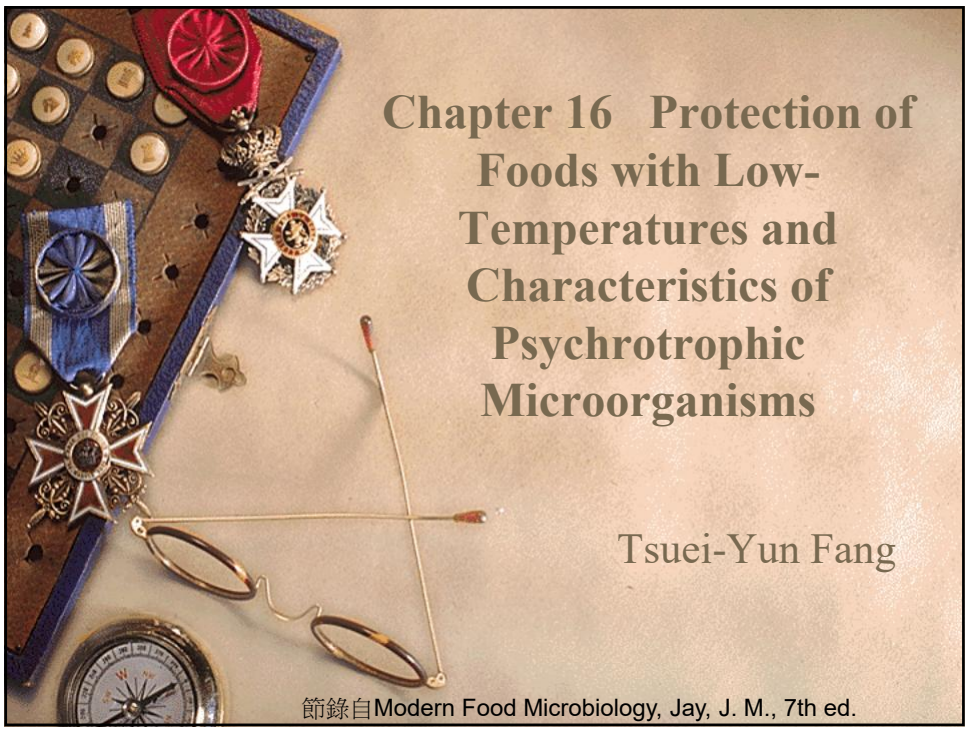
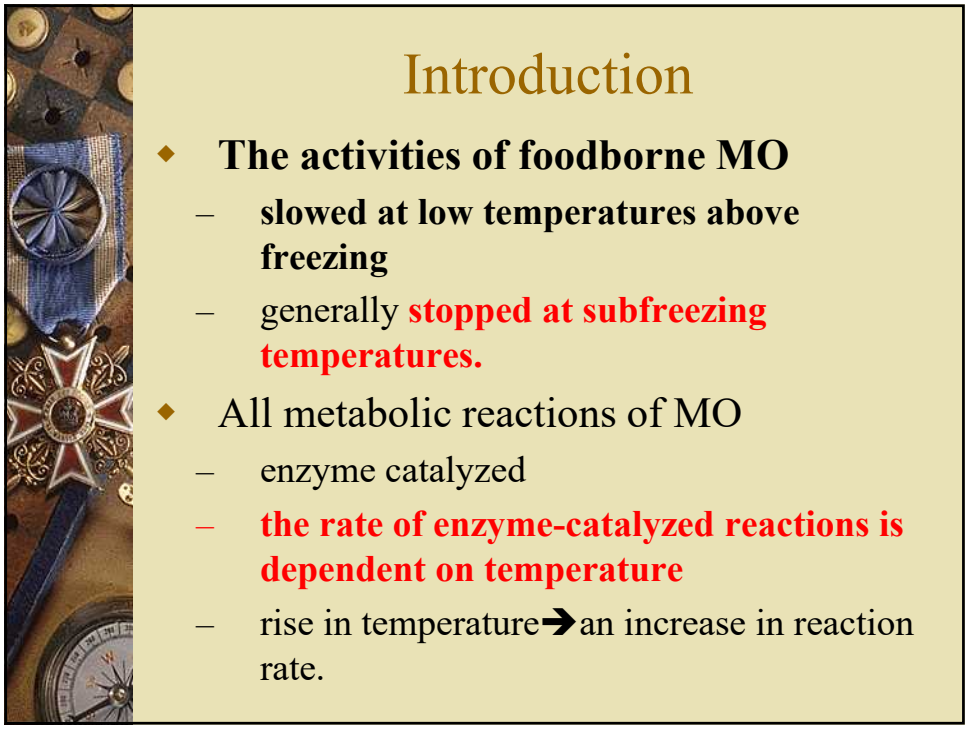

Chapter 16 Protection of Foods with Low-Temperatures and Characteristics of Psychrotrophic Microorganisms
Tsuei-Yun Fang
節錄自Modern Food Microbiology, Jay, J. M., 7th ed.


