COURSE TITLE: FOOD AND INDUSTRIAL MICROBIOLOGY
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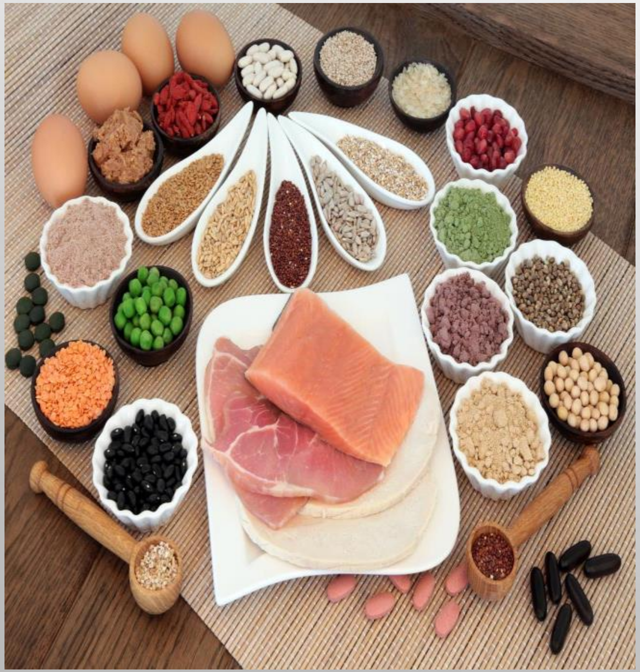
FACTORS AFFECTING GROWTH AND SURVIVAL OF MICROORGANISMS IN FOODS

Background

Bacteria exist everywhere in nature. They are in the soil, air, water and the foods we eat. When the bacteria have nutrients (food), moisture, time and favorable temperatures, they grow rapidly increasing in numbers to the point where some can cause illness. Therefore, understanding the important role temperature plays in keeping food safe is critical. If we know the temperature at which food has been handled, we can then answer the question, “Is it safe?”



- Food is a potential source of infection and is liable to contamination by microbes, at any point during its journey from the producer to the consumer.
- Food hygiene may be defined as the sanitary science which aims to produce food which is safe for the consumer and of good keeping quality.
- It covers a wide field and includes the rearing, feeding, marketing and slaughter of animals as well as the sanitation procedures designed to prevent bacteria of human origin reaching food stuff.

MICRO-ORGANISMS AND FOOD

1- The foods that we eat are rarely if ever sterile.
2- They carry microbial associations whose composition depends upon which organisms gain access and how they grow, survive and interact in the food over time.
3- The micro-organisms present will originate from the natural micro-flora of the raw material .

MICRO-ORGANISMS AND FOOD

4- And those organisms introduced in the course of harvesting, processing, storage and distribution .

5-The numerical balance between the various types will be determined by the properties of the food, its storage environment, properties of the organisms themselves and the effects of processing.

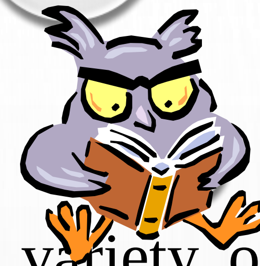
Micro-organisms manifest their presence in one of several ways.

(i) They can cause spoilage.

(ii) They can cause foodborne illness.

(iii) They can transform a food's properties in a beneficial way – food fermentation.

FACTORS AFFECTING GROWTH AND SURVIVAL OF MICROORGANISMS IN FOODS



Microorganisms are similar to more complex organisms in that they need a variety of materials from their environment to function and accomplish two primary goals--supply enough energy to manage their processes and extract building blocks to repair themselves or procreate. In addition to what they take in, microorganisms also thrive in particular environments. These environments vary as much as the organisms do themselves, and even the amount and distribution of elements in any particular environment can be very important.

Food spoilage means the original nutritional value, texture, colour, flavour, taste etc., of the food are changed / damaged, the food become harmful to people and unsuitable to consume.

