LECTURE 3

INTRINSIC AND EXTRINSIC FACTORS AFFECTING THE TYPE AND NUMBER OF MICROORGANISMS IN FOODS

Microbial growth in food is dependent on

- **Intrinsic Factors**: physical and chemical properties of the food
- **Extrinsic Factors**: Storage conditions
- **Implicit Factors**: Physiological properties of microorganisms
- **Process Factors**: heating, cutting,..

Intrinsic factors

- pH
- Water activity (a_w)
- Redox potential (Eh)
- Nutrient content
- Antimicrobial constituents
- Biological (antimicrobial) structures

Hydrogen ion concentration (pH)

- Every microorganism has a maximal and an optimal pH for growth. In general yeast and moulds are more acid tolerant than bacteria.

- The inherent pH of foods varies, although most are neutral or acidic. Foods with low pH values (below 4.5) usually are not readily spoiled by **bacteria** BUT are more susceptible to spoilage by **yeast** and **moulds** as shown in the table below:

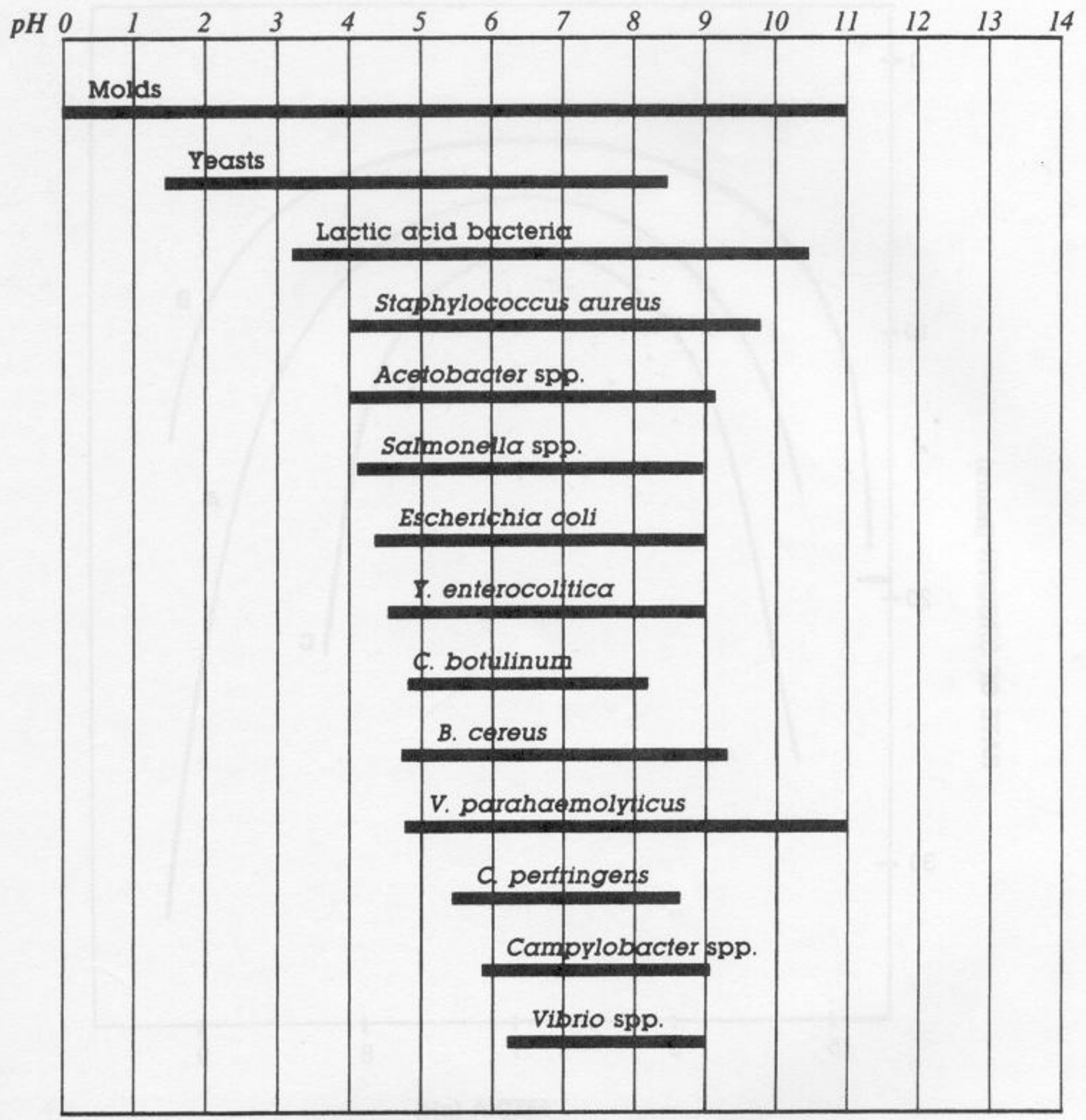


Figure 3.1. Approximate pH growth ranges for some foodborne organisms.