Introduction
The activities of foodborne MO
slowed at low temperatures above freezing
generally stopped at subfreezing temperatures.
All metabolic reactions of MO
enzyme catalyzed
the rate of enzyme-catalyzed reactions is dependent on temperature
rise in temperature➔an increase in reaction rate.

Introduction

- The **temperature coefficient** (Q_{10}) :
  - Q_{10}= Velocity at a (T + 10˚C)/Velocity at T
  - The Q_{10} for most biological systems is 1.5-2.5
- Temperature is also related to **relative humidity (R.H.)**
  - subfreezing temperatures affect R.H. as well as **pH**, and possibly **other parameters** of microbial growth as well.

DEFINITIONS

- **Psychrophile** (嗜冷生物): organisms that grow over the range of **subzero to 20˚C**, with an **optimum range of 10-15˚C**.
- **Psychrotroph** (低温性生物): organisms that can grow at temperatures between **0˚C and 7˚C** and **produce visible colonies (or turbidity) within 7-10 days**.

- **Mesophiles** ~ 15–45°C (37 °C)
- **Thermophiles** ~ 40–80°C (60 °C)
- **Hyperthermophiles** ~ 65–121°C (90°C)

DEFINITIONS

- The organisms that cause the spoilage of meats, poultry, and vegetables in the 0-5˚C range would be expected to be psychrotrophs.
- **Eurypsychrotroph** *(eurys,* "wide" or "broad"): Organisms typically form visible colonies **between 6 and 10 days**.
- **Stenopsychrotroph** *(stenos,*"narrow," "little," or "close"): Organisms typically **form visible colonies in about 5 days**.

DEFINITIONS

- **Chilling temperatures** are those between the usual refrigerator (5-7˚C) and ambient (周圍的) temperatures, **usually about 10-15˚C.** ➔ suitable for the storage of certain vegetables and fruits such as cucumbers, potatoes, and limes (萊姆,萊姆檸檬, 無籽檸檬).




DEFINITIONS

- **Refrigerator temperatures**:
  - **between 0˚C and 7˚C** (ideally no higher than 40˚F).
- **Freezer temperatures**:
  - **at or below -18˚C**.
  - generally **growth of all microorganisms is prevented** at freezer temperatures
  - Some can grow within the freezer range but at an extremely slow rate.

TEMPERATURE GROWTH MINIMA

- Bacterial species and strains that can grow at or below 7˚C
  - widely distributed among the **Gram-negative**
  - less distributed among Gram-positive genera (Tables 16-1).
- The **lowest recorded temperature of growth** for a microorganism of concern in foods is **-34˚C**, in this case a **pink yeast** (Tables 16-2).

TEMPERATURE GROWTH MINIMA

- Growth at temperatures below 0°C is more likely to be that of **yeasts and molds** than bacteria.
  - This is consistent with the growth of fungi under **lower a_w** conditions.
  - Bacteria have been reported to grow at -20°C and around -12°C.
- Foods that are likely to support microbial growth at subzero temperatures include fruit juice concentrates, bacon, ice cream, and certain fruits.
  - These products contain **cryoprotectants (冷凍保護劑)** that depress (降低) the freezing point of water.


PREPARATION OF FOODS FOR FREEZING

- The preparation of vegetables for freezing includes **selecting, sorting, washing, blanching, and packaging** prior to actual freezing.
- **Blanching** is achieved either by a **brief immersion of foods into hot water** or the **use of steam**.