Food can deteriorate as a result of two main factors:

1) Growth of microorganisms usually from surface contamination especially important in processed food

2) Action of enzymes from within cells part of normal life processes. It is important to note that many plants fresh vegetables and fruit are still alive when bought and even when eaten raw and meat from animals undergoes gradual chemical changes after slaughter. The various sources through which microorganisms gain entry into the foods are shown in Table.

Primary sources of microorganisms found in foods

Microflora present in soil and water

Microflora present in air

Microflora present on plant and plant products

Microflora present on food utensils and equipments

Microflora present in animal feeds

Microflora present on animal hides

Microflora present in intestinal tracts of humans and animals

Food handlers

A variety of intrinsic and extrinsic factors determine whether microbial growth will preserve or spoil foods.

Intrinsic Factors	Extrinsic factors	Implicit factors	Processing factors
Nutrient content	Temperature	Synergism	Irradiation
pH	Relative humidity	Antagonism	Washing
Redox potential	Gaseous atmosphere	Commensalism	Slicing
Water activity	Growth rate		Pasteurization
Antimicrobial constituents & barriers			Packaging

Microorganisms involved in food spoilage (other than canned foods) with some examples of causative organisms

Food	Type of Spoilage	Microorganisms involved
Bread	Mouldy	*Rhizopus nigricans* *Penicillium* spp. *Aspergillus niger*
	Ropy	*Bacillus subtilis*
Maple sap and syrup	Ropy	*Enterobacter aerogenes*
	Yeasty	*Saccharomyces* *Zygosaccharomyces*
	Pink	*Micrococcus roseus*
	Mouldy	*Aspergillus* *Penicillium*
Fresh fruits and vegetables	Soft rot	*Rhizopus* *Erwinia*
	Gray mold rot	*Botrytis*
	Black mold rot	*A. niger*
Fresh meat	Putrefaction	*Alcaligenes* *Clostridium* *Proteus vulgaris* *Pseudomonas fluorescens*
Cured meat	Mouldy	*Aspergillus* *Rhizopus* *Penicillium*
	Greening, slime	*Lactobacillus* *Leuconostoc*
Fish	Discoloration	*Pseudomonas*
	Putrefaction	*Alcaligenes*
Eggs	Green rot	*P. fluorescens*
	Colorless rots	*Pseudomonas* *Alcaligenes*
	Black rots	*Proteus*
Concentrated orange juice	"Off" flavor	*Lactobacillus* *Leuconostoc* *Acetobacter*
Poultry	Slime, odor	*Pseudomonas* *Alcaligenes*

Moisture content

One of the oldest methods of preserving foods is drying or desiccation; precisely how this method came to be used is not known. The preservation of foods by drying is a direct consequence of removal or binding of moisture, without which microorganisms do not grow. It is now generally accepted that the water requirements of microorganisms should be described in terms of the *water activity* (aw) in the environment. This parameter is defined by the ratio of the water vapor pressure of food substrate to the vapor

pressure of pure water at the same temperature—aw = *p/po*,

where *p* is the vapor pressure of the solution and

po is the vapor pressure of the solvent (usually water).

This concept is related to relative humidity (RH) in the following way:

RH = 100 x aw.

Pure water has an aw of 1.00, a 22% NaCl solution (w/v) has an aw of 0.86, and a saturated solution of NaCl has an aw of 0.75.The free flow of water is vital to microorganisms for their cells to exchange materials and for their metabolic processes. All microorganisms require some level of water, but a few can survive in low-moisture conditions by conserving all the water they find and by staying in a moisture-rich environment. As a general rule, though, the more moisture, the more microorganisms there will be found.

Water activity is an important property that can be used to predict food safety, stability and quality. The various applications of water activity include maintaining the chemical stability of foods, minimizing non enzymatic browning reactions and spontaneous autocatalytic lipid oxidation reactions, prolonging the desired activity of enzymes and vitamins in foods, optimizing the physical properties of foods such as texture.

