Microbiology of Food Preservation

Alejandro Castillo
Texas A&M University

Methods of food preservation

- Cold storage
- Heat processing
- Reduced $a_w$
- Acidification
- Atmosphere modification
- Addition of chemical preservatives
- Ionizing radiations

Cold Storage

- Freezing
- Refrigeration
Freezing

- Needs previous conditioning
- Freezing is applied to previously packaged foods

Vegetables
- Selecting/sorting
- Washing
- Cutting (if any)
- Blanching
- Packaging

Meats, poultry, seafood, eggs etc.
- Trimming
- Packaging

Blanching
- Enzyme inactivation
- Fixing green color in some vegetables
- Easing packaging of leafy vegetables (by wilting)
- Displacing of air from plant tissues
- Reduction of microbial counts
Freezing

- Fast freezing
  - Lower temperature to -20°C within 30 min
  - Blast freezing or immersion
- Slow freezing
- Lower temperature to -20°C within 3-72 h
- Home freezers

Effects of freezing on microorganisms:

- Sudden mortality immediately on freezing
- Continued mortality rate decreases gradually during storage
- The lower the temperature the lower the bacterial decline

Refrigeration

- The lower the temperature the lower the growth rate
- How can psychrotrophs grow at refrigeration temperatures?
**Psychrotrophs and psychrophiles**

- Greater content of unsaturated fatty acids
- Slower metabolic rate
- Better membrane transport
  - Still active at low temperatures
- Production of larger cells
  - Additional RNA
- More efficient flagella synthesis
- Favored by aeration
- Greater heat sensitivity
- Usually do not grow above 35°C
  - Inactivation of respiratory enzymes
  - Leakage of intracellular constituents

**Heat processing**

- Pasteurization
- Sterilization

**Pasteurization**

<table>
<thead>
<tr>
<th>Types of milk pasteurization</th>
<th>Acronym</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low temperature long time</td>
<td>LTLT</td>
<td>63°C/30 min</td>
</tr>
<tr>
<td>High temperature short time</td>
<td>HTST</td>
<td>72°C/15 s</td>
</tr>
</tbody>
</table>
| Ultrahigh temperature        | UHT     | 90°C/0.5 s;
                                    |         | 94°C/0.1 s;
                                    |         | 100°C/0.01 s |
Milk pasteurization
- Originally designed to target *Mycobacterium tuberculosis*
- *Coxiella burnetti* is currently the target organism

**Pasteurization**
- Thermoduric organisms
  - Organisms capable of surviving pasteurization
  - Examples: lactobacilli, micrococci, streptococci, lactococci
  - Pathogens are not thermoduric

**Factors affecting heat resistance of microorganisms**
- Water
- Fat
- Salts
  - Salts that reduce $a_w$ (Na$^+$, K$^+$)
  - More protective than those that increase $a_w$ (Ca$^{2+}$, Mg$^{2+}$)
- Carbohydrates
- pH
Factors affecting heat resistance of microorganisms (cont.)

- Proteins
- Numbers of microorganisms
- Age or microorganisms
  - Old cells (stationary phase) more resistant than younger cells (log phase)
- Growth temperature
- Inhibitory compounds
- Time and temperature
- Ultrasonic effect

Canning

- Processing of food products in hermetically sealed containers
  - Metal
  - Glass
- Canned products are commercially sterile

Spoilage organisms associated with canned products

- Mesophilic
  - Putrefactive anaerobes
    - Clostridium spp.
  - Butyric anaerobes
    - C. butyricum
  - Aciduric flat sour
    - Paenibacillus polymyxa
- Lactobacilli
- Yeasts
- Molds
Spoilage organisms associated with canned products (cont.)

