Intrinsic and Extrinsic Parameters of Foods that Affect Microbial Growth

Introduction

- As our foods are of plant and animal origin, it is worthwhile to consider those characteristics of plant and animal tissues that affect the growth of microorganisms.
- The plants and animals that serve as food sources have all evolved mechanisms of defense against the invasion and proliferation of microorganisms, and some of these remain in effect in fresh foods.
- By taking these natural phenomena into account, one can make effective use of each or all in preventing or retarding

  the microbial spoilage of the products that are derived from them.

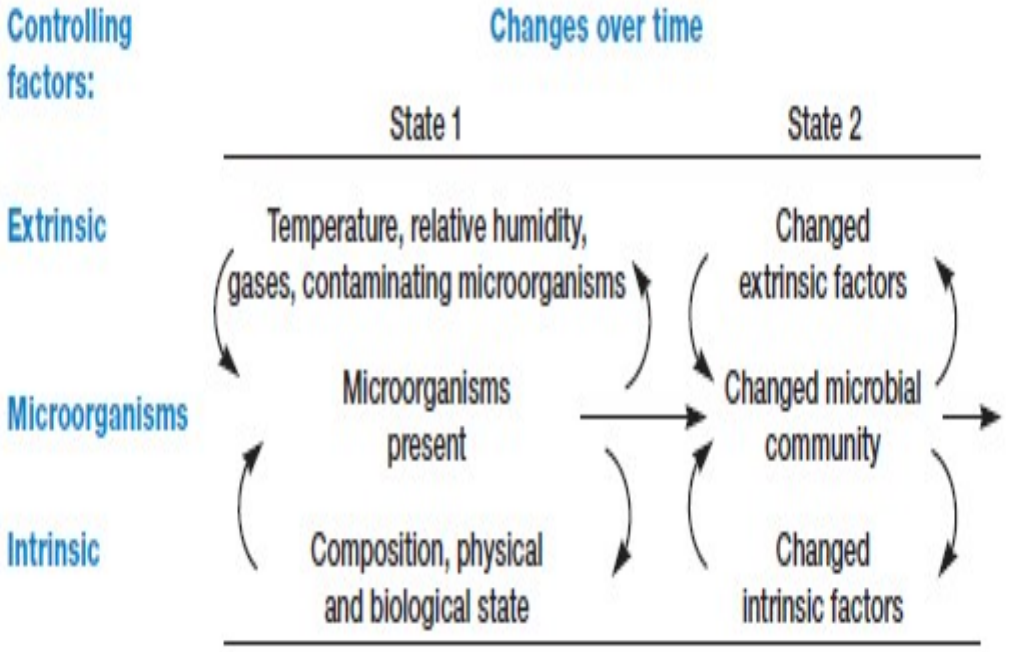


Figure 41.1 Intrinsic and Extrinsic Factors. A variety of intrinsic and extrinsic factors can influence microbial growth in foods. Time-related successional changes occur in the microbial community and the food.

INTRINSIC PARAMETERS

The parameters of plant and animal tissues that are an inherent part of the tissues are referred to as *intrinsic parameters.*

These parameters are as follows:

- pH
- Moisture content
- Oxidation-reduction potential (Eh)
- Nutrient content
- Antimicrobial constituents
- Biological structures

pH

- It has been well established that most microorganisms grow best at pH values around 7.0 (6.6-7.5), whereas few grow below 4.0.
- Bacteria tend to be more fastidious in their relationships to pH than molds and yeasts, with the pathogenic bacteria being the most fastidious.
- For example, the pH minima of certain *Lactobacilli* have been shown to be dependent on the type of acid used, with citric, hydrochloric, phosphoric, and tartaric acids permitting growth at a lower pH value than acetic or lactic acids.

pH....

- In the presence of 0.2 M NaCl, *Alcaligenes faecalis* has been shown to grow over a wider pH range than in the absence of NaCl or in the presence of 0.2M sodium citrate.
- It can be seen that fruits, soft drinks, vinegar, and wines all fall below the point at which bacteria normally grow.
- The excellent keeping quality of these products is due in great part to pH.

pH....