Minimum water activity values of spoilage microorganisms

Microbial group	Minimum a_w	Examples
Most bacteria	0.91	*Salmonella* spp. *Clostridium botulinum*
Most yeasts	0.88	*Torulopsis* spp.
Most molds	0.80	*Aspergillus flavus*
Halophilic bacteria	0.75	*Wallemia sebi*
Xerophilic molds	0.65	*Aspergillus echinulats*
Osmophilic yeasts	0.60	*Saccharomyces bisporus*

Water activity scale extends from 0 (bone dry) to 1.00 (pure water). But most foods have a water activity in the range of 0.2 for very dry foods to 0.99 for moist fresh foods. Based on regulations, if a food has a water activity value of 0.85 or below, it is generally considered as non-hazardous.

Moisture content of different food

Food	*Water Content (%)*
Meat	
Pork, raw, composite of lean cuts	53–60
Beef, raw, retail cuts	50–70
Chicken, all classes, raw meat without skin	74
Fish, muscle proteins	65–81
Fruit	
Berries, cherries, pears	80–85
Apples, peaches, oranges, grapefruit	90–90
Rhubarb, strawberries, tomatoes	90–95
Vegetables	
Avocado, bananas, peas (green)	74–80
Beets, broccoli, carrots, potatoes	85–90
Asparagus, beans (green), cabbage, cauliflower, lettuce	90–95

It should be noted that a water activity below 0.91, most bacteria including the pathogens such as *Clostridium botulinum* cannot grow. But an exception is *Staphylococcus aureus* which can be inhibited by water activity value of 0.91 under anaerobic conditions but under aerobic conditions, it requires a minimum water activity value of 0.86. Most molds and yeasts can grow at a minimum water activity value of 0.80. Thus a dry food like bread is generally spoiled by molds and not bacteria. In general, the water activity requirement of microorganisms decreases in the following order: Bacteria > Yeast > Mold. Below 0.60, no microbiological growth is possible. Thus, the dried foods like milk powder, cookies, biscuits etc are more shelf stable and safe as compared to moist or semi moist foods. Factors that affect water activity requirements of microorganisms include the following kind of solute added, nutritive value of culture medium, temperature, oxygen supply, pH, inhibitors, etc.

Water activity of the foods can be reduced by several methods: by the addition of solutes or hydrophilic colloids, cooking, drying and dehydration: (e.g. egg powder, pasta), or by concentration (e.g. condensed milk) which restrict microbial growth so as to make the food microbiologically stable and safe.

Nutrients

All microorganisms need food. The food sources can vary, but the organisms primarily extract carbon and nitrogen from substances such as proteins, fats and carbohydrates. Some microorganisms seek out and absorb such particles. Others may perform chemical reactions with surrounding elements such as carbon dioxide to gain what they need, while still others can produce their own simple sugars through photosynthesis similar to plants. Nitrogen, which is used to synthesize proteins, can be taken from the surrounding atmosphere or from other organic matter.

Many food microorganisms have the ability to utilize sugars, alcohols, and amino acids as sources of energy. Few others are able to utilize complex carbohydrates such as starches and cellulose as sources of energy. Some microorganisms can also use fats as the source of energy, but their number is quite less. Since foods are rich source of these compounds, they can be used by microorganisms also. It is because of these reasons that various food products like malt extracts, peptone, tryptone, tomato juice, sugar and starch are incorporated in microbial media. The inability to utilize a major component of the food material will limit its growth and put it at a competitive disadvantage compared to those that can. In general, molds have the lowest requirement, followed by yeasts, Gram negative bacteria, and Gram-positive bacteria

pH and buffering capacity

The pH or hydrogen ion concentration, [H+], of natural environments varies from about 0.5 in the most acidic soils to about 10.5 in the most alkaline lakes. Since the pH is measured on a logarithmic scale, the [H+] of natural environments varies over a billion-fold and some microorganisms are living at the extremes, as well as every point between the extremes. The range of pH over which an organism grows is defined by three cardinal points: the minimum pH, below which the organism cannot grow, the maximum pH, above which the organism cannot grow, and the optimum pH, at which the organism grows the best. Microorganisms which grow at an optimum pH well below neutrality (7.0) are called acidophiles. Those which grow best at neutral pH are called neutrophiles and those that grow best under alkaline conditions are called alkalophiles. In general, bacteria grow faster in the pH range of 6.0-8.0, yeasts 4.5-6.5 and filamentous fungi 3.5-6.8, with the exception of lactobacilli and acetic acid bacteria with optima between pH 5.0 and 6.0.


