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Dairy Foods Science Notes

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SHELF-LIFE OF FLUID MILK PRODUCTS
- MICROBIAL SPOILAGE -
The Evaluation of Shelf-Life

Consumers purchase pasteurized fluid milk with the belief that they are taking home a wholesome, nutritious, good quality product. We all have a definition of quality. To the consumer, quality means that the product tastes good and that it keeps well in their home refrigerator or has a long "shelf-life." *Shelf-life* can be defined as "the period of time that a product can be kept under practical storage conditions and still retain acceptable quality." In the case of pasteurized fluid milk "practical storage conditions" means held under refrigeration or less than 7.2°C (45°F) while "acceptable quality" means that product flavor, odor and appearance are satisfactory to the consumer (no consumer complaints) and that the milk is safe to drink. Current consumption and marketing patterns require that most dairy processors manufacture milk that has a shelf-life of 14 days or more. Some plants are striving for 21 days. Achieving this goal requires stringent processing parameters, rigorous product handling procedures and extreme efficacy in cleaning and sanitation programs.

Quality defects in pasteurized milk products are most often the result of microbial contamination, growth and spoilage. Microbial defects usually become evident in the finished product through shelf-life evaluations or consumer complaints. Though poor quality raw milk can result in defective products, post-pasteurization contamination with psychrotrophic spoilage bacteria is most detrimental. In most cases, product contamination is the result of insufficient cleaning and sanitation of the processing equipment and plant environment. Product contamination may occur even when it appears that a well-designed sanitation and quality control program is in place. In the absence of post-pasteurization, certain strains of microorganisms (i.e. *Bacillus* spp) that are capable of surviving pasteurization and growing under refrigeration (thermoduric psychrotrophs) can eventually grow and cause spoilage, generally later in shelf-life.

Milk product quality is generally determined by sensory, chemical and microbiological analyses. These analyses are used from when the milk leaves the cow to when the final product is consumed. To determine shelf-life potential, fluid milk is most often held at marginal refrigeration temperatures of 6.1-7.2°C (43-45°F) and evaluated by sensory and/or microbiological testing after the desired number of days (i.e. sell-by date plus 2-5 days). Though some prefer to evaluate shelf-life at more ideal holding temperatures of less than 3.3°C (38°F), marginal refrigeration temperatures allow potential product defects and sanitation deficiencies to become more evident. Sensory analyses requires that someone who is familiar with and is sensitive to milk off-flavors smell and/or taste the milk (see *Flavor and Odor Defects in Milk*). This type of evaluation is somewhat subjective, as people differ in their ability to detect off-flavors. In this regard, microbiological analyses can lend more insight into potential quality defects in dairy products. When pasteurized milk shelf-life is reduced due to microbial growth, it is most often the responsibility of the processing plant quality control personnel to determine the cause and source of contamination. Following are microbiological procedures that are used to evaluate pasteurized fluid milk quality and shelf-life.

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MICROBIOLOGICAL PROCEDURES

Standard Plate Count (SPC): Standard Limit 20,000/ml, ***Goal - less than 1,000/ml.*** (SMEDP, 16th/17th ed.) The Standard Plate Count is an estimate of the total number of aerobic bacteria present in a sample that are capable of growth on SPC media when incubated at 32°C (89.6°F) for 48 hours. The theory behind the Standard Plate Count is that individual bacteria (or tight groups or "clumps" of bacteria) will multiply and grow on SPC media to form a visible, countable colony (i.e. a colony forming unit or CFU). Colonies counted are expressed as the number of CFU per milliliter (ml) of milk (946 ml = 1 quart).

Generally the SPC of freshly pasteurized milk is less than 500/ml. Most often this initial SPC represents those bacteria that survive pasteurization (***Thermoduric Bacteria***), though gross contamination after pasteurization can also result in high counts. Initial counts higher than 1000 SPC/ml suggest a potential contamination problem either in the raw milk supply (detected by *Laboratory Pasteurization Count*) or within the processing equipment. ***It is important to note that the regulatory limit for pasteurized milk is 20,000 SPC/ml for as long as the milk is offered for sale.*** Regulatory testing is generally performed on fresh samples held well below 4.4°C (40°F), where the majority of samples tested are in compliance.

SPC Shelf-Life: Standard Limit 20,000/ml, ***Goal - less than 20,000/ml.*** The SPC is often used to evaluate the shelf-life of milk. As milk is held under refrigeration temperatures, bacteria that have the ability to grow under these conditions will increase in numbers as reflected in the SPC. These types of bacteria are referred to as ***Psychrotrophs*** and are defined as those bacteria capable of growth at temperatures at or less than 7°C (44.6°F). In general, reducing storage temperature will slow the growth of all bacteria. Refrigeration storage prevents the growth of non-psychrotrophic bacteria; growth is negligible or virtually stops at the freezing point, even for psychrotrophic bacteria. Most psychrotrophic bacteria that rapidly spoil milk do not survive pasteurization. If present in milk, they generally occur as ***post-pasteurization contaminants*** due to less than adequate sanitation practices.

