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Dairy Foods Science Notes

The Laboratory Pasteurization Count
- Thermoduric Bacteria in Raw Milk -

Milk Pasteurization. Milk pasteurization was design to provide a minimum temperature and time combination needed to inactivate the most heat-resistant, non-spore-forming, disease causing organism(s) commonly associated with raw milk. Initially the target organism was the bacterium that caused tuberculosis (*Mycobacterium bovis or M. tuberculosis*). In the 1950's, the minimum pasteurization temperature was increased to destroy a slightly more heat-resistant organism that was associated with raw milk, *Coxiella burnetti*, which causes Q-fever. The most common minimum temperatures and times used for the legal pasteurization of milk are:

161°F (72°C) for 15 seconds (High Temperature Short Time or HTST)

145°F (63°C) for 30 minutes (Batch or Vat Pasteurization)

Pasteurization conditions that destroy the "most heat resistant pathogens" ensure that other potential pathogens are killed and have the added benefit of destroying many other types of bacteria, including those that cause spoilage.

Thermoduric Bacteria. While pasteurization is effective in reducing microbial risks, some bacteria survive pasteurization; these are called *thermoduric bacteria*. Most bacteria considered natural to the cow (e.g., natural to skin, teats) as well as most mastitis-causing bacteria are not considered thermoduric. Thermoduric bacteria in milk are most commonly associated with some contamination source. A test that can determine the number of thermoduric bacteria in milk is the Laboratory Pasteurization Count (LPC). The LPC is a simple test that is often used as an indicator of the effectiveness of farm sanitation and hygiene procedures.

The Laboratory Pasteurization Count. The LPC mimics batch pasteurization: a sample of milk (5 ml) is heated to 145°F (62.8°C), held for 30 minutes at that temperature, and then immediately cooled. Bacteria that survive the lab pasteurization step (*thermoduric*) are enumerated using the Standard Plate Count procedure. While HTST and batch pasteurization are considered equivalent for pathogen kill, the ability of various thermoduric bacteria to withstand the two treatments is not necessarily the same. In most cases, however, the LPC provides a good benchmark of the relative numbers of organisms that may survive and be present in a HTST pasteurized milk sample. LPC's are generally much lower than SPC's of unheated milk; for example, raw milk with an SPC of 300,000/ml may have an LPC of less than 1000/ml. Counts >300/ml are generally considered indicative of some source of contamination, reflecting less than desirable milking conditions.

Causes of High LPC's. High LPC's are often associated with chronic/persistent cleaning failures within the milking system. Common causes of high LPC's might include leaky pumps; old and leaky pipe-line gaskets; old, cracked inflations and other rubber parts; and milk stone deposits. The bacteria commonly associated with LPC's tend to be more resistant to hot water cleanings. Build-up of milk residue due to poor cleaning efficiencies or as might be seen in a receiver jar vacuum line or a pipeline dead-end may be major sources of thermoduric bacteria. Significant levels of contamination from soiled cows may also contribute to high LPC's. Most thermoduric bacteria are not capable of significant growth under raw milk storage temperatures (e.g., <45°F) and times (2-4 days) thus their numbers generally do not increase appreciably in a bulk tank or sample.

The LPC, Milk Quality and Shelf-Life. Even though the bacteria detected in the LPC are capable of surviving pasteurization, *most* are not capable of growing under refrigeration storage; they remain dormant, some may even die off. A few strains, especially some very heat-resistant, spore-forming bacteria, are capable of cold storage growth and, if allowed, will eventually grow and cause spoilage (see *Psychrotrophic Spore Forming Bacteria*). However, the LPC does not distinguish between those that can and cannot grow under refrigeration storage; therefore the test is not a good indicator of the potential shelf-life of a milk product.

Provided with support from the NYS Dairy Promotion Advisory Board, dairy farmers dedicated to the production, manufacture & distribution of quality dairy products.

Thermoduric Bacteria in Pasteurized Milk - Technical Information on Bacterial Types:

Note: Following is a technical, summary review of the types of thermoduric bacteria commonly associated with pasteurized milk. It should be noted that some genus/species names have changed in recent years, thus the names provided in older literature may not reflect current nomenclature. An example of this is that some *Bacillus* strains have been reclassified under the genus *Paenibacillus* and others. Strains of *Paenibacillus* found in milk may have been previously recognized as strains of *Bacillus*; some strains of *Paenibacillus* may be newly recognized strains.

