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DETERMINATION OF SHELF LIFE OF FISH AND SHELLFISH

FREEZING

- It is a food preservation method by lowering temperature below freezing point
- Potentially deliver a high degree of safety, nutritional value and sensory quality
- Preventing, or at least minimizing, microbial and chemical changes



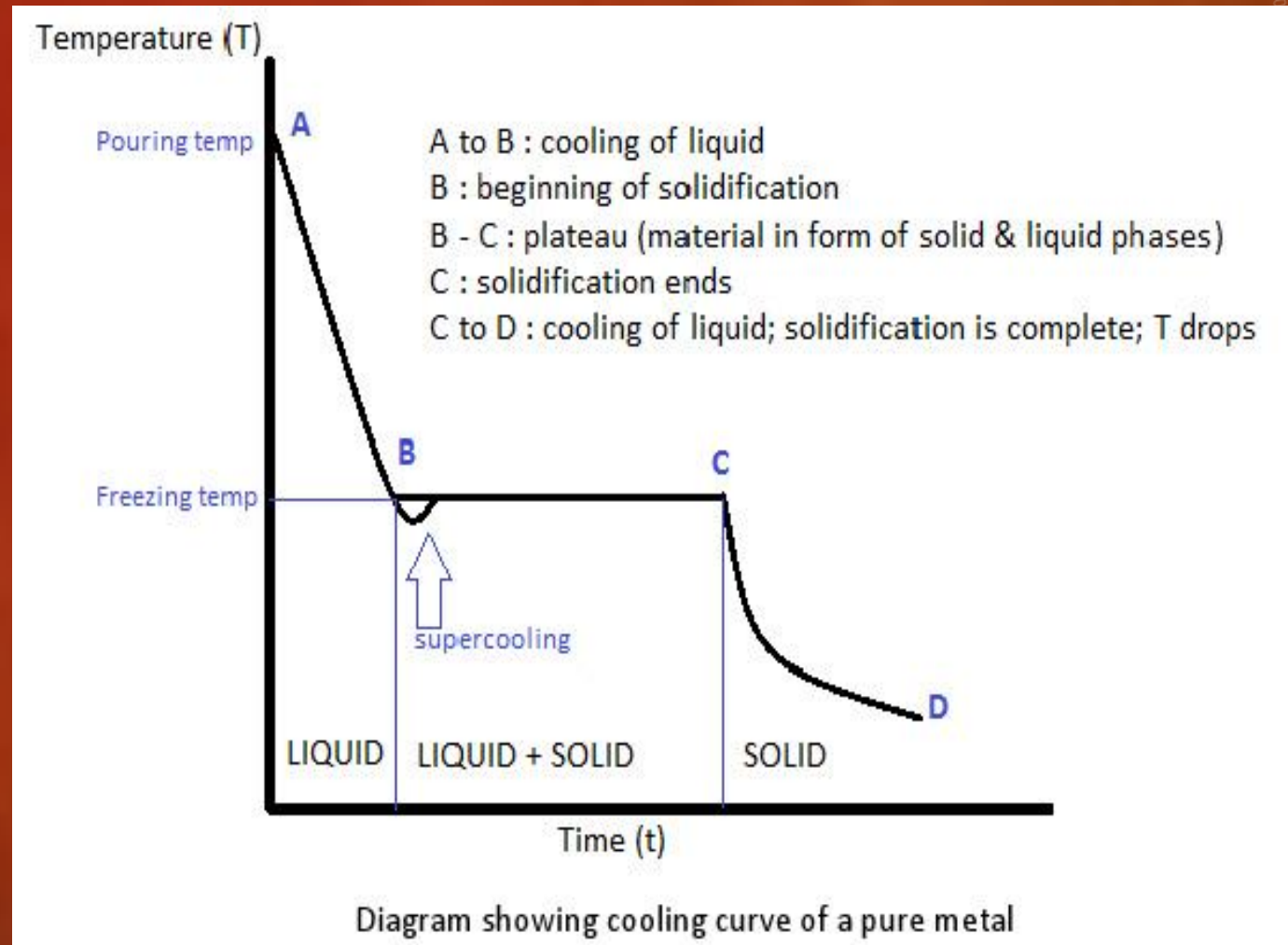
Principle

- Activities of microorganisms can be slowed at temperatures above freezing and stopped at subfreezing temperatures.
- Metabolic reactions of microorganisms are enzyme catalyzed and that the rate of enzyme-catalyzed reactions is dependent on temperature