The SPC of freshly pasteurized milk is not a good indication of the numbers of psychrotrophs present since ***most bacteria that survive pasteurization are not psychrotrophic*** (exception – see *thermoduric psychrotroph* below). Though most psychrotrophic bacteria are detected in the SPC procedure, they are indistinguishable from non-psychrotrophic bacteria. While the initial SPC may be 500/ml, only one (or less) of these bacteria may be a psychrotroph (it only takes one psychrotrophic contaminant per container to eventually cause spoilage). The actual number of psychrotrophs present can be estimated by plating the milk using the SPC procedure and incubating for 10 days at 7°C (44.6°F) instead of 32°C (89.6°F). This is a lengthy procedure and is not routinely used by most. However, in shelf-life milk stored at 6.1-7.2°C (43-45°F), the presence of psychrotrophic bacteria will become evident by an increase in the SPC over time. Non-psychrotrophic bacteria, by definition will not grow at these temperatures and will become insignificant in the overall count. This is shown in the following example:

Days at 43°F	Non-psychrotrophs/ml	Psychrotrophs/ml	Total Count (SPC)/ml
Initial	500	1	501
1	500	10	510
2	500	100	600
3	500	1,000	1,500
4	500	10,000	10,500
5	500	100,000	100,500
6	500	1,000,000	1,000,500

Generally, when the SPC exceeds 10 million CFU/ml the product will become unacceptable due to flavor defects related to bacterial growth and metabolism. The extent and type of spoilage will depend on the

strain(s) of psychrotrophic bacteria present. ***The key to preventing spoilage and extending the shelf-life of a product is to prevent post-pasteurization contamination (PPC) through a well designed quality assurance program***. ***Remember, it only takes one psychrotroph per container of milk to cause spoilage***.

Coliform Bacteria Count: Standard Not to Exceed 10/ml, *Goal - not detectable*. (SMEDP, 16th/17th ed.) Coliform bacteria are used as "indicators" of sanitation during the handling and processing of milk products. The Coliform Count is performed by plating milk samples on Violet Red Bile Agar (VRBA), which is selective for these types of Gram-negative bacteria. The plates are incubated at 32°C (89.6°F) for 24 hours after which dark red colonies are counted. Since VRBA may allow growth of non-coliform Gram-negative bacteria (generally smaller, light colored colonies), any growth would indicate a PPC concern. Certain coliform bacteria originate from the intestinal tracts of warm-blooded animals while others are environmental contaminants. ***Coliforms are killed by pasteurization, thus when present in milk, they are regarded as "indicators" of post-pasteurization contamination as a result of poor sanitation.*** Though the limit for pasteurized milk is 10/ml, the detection of any coliform bacteria suggests that some point in processing has been neglected in regard to effective cleaning/sanitation procedures**.** This procedure is routinely used for fresh pasteurized milk though it can be used in shelf-life evaluations as well. Additional tests may be used to detect low levels of coliform bacteria (see *Stress Tests*). As a rule, the detection of any coliforms in pasteurized milk indicates the potential for a shortened shelf-life.

Psychrotroph Count & Gram-Negative Bacteria Count (SMEDP, 16th/17th ed.). Psychrotrophic bacteria are generally the cause of most shelf-life problems in fluid milk. The standard psychrotroph count is determined by plating the milk using the SPC procedure and incubating for 10 days at 7°C (44.6°F). ***The most common psychrotrophic organisms implicated in the spoilage of fluid milk are Gram-negative rods, primarily belonging to the genus Pseudomonas***. As a rule, Gram-negative bacteria in general do not survive pasteurization. This is why coliform bacteria, which are one type of Gram-negative bacteria, are used as indicators of poor hygiene and post-pasteurization contamination in pasteurized milk products. Detection of coliform suggests the possibility for concurrent contamination with psychrotrophs and/or potential pathogens. The psychrotroph count will detect Gram-negative and Gram-positive psychrotrophs, though Gram-positive organisms rarely cause problems in pasteurized fluid milk (see *Thermoduric Psychrotrophs*). The Gram-negative bacteria count with Crystal Violet Tetrazolium Agar (CVTA) selects for Gram-negative bacteria. Since contamination levels are generally very low, CVTA counts are most useful when used with stress tests.

