Chapter 17 Food Protection with High-Temperature and Characteristics of Thermophilic Microorganisms

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節錄自Modern Food Microbiology, Jay, J. M., 7th ed.

Introduction

- Based on the destructive effect of high-temperature on microorganisms.
- **Pasteurization** ➔either the destruction of **all disease-producing organisms** (for example, **pasteurization of milk**) or the **destruction or reduction in the number of spoilage organisms** in certain foods, as in the **pasteurization of vinegar**.

Introduction

- The **pasteurization of milk** is achieved by heating as follows:
  - **145˚F (63˚C) for 30 min (low temperature long time, LTLT)**
  - **161˚F (72˚C) for 15 sec (high temperature short time, HTST, method)**
  - **191˚F (89˚C) for 1.0 sec**
  - 194˚F (90˚C) for 0.5 sec
  - 201˚F (94˚C) for 0.1 sec
  - 212˚F (100˚C) for 0.01 sec
- **These treatments are equivalent and are sufficient to destroy the most heat resistant of the nonsporeforming pathogenic organisms**

Introduction

- Milk pasteurization temperatures are sufficient to destroy all yeasts, molds, Gram-negative bacteria, and many Gram-positives.
- Two groups of organisms that survive milk pasteurization are: **thermodurics** (耐熱生物) and **thermophiles** (嗜熱生物).

Introduction

- **Thermoduric organisms** are those that can **survive** exposure to relatively high temperatures but **do not necessarily grow** at these temperatures.
- The nonsporeforming organisms that survive milk pasteurization generally belong to the genera ***Streptococcus*** (鏈球菌屬) and ***Lactobacillus*** (乳桿菌屬).

Introduction

- **Thermophilic organisms** are those that **not only survive** relatively high temperatures but **require high temperatures** for their growth and metabolic activities.
- The genera ***Bacillus*** (芽孢桿菌屬) and ***Clostridium*** (梭狀桿菌屬) contain the thermophiles of greatest importance in foods.

Introduction

- **Sterilization: the destruction of all viable organisms**
- Canned foods are sometimes called "**commercially sterile**" to indicate that **no viable organisms can be detected** by the usual cultural methods employed or that **the number of survivors is so low** as to be of no significance under the conditions of canning and storage.

Introduction

- The processing of **milk and milk products** can be achieved by the use of **ultrahigh temperatures** (UHT).
- The primary features of the UHT treatment include its **continuous nature**→ requiring **aseptic storage and aseptic handling** of the product downstream from the sterilizer
- The **very high temperatures** (in the range **140-150°C**) and the correspondingly **short time (a few seconds)** necessary to achieve **commercial sterility**.

Introduction

- **UHT-processed milks have higher consumer acceptability** than the conventionally heated pasteurized products, and because they are **commercially sterile**, they may be stored at room temperatures for up to 8 weeks without flavor changes.

「超高溫瞬間殺菌法」（UHT-pasteurization ， 120~130 ℃，2~5 秒), 國內市售鮮乳約 90% 均使用 UHT 設備進行殺菌程序，僅少數乳品加工廠採用 LTLT 、 HTST 的殺菌方式。依現行法令規定，超高溫瞬間殺菌乳（UHT-pasteurization milk）如採全程冷藏保存，即可標示為鮮乳；如經滅菌處理(135~150℃、1~4秒)及無菌包裝，可於常溫保存，則須標示為保久乳（農委會）

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

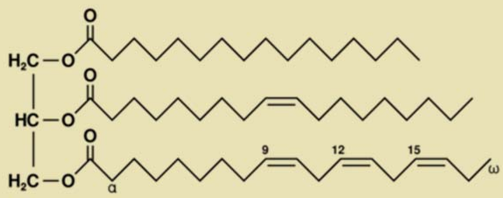
1. Water.

- The **heat resistance** of microbial cells **increases with decreasing humidity or moisture** (Table 17-1).
- **Protein denaturation** may be involved in the **mechanism of death by heat**
  - Heating of wet proteins→formation of free —SH groups →water-binding capacity of proteins increased →thermal breaking of peptide bonds easier

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

2. Fat (triglyceride, triesters of glycerol)

- Increase in the heat resistance of some microorganisms (Table 17-2). → **fat protection** ←directly affecting cell moisture.
- Heat-protective effect of **long-chain fatty acids is better** than that of short-chain acids.



FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

3. Salts.

- Depend on the kind of salt and concentration employed
- **NaCl** could **decrease water activity** and thereby **increase heat resistance** by a mechanism similar to that of drying, whereas others may increase water activity (e.g. Ca^{2+} and Mg^{2+}) and, consequently, increase sensitivity to heat.

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

4. Carbohydrates.

– Sugars causes an **increase in the heat resistance** of microorganisms→ may due to the **decrease in water activity** caused by high concentrations of sugars.

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

5. pH.

– Microorganisms are **most resistant to heat at their optimum pH of growth**, which is generally about 7.0. As the pH is lowered or raised from this optimum value, there is a consequent increase in heat sensitivity (Fig. 17-1).

– **High-acid foods require less heat** to achieve sterilization compared to foods at or near neutrality.

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

6. Proteins and Other Substances.

- Proteins have a **protective effect** on microorganisms. ➔ high-protein-content foods must be heat processed to a greater degree than low-protein-content foods
- The presence of **colloidal-sized particles** in the heating foods also offers protection against heat.

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

7. Numbers of Organisms.

- The larger the number of organisms, the higher is the degree of heat resistance (Table 17-3).
- Heat protection by large microbial populations ➔may be due to the **production of protective substances**, probably protein, excreted by the cells.

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

8. Age of Organisms.

- **Most resistant to heat ➔stationary phase of growth (old cells)**
- Less resistant ➔ logarithmic phase.
- **Heat resistance is also high** at the beginning of the **lag phase**
- **Old bacterial spores are more heat resistant** than young spores.

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

9. Growth Temperature.

- The **heat resistance of microorganisms increase as the growth temperature increases**, and this is especially true for sporeformers.
- *Salmonella senftenberg* grown at $44^{\circ}C$ was found to be approximately three times more resistant than cultures grown at $35^{\circ}C$ (Table 17-4).

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

10. Inhibitory Compounds.

- A decrease in heat resistance ← presence of heat-resistant antibiotics, SO_2, and other microbial inhibitors.
- **Heat plus antibiotics** and **heat plus nitrite** → more effective in controlling the spoilage
- Adding inhibitors to foods prior to heat treatment → reduce the amount of heat.

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

11. Time and Temperature.

- Higher the temperature → greater the killing effect of heat
- As **temperature increases → heating time decreases**.
- The **size of the heating vessel or container** and its **composition** (glass, metal, plastic) is also important. It takes longer to effect pasteurization or sterilization in large containers than in smaller ones.

FACTORS AFFECTING HEAT RESISTANCE IN MICROORGANISMS

12. Effect of Ultrasonics.

- The exposure of bacterial endospores to ultrasonic treatments just before or during heating➔ **lower spore heat resistance**.

RELATIVE HEAT RESISTANCE OF MICROORGANISMS

- **Related to their optimum growth temperatures.** Psychrophilic microorganisms are the most heat sensitive, followed by mesophiles and thermophiles.
- **Sporeforming bacteria are more heat resistant** than nonsporeformers, and **thermophilic sporeformers** are more heat resistant than mesophilic sporeformers.

RELATIVE HEAT RESISTANCE OF MICROORGANISMS

- **Gram-positive bacteria are more heat resistant** than gram-negative.
- The **asexual spores** of molds are slightly more heat resistant than mold mycelia.
- **The extreme heat resistance of bacterial endospores** is of great concern in the thermal preservation of foods.

RELATIVE HEAT RESISTANCE OF MICROORGANISMS

- Endospore resistance is believed to be due to three factors: **protoplast dehydration**, **mineralization** (礦化作用: 有機物轉換成無機物), and **thermal adaptation**. Protoplast dehydration appears to be the primary factor.

THERMAL DESTRUCTION OF MICROORGANISMS

- **Thermal Death Time (TDT)**: This is the **time necessary to kill a given number of organisms at a specified temperature**. By this method, the temperature is kept constant and **the time necessary to kill all cells** is determined.