PREPARATION OF FOODS FOR FREEZING

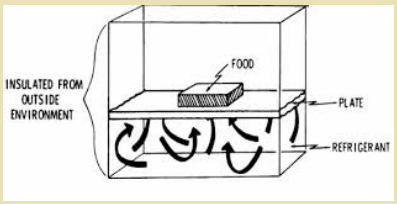
- The primary functions of blanching are as follows:
  - **Inactivation of enzymes** that might cause undesirable changes during freezing storage
  - Enhancement or **fixing of the green color** of certain vegetables
  - **Reduction in the numbers of microorganisms** on the foods
  - **Facilitating the packing** of leafy vegetables by inducing wilting
  - **Displacement of entrapped air** in the plant tissues

PREPARATION OF FOODS FOR FREEZING

- Reductions of initial microbial loads **as high as 99%** have been claimed upon blanching.
- Most vegetative bacterial cells can be destroyed at **milk pasteurization** temperature (145˚F or **62.3˚C for 30 min**).

FREEZING OF FOODS AND FREEZING EFFECTS

- The two basic ways to achieve the freezing of foods: **quick freezing** & **slow freezing**
- **Quick or fast freezing** ➔ the temperature of foods is **lowered to about -20˚C within 30 min.** This treatment is achieved by:
  - **direct immersion** or **indirect contact** of foods with the refrigerant (冷媒；冷凍劑)
  - the use of air blasts of frigid air blown across the foods.



FREEZING OF FOODS AND FREEZING EFFECTS

- **Slow freezing**: the process that the **desired temperature is achieved within 3-72 h.** This is the type of freezing utilized in the home freezer.
- **Quick freezing possesses more advantages than slow freezing,** from the standpoint of overall product quality **(Exhibit 16-1).**

FREEZING OF FOODS AND FREEZING EFFECTS

- Slow freezing favors **large extracellular ice crystals**, and quick freezing favors the formation of **small intracellular ice crystals**.
- Upon thawing, foods frozen by the slow freezing method tend to lose more **drip** (drip for meats; **leakage** in the case of vegetables) than quick-frozen foods held for comparable periods of time.

FREEZING OF FOODS AND FREEZING EFFECTS

- During the freezing of foods, **water is removed from solution** and transformed into ice crystals with high degree of purity.
- The freezing of foods is accompanied by changes such as **pH, titratable acidity, ionic strength, viscosity, osmotic pressure, vapor pressure, freezing point, surface and interfacial tension, and oxidation—reduction (O/R) potential.**

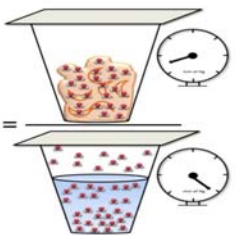
STORAGE STABILITY OF FROZEN FOODS

- The growth at and below freezing temperatures is dependent on **nutrient content, pH, and the availability of liquid water**.
- The a_w of foods is expected to decrease as temperatures fall below the freezing point.

STORAGE STABILITY OF FROZEN FOODS

- The **water activity (a_w)** of a food is the ratio between the vapor pressure of the food itself, when in a completely undisturbed balance with the surrounding air media, and the vapor pressure of distilled water under identical conditions.

$$a_w = \frac{P_{\text{water above food}}}{P_{\text{pure water}}} =$$



- For water **at 0˚C, a_w is 1.0** but falls to about **0.8 at -20˚C** and to **0.62 at about -50˚C** (Table 16-3).

STORAGE STABILITY OF FROZEN FOODS

- Organisms that grow at subfreezing temperatures must be able to grow at the reduced a_w levels.

- In fruit juice concentrates, high levels of sugars tend to maintain a_w at levels higher than would be expected in pure water➔ microbial growth at subfreezing temperatures.

STORAGE STABILITY OF FROZEN FOODS

- Not all foods freeze at the same initial point (Table 16-4). ➔the nature of its solute constituents and the relative concentration

Food	Water content (%)	Freezing point (°C)
Vegetables	78–92	−0.8 to −2.8
Fruits	87–95	−0.9 to −2.7
Meat	55–70	−1.7 to −2.2
Fish	65–81	−0.6 to −2.0
Milk	87	−0.5
Egg	74	−0.5

- Although **the metabolic activities of all microorganisms can be stopped at freezer temperatures**, **frozen foods may not able to retain the original flavor and texture**.

STORAGE STABILITY OF FROZEN FOODS

- Most frozen foods are assigned a **freezer life**.
- **The suggested maximum holding time** for frozen foods is **not based on the microbiology** but **on factors such as texture, flavor, tenderness (嫩度), color, and overall nutritional quality** upon thawing and subsequent cooking.