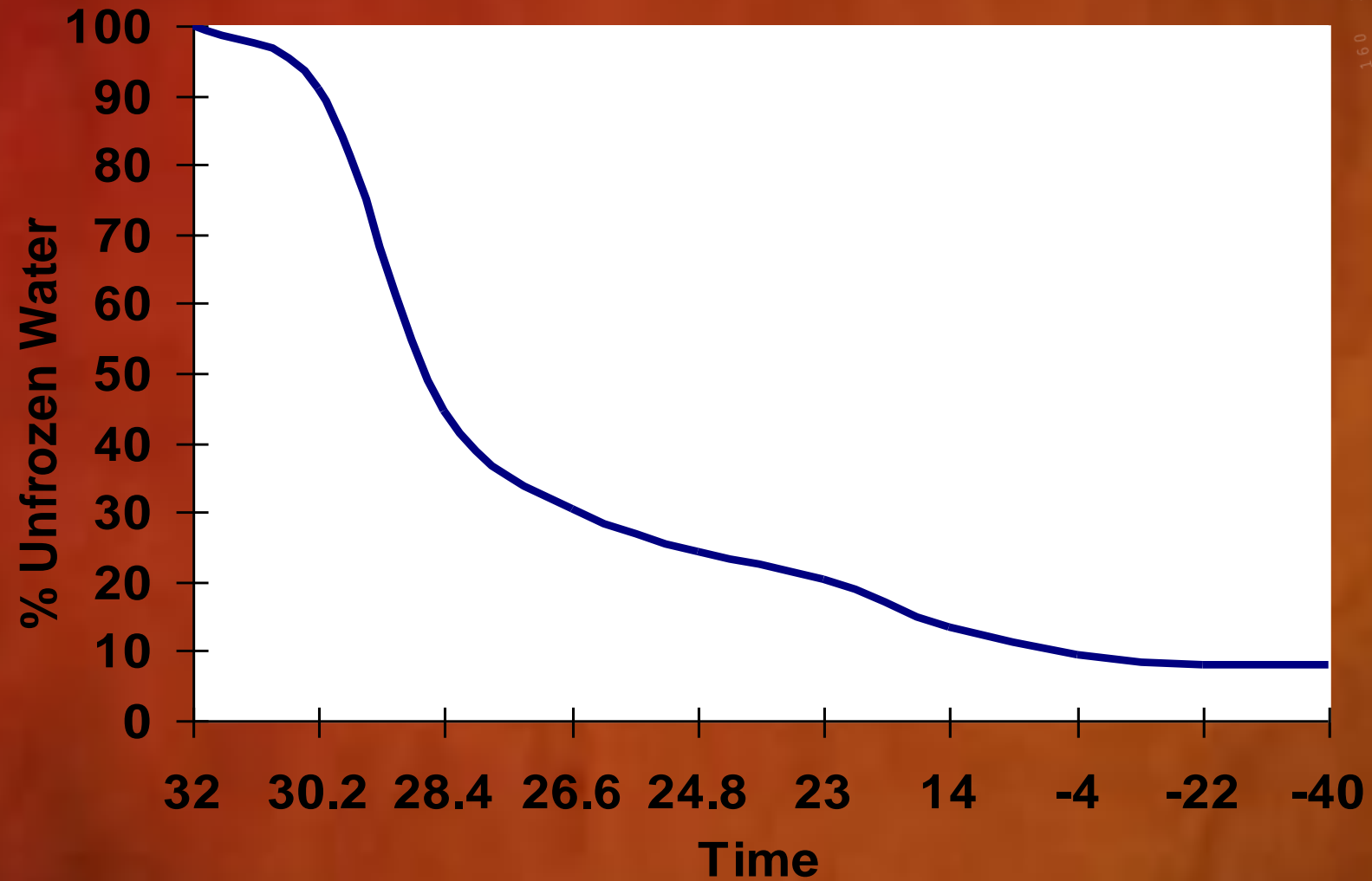
$$Q_{10} = (\text{Velocity at a given temp. } +10^{\circ}\text{C}) / \text{Velocity at } T$$