Thermoduric Psychrotrophs. Thermoduric bacteria are those that survive pasteurization or other heat treatments. Most thermoduric bacteria are not psychrotrophic, though certain bacteria that survive pasteurization are capable of growth at refrigeration temperatures. These are considered ***thermoduric psychrotrophs***. ***Thermoduric psychrotrophs occasionally spoil milk in the absence of Gram-negative post-pasteurization contamination.*** These types of organisms generally grow slower and/or begin growth later, causing problems later in shelf-life. The Laboratory Pasteurization Count (LPC - SMEDP, 16th/17th ed.) is often used to estimate the number of bacteria in a raw milk supply that will survive pasteurization. The LPC is performed by heating raw milk to 62.8°C (145°F) for 30 minutes before plating for the SPC. To detect thermoduric psychrotrophs, milk that is laboratory pasteurized is plated for psychrotrophic organisms (SPC procedure incubated at 7°C for 10 days) as well as for the standard SPC. Alternatively, the heated milk can be stored at 7°C for 10+ days and then plated for SPC. A significant increase over the initial LPC indicates the presence of thermoduric psychrotrophs. Some of the most common thermoduric psychrotrophs are spore-formers (e.g. *Bacillus*), which are more heat resistant and may require higher heat to cause spore germination. An alternative to the LPC is to heat milk at 80°C (176°F) for 10 minutes followed by rapid cooling. The milk can then be plated for psychrotrophs or the milk itself can be stored under refrigeration for 10-17+ days and then plated with the SPC. Any significant growth would indicate

potential spoilage by spore-forming psychrotrophs. Heating larger sample volumes (i.e. 200 ml) for LPC or psychrotrophic spore counts and holding under refrigeration will help detect low-level contamination.

Stress Tests. In most cases, Gram-negative psychrotrophic spoilage organisms re-contaminate product at very low levels, often less than 1 per ml, below the level of detection of most plating procedures. This still presents a major concern because one psychrotrophic bacterium with a doubling time of 6 hours can spoil a quart of refrigerated milk in less than ten days (counts of greater than 10 million CFU per ml). The SPC procedure generally is not sensitive enough to detect low-level contamination in line-samples or finished product. The number of psychrotrophs may only be a small proportion of the total SPC and are indistinguishable from non-psychrotrophic bacteria with this procedure. Therefore, finding low-level contamination is a difficult task. It generally requires a large sample size and an incubation period or "stress test" which selects for psychrotrophic organisms and allows them to increase to detectable levels.

Another advantage of stress tests is that they allow resuscitation of "injured" microorganisms, which can repair themselves during milk storage, allowing subsequent growth and possible spoilage. Microorganisms in milk may be in an injured state due to previous exposure to heat, drying, chemical sanitizers or other environmental stresses. Injured organisms are less able to grow in selective media such as VRBA or CVTA. Repair mechanisms, however, allow impaired bacteria to grow in microbiological media that may otherwise inhibit them.

To detect low-level contamination and/or injured microorganisms, a number of modified tests or stress tests, have been developed. Commonly used tests include:

Mosely Keeping Quality Test. Incubate product 7 days at 7°C. Evaluate with SPC, Coliform or Gram-negative (GN) bacteria count. Any significant increase in SPC or any coliform or GN counts, would indicate potential for reduced shelf-life. Thermoduric psychrotrophs may require 10 days or longer to show an increase.

Preliminary Incubation Pasteurized Milk Test (Virginia Tech Shelf-Life). Incubate product at 21°C for 18 hours. Evaluate with SPC, modified SPC (21°C for 48 hrs) or GN bacteria count. Significant increases in SPC or evidence of GN indicates potential for reduced shelf-life. Test should be correlated with in-house shelf-life evaluations. Thermoduric psychrotrophs may not show an increase with this time/temperature combination.

Preliminary Incubation VRBA Test for Coliform Bacteria. Incubate the 30-50 ml of product at 37°C for 6 hrs. Evaluate with the standard VRBA procedure for coliforms. Alternatively store packaged milk at room temperature for 24 hours. Any coliform or Gram-negative growth would suggest PPC and potential for reduced shelf-life.

Further information on shelf-life testing can be found in *Dairy Practices Council* (DPC) Guideline # 10.

Summary. The quality and shelf-life of pasteurized fluid milk is dependent on the quality of the raw milk and other ingredients used and on an effective cleaning and sanitation program. A shortened shelf-life is most often due to inadequacies in cleaning/sanitizing programs that are likely to result in recontamination after the pasteurization process with organisms that grow under refrigeration and are capable of spoiling milk. Some of these organisms grow relatively rapidly in milk resulting in the breakdown of milk components and subsequent conversion to compounds detected as off-flavors.

In the absence of post-pasteurization contamination, one of the most limiting factors for increasing shelf-life (or sell-by dates) are certain organisms that survive the pasteurization process and have the ability to grow and cause spoilage under refrigeration (thermoduric psychrotrophs). These organisms under proper refrigeration generally only cause defects later in shelf-life (i.e. >14 days). Because of eliminating these organisms from the raw milk supply may not be practical, extending sell-by dates beyond 21 days is not

recommended, even under the best of processing conditions. The likely occurrence of these organisms that survive pasteurization and eventually cause spoilage can be minimized through proper raw milk production and handling procedures, from the farm to the plant.

References:

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