- It is a common observation that fruits generally undergo mold and yeast spoilage, and this is due to the capacity of these organisms to grow at pH values <3.5, which is considerably below the minima for most food-spoilage and all food-poisoning bacteria.
- Most of the meats and seafoods have a final ultimate pH of about 5.6 and above. This makes these products susceptible to bacteria as well as to mold and yeast spoilage.
- Most vegetables have higher pH values than fruits, and, consequently, vegetables should be subject more to bacterial than fungal spoilage.

pH....

- With respect to the keeping quality of meats, it is well known that meat from fatigued animals spoils faster than that from rested animals and that this is a direct consequence of final pH attained upon completion of rigor mortis.
- Upon the death of a well-rested meat animal, the usual 1% glycogen is converted into lactic acid, which directly causes a depression in pH values from about 7.4 to about 5.6, depending on the type of animal.

pH…

- With respect to fish,it has been known for some time that halibut,which usually attains an ultimate pH of about 5.6, has better keeping qualities than most other fish, whose ultimate pH values range between 6.2 and *6.6.*
- Some foods are characterized by inherent acidity;others owe their acidity or pH to the actionsof certain microorganisms. The latter type is referred to as biological acidity and is displayed by products such as fermented milks, sauerkraut,and pickles.

pH….

- Regardless of the source of acidity, the effect on keeping quality appears to be the same.
- Some foods are better able to resist changes in pH than others.
- Those that tend to resist changes in pH are said to be *buffered.*
- In general, meats are more highly buffered than vegetables.

pH…

- Contributing to the buffering capacity of meats are their various proteins.
- Vegetables are generally low in proteins and, consequently, lack the buffering capacity to resist changes in their pH during the growth of microorganisms.

pH….

- The natural or inherent acidity of foods, especially fruits, may have evolved as a way of protecting tissues from destruction by microorganisms.
- It is of interest that fruits should have pH values below those required by many spoilage organisms.
- The biological function of the fruit is the protection of the plant's reproductive body, the seed.

pH….

- This one fact alone has no doubt been quite important in the evolution of present-day fruits.
- Although the pH of a living animal favors the growth of most spoilage organisms, other intrinsic parameters come into play to permit the survival and growth of the animal organism.

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	Apple juice	3.3–4.1
Broccoli	6.5	Bananas	4.5–4.7
Brussels sprouts	6.3	Figs	4.6
Cabbage (green)	5.4–6.0	Grapefruit (juice)	3.0
Carrots	4.9–5.2; 6.0	Grapes	3.4–4.5
Cauliflower	5.6	Limes	1.8–2.0
Celery	5.7–6.0	Melons (honeydew)	6.3–6.7
Corn (sweet)	7.3	Oranges (juice)	3.6–4.3
Cucumbers	3.8	Plums	2.8–4.6
Eggplant	4.5	Watermelons	5.2–5.6
Lettuce	6.0		
Olives	3.6–3.8		
Onions (red)	5.3–5.8		
Parsley	5.7–6.0		
Parsnip	5.3		
Potatoes (tubers and sweet)	5.3–5.6		
Pumpkin	4.8–5.2		
Rhubarb	3.1–3.4		
Rutabaga	6.3		
Spinach	5.5–6.0		
Squash	5.0–5.4		
Tomatoes (whole)	4.2–4.3		
Turnips	5.2–5.5		

Table 3–2 Reported Minimum pH Values for the Growth of Some Foodborne Organisms

Aeromonas hydrophila	ca.	6.0
Alicyclobacillus acidocaldarius		2.0
Bacillus cereus		4.9
Botrytis cinerea		2.0
Clostridium botulinum, Group I		4.6
C. botulinum, Group II		5.0
C. perfringens		5.0
Escherichia coli 0157:H7		4.5
Gluconobacter spp.		3.6
Lactobacillus brevis		3.16
L. plantarum		3.34
Lactococcus lactis		4.3
Listeria monocytogenes		4.1
Penicillium roqueforti		3.0
Plesiomonas shigelloides		4.5
Pseudomonas fragi	ca.	5.0
Salmonella spp.		4.05
Shewanella putrefaciens	ca.	5.4
Shigella flexneri	ca.	5.5
S. sonnei		5.0
Staphylococcus aureus		4.0
Vibrio parahaemolyticus		4.8
Yersinia enterocolitica		4.18
Zygosaccharomyces bailii		1.8