Table 3.2 *Approximate pH ranges of some common food commodities*

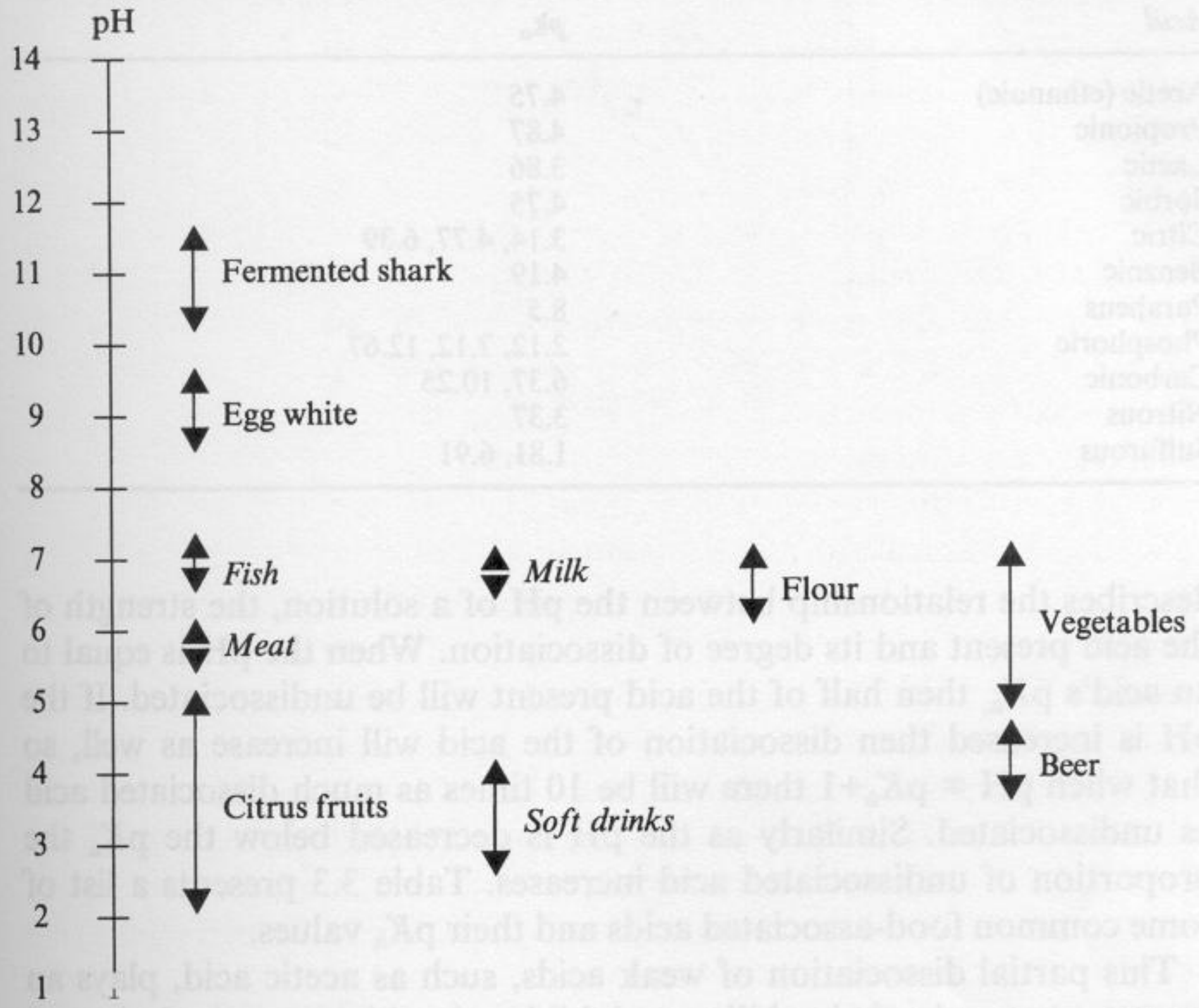


Table 3.1. Approximate pH Values of Some Fresh Fruits and Vegetables

Product	pH	Product	pH
Vegetables		**Fruits**	
Asparagus (buds and stalks)	5.7–6.1	Apples	2.9–3.3
Beans (string and Lima)	4.6–6.5	[apple cider	3.6–3.8]
Beets (sugar)	4.2–4.4	Bananas	4.5–4.7
Broccoli	6.5	Figs	4.6
Brussels sprouts	6.3	Grapefruit (juice)	3.0
Cabbage (green)	5.4–6.0	Limes	1.8–2.0
Carrots	4.9–5.2; 6.0	Melons (honeydew)	6.3–6.7
Cauliflower	5.6	Oranges (juice)	3.6–4.3
Celery	5.7–6.0	Plums	2.8–4.6
Corn (sweet)	7.3	Watermelons	5.2–5.6
Cucumbers	3.8	Grapes	3.4–4.5
Eggplant	4.5		
Lettuce	6.0		
Olives	3.6–3.8		
Onions (red)	5.3–5.8		
Parsley	5.7–6.0		
Parsnip	5.3		
Potatoes (tubers & sweet)	5.3–5.6		
Pumpkin	4.8–5.2		
Rhubarb	3.1–3.4		
Spinach	5.5–6.0		
Squash	5.0–5.4		
Tomatoes (whole)	4.2–4.3		
Turnips	5.2–5.5		

- A food with inherently low pH would therefore tend to be more stable microbiologically than a neutral food. The excellent keeping quality of soft drinks, fermented milks, sauerkraut and pickles is due to their restrictive pH. Some foods have a low pH because of inherent acidity; others e.g. the fermented products have a low pH because of developed acidity from the accumulation of lactic acid during fermentation.
