Microbiology of primary food commodities

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Milk and milk products

Microbiology of milk and milk products

- Raw milk
- Pasteurized milk
- Dried products
- Butter
- Frozen dairy products
- Concentrated products
- Fermented dairy products

Raw milk

- Microbiota of milk from healthy cows
  - Micrococcus, Staphylococcus, Lactic acid cocci
    (Lactococcus, Streptococcus)
  - Counts usually <10^3/ml
- Microbiota of milk from cows with mastitis
  - Contagious mastitis
    - Staphylococcus aureus, Streptococcus agalactiae
  - Environmental mastitis
    - Coliforms, Pseudomonas, other streptococci
  - Counts >10^3 in the bulk tank (if not separated)
Raw milk

Sources of microorganisms
- Cow surfaces (hides, udders)
  - Organisms from manure, soil, feed, water
    - Gram negatives and Gram positives
  - Few microorganisms in the teat sinus
    - Gram positive
- Uncleaned equipment and utensils
  - Gram-positive thermoduric organisms
  - Thermoduric bacteria affect the microbiological quality of pasteurized milk

Raw milk

- Increase in microbial counts in raw milk is usually due to psychrotrophs
  - Pseudomonas, Flavobacterium, Alcaligenes
  - Some coliforms
- Spoilage of refrigerated milk consists usually of bitter, rancid, fruity flavors
  - Due to putrefaction
  - Caused by psychrotrophs
- Spoilage of milk at room consists usually of souring
  - Due to fermentation
  - LAB

Raw milk

Food safety issues
- Most outbreaks of Campylobacter enteritis are associated with consumption of raw milk
- M. tuberculosis and M. paratuberculosis are of concern in raw milk
Pasteurized milk

- Initial microbiota consisting of thermoduric and sporeforming organisms
- Types and numbers of bacteria depend on the microbial load before pasteurization
- Common thermoduric organisms
  - *Bacillus, Micrococcus, Lactococcus, Microbacterium, Corynebacterium, Arthrobacter*
  - All Gram positive

Spoilage

- Usually associated with Gram-negative psychrotrophs
  - Bitter, rancid, fruity, or unclean flavor
- Gram-positive, psychrotrophic sporeformers (*Bacillus spp.*), can grow and cause spoilage (sweet curdling)

Microbiological standards for raw and pasteurized milk

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Raw milk</th>
<th>Pasteurized milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Cooled to ≤ 10°C within ≤ 4 h of the commencement of the first milking, and to ≤ 7°C within 2 hours of completion of milking. Blend temperature after first and subsequent milkings must not exceed 10°C</td>
<td>Cooled to and maintained at ≤ 7°C</td>
</tr>
<tr>
<td>APC</td>
<td>100,000/ml before commingling, 300,000/ml after commingling</td>
<td>20,000/ml o gm</td>
</tr>
<tr>
<td>Coliforms</td>
<td>N/A</td>
<td>≤ 10/ml</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>N/A</td>
<td>350 mU/ml</td>
</tr>
</tbody>
</table>

Grade “A” Pasteurized Milk Ordinance: 2001 Revision
Temperature/time chart for milk pasteurization
(from Grade "A" Pasteurized Milk Ordinance)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>63°C (145°F)*</td>
<td>30 minutes</td>
</tr>
<tr>
<td>72°C (161°F)*</td>
<td>15 seconds</td>
</tr>
<tr>
<td>89°C (191°F)</td>
<td>1.0 second</td>
</tr>
<tr>
<td>90°C (194°F)</td>
<td>0.5 seconds</td>
</tr>
<tr>
<td>94°C (201°F)</td>
<td>0.1 seconds</td>
</tr>
<tr>
<td>96°C (204°F)</td>
<td>0.05 seconds</td>
</tr>
<tr>
<td>100°C (212°F)</td>
<td>0.01 seconds</td>
</tr>
</tbody>
</table>

* Increase temperature by 3°C (5°F) if milk contains ≥ 10% fat or any added sweeteners

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**Phosphatase test**

**Scharer’s Method**

\[
\text{Diphenyl disodium phosphate} \xrightarrow{\text{Alkaline phosphatase}} \text{Phenol} + \text{2,6 dichloroquinone chloroimide} + \text{Indophenol (blue color)}
\]

**Rutgers Method**

\[
\text{Phenolphthalein monophosphate} \xrightarrow{\text{Alkaline phosphatase}} \text{Phenolphthalein} + \text{H}_3\text{PO}_4 + \text{NaOH} \xrightarrow{\text{Pink color}}
\]
**Pasteurized milk**