- The Q_{10} for most biological systems is 1.5-2.5, so that for each 10°C rise in temperature within the suitable range, there is a twofold increase in the rate of reaction & vice versa

FREEZING CURVE



UNFROZEN WATER



TEMPERATURE GROWTH MINIMA

- Bacterial species and strains that can grow at or below 7°C are rather widely distributed among the Gram-negative and less so among Gram-positive genera
- The lowest recorded temperature of growth for a microorganism of concern in foods is -34°C , in this case a pink yeast.
- 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 water activity (a_w) conditions.
- Bacteria have been reported to grow at -20°C and around -12°C

Table 16–2 Minimum Reported Growth Temperatures of Some Foodborne Microbial Species and Strains that Grow at or Below 7°C

<i>Species/Strains</i>	<i>Temperature (°C)</i>	<i>Comments</i>
Pink yeast	–34	
Pink yeasts (2)	–18	
Unspecified molds	–12	
<i>Vibrio</i> spp.	–5	True psychrophiles
<i>Cladosporium cladosporioides</i>	–5	
<i>Yersinia enterocolitica</i>	–2	
<i>Bacillus psychrotolerans</i>	–2 to 1	
<i>Acetobacterium bakii</i>	1.0	
<i>Carnobacterium viridans</i>	2.0	
<i>Clostridium algidixylanolyticum</i>	2.5	
<i>Janthinobacterium agaricidamnosum</i> 2.0	2.0	
<i>Frigoribacterium faeni</i>	2.0	
<i>Lactobacillus algidus</i>	0	
<i>Bacillus psychrodurans</i>	–2 to 0	
Unspecified coliforms	–2	

Table 16–2 Minimum Reported Growth Temperatures of Some Foodborne Microbial Species and Strains that Grow at or Below 7°C

<i>Species/Strains</i>	<i>Temperature (°C)</i>	<i>Comments</i>
<i>Brochothrix thermosphacta</i>	−0.8	Within 7 days; 4°C in 10 days
<i>Aeromonas hydrophila</i>	−0.5	
<i>Enterococcus</i> spp.	0	Various species/strains
<i>Leuconostoc carnosum</i>	1.0	
<i>L. gelidum</i>	1.0	
<i>Listeria monocytogenes</i>	1.0	
<i>Thamnidium elegans</i>	~ 1	
<i>Leuconostoc</i> sp.	2.0	Within 12 days
<i>L. sakei/curvatus</i>	2.0	Within 12 days; 4°C in 10 days
<i>Lactobacillus alimentarius</i>	2.0	
<i>C. botulinum</i> B, E, F	3.3	
<i>Pantoea agglomerans</i>	4.0	
<i>Salmonella</i> Panama	4.0	In 4 weeks
<i>Bacillus weihenstephanensis</i>	4.0	
<i>Serratia liquefaciens</i>	4.0	
<i>Vibrio parahaemolyticus</i>	5.0	
<i>Vagococcus salmoninarum</i>	5.0	
<i>Salmonella</i> Heidelberg	5.3	

Table 16–2 Minimum Reported Growth Temperatures of Some Foodborne Microbial Species and Strains that Grow at or Below 7°C

<i>Species/Strains</i>	<i>Temperature (°C)</i>	<i>Comments</i>
<i>Pediococcus</i> sp.	6.0	Weak growth in 8 days
<i>Lactobacillus brevis</i>	6.0	In 8 days
<i>Weissella viridescens</i>	6.0	In 8 days
<i>Salmonella</i> Typhimurium	6.2	
<i>Staphylococcus aureus</i>	6.7	
<i>Klebsiella pneumoniae</i>	7.0	
<i>Bacillus</i> spp.	7.0	165 of 520 species/strains
<i>Salmonella</i> spp.	7.0	65 of 109, within 4 weeks

Source: Data from Bonde,⁹ Mossel et al.,⁴⁹ Reuter,⁵⁷ and the literature.