- Moulds can grow over a wider range of pH values than most yeast and bacteria most fermentative yeast are favoured by a pH of about 4.0 to 4.5 as in fruit juices. A film of yeast grows well on acid foods such as sauerkraut and pickles.

Moisture Requirements: The Concept of Water Activity:

- Microorganisms have an absolute demand for water, for without water no growth can occur the exact amount of water needed for growth of microorganisms varies. This water requirement is best expressed in terms of available water or water activity (aw) the vapour pressure of the solution (of solutes in water in most foods) divided by the vapour pressure of the solvent usually water.

- The water activity for pure water would be 1.00 and for a 1M solution of ideal solute, the aw would be 0.9823. The aw would be in equilibrium with a relative humidity (RH) of the atmosphere about the food. Solutes and ions tie up water in solution therefore an increase in the concentration of dissolved substances such as sugars and salts has an effect of drying of the material.

- Not only water is tied with the solutes, but water tends to leave the microbial cells by Osmosis if there is a higher concentration of solutes outside cells than inside.

Moisture content/Water activity: a_w

- Moisture content = (% water / 100 g of the product)

Ex:		
	bananas	75.7%
	asparagus	91.7%
	pasta	10.4%

- Water activity = index of availability of water to microbial growth

$$a_w = P / P_0 \longleftrightarrow \text{Relative humidity RH} = 100 \times a_w$$