pH Food Chart
COLA
Soda
Purified Water
Most Spring Water
Tap Water
Sea Water
ALKA
3
4
5
6
7
8
9
10
Acidic
Neutral
Alkaline
pH
Acidic
pH SPECTRUM
Alkaline
pH

Approximate pH ranges of some common food commodities

Product	pH
Citrus fruits	2.0-5.0
Soft drinks	2.5-4.0
Apples	2.9-3.3
Bananas	4.5-4.7
Beer	3.5-4.5
Meat	5.6-6.2
Vegetables	4.0-6.5
Fish (most spp)	6.6-6.8
Milk	6.5-6.8
Wheat flour	6.2-6.8
Egg white	8.5-9.5
Fermented shark	10.5-11.5

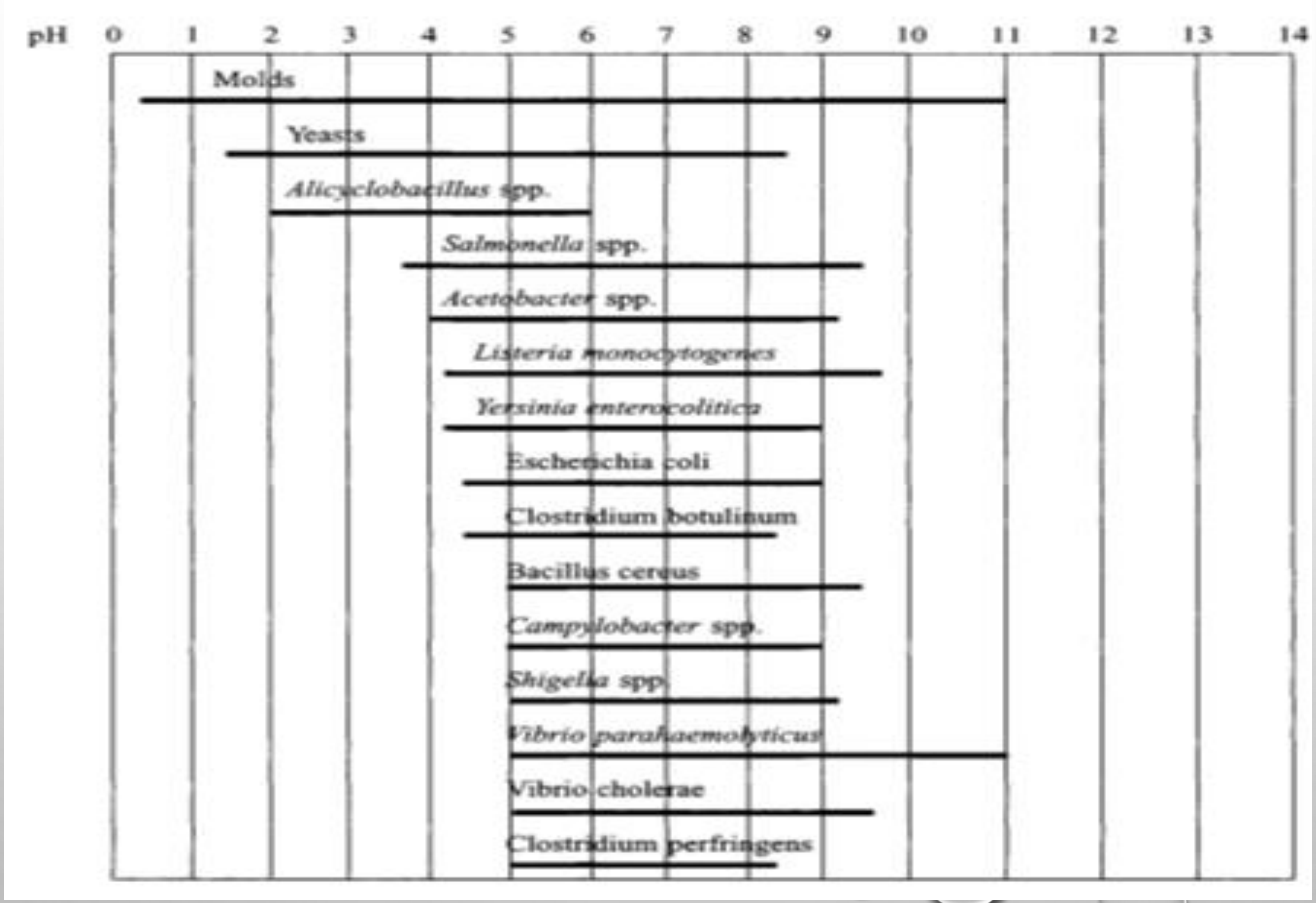
Approximate pH ranges of different microbial groups