THERMAL DESTRUCTION OF MICROORGANISMS

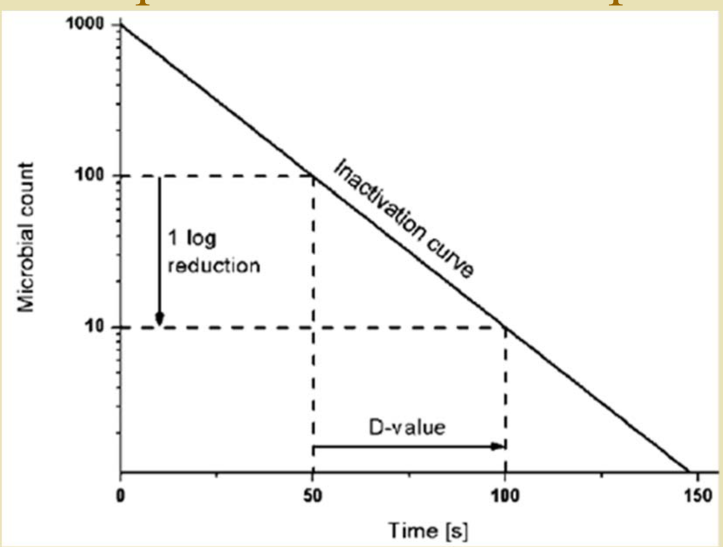
General procedure for determining TDT:

1. Place a **known number of cells or spores** in a sufficient number of sealed containers.
2. The organisms are then **placed in an oil bath** and **heated for the required time period**.
3. At the end of the heating period, containers are **removed and cooled quickly in cold water**.
4. The organisms are then **placed on a suitable growth medium**.
5. **Grow and then plate count.** Death is defined as the inability of the organisms to form a visible colony.

THERMAL DESTRUCTION OF MICROORGANISMS

- **Thermal Death Point**: the temperature necessary to kill a given number of microorganisms in a fixed time, usually 10 min.
- **D Value**: the **decimal reduction time (九成滅菌時間)**, or the **time required to destroy 90% of the organisms.**
  - = minutes required for the destruction curve to traverse one log cycle
  - = 1/(the slope of the destruction curve) (Fig. 17-2).
  - – When D is determined at $250^{\circ}F$, it is often expressed as Dr.

slope= 1/D → D=1/slope

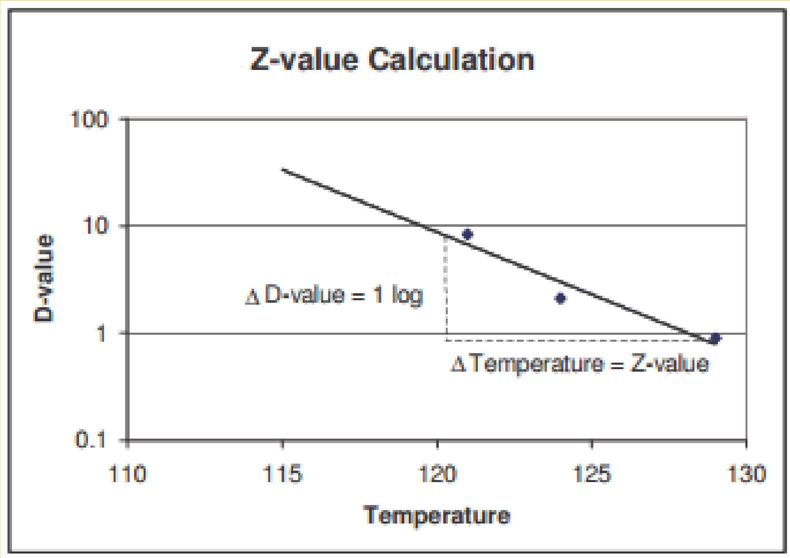


THERMAL DESTRUCTION OF MICROORGANISMS

- D value is the measure of death rate of microorganisms
- D value reflects **the resistance of an organism to a specific temperature** and can be used to **compare the relative heat resistance** among different organisms/spores
- D value for the same organism varies depending on the food type
- D value is **lower in acid foods** and **higher in presence of high proteins**

THERMAL DESTRUCTION OF MICROORGANISMS

- **Thermal Death Time Curve.**
  - A curve used to calculate z value (Fig. 17-3).
  - D value in minutes is plotted on the semilog paper along the log scale (y-axis), and the temperature (in Fahrenheit) of heating is plotted along the linear axis (x-axis).