P= vapour pressure above the product

P_0= vapour pressure of pure water

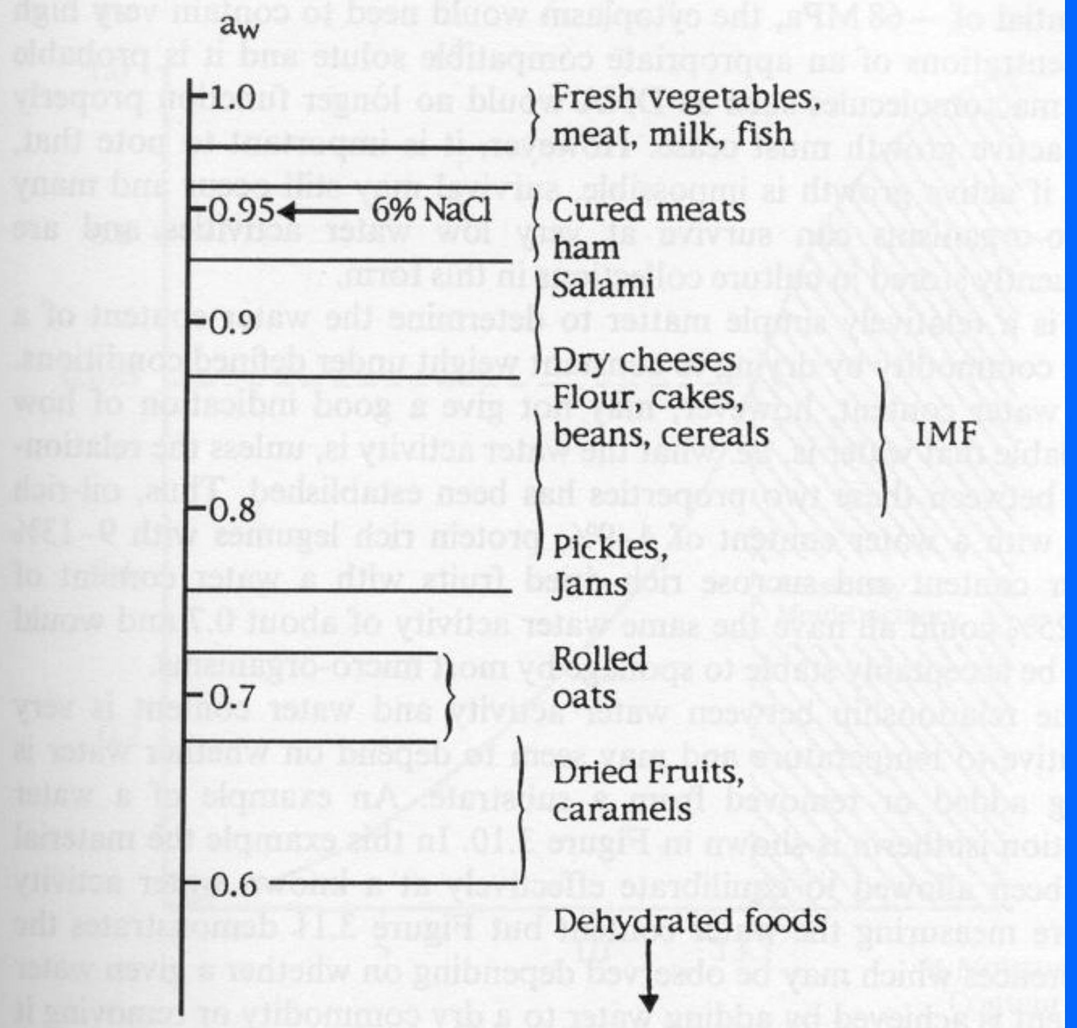


Figure 3.9 *Range of* a_w *values associated with a number of food commodities*

The lowest aw values permitting growth of spoilage organisms

Group of organisms	**Minimal aw value**
Most spoilage bacteria	0.91
Most spoilage yeast	0.88
Most spoilage moulds	0.80

Oxidation – Reduction Potential (Eh)

- Microorganisms display varying degrees of sensitivity to the oxidation-reduction potential of their growth medium.

- Eh of substance is defined as the ease with which the substrate loses or gains electrons. When an element or compound loses electrons, the substrate is said to be oxidized, where as a substrate that gains electrons becomes reduce

$$Cu \underset{\text{Oxidation}}{\overset{\text{Reduction}}{\rightleftarrows}} Cu^{+} + e^{-}$$

- Oxidation may also be achieved by addition of oxygen. e.g.

$$2Cu + O_2 \rightarrow 2Cuo$$

- Substance that readily gives up electrons: Good reducing agents Substance that readily takes up electrons: Good oxidizing agents

- When electrons are transferred from one compound to another, a potential difference is created between the two compounds. The potential difference is measured in millivolts (mV). The more highly oxidized a substance is the more positive will be its electrical potential the more highly reduced a substance is the more negative will be its electrical potential.
- When the concentration of **oxidant** and **reductant** is equal, a **zero** electrical potential exists.

- Aerobic microorganisms require positive Eh values (oxidized) for growth, whereas anaerobes require negative Eh values (reduced).

- Among the substances in foods that help to maintain reducing conditions are SH groups in meats and ascorbic acid and reducing sugars in fruit and vegetables.

The redox potential (Eh) of a food is determined by the following:

- The **characteristic redox potential (Eh)** of the original food
- The **poising capacity**; that is the resistance to changes in potential to the food
- The **oxygen tension** of the atmosphere about the food
- The **access that the atmosphere** has to the food
- With regard to the Eh of foods, plant foods, especially plant juices tend to have Eh values from 300mV- 400mV therefore aerobic bacteria and molds are the common cause of spoilage of these products. Solid meats have Eh values around 200mV, in minced meat Eh is generally 200mV. Cheeses of various types have Eh values on the negative side, from -20 to -200mV

Nutrient Content:

- In order to grow and function normally the microorganisms of importance in foods require the following:
- Water
- Source of energy
- Sources of nitrogen
- Vitamins and related growth factors
- Minerals

As sources of energy, food borne microorganisms may utilize sugar, alcohols and amino acids. Some few microorganisms are able to utilize complex carbohydrates such as starches and cellulose as sources of energy by first degrading these compounds to simple sugar. Fats are used also by microorganisms as sources of energy but these compounds are attacked by a relatively small number of microbes in foods.