STORAGE STABILITY OF FROZEN FOODS

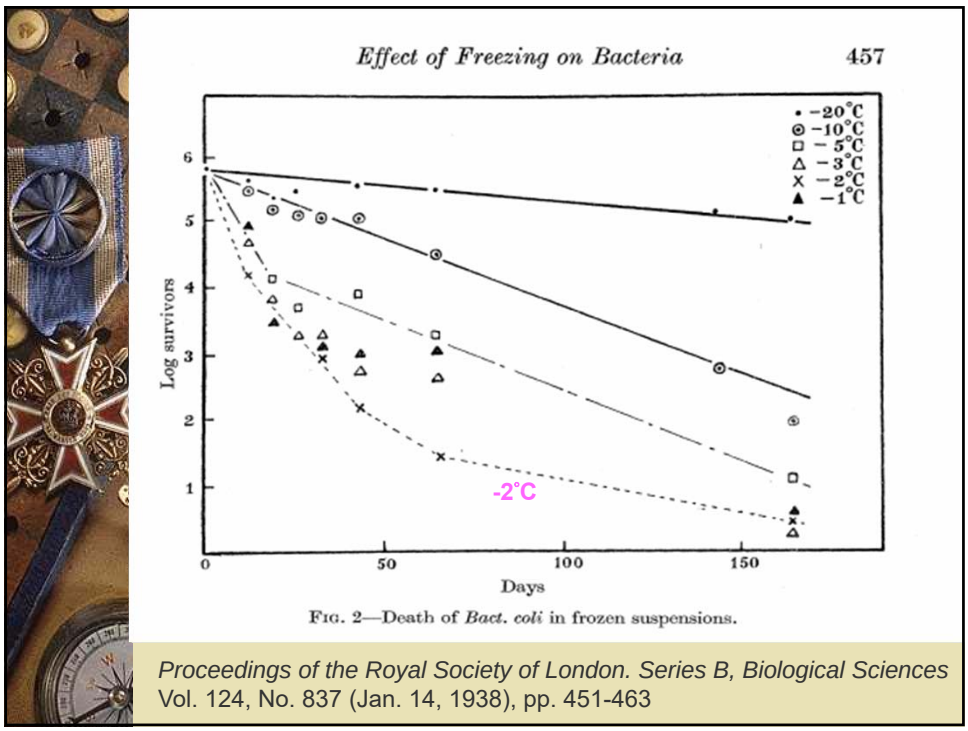
- Some foods that are **improperly wrapped during freezer storage** undergo **freezer burn** (凍燒), characterized by **a browning of light-colored foods** such as the skin of chicken meat.
  - The browning results from **the loss of moisture at the surface**, leaving the product **more porous** than the original at the affected site.
- **Freezer burn is irreversible**

EFFECT OF FREEZING ON MICROORGANISMS

- Freezing is one means of preserving microbial cultures, with **freeze drying** being perhaps the best method known.
- Bacteria differ in their capacity to survive during freezing.
  - Among the food-poisoning bacteria, **salmonellae** (沙門氏菌) **are less resistant** than *Staphylococcus aureus* or vegetative cells of clostridia (梭菌), whereas **endospores and food-poisoning toxins** are apparently **unaffected by low temperatures**.

EFFECT OF FREEZING ON MICROORGANISMS

- Facts happens upon freezing:
  - **A sudden mortality** immediately on freezing, varying with species.
  - The proportion of cells surviving immediately after freezing **die gradually** when stored in the frozen state.
  - This **decline** in numbers is **relatively rapid at temperatures just below the freezing point**, **especially about -2˚C**, but less so at lower temperatures, and it is usually slow below -20˚C.



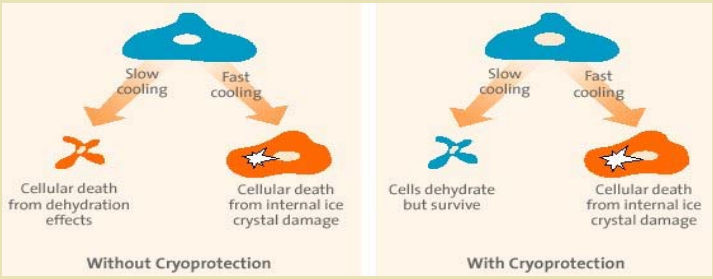
Proceedings of the Royal Society of London. Series B, Biological Sciences
Vol. 124, No. 837 (Jan. 14, 1938), pp. 451-463

EFFECT OF FREEZING ON MICROORGANISMS

- Low freezing temperature of about -20˚C are less harmful to microorganisms than the medium range of temperatures, such as -10˚C. Temperatures below **-24˚C** seem to have no additional effect.
- **Food constituents** such as egg white, sucrose, corn syrup, fish, glycerol, and undenatured meat extracts **➔increase freezing viability**
- **Acid conditions➔ decrease cell viability**.