THERMAL DESTRUCTION OF MICROORGANISMS

z Value:

- **the number of degree temperature** (in Fahrenheit) required for the thermal death time curve to pass through one log cycle.

  =1/(the slope of the TDT curve) (Fig. 17-3).

  – z provides information on the **relative resistance of an organism** to different destructive temperatures.

THERMAL DESTRUCTION OF MICROORGANISMS

- **z Value**:
- Example: If adequate heat process is achieved at 150°F for 3 min and Z value was determined as 17°F, the equivalent heat process will be either 30 min at 133°F or 0.3 min at 167°F.

F Value.

- This value is **the equivalent time (in min)** at 250°F (121°C) of all heat considered ➔to destroy a specific number of spores or vegetative cells of a particular organism.
- F_0 = Dr(log a - log b), where a, b are the numbers of cells in the initial and final populations, respectively.

12-D concept

- 12D concept is used mainly in low acid canned foods (pH >4.6) where ***Clostridium botulinum*** is a serious concern
- The minimum heat process that reduce the probability of survival of the most resistant ***C. botulinum*** **spores to 10^{-12} in canned food**.

SOME CHARACTERISTICS OF THERMOPHILES

- The growth temperature of **thermophiles has a minimum of around 45˚C**, an **optimum between 50˚C and 60˚C**, and **a maximum of 70˚C or above**.
- Five important thermophile genera in foods: ***Bacillus*** (芽孢桿菌屬), ***Alicyclobacillus*** (環脂酸芽孢桿菌屬)**,** ***Geobacillus*** (土芽孢桿菌屬), ***Clostridium*** (梭菌屬,又稱梭狀芽孢桿菌屬)**, and** ***Thermoanaerobacterium*** (熱厭氧桿菌屬)**.**

SOME CHARACTERISTICS OF THERMOPHILES

1. Enzymes

- Three groups of thermophlic enzymes:
  - Enzymes are **stable at the temperature of production** but can be inactivated at higher temperatures (e.g., ATPase).
  - Enzymes are **inactivated at the temperature of production in the absence of specific substrates** (e.g., catalase).
  - Enzymes are **highly heat resistant** (e.g., alpha-amylase).

SOME CHARACTERISTICS OF THERMOPHILES

1. Enzymes

- **Enzymes obtained from thermophiles are more heat resistant** than those enzymes from mesophiles.
- Possible reasons for enzymes become heat resistant: **higher levels of hydrophobic amino acids** and **binding of metal ions**, such as divalent ions (Mg^{2+}) .

SOME CHARACTERISTICS OF THERMOPHILES

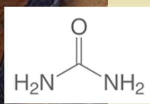
2. Ribosomes

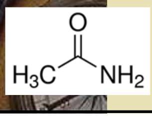
- Thermal stability of ribosomes is correlated to the maximum growth temperature of microorganisms.
- The **increased G-C content in RNA → more stable structure** through more extensive hydrogen bonding →higher thermal resistance.

SOME CHARACTERISTICS OF THERMOPHILES

3. Flagella

- **Thermophilic flagella are more heat stable** than those of mesophiles, for example, thermophilic flagella stay intact at temperatures as high as 70˚C; the mesophilic flagella fall off at 50˚C.
- **Thermophilic flagella are more resistant to urea and acetamide➔** suggesting more **effective hydrogen** bonding occurs in thermophilic flagella .





OTHER CHARACTERISTICS OF THERMOPHILIC MICROORGANISMS

1. **Nutrient Requirements: Thermophiles have a higher nutrient requirement** than mesophiles when growing at thermophilic temperatures.
2. **Oxygen Tension:**
   - As the **growth rate of microorganisms increases ➔ the oxygen demand increases**. But the decreased solubility of oxygen cannot meet the demand.
   - This is one of the most important limiting factors of thermophilic growth in culture media.

