Intrinsic and Extrinsic Parameters of Foods That Affect Microbial Growth

- As our foods are of plant and/or 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.

INTRINSIC PARAMETERS

- The parameters of plant and animal tissues that are an inherent part of the tissues are referred to as intrinsic parameter. These parameters are as follows:

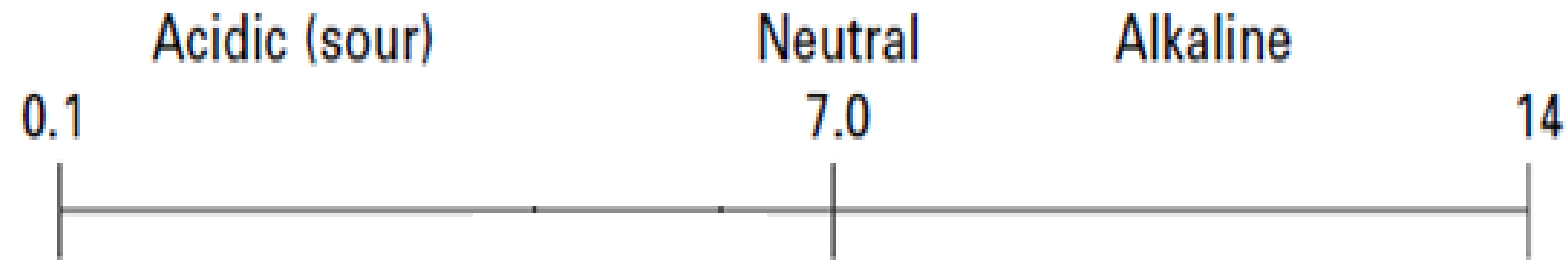
1. pH
2. Moisture content
3. Oxidation–reduction potential (Eh)
4. Nutrient content
5. Antimicrobial constituents
6. Biological structures

pH

- Most spoilage bacteria grow best near neutral pH
- Pathogenic bacteria even more narrow in tolerance range of near neutral
- Yeast and moulds have much greater tolerance to acidic (lower) 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.

Sensitivity to acidity: Bacteria > yeasts> molds



pH scale

pH	Optimum	Min	Max
Bacteria	6-8	4.5-5	9
Yeast	4.5-6	2-3	11
Mold	3.5-4	2-3	11

pH minima and maxima of microorganisms should not be taken to be precise boundaries, as the actual values are known to be dependent on other growth parameters.

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.