Thermophilic

- Anaerobic sulfide producers
  - Desulfotomaculum nigrificans
- Flat-sour spores
- Geobacillus stearothermophilus,
  Bacillus coagulans
- Anaerobic non-sulfide producers
  - Thermoanaerobacterium
  - thermosaccharolyticum

Canning

<table>
<thead>
<tr>
<th>pH category</th>
<th>Examples of foods</th>
<th>Spoilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low acid</td>
<td>Meats, seafood,</td>
<td>Non-sporeforming mesophiles (yeasts,</td>
</tr>
<tr>
<td>&gt; 4.6</td>
<td>milk, some</td>
<td>malts, lactic acid bacteria</td>
</tr>
<tr>
<td>Acid</td>
<td>Tomatoes, pears,</td>
<td>Thermophilic spoilers</td>
</tr>
<tr>
<td>3.7-4.0</td>
<td>figs</td>
<td></td>
</tr>
<tr>
<td>High acid</td>
<td>Fruits and fruit /</td>
<td>Non-sporeforming mesophiles (yeasts,</td>
</tr>
<tr>
<td>&lt; 4.0-3.7</td>
<td>vegetable products</td>
<td>malts, lactic acid bacteria</td>
</tr>
</tbody>
</table>

Reduced $a_w$

- Drying
- Concentration
Foods of low and intermediate moisture

- Low moisture
  - Dry, dessicated
  - <20% moisture
  - $a_w$ 0.00 – <0.60
- Intermediate moisture
  - 15-50% moisture
  - $a_w$ 0.60 – 0.85

These foods are shelf stable

Effect of drying on microorganisms

<table>
<thead>
<tr>
<th>$a_w$</th>
<th>Microorganisms Involved</th>
<th>Time to spoilage at room temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.9</td>
<td>Bacteria</td>
<td>Hours</td>
</tr>
<tr>
<td>0.80-0.85</td>
<td>Molds and yeasts</td>
<td>1-2 weeks</td>
</tr>
<tr>
<td>&gt;0.65 - 0.7</td>
<td>Molds and yeasts</td>
<td>Months</td>
</tr>
<tr>
<td>0.60-0.65</td>
<td>Molds and yeasts</td>
<td>&gt;2 years</td>
</tr>
<tr>
<td>&lt;0.60</td>
<td>N/A</td>
<td>No microbial spoilage</td>
</tr>
</tbody>
</table>

Drying

- Freeze drying
  - Lyophilization
- Hot vacuum drying
- Hot air drying
- Sun drying

Drying depends on temperature and pressure conditions
Effect of the $a_w$ adjustment method

Ways of adjusting the $a_w$ of a food:

- **Adsorption**
  - Adding water to dry foods to adjust $a_w$
  - Freeze dried foods
  - Egg beaters

- **Desorption**
  - Eliminate water from the food system
  - Hot vacuum dried or sun dried foods
  - At similar moisture content (% water), desorption tends to produce lower $a_w$

Concentration

- Adding solutes to the food system
  - Salting (dry fish)
  - Adding sugar (jellies, syrups)
- Adding solutes and eliminating water
  - Caramelized condensed milk (cajeta)
- The solute plays an important role in $a_w$ reduction

Acidification

- Low pH
- Organic acids
Acidification

- Fermented foods
- Acidified foods
  - Acidic foods vs. acidified foods

Atmosphere modification

- Controlled atmospheres
- Modified atmospheres

Controlled atmospheres

- Substitution of air by a gas mixture
- Controlling gas concentration during storage
- Usually includes CO₂
  - Greater inhibition of microorganisms by CO₂ at refrigeration temperatures
  - Lower APC in steaks during storage in 100% CO₂ compared to 100% N₂ or air
  - Shelf life of lean beef inoculated with PMA and stored at 5°C in 85% O₂ + 15% CO₂ was 9 days longer than beef stored in air.
- Reducing O₂ concentration may affect food quality
  - Red meats

Greater inhibition of microorganisms by CO₂ at refrigeration temperatures
Lower APC in steaks during storage in 100% CO₂ compared to 100% N₂ or air
Shelf life of lean beef inoculated with PMA and stored at 5°C in 85% O₂ + 15% CO₂ was 9 days longer than beef stored in air.
Reducing O₂ concentration may affect food quality
Red meats
Effect of CO$_2$ on microorganisms in meat packs

