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

Range of Conditions for Ethylene Oxide Sterilization (From Hoxley, 1989)

Factor	Conditions
[Ethylene oxide] (mg mL^{-1})	250 - 1500
Temperature (^oC)	30 - 65
Exposure time (h)	1 - 30

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^{o}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 subtilus* 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 susbtances, 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 convcentration 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)

Food	Benzoic acid & Na Benzoate	Methyl- & Propyl-Paraben	Sorbates	Propio-nates	Sulphites	Acetates & diacetates	Nitrite & nitrate
Carbonated beverages							
Fruit juices							
Wine & beer							
Cheese							
Margarine							
Pastries							
Pie fillings							
Sausage							
Salad dressings							
Dried fruits; vegetables							
Fresh fruits; vegetables							
Pickles							

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

Preservative Agent	Pharmaceutical Products			
	Injectable	Opthalmic	Topical	Oral
Benzalkonium chloride				
Benzoic acid (+ salts)				
Benzyl alcohol				
Bronopol				
Cetrimide				
Sulfites, inorganic				
Chlorhexidine				
Cresol				
Ethanol				
Parabens (+ salts)				
Phenol				
Sorbic acid				

4. Inactivation of Microorganisms by Radiation

Method of choice for heat-labile materals 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

b. Accelerated β-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 γ-ray irradiation

Applications of Food Irradiation (ACSH, 1985)

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

Effects of Radiation 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

- 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 γ-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

Growth Rate of *Pseudomonas fragi* at Various Temperatures (Nickerson & Sinskey, 1972)	
Temperature ºC	Ave. Exponential Generation Time (Min)
0	667
2.5	462
5.0	300
7.5	207
10.0	158
20.0	65

Growth of Microorganisms at Low Temperature

- **Selection**

Types of Bacteria Causing Spoilage in Chicken Meat (Tompkin (1973)			
	Spoilage Flora at Each Temperature, %		
	1ºC	**10ºC**	**15ºC**
Pseudomonas	90	37	15
Acinetobacter	7	26	34
Enterobacteriaceae	3	15	27
Streptococcus		6	8
Aeromonas		4	6
Others		12	10

Cooling can select for psychrophilic microorganisms

Growth of Microorganisms at Low Temperature 2

- **Psychrophilic microorganisms**

Low Temperature Growth of Some Foodborne Bacterial Pathogens (After Frazier & Westhoff, 1988)	
Microorganism	**Min. Temperature for Growth (ºC)**
Aeromonas hydrophila	1 - 5
Bacillus cereus	7
Campylobacter jejuni	27
Clostridium botulinum (E)	3.3
Clostridium perfringens	20 (most strains)
Escherichia coli	4
Listeria monocytogenes	3
Plessiomonas shigelloides	8
Salmonella	5.2
Staphylococcus aureus	10
Vibrio parahaemolyticus	5
Yersinia enterocolitica	1 - 7

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

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

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

During frozen storage, chemical and enzymatic reactions proceed slowly

- **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

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 microganisms**
  - Slow continuous decrease in numbers of viable cells

Methods of Product Preservation 7

6. Inhibition of Growth Rate by Restricting Availability of Water

A_w		
1.000	Water; Fresh meat	
0.995	Bread	Nearly all microbes grow most rapidly in this range
0.990		
0.900	Raw ham	Few bacteria multiply below this. Exceptions includes halophiles and Staphylococci
0.850	Dried fruit	
0.750	Satd. NaCl	

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

Drying

- **Requirement for drying depends on the nature of the product** ***e.g.*** **grains (stable); fruits (unstable)**
- **Drying has to reduce** ***Aw*** **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 Aw 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 Aw but not moisture content

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