Antimicrobial Constituents

Some foods cannot easily be attacked by microorganisms due to the presence of naturally occurring substances that have antimicrobial activity

- Example is the presence of essential oils in some species. Among these are:-
  i. Eugenol in cloves
  ii. Allicin in garlic
  iii. Cinamic aldehyde and eugenol in cinnamon lactoferrin, conglutinin and lactoperoxidase system in cow's milk. Under certain conditions milk **casein** as well as some **free fatty acids** have antimicrobial activity

- Eggs contain **lysozymes**. Thi enzyme along with **conalbumin**, provides fresh eggs with a fairly efficient antimicrobial system.

- Fruits, vegetables, tea, molasses contain **hydroxycinnamic** acid derivatives which show antibacterial activity.

Biological Structures

Some foods are naturally covered and these covering provide excellent protection against the entry and subsequent damage by spoilage organisms.

Testa of seeds, the **outer covering of fruits** the **shell of nuts**, the hide of animals and the **egg shells**.

- In nuts, the **shell prevent the entry of all organisms** but once cracked nutmeats are subject to spoilage by molds

- The **egg shell and membranes if intact prevent the entry of nearly all microorganisms** when stored under the proper conditions of humidity and temperature.

- Fruits and vegetable with **damaged covering undergo spoilage much faster than those not damaged**

- The **skin covering of fish and meats such as beef pork** prevents the contamination and spoilage of these foods partly because it tends to dry out faster than freshly cut surfaces.

EXTRINSIC PARAMETERS

These are the properties of the storage environment that affect both the foods and their microorganisms. These are:

- Temperature of storage
- Relative humidity of the environment
- Presence and concentration of gases
- Presence and activities of other microorganisms

Temperature

Individual or groups of microorganisms grow over a wide range of temperature. Depending on their temperature requirements for growth, microorganisms can be placed in three different groups

(i) Psychrotrophs:

Are those organisms that grow well at or below 7°C and their optimum temperature is between 20°C and 30°C. The lowest temperature at which a microorganism has been reported to grow is -34°C.; the highest somewhere in excess of 100°C.

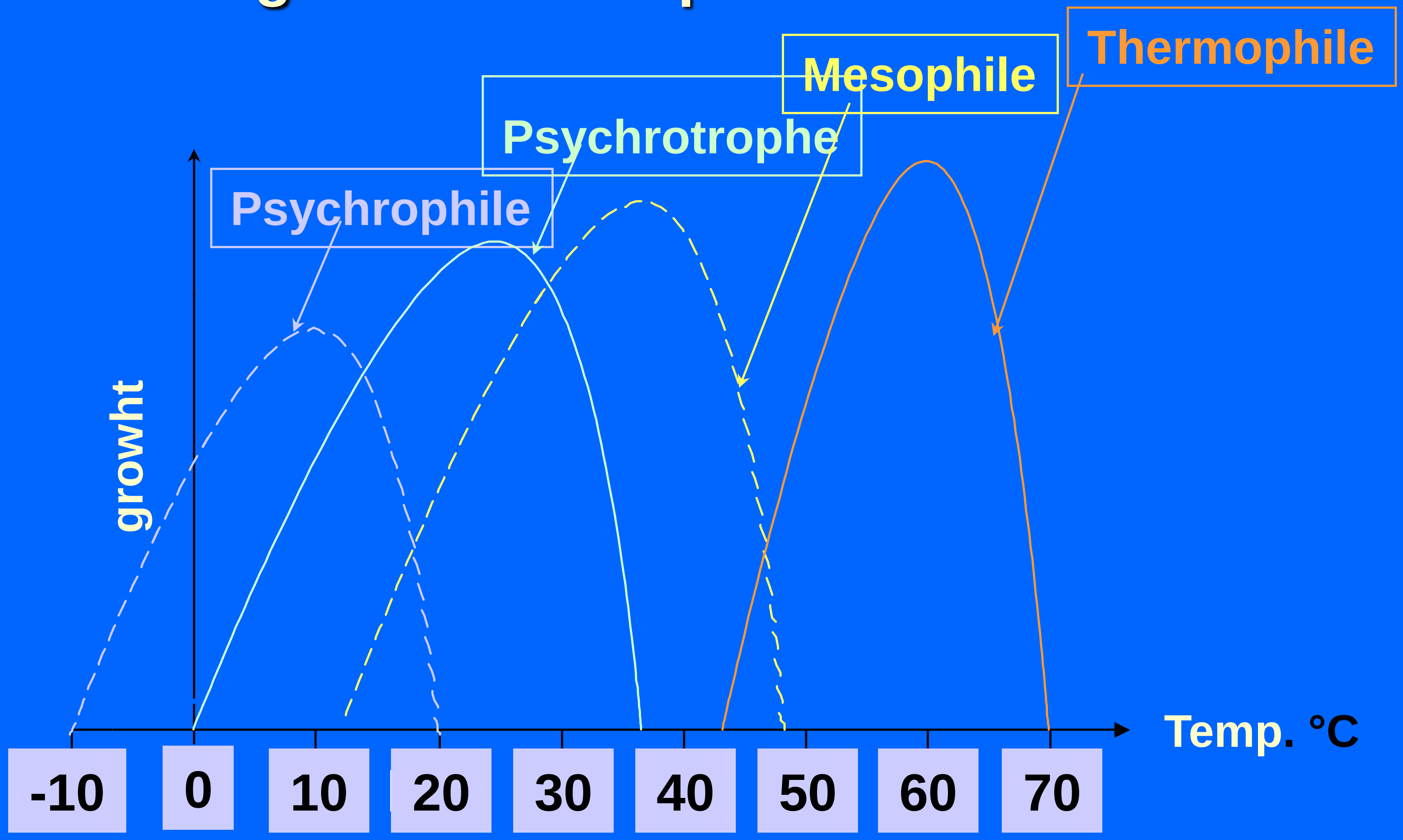
(ii) Mesophiles:

Those microorganisms that grow well between 20°C and 45°C with optima between 30°C and 40°C (E.g. Enterococcus feacalis)

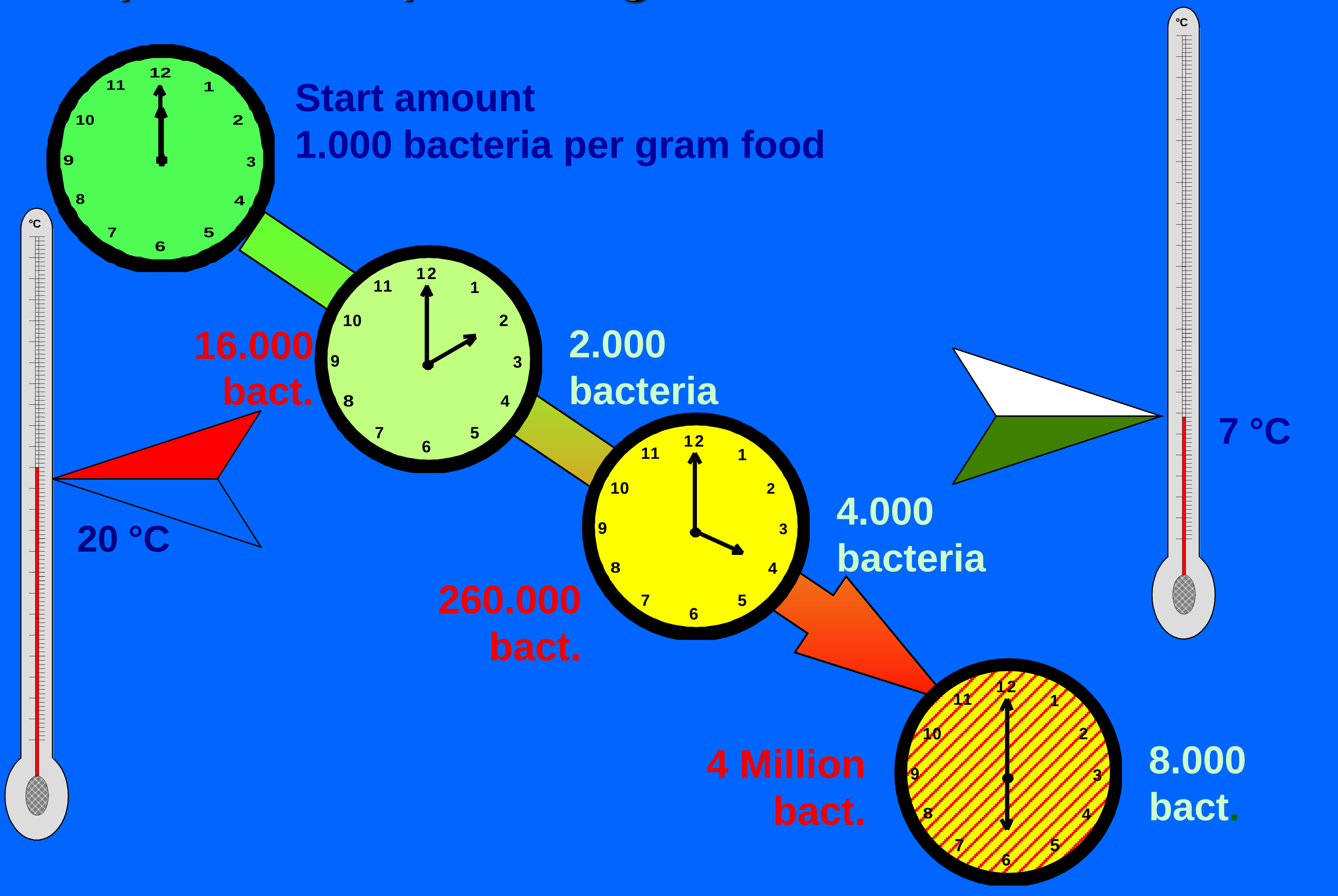
(iii) Thermophiles:

Those microorganisms that grow well at and above 45°C with optima between 55°C and 65°C

Micro-organisms - Temperature and Growth



Temperature dependent growth



- The psychrotrophs found most commonly on foods are those that belong to the genera *Pseudomonas* and *Enterococcus*. These organisms grow well at refrigerator temperatures and cause spoilage of meats poultry, eggs and other foods normally held at this temperature.

- Thermophilic bacteria of importance in foods belong to the genera *Bacillus* and *Clostridium*. Although a few species of this genera are thermophillic, they are of great importance in the canning industry.

- Molds are able to grow over a wider range of temperature than bacteria many molds are able to grow at refrigeration temperatures (e.g. some strains of *Aspergillus* in eggs, sides of beef and fruits.

- Yeast grow over the psychrotrophic and mesophillic temperature ranges but generally not within the thermophillic range

- Temperature of storage is the most important parameter that affects the spoilage of highly perishable foods.

Relative Humidity of Environment (RH)

- When foods with low a_w values are placed in environments of high R.H the foods pick up moisture until equilibrium has been established.

- Likewise foods with a high a_w lose moisture when should bear in mind that there is a relationship between R.H and temperature the higher the temperature, the lower the R.H and vice versa.

- Foods that undergo surface spoilage from moulds, yeasts and certain bacteria should be stored under condition of low R.H

- Improperly rapped meats such as whole chickens and beef cuts tend to suffer much spoilage in the refrigerator before deep spoilage occurs. This is due to high R.H of the refrigerator and the fact that meat spoilage microorganisms are aerobic in nature.

Presence of Concentration of Gases in The Environment

Controlled atmosphere (modified atmosphere storage)

- This is the atmosphere containing increased amounts of CO_2 up to about 10% CO_2 is applied from mechanical sources or by use of solid CO_2
- Modified atmosphere is employed in storage of fruits e.g. apples and pears. CO_2 retard fungal rotting of fruits
- CO_2 atmospheres extend the storage life of meat carcasses. In general the inhibitory effects of CO_2 at lower temperatures, and the pH of meats stored in high- CO_2 environments tends to be slightly lower than that of air – stored due to carbonic acid formation.

- Gram-negative bacteria are more sensitive to CO_2 than gram-positive.

- Ozone (O_3); when added to food storage environment has a preservative effect on certain foods. At levels of several parts per million (ppm) O_3 has been found to be effective against a variety of microorganisms O_3 should not be used on high-lipid content foods because it would cause an increase in rancidity as it is a strong oxidizing agent

Presence and Activities of Other Microorganisms

Some food borne organisms produce substances that are either inhibitory or lethal to others. These substances include antibiotics, bacteria, hydrogen peroxide and organic acids.

Antibiotics:

- These are secondary metabolites produce by microorganisms that inhibit or kill a wide spectrum e.g. nicin and natamycin.

General microbial interference

- Refers to general nonspecific inhibition or destruction of one microorganism by other members of the same habitat or environment

Lactic antagonism:

- This is a specific example of microbial interference, it is the phenomenon of a lactic acid bacterium inhibiting or killing closely related and food poisoning or food spoilage organisms when in mixed culture.
- In this case the bacteriocins, pH depression, organic acids, H_2O_2 diacetyl and other products effect inhibition of pathogens and food – spoilage organisms.

COMBINED INTRINSIC AND EXTRINSIC PARAMETERS THE HURDLE CONCEPT

- In the **hurdle concept**, multiple factors or techniques are employed to effect the control of microorganisms in foods, while under intrinsic and extrinsic parameters; the effect of single factors on the growth of microorganisms is presented.