Some Bacterial Genera that Contain Species/Strains Known to Grow at or Below 7°C

<i>Gram Negatives</i>	<i>Relative Numbers</i>	<i>Gram Positives</i>	<i>Relative Numbers</i>
<i>Acinetobacter</i>	XX	<i>Bacillus</i>	XX
<i>Aeromonas</i>	XX	<i>Brevibacterium</i>	X
<i>Alcaligenes</i>	X	<i>Brochothrix</i>	XXX
<i>Alteromonas</i>	XX	<i>Carnobacterium</i>	XXX
<i>Cedecea</i>	X	<i>Clostridium</i>	XX
<i>Chromobacterium</i>	X	<i>Corynebacterium</i>	X
<i>Citrobacter</i>	X	<i>Deinococcus</i>	X
<i>Enterobacter</i>	XX	<i>Enterococcus</i>	XXX
<i>Erwinia</i>	XX	<i>Kurthia</i>	X
<i>Escherichia</i>	X	<i>Lactobacillus</i>	XX
<i>Flavobacterium</i>	XX	<i>Lactococcus</i>	XX
<i>Halobacterium</i>	X	<i>Leuconostoc</i>	X
<i>Hafnia</i>	XX	<i>Listeria</i>	XX
<i>Klebsiella</i>	X	<i>Micrococcus</i>	XX
<i>Moraxella</i>	XX	<i>Pediococcus</i>	X
<i>Morganella</i>	X	<i>Propionibacterium</i>	X
<i>Photobacterium</i>	X	<i>Vagococcus</i>	XX
<i>Pantoea</i>	XX	<i>Macrococcus</i>	X
<i>Proteus</i>	X	<i>Paenibacillus</i>	X
<i>Providencia</i>	X	<i>Staphylococcus</i>	X

Relative importance and dominance as psychrotrophs: X = minor, XX = intermediate, XXX = very significant

CONTD...

<i>Gram Negatives</i>	<i>Relative Numbers</i>
<i>Pseudomonas</i>	XXX
<i>Psychrobacter</i>	XX
<i>Salmonella</i>	X
<i>Serratia</i>	XX
<i>Shewanella</i>	XXX
<i>Vibrio</i>	XXX
<i>Burkholderia</i>	X
<i>Chryseobacterium</i>	X
<i>Frigoribacterium</i>	XX
<i>Janthinobacterium</i>	XX
<i>Acetobacterium</i>	XX
<i>Yersinia</i>	XX

Relative importance and dominance as psychrotrophs: X = minor, XX = intermediate, XXX = very significant

EFFECT OF FREEZING ON MICROORGANISMS

Ingram summarized that :

1. There is a sudden mortality immediately on freezing,
2. The proportion of cells surviving die gradually in storage period
3. This decline in numbers is relatively rapid at temperatures just below the freezing point(-2°C), but less so at lower temperatures(-20°C).

EVENTS THAT ARE KNOWN TO OCCUR WHEN CELLS FREEZE:

- Depletes them of usable liquid water and thus dehydrates them.
- Increase in the viscosity of cellular matter.
- Loss of cytoplasmic gases such as O_2 and CO_2 thus suppresses respiratory reactions.
- Changes in pH of cellular matter ranging from 0.3 to 2.0 pH units.
- Temperature shock
- Metabolic injury to some microbial cells

- Effects concentration of cellular electrolytes.
- General alteration of the colloidal state of cellular protoplasm.(dynamic colloidal state of protein)
- Some denaturation of cellular proteins. some -SH groups disappear and such groups as lipoproteins break apart from others.
- According to Mazur the response of microorganisms to subzero temperatures appears to be largely determined by solute concentration and intracellular freezing