- Standards are not useful for predicting keeping quality
- Shelf life is considered to be related to contamination occurring post pasteurization
- The Mosseley Keeping Quality Test is better at predicting shelf life

**Mosseley Keeping Quality Test**

- A sample of freshly packaged milk is held at 7°C (45°F) for 5 days
- After this holding period, a Standard Plate Count is performed to determine the microbiological condition of the sample
- Use of charts to predict the shelf life of milk at a selected level of confidence and temperature of storage

**Relationship of Bacterial Counts to Shelf-life at 4.4°C**

<table>
<thead>
<tr>
<th>Confidence Level (%)</th>
<th>Days of Shelf-life</th>
<th>≥10</th>
<th>≥15</th>
<th>≥20</th>
<th>≥25</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td></td>
<td>813</td>
<td>42</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>6,650</td>
<td>355</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>15,800</td>
<td>4,560</td>
<td>244</td>
<td>----</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>519,000</td>
<td>28,300</td>
<td>6,530</td>
<td>82</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>2,480,000</td>
<td>135,000</td>
<td>7,330</td>
<td>395</td>
</tr>
</tbody>
</table>

*Colonies per ml after storage for 5 days at 7°C.*
Relationship of Bacterial Counts to Shelf-life at 7°C

<table>
<thead>
<tr>
<th>Confidence Level (%)</th>
<th>Days of Shelf-life</th>
<th>( \geq 8 )</th>
<th>( \geq 12 )</th>
<th>( \geq 16 )</th>
<th>( \geq 20 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>*273</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>90</td>
<td>2,430</td>
<td>145</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>80</td>
<td>33,400</td>
<td>2,040</td>
<td>123</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>70</td>
<td>220,000</td>
<td>13,500</td>
<td>824</td>
<td>49</td>
<td>----</td>
</tr>
<tr>
<td>60</td>
<td>1,100,000</td>
<td>67,090</td>
<td>4,150</td>
<td>253</td>
<td>----</td>
</tr>
</tbody>
</table>

*Colonies per ml after storage for 5 days at 7°C.

Moseley Keeping Quality Test

Tables can also be used as guidelines for establishing shelf-life goals.

- Example: a shelf-life of 20 days is desired at 4.4°C (40°F) with 70% assurance that the sample will keep for 20 days, a goal of 6,530 bacterial colonies/ml of milk should be maintained.

Modified Early Detection Test

- Test to determine potential shelf-life of pasteurized milk
  - Sterilize screw cap test tube
  - Add 1 ml of sterile 5.5% sodium desoxycholate
  - Add 9 ml of milk sample
  - Add 1 ml of sterile Trypticase Soy Broth (TSB)
  - Add 1 ml of sterile 0.005% resazurin solution
  - Tighten cap and mix by inverting
  - Incubate tube at 32°C for 16 hours
  - Invert tube once and read color
Modified Early Detection Test

- **Results:**
  - No color change (purple)
    - Milk of good keeping quality with very low psychrotrophic contamination and an expected shelf-life of 12 to 14 days
  - Pink color
    - Milk with moderate psychrotrophic contamination and fair to poor keeping quality
  - White color
    - Heavily contaminated milk with expected poor keeping quality

Dried dairy products

- Milk
- Skimmed milk (nonfat dry milk)
- Buttermilk
- Whey
- Cheese
- Certain fermented products

Dried dairy products

- Shelf stable due to low $a_w$
- Preheating reduces some microorganisms, mostly psychrotrophic Gram negatives, coliforms, yeasts and molds
  - Their presence in dry product indicates contamination post drying
- Typical microbiota include thermoduric micrococci, thermoduric streptococci, corinebacteria and aerobic sporeformers
- Depending on the temperature used for processing there are:
  - Low-heat, medium-heat or high-heat products
- Their microbiological quality depends on the quality of the raw product
Dried dairy products

- Dry products are often used as ingredients
- When used for direct consumption after rehydrating, food safety concerns emerge
  - *Salmonella* outbreaks associated with consumption of dried milk
  - *S. aureus* poisoning in desserts including dry milk then temperature abused

Butter

- Made by creating a water-in-oil emulsion by churning cream
  - Churning causes a phase inversion of cream
  - Water is forced into the lipid structure
  - Moisture is dispersed as fine droplets throughout butter
  - Droplets must be small and evenly distributed
  - If uneven distribution of water, areas of higher water content may permit bacterial growth
- May be salted or unsalted
- May or may not be added with starters
  - *L. lactis*, *L. cremoris*, or *Leuconostoc* spp.