Events occurred when cells freezed

1. The water that freezes is the so-called **free water**. Upon freezing, the free water forms **ice crystals**. In **slow freezing**, ice crystals are **extracellular**; in **fast freezing**, they are **intracellular**. Bound water remains unfrozen. The freezing of cells depletes them of usable liquid water and thus **dehydrates** them.



Events occurred when cells freezed

2. Freezing results in **an increase in the viscosity of cellular matter**, a direct consequence of water being concentrated in the form of ice crystals.

3. Freezing results in **a loss of cytoplasmic gases** such as O_2 and CO_2. A loss of O_2 to aerobic cells suppresses respiratory reactions.

Events occurred when cells freezed

4. Freezing causes **changes in pH** of cellular matter. The pH may Increase or decrease from 0.3 to 2.0 pH units upon freezing and thawing.

5. Freezing **affects concentration of cellular electrolytes.** This effect is also a consequence of free water in the form of ice crystals.

Events occurred when cells freezed

6. Freezing causes a general **alteration of the colloidal state of cellular protoplasm** (原生質). A proper amount of water is necessary to maintain this state.
7. Freezing causes some **denaturation of cellular proteins.**

Events occurred when cells freezed

8. Freezing induces **temperature shock** in some microorganisms. More cells die when **the temperature decline above freezing** is sudden than when it is slow.

- 9. Freezing causes **metabolic injury** to some microbial cells such as certain *Pseudomonas* spp. (假單胞菌屬)

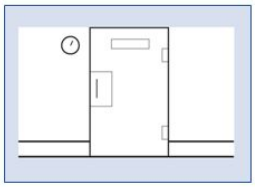
「sp.」是指某一個未知的種名...但確定是一個種.
「spp.」不確定是否只有一個種...通常指他所研究的對象範圍.

Effect of Thawing

- **Repeated freezing and thawing** will destroy bacteria by **disrupting cell membranes**. **The faster the thaw, the greater the number of bacterial survivors.** This is not entirely clear.

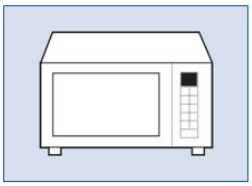
- Microorganisms die may not upon freezing but, rather, during the thawing process.

The Four Acceptable Methods for Thawing Food



In a refrigerator, at 41°F (5°C) or lower

Submerged under running potable water, at a temperature of 70°F (21°C) or lower



In a microwave oven, if the food will be cooked immediately after thawing

As part of the cooking process

Effect of Thawing

- Most frozen-foods processors advise against the refreezing of foods once they have been thawed. **Foods from the frozen state spoil faster than similar fresh products.**
- There are **textural changes associated with freezing** that would seem to aid the invasion of surface organisms into deeper parts of the product and, consequently, **facilitate the spoilage process**.

Effect of Thawing

- Freezing has the effect of **destroying many thermophilic and some mesophilic organisms**, making for less competition among the survivors upon thawing.
  - A greater relative number of **psychrotrophs on thawed foods might increase the spoilage rate**.

SOME CHARACTERISTICS OF PSYCHROTROPHS AND PSYCHROPHILES

1. There is an increase in unsaturated fatty acid residues

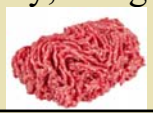
- The usual lipid content of most bacteria is between 2% and 5% in the cell membrane.
- **An increase in the degree of unsaturation of fatty acids** in lipids leads to **a decrease in the lipid melting point** → maintaining the lipid in **a liquid and mobile state** → **allowing membrane activity to continue to function** ← **lipid solidification** theory.

SOME CHARACTERISTICS OF PSYCHROTROPHS AND PSYCHROPHILES

2. Psychrotrophs synthesize high levels of polysaccharides.

- The **production of extracellular dextrans** by *Leuconostoc* (明串珠菌屬) and *Pediococcus* (片球菌屬) spp. are favored at temperatures below the growth optima of these organisms.
  - Dextransucrase converts sucrose into dextran.
  - **Dextransucrase** is very rapidly inactivated at temperatures in excess of 30°C.