Table 3–3 Approximate pH Values of Dairy, Meat, Poultry, and Fish Products

Product	*pH*	*Product*	*pH*
Dairy products		**Fish and shellfish**	
Butter	6.1–6.4	Fish (most species)*	6.6–6.8
Buttermilk	4.5	Clams	6.5
Milk	6.3–6.5	Crabs	7.0
Cream	6.5	Oysters	4.8–6.3
Cheese (American mild and cheddar)	4.9; 5.9	Tuna fish	5.2–6.1
		Shrimp	6.8–7.0
		Salmon	6.1–6.3
Meat and poultry		White fish	5.5
Beef (ground)	5.1–6.2		
Ham	5.9–6.1		
Veal	6.0		
Chicken	6.2–6.4		

*Just after death.

pH Effects

- Adverse pH affects at least two aspects of a respiring microbial cell: the functioning of its enzymes and the transport of nutrients into the cell.
- The cytoplasmic membrane of microorganisms is relatively impermeable to H+ and OH ions.
- Their concentration in the cytoplasm therefore probably remains reasonably constant despite wide variations that may occur in the pH of the surrounding medium.

Moisture Content

- One of the oldest methods of preserving foods

  is drying or desiccation.
- 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.*

Miosture content….

- *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.

Miosture content….

- The aw of most fresh foods is above 0.99. The minimum values reported for the growth of some microorganisms in foods.

  In general, bacteria require higher values of aw for growth than fungi, with gram-negative bacteria having higher requirements than gram positives. Most spoil-age bacteria do not grow below aw = 0.91,whereas spoilage molds can grow as low as 0.80.

Miosture content….

- With respect to food-poisoning bacteria, *Staphylococcus aureus has been found to grow as low* as 0.86, whereas *Clostridium botulinum does not* grow below 0.94.
- Just as yeasts and molds grow over a wider pH range than bacteria, the same is true for aw. The lowest reported value for bacteriaof any type is 0.75 for halophilic (literally,"salt-loving") bacteria, whereas xerophilic ("dryloving“)molds and

Miosture content….

- osmophilic (preferring highosmotic pressures) yeasts have been reported to grow at aw values of 0.65 and 0.61, respectively When salt is employed to control aw, an extremely high level is necessary to achieve aw values below 0.80.

Table 3–4 Relationship between Water Activity and Concentration of Salt Solutions

Water Activity	*Sodium Chloride Concentration*	
	Molal	*Percent, w/v*
0.995	0.15	0.9
0.99	0.30	1.7
0.98	0.61	3.5
0.96	1.20	7
0.94	1.77	10
0.92	2.31	13
0.90	2.83	16
0.88	3.33	19
0.86	3.81	22

Table 3–5 Approximate Minimum a_w Values for Growth of Microorganisms Important in Foods

Organisms	a_w	*Organisms*	a_w
Groups		**Groups**	
Most spoilage bacteria	0.9	Halophilic bacteria	0.75
Most spoilage yeasts	0.88	Xerophilic molds	0.61
Most spoilage molds	0.80	Osmophilic yeasts	0.61
Specific Organisms		**Specific Organisms**	
Clostridium botulinum, type E	0.97	*Candida scottii*	0.92
Pseudomonas spp.	0.97	*Trichosporon pullulans*	0.91
Acinetobacter spp.	0.96	*Candida zeylanoides*	0.90
Escherichia coli	0.96	*Geotrichum candidum*	ca. 0.90
Enterobacter aerogenes	0.95	*Trichothecium* spp.	ca. 0.90
Bacillus subtilis	0.95	*Byssochlamys nivea*	ca. 0.87
Clostridium botulinum, types A and B	0.94	*Staphylococcus aureus*	0.86
Candida utilis	0.94	*Alternaria citri*	0.84
Vibrio parahaemolyticus	0.94	*Penicillium patulum*	0.81
Botrytis cinerea	0.93	*Eurotium repens*	0.72
Rhizopus stolonifer	0.93	*Aspergillus glaucus**	0.70
Mucor spinosus	0.93	*Aspergillus conicus*	0.70
		Aspergillus echinulatus	0.64
		Zygosaccharomyces rouxii	0.62
		Xeromyces bisporus	0.61

*Perfect stages of the *A. glaucus* group are found in the genus *Eurotium*.

