2. Inactivation of Microorganisms by Gaseous Chemicals

- Many chemicals which can be generated in a gaseous phase have microbiocidal activity
  - e.g. Ethylene oxide, formaldehyde, propylene oxide, methyl bromide, -propiolactone, peracetic acid, chlorine dioxide, and ozone
- Most commonly used is ethylene oxide, followed by formaldehyde
- Useful for sterilizing heat-sensitive materials

a. Ethylene oxide

- Used for plastics which cannot stand irradiation
- Ethylene oxide is flammable and toxic. Max. exposure level in air of 5 ppm over 8 h (U.K. Health and Safety Executive, 1989)
- No standard set of conditions. Validation is individually developed for each product (U.K. Dept. of Health, 1990)
- Efficacy affected by time of exposure, temperature, humidity, gas concentration and pressure, gas penetration and distribution

<table>
<thead>
<tr>
<th>Range of Conditions for Ethylene Oxide Sterilization (From Hoxley, 1989)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
</tr>
<tr>
<td>Ethylene oxide (mg mL⁻¹)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>Exposure time (h)</td>
</tr>
</tbody>
</table>

b. Formaldehyde

The Low-Temperature Steam and Formaldehyde (LTSF) Process

- Toxic gas. Max. exposure level in air over 8 h of 2 ppm (U.K. Health and Safety Executive, 1989)
- Temperatures between 70 and 80°C
- Formaldehyde concentration around 14 mg L⁻¹ with steam
- Efficacy affected by time of exposure, temperature, humidity, gas concentration and pressure, gas penetration and distribution
- A less penetrating gas than ethylene oxide therefore limiting packaging materials to principally paper and cotton fabric

Gas Processes

Important features

- Operational safety for operators
- Evacuation of chamber and entrapped air
- Control of humidity
- Degassing at the end of the cycle
- Use of biological indicators. Spores of Bacillus subtilis var. niger (Dadd et al., 1983); Bacillus stearothermophilus (U.K. Dept. of Health, 1980)
3. Inactivation of Microorganisms by Chemicals (Preservatives)

"A food additive is a substance or mixture of substances, other than the basic food stuff, which is present as a result of any aspect of production, processing, storage or packaging. The term does not include chance contamination" (WHO, 1965)

Chemical preservatives
Added to prevent deterioration or decomposition of products
- Microbiostats and microbiocides
- Chemistats; modifiers; stabilizers; coating agents

The Ideal Antimicrobial Preservative
(After Croshaw, 1977; Orth & Lutes, 1985)

a. Broad spectrum of activity
   A single agent is ideal

b. Effective and stable over a range of pH
   Potency is maintained with stability. Function is maximized when effectiveness is maintained over a wide pH range

c. Compatible with other product ingredients and packaging
   Should not alter the chemical properties of the product. Preservative potency should not be lost

d. Does not affect the properties of the product
   Appearance, color, clarity, viscosity, texture, taste, aroma.

e. Has a suitable oil/water partition coefficient
   Ensures sufficient preservative concentration in the aqueous phase

f. Inactivates microorganisms quickly
   Prevents microbial adaptation. Should kill rather than inhibit

g. Safe to use
   Safe at usage concentration as well as in pure or concentrate form. Non-toxic; non-irritant; non-sensitizing

h. Complies with regulations

i. Cost-effective
   An effective concentration should add little to the cost of the product

Concept of the Preservative System

- Preservative action is often considered to be solely due to the added agent.
- However, the preservative system of a product involves both the agent and the physicochemical constitution of the product (Orth et al., 1987)
- pH; water activity; nutrient availability; surfactant concentration; sequestering agents; non-aqueous components
The Use of Antimicrobial Preservatives in Foods
(After Frazier & Westhoff, 1988)

<table>
<thead>
<tr>
<th>Food</th>
<th>Benzoic acid &amp; Na Benzoate</th>
<th>Methyl- &amp; Propyl-Paraben</th>
<th>Sorbates Propionate</th>
<th>Sulphites</th>
<th>Acetates &amp; diacetates</th>
<th>Nitrite &amp; nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonated beverages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit juices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine &amp; beer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margarine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pastry</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pie fillings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sausages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltdessings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried fruits, vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh fruits, vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pickles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inactivation of Microorganisms by Chemicals (Preservatives) 3

The Use of Antimicrobial Preservatives in Pharmaceuticals
(After Wallhaeusser, 1974; Akers, 1984; Chapman, 1987; Bloomfield, 1988)