- **Slime formation** is characteristic of the bacterial spoilage of frankfurters (法蘭克福香腸), fresh poultry, and ground beef.



SOME CHARACTERISTICS OF PSYCHROTROPHS AND PSYCHROPHILES

3. Pigment production is favored.

- A very large number of **marine psychrotrophs (and perhaps psychrophiles) are pigmented**.
- This is true for bacteria as well as yeasts.
- On the other hand, **none of the more commonly studied thermophiles is pigmented**.

SOME CHARACTERISTICS OF PSYCHROTROPHS AND PSYCHROPHILES

4. Some strains display differential substrate utilization.

- Psychrotrophs that fermented glucose and other sugars with the formation of **acid and gas at 20˚C and lower but produced only acid at higher temperatures.**
- A **temperature-sensitive formic hydrogenase system** is involved.

THE EFFECT OF LOW TEMPERATURES ON MICROBIAL PHYSIOLOGIC MECHANISMS

1. **Psychrotrophs have a slower metabolic rate.**

- As the temperature is decreased, **the rate of protein synthesis is known to decrease**, and this occurs in the absence of changes in the amount of cellular DNA.
- Low temperatures may **influence the fidelity of the translation of mRNA** during protein synthesis.
- The minimum growth temperature may be determined by the **structure of the enzymes** and **cell membrane**, as well as by **enzyme synthesis**.

THE EFFECT OF LOW TEMPERATURES ON MICROBIAL PHYSIOLOGIC MECHANISMS

2. **Psychrotroph membranes transport solutes more efficiently.**

- The temperature at which **transport permeases** are inactivated determines the minimum growth temperature of mesophiles.
- Psychrotrophs tend to possess in their membrane lipids that enable the membrane to be more fluid.
  ➔facilitate membrane transport at low temperatures.

THE EFFECT OF LOW TEMPERATURES ON MICROBIAL PHYSIOLOGIC MECHANISMS

3. Some psychrotrophs produce larger cells.

- Psychrotrophic organisms are generally regarded as having **higher levels of both RNA and proteins**.

4. Flagella synthesis is more efficient.

- Examples of the more efficient production of flagella at low temperatures include *E. coli*, *Salmonella* paratyphi B (副傷寒桿菌 B), and other organisms, including some psychrophiles.

THE EFFECT OF LOW TEMPERATURES ON MICROBIAL PHYSIOLOGIC MECHANISMS

5. Psychrotrophs are favorably affected by aeration.

- Plate counts are higher at low temperatures than at temperatures of 30°C and above. ➔ due to the **increased solubility and the availability of O_2**. ➔Equally high cell yields can be obtained at both low and high incubation temperatures when O_2 is not limiting.
- This greater availability of O_2 in refrigerated foods exerts selectivity on the spoilage flora of such foods. Many psychrotrophic bacteria studied are aerobes or facultative anaerobes.

THE EFFECT OF LOW TEMPERATURES ON MICROBIAL PHYSIOLOGIC MECHANISMS

6. Some psychrotrophs display an increased requirement for organic nutrients.

- In one study, the generation times for unidentified aquatic bacterial isolates in low-nutrient media were two to three times longer than in high-nutrient media.

Three basic mechanisms by which low temperature could affect solute uptake:

- **1) inactivation of individual permease proteins** at low temperature as a result of low-temperature-induced conformational changes
- **2) changes in the molecular architecture of the cytoplasmic membrane that prevent permease action**
- **3) a shortage of energy** required for the active transport of solutes

(Although the precise mechanisms are not clear, the second mechanism seems the most likely.)

NATURE OF THE LOW HEAT RESISTANCE OF PSYCHROTROPHS

- Psychrotrophic microorganisms are generally **unable to grow much above 30-35˚C.**
- **Many respiratory enzymes are inactivated** at the temperatures of maximal growth of various psychrotrophic types (Table 16-8).
- The **thermal sensitivity of certain enzymes of psychrotrophs** is at least one of the factors that limit the growth of these organisms to low temperatures.

NATURE OF THE LOW HEAT RESISTANCE OF PSYCHROTROPHS

- When some psychrotrophs are subjected to **temperatures above their growth maxima, cell death is accompanied by the leakage of various intracellular constituents.**
- The leakage substances consist of proteins, DNA, RNA, free amino acids, and lipid phosphorus.
- **The destruction at relatively low temperatures** is characteristic of psychrotrophs.