Butter

- Sources of microorganisms:
  - Raw materials, equipment and utensils and work environment
- Yeasts and molds can grow on the surface of butter causing spoilage
  - *Geotrichum*, *Candida*
  - Discoloration, off flavors
- Cream may be pasteurized
  - Reduction of heat-sensitive, vegetative cells
  - Surviving bacteria may cause spoilage
    *Pseudomonas*, *Streptococcus*
    - off flavors (putrid, rancid, fishy, malty)
Butter

Food safety issues
- Outbreak of *S. aureus* poisoning in 1970
- Growth of *S. aureus* in butter was affected by salt concentration and storage temperature
- Temperature abuse of cream can lead to outbreaks
  - *S. aureus* can grow and produce toxin in the cream before butter making

Frozen products

- Ice cream
- No microbial growth at freezing temperatures
- Low microbial counts if good-quality ingredients are used
- Pathogens can survive
- Several outbreaks of *Salmonella* infection and *S. aureus* poisoning have been reported

Concentrated products

Products:
- Evaporated milk
- Condensed milk
- Sweetened condensed milk
Process:
- Pasteurization, preheating, evaporation, cooling
- Microbiota includes thermoduric and spore-forming bacteria
  - *Bacillus*, *Micrococcus*, *Lactobacillus*, *Corynebacterium*, *Microbacterium*, *Streptococcus*, *Arthrobacter*
  - Aw is high enough to support microbial growth (must be refrigerated)
- Sweetened products are different: canned and concentrated.
  Spoiled by osmophilic yeasts such as *Torula*
Fermented products

Starter cultures
- Added to modify aroma and texture
- LAB are commonly used but not the only starters
- Some starters will produce acid
  - Propionibacterium shermanii in Swiss cheese
- Some starters will coagulate proteins
  - Thickening of yogurt by Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus
- Some starters will produce a specific aroma
  - Penicillium roquefortii in roquefort cheese

Fermented products

- Cheeses
- Fresh or unripened
  - Cottage, Cream, Mozzarella, Neufchatel, several Mexican-style cheeses
- Ripened
  - Soft surface ripened
    - Camembert, Brie
  - Semisoft
    - Muenster, Gouda, Edam, Roquefort, Blue
  - Hard
    - Cheddar, Swiss, Ementaler, Gruyere
  - Hard-grating
    - Romano, Parmesan

Fermented products

Cheeses
- Microbial spoilage limited by combined effect of salt, acid and bacterial activity
- Some cheeses may permit growth of anaerobic sporeformers, causing gassy spoilage
- Fresh cheeses have a pH >5.0 and aw >0.9 therefore may be spoiled by Gram-negative psychrotrophic bacteria
  - Pseudomonas, Flavobacterium, Alcaligenes
Fate of *Salmonella* during fresh cheese making

<table>
<thead>
<tr>
<th>Milk used</th>
<th>Whey</th>
<th>Curd</th>
<th>Milk</th>
<th>Days of storage of end product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Pasteurized</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>


Antagonism of LAB against 3 pathogens

10 strains each of 3 LAB
13 strains each of 3 pathogens
Upper circles: LAB conc. 1 log lower than pathogens
Lower circles: LAB conc. 1 log higher than pathogens


Fermented Milk Products

- **Yogurt**
  - **Cultures**
    - *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*
  - **Symbiotic growth**
    - *L. delbrueckii* subsp. *bulgaricus* stimulates *Streptococcus thermophilus* down to pH 4.6-4.8
    - *Streptococcus thermophilus* stimulates *Lactobacillus bulgaricus* to pH 4.1-4.3
Fermented Milk Products

- Yogurt
  - Heat treatment of milk
    - 185°F (85°C) - 30 min
    - 195°F (90.5°C) - 3-5 min
  - Inoculation
    - Bulk starter 1-5%, from frozen concentrate or frozen concentrate as recommended

Fermented Milk Products

Food safety issues:
- Outbreaks have been associated with cheeses
  - *Salmonella* serovar Zanzibar in goat milk cheese
  - *L. monocytogenes* in Mexican-style cheese
- No outbreaks associated with yogurt
- *Salmonella* and *S. aureus* die off during cheese aging
  - Due to antagonistic activity by starters, salt and acid