<table>
<thead>
<tr>
<th>Preservative Agent</th>
<th>Pharmaceutical Products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injectable</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td></td>
</tr>
<tr>
<td>Benzoic acid (+ salts)</td>
<td></td>
</tr>
<tr>
<td>Benzy alcohol</td>
<td></td>
</tr>
<tr>
<td>Bronopol</td>
<td></td>
</tr>
<tr>
<td>Calamine</td>
<td></td>
</tr>
<tr>
<td>Sulphates, inorganic</td>
<td></td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td></td>
</tr>
<tr>
<td>Cresol</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
</tr>
<tr>
<td>Parabens (+ salts)</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td></td>
</tr>
<tr>
<td>Sorbic acid</td>
<td></td>
</tr>
</tbody>
</table>

Methods of Product Preservation 5

4. Inactivation of Microorganisms by Radiation

Method of choice for heat-labile materials which can withstand radiation

- **Radappertization**
  "Radiation sterilization"; high-dose treatment; shelf-stable products

- **Radurization**
  "Radiation pasteurization"; low-dose treatment; extended product shelf-life

- **Radicidation**
  "Radiation pasteurization" for elimination of a particular microorganism

Irradiation Treatment

**a. Gamma Rays**

- Cobalt-60 (or less commonly, Caesium-137)
- γ-rays bombard the material, resulting in emission of lower-energy photons and electrons
- The electrons undergo further reactions which cause ionization of molecules within the microorganisms
- Rays are omni-directional
Irradiation Treatment 2

b. Accelerated $\beta$-Particles

- Particles are accelerated by electrical devices
- The higher the acceleration, the greater the penetrating power
- High-speed electrons cause ionization of molecules within the microorganisms
- Beam is focused
- Not as widely used as $\gamma$-ray irradiation

Effects of Irradiation on Foods

- Doses high enough to sterilize
  - Undesirable side reactions or secondary changes
    - E.g. color, odors, tastes

These changes include

- In meat, a rise in pH, destruction of glutathione, and ↑ carbonyl compounds, $H_2S$ and methyl mercaptan
- In fats and lipids, destruction of natural antioxidants, oxidation followed by polymerization, ↑ carbonyl compounds

Applications of Food Irradiation (ACSH, 1985)

<table>
<thead>
<tr>
<th>Type of Food</th>
<th>Dose (KGrays)</th>
<th>Effect of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat, poultry, fish, shellfish, some vegetables, baked goods, prepared foods</td>
<td>20 - 70</td>
<td>Sterilization. Treated product can be stored at room temperature</td>
</tr>
<tr>
<td>Spices and other seasonings</td>
<td>8 - 30</td>
<td>Reduces number of microbes and insects. Replaces chemicals</td>
</tr>
<tr>
<td>Meat, poultry, fish</td>
<td>1 - 10</td>
<td>Delays spoilage by reducing numbers.</td>
</tr>
<tr>
<td>Strawberries and some other fruits</td>
<td>1 - 4</td>
<td>Extends shelf-life by delaying mold growth</td>
</tr>
<tr>
<td>Grain, fruit, vegetables, and other foods subject to insect infestation</td>
<td>0.1 - 1</td>
<td>Kills insects.</td>
</tr>
<tr>
<td>Bananas, avocados, mangoes, papayas, guavas, and certain other non-citrus fruits</td>
<td>0.25 - 0.35</td>
<td>Delays ripening</td>
</tr>
<tr>
<td>Potatoes, onions, garlic</td>
<td>0.05 - 0.15</td>
<td>Inhibits sprouting</td>
</tr>
<tr>
<td>Pork</td>
<td>0.08 - 0.15</td>
<td>Inhibits Trichinae</td>
</tr>
<tr>
<td>Grain, dehydrated vegetables, other foods</td>
<td>Various</td>
<td>Desirable physical &amp; Chemical changes</td>
</tr>
</tbody>
</table>

Effects of Radiation on Foods 2

- In vitamins, reduction in levels of thiamine, pyridoxine, and vitamins B$_{12}$, C, D, E, and K. Riboflavin and niacin are fairly stable.
- Destruction of many food enzymes requires 5 - 10 times the dose needed to kill microorganisms. Enzyme action may continue after irradiation unless the product is blanched.
- There is no indication of the production of radioactivity with electron beams below 11 meV or with $\gamma$-rays from Cobalt-60.
5. Inhibition of Growth Rate by Cooling

- **Temperature**
  
  Energy is required for the reactions associated with growth

- **Cooling**
  
  Removes energy thus slowing reactions and growth

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Ave. Exponential Generation Time (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>667</td>
</tr>
<tr>
<td>2.5</td>
<td>462</td>
</tr>
<tr>
<td>5.0</td>
<td>300</td>
</tr>
<tr>
<td>7.5</td>
<td>207</td>
</tr>
<tr>
<td>10.0</td>
<td>158</td>
</tr>
<tr>
<td>20.0</td>
<td>85</td>
</tr>
</tbody>
</table>