Microbe	Minimum	Optimum	Maximum
Most Bacteria	4.5	6.5 – 7.5	9.0
Yeasts	1.5 – 3.5	4.0 – 6.5	4.0 – 6.5
Molds	1.5 – 3.5	4.5 – 6.8	8.0 – 11.0

Approximate pH ranges of different types of food

Food type	Range of pH values
Beef	5.1 - 6.2
Chicken	6.2 – 6.4
Milk	6.3 – 6.8
Cheese	4.9 - 5.9
Fish	6.6 - 6.8
Oyster	4.8 - 6.3
Fruits	< 4.5 (most < 3.5)
Vegetables	3.0 – 6.1

Approximate pH growth ranges for some foodborne organisms.



Redox potential (Eh)

Microorganisms display varying degrees of similarity to Oxidation Reduction potential of their growth medium. The O/R potential is the measure of tendency of a revisable system to give or receive electrons. When an element or compound loses electrons, it is said to be oxidized, while a substrate that gains electrons becomes reduced. Thus, a substance that readily gives up electrons is a good reducing agent, while one that readily gains electrons is a good oxidizing agent. When electrons are transferred from one compound to another, a potential difference is created between the two compounds and is expressed in as milk volts (mV). If a substance is more highly oxidized, the more positive will be its electrical potential and vice versa. The O/ R potential of a system is expressed as Eh. Aerobic microorganisms require positive Eh values for growth while anaerobic microorganisms require negative Eh values (reduced). Oxygen is the most powerful of redox couple present in food system and if the food is stored in the presence of air, high positive potential will result. Thus, increasing the exposure to oxygen in air by mincing, cutting, chopping, grinding of food will increase the Eh. Finally, microbial growth in the food reduces the Eh due to oxygen depletion. The decrease in Eh due to microbial activity forms the basis of some tests used frequently in raw milk such as platform MBRT test.

Microorganisms can be grouped into categories based on their requirement on intolerance to oxygen

1) Aerobes • Grow in the presence of air that contains molecular oxygen. • Obligate aerobes require oxygen for growth and carry out aerobic respiration.

2) Microaerophiles • Grow only at reduced concentrations of molecular oxygen - 5% of atmospheric oxygen concentration (20%)

3) Facultative anaerobes • Can grow in the presence or absence of air. If oxygen is not available, they will carry out anaerobic respiration

4) Anaerobes • Do not require oxygen for growth, therefore only in the absence of air. • Strict anaerobes are sensitive to oxygen and even to a brief exposure to oxygen will kill such organisms e.g. Clostridium spp.