EFFECT IN BACTERIA

- Bacterial spores are virtually unaffected by freezing
- Most vegetative gram-positive bacteria are relatively resistant
- Gram-negatives bacteria show the greatest sensitivity
- salmonellae are less resistant than *Staphylococcus aureus* or vegetative cells of clostridia
- cocci being generally more resistant than Gram-negative rods

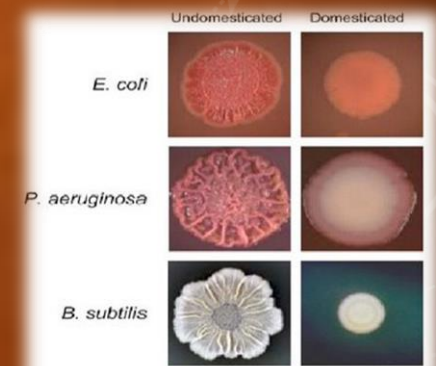
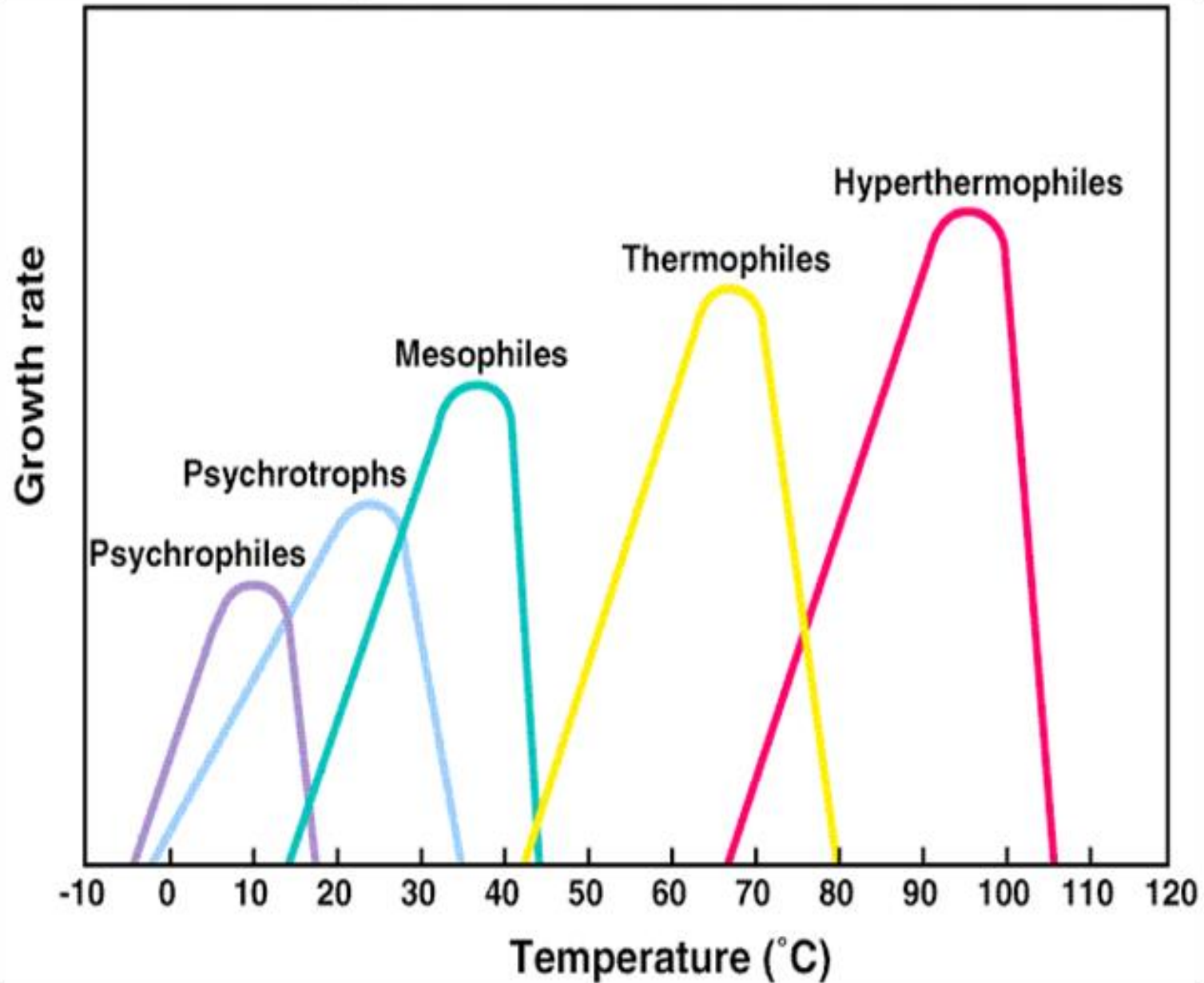
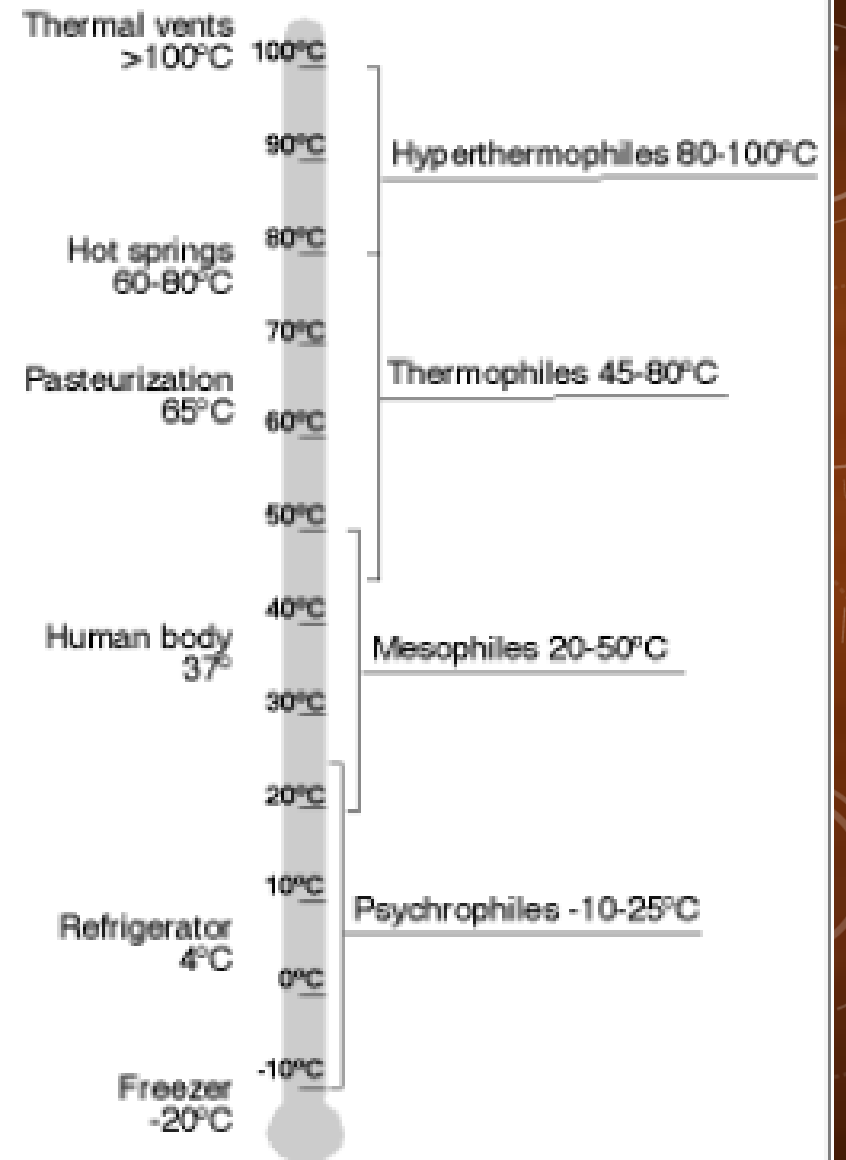