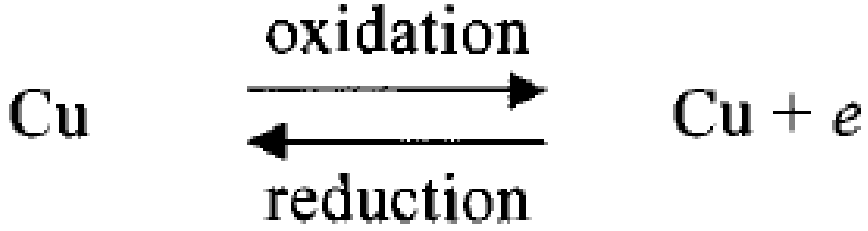
Effects of Low aW

- The general effect of lowering aW below optimum is to increase the length of the lag phase of growth and to decrease the growth rate and size of final population.
- This effect may be expected to result from adverse influences of lowered water on all metabolic activities because all chemical reactions of cells require an aqueous environment.

Oxidation-Reduction Potential

- It has been known for many years that microorganisms display varying degrees of sensitivity to the oxidation-reduction potential (O/R, Eh)of their growth medium.
- The O/R potential of a substrate may be defined generally 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, whereas a substrate that gains electrons becomes reduced:

Oxidation-Reduction Potential....



Oxidation may also be achieved by the addition of oxygen, as illustrated in the following reaction:

$$2\,Cu + O_2 \rightarrow 2CuO$$

Oxidation-Reduction Potential….

- Therefore, a substance that readily gives up electrons is a good reducing agent, and one that readily takes up electrons is a good oxidizing agent.
- When electrons are transferred from onecompound to another, a potential difference is created between the two compounds.
- This difference may be measured by use of an appropriate instrument, and expressed as millivolts (mV).

Oxidation-Reduction Potential….

- 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.
- The O/R potential of a system is expressed by the symbol Eh.
- Aerobic microorganisms require positive Eh values (oxidized) for growth, whereas anaerobes require negative Eh values (reduced)

Oxidation-Reduction Potential….

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

The O/R potential of a food is determined by the following:

- The characteristic O/R potential of the original food
- The poising capacity; that is, the resistance to change in potential of the food
- The oxygen tension of the atmosphere about the food
- The access that the atmosphere has to the food