- Microbiota is shifted
- Predominant PMA (aerobic, CO$_2$ sensitive) to lactic acid bacteria (LAB, facultatively anaerobic, CO$_2$ resistant)
- PMA are putrefactive, LAB are fermentative
  - Some sensorial change is still accepted by consumers

Modified atmosphere packaging (MAP)

- Substitution of air by a gas mixture
- Vacuum packaging
  - 80% of fresh beef in the U.S. is vacuum packaged
  - Not all O$_2$ is removed
  - The O$_2$ remaining is consumed by the microbiota and the food
  - CO$_2$ is also produced by metabolic activity
    - Inhibitory for microorganisms
- Careful selection of packaging film

Spoilage of vacuum packaged meats

- Long-term refrigeration spoilage associated with Brocchothrix thermosphacta and lactobacilli
- Clostridium laramie is also associated with gassy spoilage in vacuum packaged meats
Food safety concerns

- Some researchers believe that MAP results in a selection of potential pathogens by inhibiting competitive biota
  - Aeromonas hydrophila
  - Listeria monocytogenes
- A. hydrophila survives and grows better in meat under air packaging compared to vacuum

Addition of chemical preservatives

GRAS substances

- Chemical additives must be listed as GRAS by FDA regulations
  - GRAS = Generally Recognized as Safe
- There is a maximum tolerance for their concentration in foods
- Different preservatives target specific organisms
- The effect of some preservatives is affected by food characteristics such as pH
**Benzoates and parabens**

- **Parabens**
  - Effect depends on pH
- **Benzoic acid**
  - Effect does not depend on pH

**Other organic acid derivatives**

- **Sorbates**
  - Used as sorbic acid or as calcium or potassium sorbate (salts more soluble than acid)
  - Widely used as a preservative
  - One half the toxicity of NaCl
  - pH dependant
  - No effect above pH 6.0
  - May change the taste of foods if added in excess (usually no more than 0.2%)
  - Effective against spoilage and pathogenic bacteria
- **Propionates**
  - Lower tendency to dissociate
  - Effective in low-acid foods

**SO₂ and sulfites**

- SO₂ used as a gas or in liquid form
- Used as preservative in wines, dried fruits, juices, molasses
- Effect greatly favored by low pH
- Bacteriostatic against Acetobacter and LAB
  - Juices and beverages
- SO₂ can change to sulfurous acid derivatives
  - SO₃⁻ then to sulfites, bisulfites and metabisulfites
- Sulfites are used as preservatives in juices and beverages
  - Also to prevent melanosis in shrimp
Nitrites and Nitrates

- Used in curing formulas for meat
- Stabilize red color of meat
- Inhibit spoilage and pathogenic organisms
- Contribute to flavor development
- NO₂ and NO₃ change to HNO₂ then to NO

NO₂ and Clostridium botulinum

- Prevents germination of spores of C. botulinum
- Also effective against S. aureus
- Added to cheeses to prevent gassy spoilage caused by clostridia

The Perigo factor

- Question:
  - Why is C. botulinum almost totally absent in cured, canned and vacuum packaged meats?
- Perigo and Roberts (1968) found that NO₂ was 10 times more effective if added before heat treatment
  - A chemical factor is thought to be produced during heating that enhances the inhibitory effect of NO₂
- Several researchers have reported that the antibotulinal effect is interdependent with NO₂ with other curing ingredients, pH, heat treatment, and temperature/time of storage
Antimicrobial mechanism
- NO₂ inhibits *C. botulinum* by interfering with iron-sulfur enzymes, thus preventing ATP synthesis
  - Ferredoxin
- Bacteria that lack ferredoxin are resistant to NO₂
  - LAB lack ferredoxin
  - Lactobacillus and other LAB spoilage in cured meats is reported
    - Green ham (Sam I am)

Other antimicrobials
- Ethylene oxide
  - Highly toxic, but no residues
- Antibiotics and bacteriocins
  - Nisin, subtilin, tylosin
- Antifungals
  - Natamycin, benomyl, thiabendazole