Figure 2. Examples of the differences in colony morphology between undomesticated and domesticated strains of three bacterial species.



Temperatures in which Bacteria Exist



psychrophile

- The term psychrophile was coined by Schmidt-Nielsen(1902) for microorganisms that grow at 0°C .
- This term is now applied to organisms that grow over the range of subzero to 20°C , with an optimum range of $10\text{-}15^{\circ}\text{C}$.
-

Psychrophilic organisms are important in low temperature preservation of foods basic mechanism of their ability to grow are categorised into-

1. Temperature induced changes in the production of metabolic end products
2. Temperature effects on physiologic mechanisms

1. TEMPERATURE INDUCED CHANGES IN THE PRODUCTION OF METABOLIC END PRODUCTS

There is an increase in unsaturated fatty acid residues.

- synthesize neutral lipids and phospholipids with unsaturated fatty acids
- 50% increase in the content of UFA from mesophilic
- maintaining the lipid in a liquid and mobile state, thereby allowing membrane activity to continue to function.

Psychrotrophs synthesize high levels of polysaccharides

- The production of **extracellular dextrans** by *Leuconostoc* and *Pediococcus* spp. are known to be favored at temperatures below the growth optima of these organisms.
- **dextranase** is very rapidly inactivated at temperatures in excess of 30°C.

Pigment production is favored

This effect appears to be confined to those organisms that synthesize phenazine and carotenoid pigments.

Produces **heat-sensitive enzyme** that catalyzes the coupling of a monopyrrole and bipyrrrole precursor to give prodigiosin (the **red pigment**).

Some strains display differential substrate utilization

- Psychrotrophs that ferments glucose and other sugars with the formation of acid and gas at 20°C and lower, but produced only acid at higher temperatures.
- The latter was ascribed to a temperature-sensitive formic hydrogenase system.

2. LOW TEMPERATURES ON MICROBIAL PHYSIOLOGIC MECHANISMS

Psychrotrophs have a slower metabolic rate.

- Psychrotrophic growth decreases more slowly than that of mesophilic with decreasing temperatures.
- As the temperature is decreased, the rate of protein synthesis is known to decrease.
- increase in intramolecular hydrogen bonding that occurs at low temperatures, leading to increased folding of enzymes with losses in catalytic activity
- On the other hand, the decrease in protein synthesis appears to be related to a decreased synthesis of individual enzymes at low growth temperatures

The rate of synthesis at 0°C about 350 times slower than at 37°C

Psychrotroph membranes transport solutes more efficiently.

- upon lowering the growth temperature of mesophiles within the psychrotrophic range, solute uptake is decreased
 - inactivation of permease proteins
 - changes in cytoplasmic membrane
- In psychrotrophs greater mobility of membrane (consists UFA) may be expected to facilitate membrane transport at low temperatures.

Some psychrotrophs produce larger cells.

- Yeasts, molds, and bacteria have been found to produce larger cell sizes when growing under psychrotrophic condition.
- Increased cell size was believed to be due to **increases in RNA** of cells.

Flagella synthesis is more efficient.

Psychrotrophs are favorably affected by aeration

Some psychrotrophs display an increased requirement for organic nutrients.

Freezing effect on virus

- Treatments commonly applied to produce (i.e., freezing, frozen storage, and washing with different sanitizers) are apparently unable to completely remove or inactivate enteric viruses .
- Koopmans and Duizer , have addressed enteric virus survives after freezing. viruses remained viable for periods exceeding the shelf-lives of the products.
- HAV and rotavirus (RV) numbers remained the same in all the tested frozen products during 90 days of storage
- Freezing reduced the numbers of most viruses on all produce by less than one \log_{10} unit, numbers of FCV(feline calicivirus) progressively declined during the frozen storage.

Contd ...

- Freeze-drying is widely used for the preservation of foods, but no data on its effects on enteric virus inactivation have been available previously, except for a reported 3- to 4- \log_{10} unit reduction in poliovirus (PV) levels.
- reduction in HAV infectivity after freeze-drying was less than 2 \log_{10} units
- freeze-drying was significantly ($P < 0.05$) more effective for reducing the level of HuNoV GII than for reducing the level of HuNoV GI

FREEZING EFFECT ON PARASITES :

- Cryopreservation in 20% glycerol increased the time period which nematodes could withstand freezing conditions, whereas all nematodes died in water at -20°C .
- Freezing significantly ($p < 0.05$) reduced nematode infectivity on day 10 and 20 but not day 1.
- Infectivity for both frozen and control declined significantly between day 1 and day 10 ($p < 0.01$) but not between day 10 and day 20.