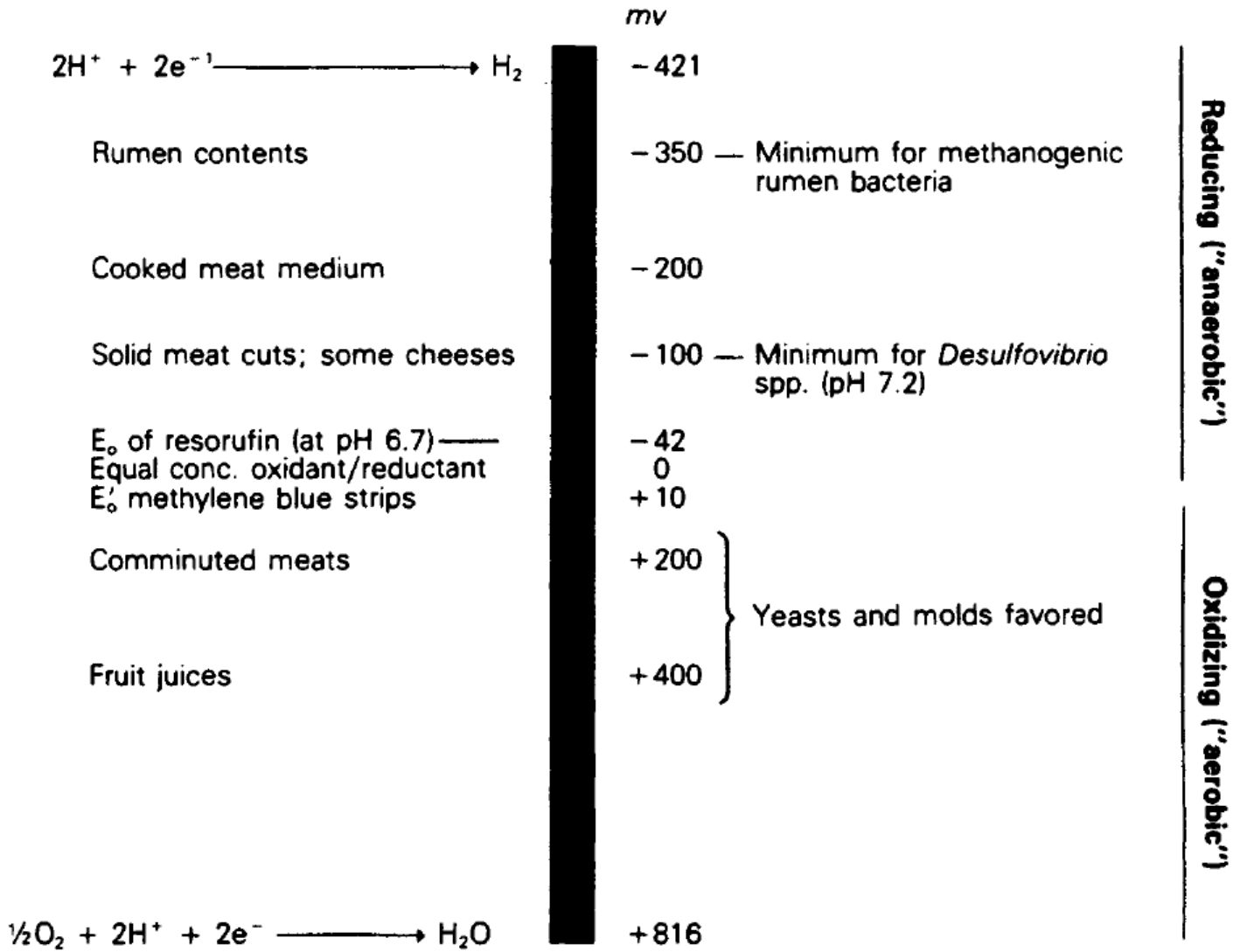
Oxidation-Reduction Potential….

- With respect to Eh requirements of microorganisms, some bacteria require reduced conditions for growth initiation (Eh of about -200mV), whereas others require a positive Eh for growth.
- In the former category are the anaerobic bacteria such as the genus *Clostridium; in*

  the latter belong aerobic bacteria such as some members of the genus *Bacillus.*

Oxidation-Reduction Potential….

- *Some aerobic* bacteria actually grow better under slightly reduced conditions, and these organisms are often referred to as *microaerophiles.*
- *Examples of* microaerophilic bacteria are lactobacilli and campylobacters.
- Some bacteria have the capacity to grow under either aerobic or anaerobic conditions. Such types are referred to as *facultative anaerobes.*
- *Most molds and yeasts encountered* in and on foods are aerobic, although a few tend to be facultative anaerobes.



igure 3–3 Schematic representation of oxidation-reduction potentials relative to the growth of certain microrganisms.

Eh Effects

- Microorganisms affect the Eh of their environments during growth just as they do pH.
- This is true especially of aerobes, which can lower the Eh of their environment while anaerobes cannot.
- As aerobes grow, O_2 in the medium is depleted, resulting in the lowering of Eh.
- Growth is not slowed, however, as much as might be expected due to the ability of cells to make use of O_2-donating or hydrogen-accepting substances in the medium.

Eh Effects….

- The result is that the medium becomes poorer in oxidizing and richer in reducing Substances.
- The Eh of a medium can be reduced by microorganisms by their production of certain metabolic byproducts such as H_2S, which has the capacity to lower Eh to -300 mV Because H_2S reacts readily with O_2, it will accumulate only in anaerobic environments.

