to destroy *Mycobacterium tuberculosis* and *C. burnetii* which are known as the most heat-resistant pathogens in milk. Pasteurization process is performed in two ways: batch and continuous operations. In batch operations, milk is heated at lower temperatures (i.e., 63–65°C) for longer times (i.e., 30 min). This process is also called low-temperature long-time (LTLT) process. In continuous pasteurization systems, milk is subjected to heat treatment at higher temperatures (i.e., 72°C) for shorter time (i.e., 15 s). This process is called high-temperature short-time (HTST) pasteurization. In continuous system, the milk is passed through a plate heat exchanger and kept in holding tubes. Pasteurization systems are designed to provide a 5 log reduction of the microbial load of raw milk Although pasteurization

aims to destroy the most thermo-tolerant pathogen, *C. burnetii* , due to the differences in activation kinetics of different strains in raw milk, the changes in pasteurization conditions may be considered. For example, a slight increase in pasteurization temperature and/or a slight extension of holding time (i.e., 20–30 s) is recommended to inactivate heat-resistant strains of *L. monocytogenes , E. coli* and *Campylobacter spp.*

Extended shelf-life (ESL)

Milk Ultra-pasteurization (i.e., 120–135°C for 1–4 s) is one of the approaches to extend the shelf-life of processed liquid milk. It is a continuous heat treatment between HTST pasteurization and UHT sterilization. The extended shelf-life (ESL) milk is defined as ‘‘milk showing a negative reaction to the peroxidase test’’ and must be labeled as ‘‘high temperature pasteurized milk’’. A broader definition made by was as follows: “ESL products are products that have been treated in a manner to reduce the microbial count beyond normal pasteurization, packaged under extreme hygienic conditions, and which have a defined prolonged shelf-life under refrigeration conditions”. Ultra-pasteurization is applied to milk before or after packaging. All microorganisms including vegetative cells and endo-spores are destroyed after this treatment. The aroma and flavor of ESL milk is slightly less preferable by the consumers comparing to the HTST pasteurized milk. Commercial ESL milk has a shelf-life varying from 30 days to 90 days at 4°C. The combination of microfiltration and pasteurization has proved to be a very efficient way of producing ESL milk. Microfiltration is a common membrane technology employed in removing raw milk microflora and improves organoleptic properties of dairy products. Cross-flow microfiltration is operated at lower temperatures and makes efficient control of microbial growth possible, leading to extending shelf-life of liquid milk

Sterilization (in-container and UHT)

High heat treatment applied in the manufacture of long shelf-life liquid milk is called ‘‘sterilization’’. In this process, all pathogenic and non-pathogenic microorganisms and spores are destroyed, as well as most milk enzymes. Therefore, sterilized milk can be stored for several months at ambient temperatures.

In-container sterilization of milk, the temperatures vary from 105°C to 120°C for 20–40 min. In this process, milk is filled into cans or bottles, closed with crimped metal cap and then sterilized in a batch or continuous retort. The aim of this process, also called conventional sterilization, is to ensure a 9 log reduction in the spores of thermophilic bacteria or 12 log reduction of Clostridium botulinum. The major drawback of in-container sterilization is the development of cooked flavor triggered by Maillard reaction in milk.

Microflora of processed liquid milk

Factors determining shelf-life and quality of pasteurized milk include microbiological and enzymatic profile of raw milk, types of microorganisms and spores withstanding heat treatment and types and incidences of microorganisms contaminated to pasteurized milk after processing. Critical level of microbial counts for detectable sensory deterioration in pasteurized milk is 10^6–10^7 cfu ml–. Pasteurized milk may contain two groups of microorganisms: spoilage or pathogenic. Major source of spoilage microorganisms in pasteurized milk is the post-pasteurization contamination. Heat-resistant bacteria surviving pasteurization are

another source of microorganisms in processed liquid milk. Gram-negative psychrotrophic bacteria are the most common post-production contaminants in pasteurized milk. These bacteria widely contaminate milk during filling.

Gram-negative psychrotrophs show certain degrees of resistance against sanitation solutions and may well-colonize on stainless steel surfaces and rubber gaskets that contact with milk. Shelf-life of contaminated pasteurized milk varies depending on contamination level, storage temperature and time, and generation time of contaminant microorganism. *Serratia, Enterobacter, Citrobacter, Hafnia, Pseudomonas, Alcaligenes and Flavobacterium* are among the most common spoilage microorganisms in pasteurized milk. *Pseudomonas spp.* can spoil pasteurized milk stored at 4 or 7°C when they are present in milk at level of 10^3 cfu ml^{-1}. Common species of Pseudomonas isolated from pasteurized milk include *P. fluorescens , P. putida , P. maltophilia , P. aureofaciens , P. cepacia and P. pseudomallei.* Psychrotrophs can grow very rapidly under refrigeration conditions; thus, very low level of initial contamination of these bacteria may shorten the shelf-life of pasteurized milk. Common sensory problems caused by Pseudomonas spp. in pasteurized milk are development of rancidity, bitterness, fruity and unclean off-flavors, as well as coagulation. Among the thermoduric Gram-positive bacteria with standing pasteurization conditions are *Microbacterium spp*., *Micrococcus spp*., *Enterococcus spp., Streptococcus spp., Lactobacillus spp. and Corynebacterium spp. Coryneform, Micrococci and Streptococci* cannot grow in milk stored below 6°