SURVIVAL OF STEINERNEMATID NEMATODES EXPOSED TO FREEZING

(I. M. BROWN^{1*} and R. GAUGLER¹, Department of Entomology, Rutgers University, New Brunswick, NJ 08903-0231, U.S.A).

CONTD. .

- Survivors of all species frequently exhibited **sluggish behavior** and **extensive vacuolization**, suggesting sublethal freezing injury.
- **Cestode(tapeworm) are more sensitive than Nematode(roundworm) & roundworm more sensitive than trematodes(flukes)**
- USFDA set out few regimes to kill parasites, such as-
 - Frozen storage at - 200C or below for a total of 7 days.
 - Frozen storage at -350C or below for 15 hours after solidifying
 - Freezing at -350C and storage at -200C or below for 24 hours

Microbial standard for seafood as per EU norms

For finfish & shell fish

Product	APC	Faecal coliforms/g m	S.areus/gm	Sulphite redcers/gm	Salmonella in 25 gms	V.cholera	L.monocytogenase
Chilled, quick frozen, peeled & cooked	M=10,000 M=100,000 n=5,c=2	m=100 M=100 n=5,c=2	m=100 M=1000 n=5,c=2	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0
Chilled fish protein breaded or not	M=50,000 M=500,000 n=5,c=2	m=10 M=100 n=5,c=2	m=100 M=1000 n=5,c=2	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0
Frozen crustaceans	m=50,000 M=500,000 n=5,c=2	m=100 M=1000 n=5,c=2	m=100 M=1000 n=5,c=2	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0	Absent in 25 gm n=5,c=0

n=number of units comprising sample,
m=no. of sampling units giving bacterial counts between M & m

Compilation of USFDA,EU & Japanese standards

For Shell fish & Bivalves

Raw products	
TPC at 370C	5*10 ⁵ /gm
Faecal coliforms	20/gm
E.Coli	20/gm
Coagulase +ve stphylococci	100/gm
Salmonella	Absent in 25 gm
Vibrio cholerae	Absent in 25 gm
L.Monocytogenase	Absent in 25 gm

Cooked products	
TPC at 370C	1*10 ⁵ /gm
Faecal coliforms	<1/gm
E.Coli	<1/gm
Coagulase +ve stphylococci	100/gm
Salmonella	Absent in 25 gm
Vibrio cholerae	Absent in 25 gm
L.monocytogenase	Absent in 25 gm

CONCLUSION



- Quality of the final products always depends on the Quality of Raw material as-
 1. It is generally the case that total numbers of micro-organisms decrease
 2. Cannot be completely destroy all micro-organisms in foods
- Though inactivation of Parasites and Bacteria are possible to some extend but virus inactivation can notbe achived by freezing.

REFERENCES

- Inactivation of Enteric Viruses in Minimally Processed Berries and Herbs (S. Butot, T. Putallaz, R. Amoroso, and G. S´anchez)
- SURVIVAL OF STEINERNEMATID NEMATODES EXPOSED TO FREEZING(I. M. BROWN1* and R. GAUGLER1,Department of Entomology, Rutgers University, New Brunswick, NJ 08903-0231, U.S.A).
- **Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs**(S. Butot, T. Putallaz, G. S´anchez)
- Fish processing by George M. Hall
- Textbook of Fish Processing Technology by Dr. K. Gopakumar
- Mordern Food Microbiology by James M Jay

THANK YOU

The background is a solid orange color with a subtle gradient. It features several faint, light-colored technical diagrams. On the right side, there is a large circular diagram with concentric circles and radial lines, resembling a gauge or a scale, with numbers ranging from 80 to 210. Below it is another circular diagram with dashed lines and arrows. In the bottom left corner, there is a partial circular diagram with arrows. At the top center, there is a small circular diagram with a curved arrow.