- The hurdle concept is applied in food preservation is also described in different ways such as; Barrier Technology, Combination Preservation or Combined Methods.

- In this technique, in order to grow the organisms must "hurdle" a series of barriers. A large number of factors are known that can be applied to food systems as hurdles.

Example of the Hurdle Effect

- In preventing germination of spores of photolytic or group 1 strains of *Clostridium botulinum*, among the intrinsic and extrinsic parameters that are known are as follows: pH<4.6, aw <0.99, 10% Nacl, 120ppm, NaNO2, incubation temperature and <10^{o}C.

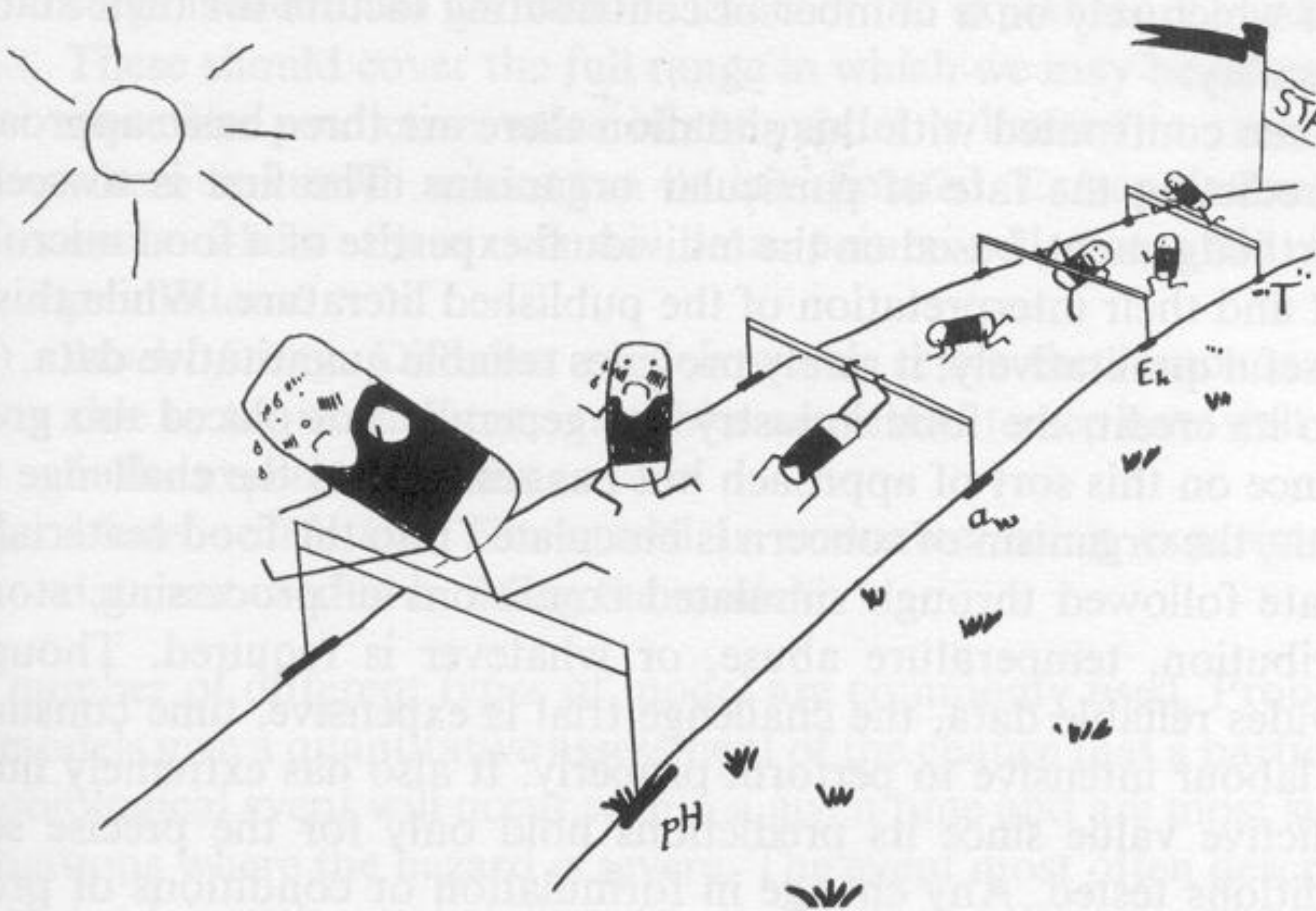


Figure 3.14 *The hurdle effect*