Aerobes	Between +500 and +300 mV
Anaerobes	Between +100 and -250 mV
Facultative anaerobes	Between +300 and -100 mV

Antimicrobial constituents

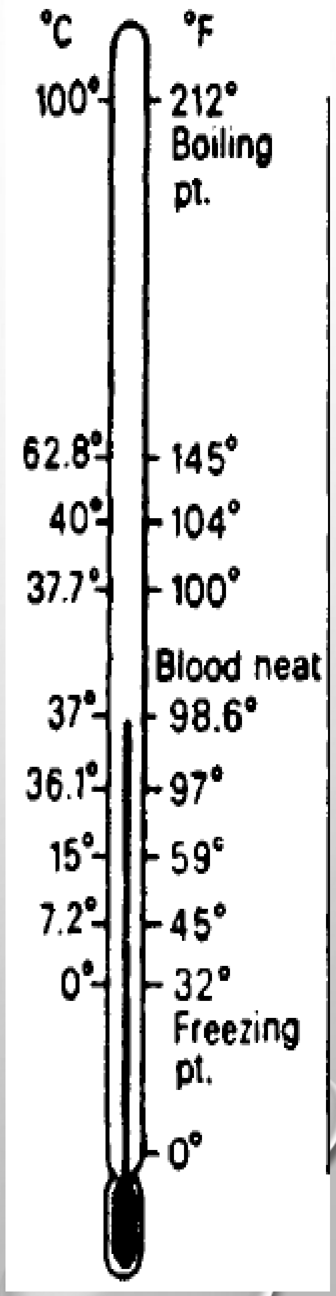
Chemical compounds having pharmacological and biological activity and produced by living organisms are called natural products. Living organisms produce primary and secondary metabolites. Primary metabolites are the products that have essential function in the organism, while secondary metabolites could simply be waste products or could have some important function in their producers. Secondary metabolites possessing antimicrobial activity are called the natural antimicrobials and could be extracted from different sources like plants (fruits, vegetables, seeds, herb, and spices), animals (eggs, milk, and tissues), and microorganisms (fungi and bacteria). With special reference to plants, secondary metabolites are found to be healthy ingredients that work as antimicrobials or disease-controlling agents. Owing to the potential of antimicrobials against pathogenic and spoilage microorganisms, these secondary metabolites gain much importance for the application in food products. Some foods can resist the attack by microorganisms due to the presence of certain naturally occurring substances which possess antimicrobial activity such as essential oils in spices (eugenol in cloves and cinnamon, allicin in garlic, cinnamic aldehyde in cinnamon, thymol in sage); lactaferrin, lactoperoxidase and lysozyme in milk; and ova transferrin, avidin, lysozyme and Ovo flavoprotein in hen's egg albumin. Similarly, casein as well as free fatty acids found in milk also exhibit antimicrobial activity.

Biological Structures

The natural covering of some foods provides excellent protection against the entry and subsequent damage by spoilage organisms. In this category are such structures as the testa of seeds, the outer covering of fruits, the shell of nuts, the hide of animals, and the shells of eggs. In the case of nuts such as pecans and walnuts, the shell or covering is sufficient to prevent the entry of all organisms. Once cracked, of course, nutmeats are subject to spoilage by molds. The outer shell and membranes of eggs, if intact, prevent the entry of nearly all microorganisms when stored under the proper conditions of humidity and temperature. Fruits and vegetables with damaged covering undergo spoilage much faster than those not damaged. The skin covering offish and meats such as beef and pork prevents the contamination and spoilage of these foods, partly because it tends to dry out faster than freshly cut surfaces.