Thermoduric Strains: The initial microflora of freshly pasteurized milk usually reflects the Gram-positive thermoduric organisms present in the raw milk. Gram-negative bacteria generally do not survive pasteurization (Cousin, 1982), unless total bacterial numbers in the raw milk exceed the thermal destruction capability of the pasteurization process (Patel and Blankenagel, 1972). Thermoduric bacteria isolated from processed milk products include strains of *Microbacterium*, *Micrococcus*, *Enterococcus*, *Streptococcus*, *Arthrobacter* and *Lactobacillus*; and spore-formers such as *Bacillus*, *Paenibacillus* and *Clostridium* (Olson and Mocquat, 1980; Martin, 1981; Hull et al., 1992; Kikuchi et al., 1996; Ralyea et al., 1998). *Bacillus* (and *Paenibacillus*) species tend to be isolated most often and may present quality concerns. *B. licheniformis*, *B. cereus*, *B. circulans* and *B. subtilis* are species that have been commonly isolated from freshly pasteurized products (Westhoff, 1981; Hull et al., 1992; Ternstrom et al, 1993; Crielly et al., 1994). Specific processing parameters used for pasteurization will affect the relative proportions of bacterial types that survive pasteurization (Cromie et al., 1989). Higher temperatures and/or longer times will tend to select for the spore-forming bacteria. Although farm raw milk is generally considered to be a principal source of thermoduric organisms in pasteurized milk, an improperly cleaned dairy plant processing system may also contribute large numbers; contamination can occur before, during or after the pasteurization process (Te Giffel, et al, 1997).

Thermoduric Psychrotrophs: Most thermoduric strains of bacteria are not capable of reproducing in pasteurized milk under conditions of refrigerated storage. However, psychrotrophic strains of *Bacillus*, *Micrococcus*, *Enterococcus*, *Corynebacterium*, *Microbacterium*, *Arthrobacter* and *Lactobacillus* have been identified (Cousin, 1982; Meer et al., 1991). Strains of *B. cereus* (and related organisms) have been implicated as the cause of "sweet-curdling" of milk and "bitty" cream (Overcast & Atmaram, 1974; Meer et al., 1991). Other milk defects that have been associated with *Bacillus* and other psychrotrophic spore-formers include bitter, yeasty, unclean, and rancid off-flavors as well as coagulation of the milk proteins. Psychrotrophic Gram-positive organisms other than *Bacillus* spp. also may be responsible for limiting the shelf-life of pasteurized milk. In a study that evaluated the dominant microflora of 106 commercial pasteurized milk samples with total bacterial counts exceeding 10^6 cfu/ml after 14 days at 6.1°C, a variety of organisms were found. Although the predominant bacteria were Gram-negative rods, Gram-positive cocci were isolated from 5 of the samples tested. *Bacillus* strains were not found in this study (Kozlowski et al. 1993). In another study (Ternström et al. 1993), strains of *Leuconostoc*, *Lactobacillus*, *Enterococcus* and other unidentified Gram-positive bacteria were found in approximately 7% of milk samples that had numbers of 10^7 cfu/ml after two weeks at 7°C. Most thermoduric psychrotrophs, especially the spore formers, tend to grow slower and/or later in milk products. Therefore they generally cause quality concerns later in shelf-life and only become predominant in the absence of faster growing post-pasteurization contaminants such *as Pseudomonas* spp.

Bacillus cereus as a Potential Foodborne Pathogen. Although commonly present in milk, *B. cereus*, a potential foodborne pathogen, may not grow as well at lower refrigeration temperatures (<5°C) as other *Bacillus* spp. such as *B. polymyxa* and *B. circulans* (Langeveld & Cuperus, 1980; Ternström et al., 1993). In a comparison of microbial numbers of duplicate milk samples held for 3 weeks at 5°C and for 2 weeks at 7°C, it was found that 66% and 86%, respectively, had bacteria counts of $>10^7$ cfu/ml, (Ternström et al. 1993). In product held at 5°C, *B. polymyxa* was isolated from 17% of the milks that had reached 10^7 cfu/ml, whereas *B. cereus* was not isolated. In product held at 7°C, *B. cereus* was isolated from 18% of the samples, suggesting that the increased temperature influenced the relative abilities of the organisms to reproduce. The possibility of foodborne illness caused by *B. cereus* and related organisms cannot be ruled out, especially with longer product sell-by dates and the potential for temperature abuse.

References Page 1: Standard Methods for the Examination of Dairy Products, 15th edition. 1985. APHA.
Murphy, S. C., & K. J. Boor. 2000. Dairy, Food and Env. Sanit. 20:606-611.
Boor. K. J., et al., 1998. Journal of Dairy Science 81:1743-1748.

References Page 2: Adapted from K. J. Boor & S. C. Murphy, 2002. Microbiology of Market Milks. In *Dairy Microbiology Handbook*. John Wiley & Sons, NY. Complete citation of references listed is available on request.

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