Nutrient Content

In order to grow and function normally, the microorganisms of importance in foods require the following:

- water
- source of energy
- source of nitrogen
- vitamins and related growth factors
- minerals

Nutrient Content….

- With respect to the other four groups of substances (except water- discussed previously), molds have the lowest requirement, followed by yeasts, gram-negative bacteria, and gram-positive bacteria.
- As sources of energy, food borne microorganisms may utilize sugars, 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 sugars.
- Fats are used also by microorganisms as sources of energy, but these compounds are attacked by a relatively small number of microbes in foods.

Nutrient Content....

- The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids.
- A large number of other nitrogenous compounds may serve this function for various types of organisms.
- Some microbes, for example, are able to utilize nucleotides and free amino acids, whereas others are able to utilize peptides and proteins.
- In general, simple compounds such as amino acids will be utilized by almost all organisms before any attack is made on the more complex compounds such as high-molecular-weight proteins.
- The same is true of polysaccharides and fats.

Nutrient Content….

- Microorganisms may require B vitamins in low quantities, and almost all natural foods tend to have an abundant quantity for those organisms that are unable to synthesize their essential requirements.
- In general, gram-positive bacteria are the least synthetic and must therefore be supplied with one or more of these compounds before they will grow.
- The gram-negative bacteria and molds are able to synthesize most or all of their requirements.
- Consequently, these two groups of organisms may be found growing on foods low in B vitamins.
- Fruits tend to be lower in B vitamins than meats, and this fact, along with the usual low pH and positive Eh of fruits, helps to explain the usual spoilage of these products by molds rather than bacteria.

Antimicrobial Constituents

- The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances that have been shown to have antimicrobial activity.
- Some species are known to contain essential oils that possess antimicrobial activity.
- Among these are eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage,and carvacrol (isothymol) and thymol in oregano.
- Cow's milk contains several antimicrobial substances, including lactoferrin, conglutinin, and the lactoperoxidase system .
- Raw milk has been reported to contain a rotavirus inhibitor that can inhibit up to 10^6 pfu (plaque forming units)/mL. It is destroyed by pasteurization.
- Milk casein as well as some free fatty acids have been shown to be antimicrobial under certain conditions.

Antimicrobial Constituents….

- Eggs contain lysozyme, as does milk, and this enzyme, along with conalbumin, provides fresh eggs with a fairly efficient antimicrobial system.
- The hydroxycinnamic acid derivatives (p-coumaric, ferulic, caffeic, and chlorogenic acids) found in fruits, vegetables, tea, molasses,and other plant sources all show antibacterial and some antifungal activity.
- Lactoferrin is an ironbinding glycoprotein that is inhibitory to a number of foodborne bacteria.
- Its antimicrobial activity is antagonized by citrate.

Antimicrobial Constituents….

- Ovotransferrin appears to be the inhibitory substance in raw egg white that inhibits *Salmonella enteritidis.*
- Cell vacuoles of cruciferous plants (cabbage, Brussels sprouts, broccoli, turnips, etc.) contain *glucosinolates, which upon injury or mechanical* disruption, yield isothiocyanates.
- Some of the latter possess antifungal as well as antibacterial

  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 of fish 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.

EXTRINSIC PARAMETERS

- The extrinsic parameters of foods are those properties of the storage environment that affect both the foods and their microorganisms.

  Those of greatest importance to the welfare of foodborne organisms are as follows:
- Temperature of storage
- Relative humidity of environment
- Presence and concentration of gases
- Presence and activities of other microorganisms

Temperature of storage

- Microorganisms, individually and as a group grow over a very wide range of temperatures.
- Therefore, it would be well to consider at this point the temperature growth ranges for organisms of importance in foods as an aid in selecting the proper temperature for the storage of different types of foods.
- Those organisms that grow well at or below 7°C and have their optimum between 20°C and 30°C are referred to as ***psychrotrophs***.