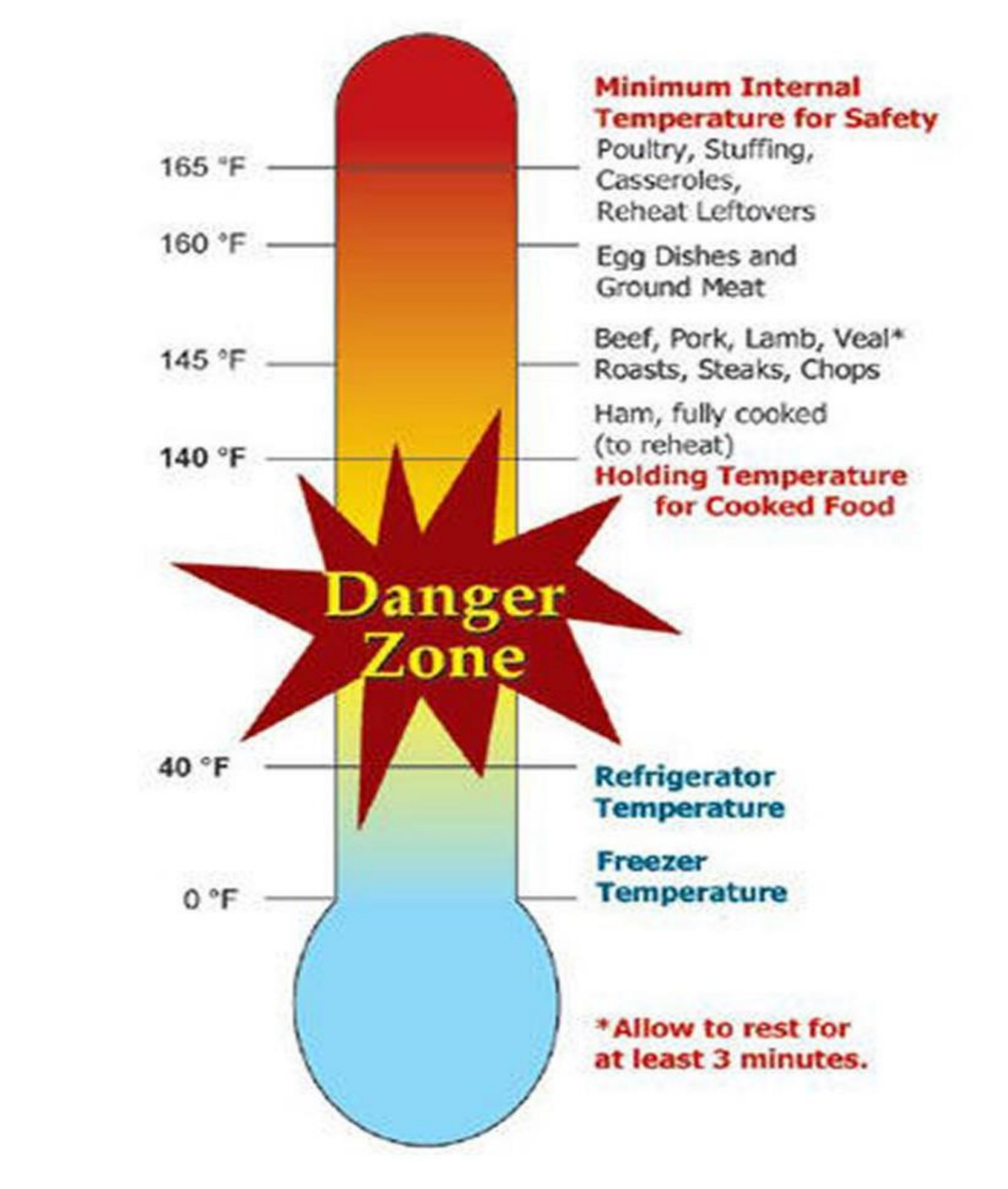
Taken together, these six intrinsic parameters represent nature's way of preserving plant and animal tissues from microorganisms. By determining the extent to which each exists in a given food, one can predict the general types of microorganisms that are likely to grow and, consequently, the overall stability of this particular food. Their determination may also aid one in determining age and possibly the handling history of a given food.