Growth Rate of *Pseudomonas fragi* at Various Temperatures (Nickerson & Sinskey, 1972)

- Cooling can select for psychrophilic microorganisms

### Growth of Microorganisms at Low Temperature

#### Psychrophilic microorganisms

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Min. Temperature for Growth (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>1 - 5</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>7</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>27</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> (E)</td>
<td>3.3</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>20 (most strains)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Plesiomonas shigelloides</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>5.2</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>1 - 7</td>
</tr>
</tbody>
</table>

- **Selection**
  
  Types of Bacteria Causing Spoilage in Chicken Meat (Tompkin (1973))

<table>
<thead>
<tr>
<th>Spoilage Flora at Each Temperature, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°C</td>
</tr>
<tr>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Acinetobacter</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Streptococcus</td>
</tr>
<tr>
<td>Aeromonas</td>
</tr>
<tr>
<td>Others</td>
</tr>
</tbody>
</table>

Cooling can select for psychrophilic microorganisms

### Growth of Microorganisms at Low Temperature 3

#### a. Chilling or Cold Storage

- Temperatures not far above freezing
- Ice or mechanical refrigeration
- Temporary preservation only

- Factors to be considered include the temperature, relative humidity, air velocity and composition of the gaseous atmosphere in the storeroom
Growth of Microorganisms at Low Temperature

b. Freezing or Frozen Storage

- **Slow Freezing**
  - -15 to -29°C achieved by freezing in air. Freezing time may be from 3 to 72 h

- **Quick Freezing**
  - Variously defined but generally freezing time is <30 min.
  - i. Immersion in refrigerant e.g. fish in brine; berries in syrup
  - ii. Indirect contact e.g. exchanging with refrigerant at -17.8 to -45.6°C
  - iii. Air-blast e.g. air at -17.8 to -34.4°C blown against product

Freezing or Frozen Storage 2

The Superiority of Quick Freezing Over Slow Freezing

- Smaller ice crystals are formed
  => less cell damage

- Shorter period of solidification
  => less time for diffusion of soluble materials and separation of ice

- Prompt prevention of microbial growth

- More rapid arrest of enzymatic activity

Changes During Frozen Storage 2

- **Dessication of the product**
  - Freezer burn - When ice evaporates from an area at the surface
  - Produced on fruits, vegetables, meat, poultry and fish

- **Death of microorganisms**
  - Slow continuous decrease in numbers of viable cells

Changes During Frozen Storage

- **Meat, poultry and fish**
  - Proteins become irreversibly dehydrated
  - Myoglobin of meat may be oxidized
  - Fats may become oxidized and hydrolysed

- **Metacryotic liquid**
  - Unfrozen, concentrated solution of sugars, salts etc. may ooze from fruits and their concentrates

- **Fluctuation in storage temperature may result in the growth of ice crystals**
  => cell damage
6. Inhibition of Growth Rate by Restricting Availability of Water

<table>
<thead>
<tr>
<th>Aw</th>
<th>Fresh meat</th>
<th>Bread</th>
<th>Raw ham</th>
<th>Dried fruit</th>
<th>Salt NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>Nearly all microbes grow most rapidly in this range</td>
<td>Few bacteria multiply below this</td>
<td>Few bacteria multiply below this</td>
<td>Few bacteria multiply below this</td>
<td>Exceptions includes halophiles and Staphylococci</td>
</tr>
</tbody>
</table>

Methods

- **Drying**
  - Removes water
- **Addition of solutes**
  - Salt; sugar. Reduces $A_w$. Osmolysis can occur.
- **Formation of gels**
  - Hydrophilic gels make water unavailable
- **Crystallize water**
  - Freezing makes free water unavailable

Methods 2

**Drying**

- Requirement for drying depends on the nature of the product *e.g.* grains (stable); fruits (unstable)
- Drying has to reduce $A_w$ to a level consistent with the shelf-life desired
- **Processes**
  - Solar drying: Fruits; fish; meats; grains
  - Mechanical drying: All types; liquids
  - Freeze drying: Heat sensitive products
- **Terms**
  - Sun-dried; dehydrated or desiccated; condensed or evaporated

Reduced $A_w$ Products

- Intermediate-moisture products contain 20 - 40% water
  - soft candies; jams; honey; dried fruits; some bakery items; meats (pepperoni; hams)

Additional Treatment

Drying may be combined with other treatments such as the addition of solutes to reduce $A_w$ but not moisture content

- *e.g.* Dog food: $A_w$ 0.83 - 0.85; pasteurized; preservative added; moisture content 25 - 27%