Temperature of storage…

- Those that grow well between 20°C and 45°C with optima between 30°C and 40°C are referred to as *mesophiles, whereas those*that grow well at and above 45 °C with optima between 55°C and 65°C are referred to as ***thermophiles.***
- With regard to bacteria, psychrotrophic species and strains are found among the following genera (*Alcaligenes, Shewanella, Brochothrix, orynebacterium, Flavobacterium, Lactobacillus, Micrococcus, Pseudomonas, Psychrobacter, Enterococcus,* and others).
- 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, fish, poultry, eggs, and other foods normally held at this temperature.

Temperature of storage….

- Mesophilic species and strains are known among all genera
- They may be found on foods held at refrigerator temperatures.
- They apparently do not grow at this temperature but do grow at temperatures within the mesophilic range if other conditions are suitable.
- It should be pointed out that some organisms can grow over a range from 0^0C and 30^0C or above.
- One such organism is *Enterococcus faecalis.*

Temperature of storage….

- Most thermophilic bacteria of importance in

  foods belong to the genera *Bacillus and Clostridium.*
- Although only a few species of these genera are thermophilic, they are of great interest to the food microbiologist and food technologist in the canning industry.

Temperature of storage….

- Just as molds are able to grow over wider ranges of pH, osmotic pressure, and nutrient content, they are also able to grow over wide ranges of temperature as do bacteria.
- Many molds are able to grow at refrigerator temperatures, notably some strains of *Aspergillus, Cladosporium,* and *Thamnidium, which may be found growing* on eggs, sides of beef, and fruits.
- Yeasts grow over the psychrotrophic and mesophilic temperature ranges but generally not within the thermophilic range.

Relative Humidity of Environment

- The RH of the storage environment is important both from the standpoint of aw within foods and the growth of microorganisms at the surfaces.
- When the aw of a food is set at 0.60, it is important

  that this food be stored under conditions of RH that do not allow the food to pick up moisture from the air
- and thereby increase its own surface and subsurface aw to a point where microbial growth can occur.

Relative Humidity of Environment….

- When foods with low *aW* values are placed in environments of high RH, the foods pick up moisture until equilibrium has been established.
- Likewise, foods with a high aW lose moisture when placed in an environment of low RH.
- There is a relationship between RH and temperature that should be borne in mind in selecting proper storage environments for foods.
- In general, the higher the temperature, the lower the RH, and vice versa.

Presence and Concentration of Gases in the Environment

- Carbon dioxide (CO_2) is the single most important atmospheric gas that is used to control microorganisms in foods,
- Ozone (O_3) is the other atmospheric gas that has antimicrobial properties, and it has been tried over a number of decades as an agent to extend the shelf life of certain foods.
- It has been shown to be effective against a variety of microorganisms,
- but because it is a strong oxidizing agent,it should not be used on high-lipid-content foods since it would cause an increase in rancidity.

Presence and Activities of Other Microorganisms

- Some foodborne organisms produce substances that are either inhibitory or lethal to others; these include antibiotics, bacteriocins, hydrogen peroxide, and organic acids.
- Some members of the food biota on others is well established, and this is discussed under the heading

General Microbial Interference

- This phenomenon refers to the general nonspecific inhibition or destruction of one microorganism by other members of the same habitat or environment.
- Whereas lactic antagonism is a specific example of microbial interference, there are other less well-defined ways in which inhibition occurs.

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.
- Barrier technology, combination preservation, and combined methods are among some of the other descriptions of this concept.
- Referred to as "hurdle technology" since the mid-1980s by L. Leistner in Germany, the practice has been applied to some foods for over a century.

COMBINED INTRINSIC AND EXTRINSIC PARAMETERS: THE HURDLE CONCEPT….

- A simple example is demonstrated in preventing the germination of spores of proteolytic or group 1 strains of *C. botulinum.*
- *Among the intrinsic* and extrinsic parameters that are known to prevent their germination and growth are pH <4.6, aW <0.94, 10% NaCl, -120 ppm $NaNO_2$, incubation temperature <10°C, and a large aerobic biota.
- Foods that employ the hurdle concept in their formulation would embody a series of the above, thus making for a multitargeted approach to preventing germination and growth of these spores.
- In order to grow, the organism must "hurdle" a series of barriers.
- A large number of factors are known that can be applied to food systems as hurdles, and more and more shelf stable foods of the future are likely to embody this concept.