Temperature On the basis of temperature preferences microorganisms are categorized as psychrophiles, psychrotrophs, mesophiles and thermophiles. For example, organisms with an optimum temperature near 37°C are called mesophiles. Organisms with an optimum temperature between about 45°C and 70°C are thermophiles e.g, Bacillus, Clostridium etc. Some archaebacteria with an optimum temperature of 80°C or higher and a maximum temperature as high as 115°C, are now referred to as extreme thermophiles or hyperthermophiles. The cold loving organisms are psychrophiles defined by their ability to grow at 0°C. A variant of a psychrophile (which usually has an optimum temperature above 10°C) is a psychrotroph, which grows at below 7°C but displays an optimum temperature in the mesophile range, nearer room temperature. Psychrotrophs are the problematic group of microorganisms during food storage in refrigerators since they continue to grow in the refrigerated environment where they spoil the food. Of course, they grow slower at 2°C than at 25°C. In food microbiology mesophilic and psychrotrophic organisms are of greatest importance.



The Danger Zone (40 to 140 °F)

Bacteria grow most rapidly in the range of temperatures between 40 and 140 °F, doubling in number in as little as 20 minutes. This range of temperatures is often called the “Danger Zone.” That’s why perishable foods should never be left out of refrigeration over two hours. If the temperature is above 90 °F, food should not be left out more than one hour. Properly handled food stored in a freezer at 0 °F will always be safe. Freezing keeps food safe by slowing the movement of molecules, causing bacteria to enter a dormant stage. Once thawed, these bacteria can again become active and multiply to levels that may lead to foodborne illness. Because bacteria on these foods will grow at about the same rate as they would on fresh food, thawed foods should be handled as any other perishable food



Relative humidity of the storage environment

Relative humidity of air is defined as the ratio of the vapor pressure of air to its saturation vapor pressure. The equilibrium relative humidity (ERH) of a food product is defined as relative humidity of the air surrounding the food that is in equilibrium with its environment. When the equilibrium is obtained, the ERH (in percent) is equal to the water activity multiplied by 100, i.e. ERH (%) = aw × 100. When a food is exposed to a constant humidity, the product will gain or lose moisture until the ERH is reached. The moisture migration significantly affects the physical and chemical properties of the food.

Relative humidity and water activity are interrelated. When foods with low aw are stored in environment of high humidity, water will transfer from the gas phase to the food and thus increasing aw of the food leading to spoilage by the viable flora. There is a relationship between temperature and humidity which should be kept in mind. In general, the higher the temperature, lower is the relative humidity and vice versa. Foods that undergo surface spoilage from molds, yeasts, and some bacteria should be stored in conditions of low relative humidity to increase their shelf life. This can also be done by proper wrapping of the food material also.

Gaseous atmosphere

Modified atmosphere packaging (**MAP**) is a **packaging** system that involves changing the gaseous atmosphere surrounding a food product inside a pack, and employing **packaging** materials and formats with an appropriate level of gas barrier to maintain the changed atmosphere at an acceptable level for preservation of the food. Modified Atmosphere Packaging (MAP) is a long established and continuously increasing technique for extending the shelf-life of fresh food products. MAP requires specialized machinery to flush out air from the packaging and replace it with a different gas or gas mixture. The MAP packaging aims to provide longer shelf-life, maintain sensory attributes like colour or appearance and achieve the food safety of the product. The normal composition of air is 21% oxygen, 78% nitrogen and less than 0.1% carbon dioxide. Modification of the atmosphere within the package by reducing the oxygen content while increasing the levels of carbon dioxide and/or nitrogen has been shown to significantly extend the shelf-life of perishable foods at chill temperatures.

Oxygen is one of the most important gases which come in contact with food influence the redox potential and finally the microbial growth. The inhibitory effect of CO_2 on the growth of microorganisms is applied in modified atmosphere packaging of foods. The storage of foods in atmosphere containing 10% of CO_2 is referred to as “Controlled Atmosphere”. This type of treatment is applied more commonly in case of fruits such as apples and pears. With regards to the effect of CO_2 on microorganisms, molds and Gram negative bacteria are the most sensitive, while the Gram positive bacteria, particularly the lactobacilli tend to be more resistant.

THANK YOU