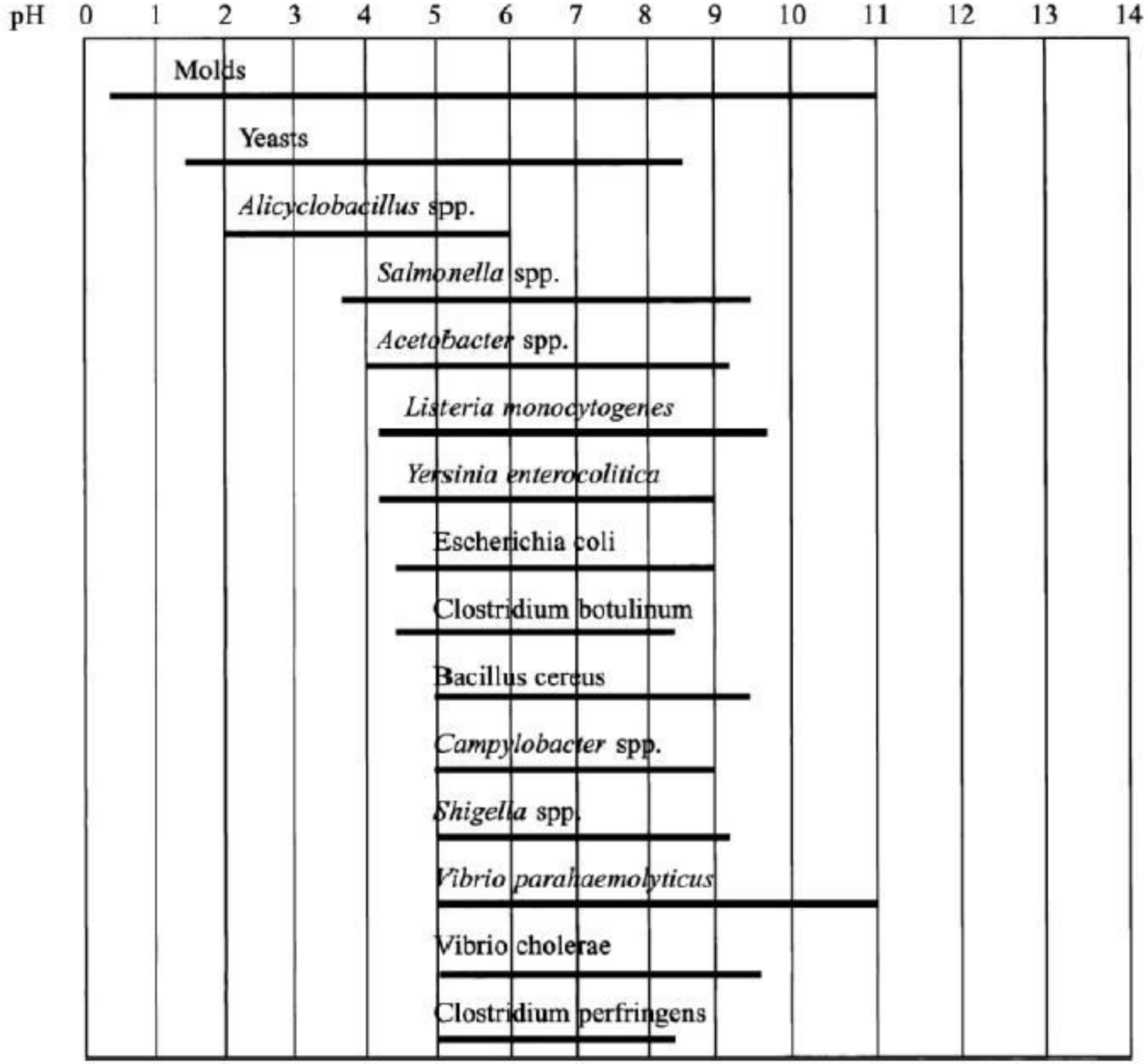


Figure 3–1 Approximate pH growth ranges for some foodborne organisms. The pH ranges for *L. monocytogenes* and *S. aureus* are similar.

Table 3-5. Approximate pH values permitting the growth of selected pathogens in food.

Microorganism	Minimum	Optimum	Maximum
Clostridium perfringens	5.5 - 5.8	7.2	8.0 - 9.0
Vibrio vulnificus	5.0	7.8	10.2
Bacillus cereus	4.9	6.0 -7.0	8.8
Campylobacter spp.	4.9	6.5 - 7.5	9.0
Shigella spp.	4.9		9.3
Vibrio parahaemolyticus	4.8	7.8 - 8.6	11.0
Clostridium botulinum toxin	4.6		8.5
Clostridium botulinum growth	4.6		8.5
Staphylococcus aureus growth	4.0	6.0 - 7.0	10.0
Staphylococcus aureus toxin	4.5	7.0 - 8.0	9.6
Enterohemorrhagic *Escherichia coli*	4.4	6.0 - 7.0	9.0
Listeria monocytogenes	4.39	7.0	9.4
Salmonella spp.	4.2[1]	7.0 - 7.5	9.5
Yersinia enterocolitica	4.2	7.2	9.6