Sources of contamination in pasteurized milk

Process equipment

The primary source of contamination of pasteurized milk is filling equipment. The psychrotrophic bacteria usually penetrate to the filler from vacuum or bulk tanks. In the case that the tanks are used for storing pasteurized milk prior to filling, the tanks may well be a source of contamination as well. Microscopic gaps on tank surfaces decrease the efficiency of cleaning and sanitation. Especially *Pseudomonas spp*. are prone to adsorb on tank and plate surfaces. Some strains of Pseudomonas spp. form polysaccharide fibrils and colonize on tank surfaces. An efficient sanitation is needed to remove these colonies from the surfaces. Highly hydrophobic spores of some Bacillus spp. (i.e., B. *thermoleovorans, B. coagulans, B. pumilis*) and *Geobacillus stearothermophilus* adhere on the stainless steel surfaces and when the conditions are suitable, the spores germinate and then coloniz.

Air

Bacteria occupy the largest stake of air microflora (i.e., 85% of total air flora) which are mainly Gram-positive types, followed by molds (~10% of total flora) and yeast (~5% of total flora). Air enters into plants through ventilation system, floor drains, personnel and other openness. Products are exposed to contamination by air mostly during packaging. Besides, residue of product in areas which are not cleaned properly is also an important source of air contamination. Although the numbers of psychrotrophic bacteria in air is fairly low, it may still pose a problem in the processed mil. The generation time of Gram-negative psychrotrophic bacteria is 4 to 5 hours and the number of this group of bacteria can reach at 10^7 cfu ml–1 within 7–10 days at 7ºC. Milk containing psychrotrophic bacteria at level of <10 cfu ml^{-1}, is spoiled within 7–11 days under refrigerated storage.

Packaging materials

Production conditions of packaging materials have been improved greatly during the last decades. The number of bacteria associated with unit carton surfaces is <5000 cfu cm–2. Gram-positive bacteria are the most frequently present group of bacteria on ESL milk package . Generally, flavored pasteurized milk is spoiled faster than unflavored pasteurized milk. It was demonstrated that the chocolate powder used in the production of chocolate-flavored pasteurized milk stimulated the growth of bacteria in milk but it did not introduce additional microbes into the milk. It was reported that the growth of *L. monocytogenes* in chocolate milk was more pronounced than skim and whole milk and whipping cream.

Table 5.2 The comparison of different heat treatments to milk.

	HTST pasteurization	Ultra-pasteurization (ESL milk)	UHT
Reference enzymes	Phosphatase (–) Lactoperoxidase (+)	Phosphatase (–) Lactoperoxidase (–)	Phosphatase (–) Lactoperoxidase (–)
Storage conditions	Refrigerated	Refrigerated	Room temperature
Packaging	Clean	Ultra-clean or aseptic	Aseptic
Shelf-life	10–14 days	30–60 days	>6 months
Flavor	Little cooked flavor	Mild cooked flavor	Definite cooked flavor
Lactulose (mg l^{-1})	Trace	20–≤40	80–500
Furosine (mg g^{-1} protein)	Trace	200	400–1200
α-lactalbumin denaturation (%)[a]	~5	~5	~30–80
β-lactoglobulin denaturation (%)[b]	~13	~22	~60–100
Immunoglobulin denaturation (%)	~67	~100	~100

[a]Assuming concentration in raw milk: 1200 mg l^{-1}
[b]Assuming concentration in raw milk: 3000 mg l^{-1}
Adapted from Lewis and Deeth (2009)

Table 5.1 Types of heat treatment applied to milk and their effects on microbial population.

Process	Process conditions	Heat exchanger	Target	Typical microbial quality
Thermization	57–68°C/15 s	PHE	Psychrotrophs	3–4 log reduction in psychrotrophs
Pasteurization	63°C/30 min	Batch (Vat)	Vegetative pathogens	Total plate count <100,000 cfu ml^{-1}
Pasteurization	72–74°C/15–30 s	PHE	Vegetative pathogens	Total plate count <100,000 cfu ml^{-1}
Heating for yogurt milk	85–95°C/2–30 min	PHE/THE	All pathogens and most spores	-
Preheating for producing milk powder	70°C/5 min or 135°C/1 s	PHE/THE/Other	Depends on treatment	-
Ultra pasteurization (ESL)	120–130°C/<1–5 s	PHE/THE	Vegetative bacteria and most spores	-
UHT (indirect)	135–140°C/3–5 s	PHE/THE/SSHE	All bacteria and spores	Total plate count <100 cfu ml^{-1} after 15 days at 30°C
UHT (direct)	135–140°C/3–5 s	Steam infusion/injection system	All bacteria and spores	Total plate count <100 cfu ml^{-1} after 15 days at 30°C
In-container sterilization	115–120°C/10–20 min	Batch or retorting	All bacteria and spores	Total plate count <100 cfu ml^{-1} after 15 days at 30°C

After: Kelly et al. (2012)

PHE: Plate heat exchanger; **THE**: Tubular heat exchanger; **SSHE**: Scraped surface heat exchanger