OTHER CHARACTERISTICS OF THERMOPHILIC MICROORGANISMS

3. Cellular Lipids.

- The state of cellular lipids affects thermophilic growth.
- Mesophiles growing above their maximum range showed **decreases in lipid content** and **more lipid saturation.** ➔ **saturated branched-chain fatty acids** are preferred
- The **decrease in the proportion of unsaturated fatty acids** ⬅**growth temperatures increase**

OTHER CHARACTERISTICS OF THERMOPHILIC MICROORGANISMS

4. Cellular Membranes.

- The **leakage** of ultraviolet light-absorbing and other material from cells undergoing "**cold shock**" ➔membrane related to high-temperature death.
- Most animal die when body temperatures reach between 40°C and 45°C and most psychrophilic bacteria are killed at about this temperature range➔ may due to the **melting of lipid constituents** of the cell or cell membrane.
- **Cellular membrane integrity** ➔critical to growth and survival at thermophilic temperature

OTHER CHARACTERISTICS OF THERMOPHILIC MICROORGANISMS

5. Effect of Temperature.

- Thermophiles do not grow as fast at their optimum temperatures as mesophiles do.
- **Thermophile enzymes are inherently less efficient** than mesophiles because of thermal stability ← discard growth efficiency to survive

6. Genetics.

- The genetic loci for streptomycin resistance and that for growth at $55^{\circ}C$ were closely linked.
- The precise mechanisms about the high-temperature phenomenon remain mystery.

CANNED FOOD SPOILAGE

- Due to the following improper handling: **underprocessing, inadequate cooling, leakage through seams,** and **preprocess spoilage.**
- The spoilage of canned food can be classified based on acidity.

CANNED FOOD SPOILAGE

1. Low acid food

a) **4.6< pH <6.8**

b) Found in meat and marine products, milk, some vegetables (corn, lima beans), meat and vegetable mixtures, and so on.

c) Spoiled by

- **thermophilic flat-sour group**: *Geobacillus stearothermophilus, Bacillus coagulans*
- **sulfide spoilers**:*Clostridium nigrificans, C. bifermentans*
- **gaseous spoilers**: *Thermoanaerobacterium thermosaccharolyticum*

CANNED FOOD SPOILAGE

2. Acid food

a) **3.7- 4.0< pH <4.6**

b) Found in fruits such as tomatoes, pears, and figs.

c) Spoiled by

- **Thermophilic spoilers**: *B. coagulans* types
- **Mesophiles**: *B. polymyxa, Paenibacillus macerans, C. pasteurianum, C. butyricum,* lactobacilli, and others.

CANNED FOOD SPOILAGE

3. High acid food

a) **pH < 4.0-3.7**

b) Found in fruits, and fruit/vegetable products, such as grapefruit, pickles, and so forth.

c) Spoiled by **nonsporeforming mesophiles: yeasts, molds, lactic acid bacteria**

CANNED FOOD SPOILAGE

Canned food spoilage organisms may be characterized as: (also see Table 17-15)

1. Mesophilic organisms

- Putrefactive (致腐敗的) anaerobes
- Butyric anaerobes
- Aciduric flat sours
- Lactobacilli
- Yeasts
- Molds

2. Thermophilic organisms

- Thermophilic anaerobes producing sulfide
- Flat-four spores
- Thermophilic anaerobes not producing sulfide

Appearance of canned food spoilage

- The normal appearance is **flat** or **slightly concave (凹)**.

1. Flipper (急跳罐，輕度膨罐):

- the can looks normal but one end of the can be made convex (凸) by striking or heating the can.
- Not always represent microbial spoilage.

2. Springer (彈性罐):

- both ends may be bulged but one can be pushed back into place, or else a can with one end bulged that when pressed in, pops out the other end.
- Not always represent microbial spoilage.

Appearance of canned food spoilage

3. Soft swell (軟膨罐):

- Both ends bulged that may be dented by pressing with the fingers.
- Often represent **microbial spoilage**.

4. Hard swell (硬膨罐):

- Both ends bulged, so that neither end can be dented by hand.
- Often represent **microbial spoilage**.
- In high-acid foods, it is referred to **hydrogen swells (氫脹罐)**, which result from the release of hydrogen gas.
- Two other common gases in cans of spoiled foods are **CO_2** and **H_2S**

Appearance of canned food spoilage

5. "Leakage-type"spoilage**:**

- The organisms enter cans **at the start of cooling through faulty seams**, which generally result from **can abuse**.
- The organisms that cause leakage-type spoilage can be found either on the cans or in the cooling water.
- This problem is minimized if the cannery cooling water contains <100 bacteria/ml.