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

Aeromonas hydrophila	ca. 6.0
Asaia siamensis	3.0
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
L. sakei	3.0
Lactococcus lactis	4.3
Listeria monocytogenes	4.1
Penicillium roqueforti	3.0
Propioniibacterium cyclohexanicum	3.2
Plesiomonas shigelloides	4.5
Pseudomonas fragi	ca. 5.0
Salmonella spp.	4.05
Shewanella putrefaciens	ca. 5.4
Shigella flexneri	5.5–4.75
S. sonnei	5.0–4.5
Staphylococcus aureus	4.0
Vibrio parahaemolyticus	4.8
Yersinia enterocolitica	4.18
Zygosaccharomyces bailii	1.8

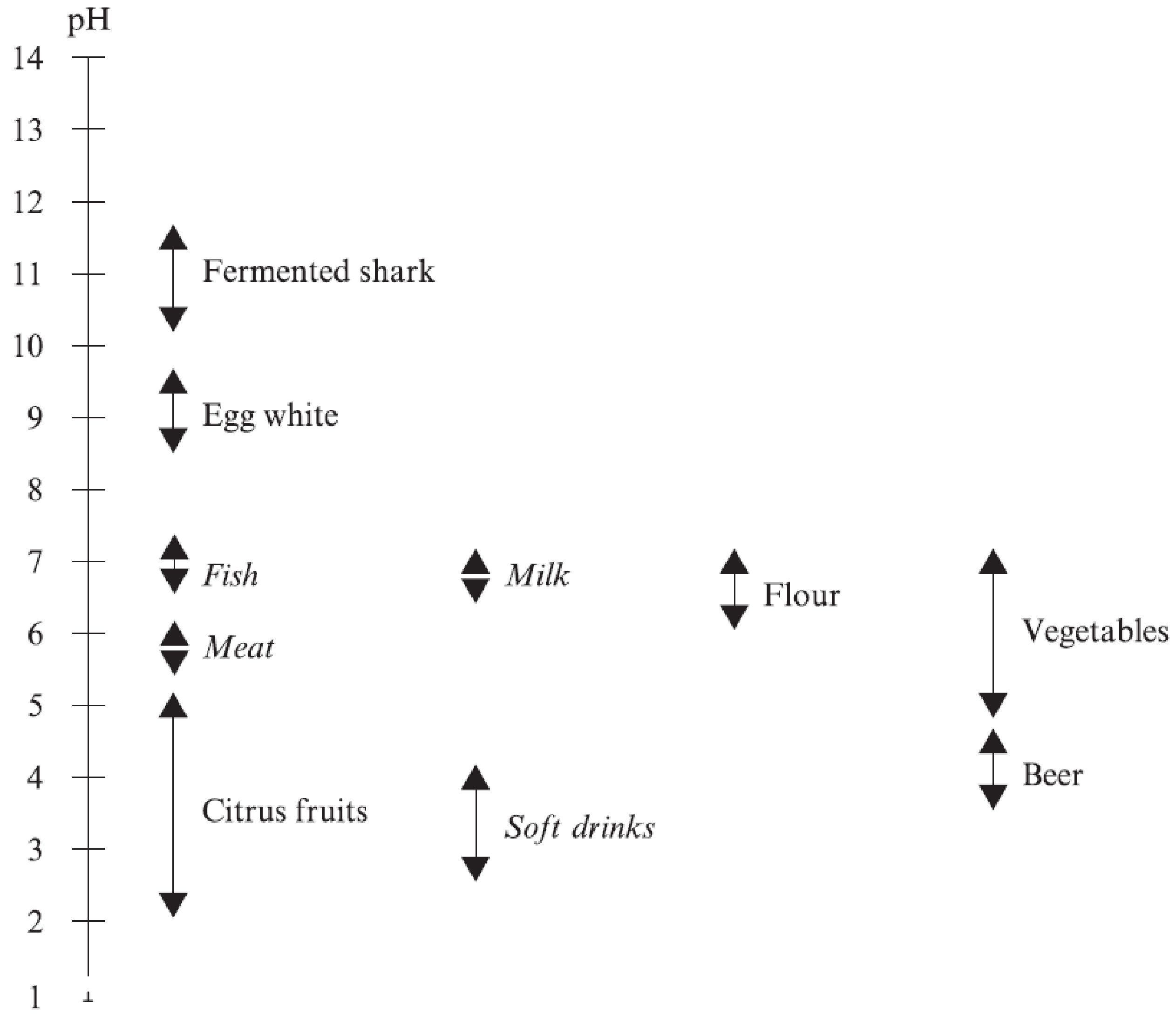
- With respect to temperature, the pH of the substrate becomes more acid as the temperature increases.
- Concentration of salt has a definite effect on pH growth rate curves, where it can be seen that the addition of 0.2 M NaCl broadened the pH growth range of *Escherichia coli* .
- However, when the salt content exceeds this optimal level, the pH growth range is narrowed.
- Young cells are more susceptible to pH changes than older or resting cells.
- When microorganisms are grown on either side of their optimum pH range, an increased lag phase results.

- Some food such as fruits and vinegar fall below the point at which bacteria normally grow.
- 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 vegetables have higher pH values than fruits, and, consequently, vegetables should be subject more to bacterial than fungal spoilage.

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 *Approximate pH ranges of some common food commodities*



- Most of the meats and sea foods 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.
- With respect to the keeping quality of meats, it is well established 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 to lactic acid, which directly causes a depression in pH values from about 7.4 to about 5.6, depending on the type of animal.

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
Meat and poultry		Salmon	6.1–6.3
Beef (ground)	5.1–6.2	White fish	5.5
Ham	5.9–6.1		
Veal	6.0		
Chicken	6.2–6.4		
Liver	6.0–6.4		

*Just after death.

- Some foods such as fruit are characterized by "**inherent acidity**" or "**natural acidity**".
- The natural or inherent acidity of foods, especially fruits, may have evolved as away of protecting tissues from destruction by microorganisms.
- Others owe their acidity or pH to the actions of certain microorganisms. This type is referred to as "**biological acidity**" and is displayed by products such as fermented milks (e.g yoghourt) and pickles.

- 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**".
- Meats are more highly buffered than vegetables. 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
- Although acidic pH values are of greater use in inhibiting microorganisms, alkaline values in the range of pH 12–13 are known to be destructive, at least to some bacteria.
- For example, the use of $CaOH_2$ to produce pH values in this range has been shown to be destructive to *Listeria monocytogenes* and other foodborne pathogens.

- 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.


Halibut
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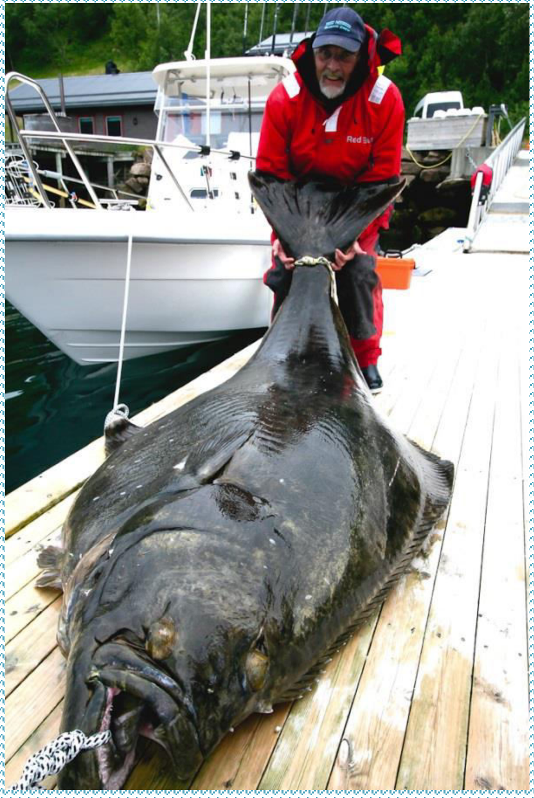


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- **Effects of adverse pH on microorganisms:**
  - Disruption of cellular enzymes
  - Disruption of the transport of nutrients into the cell
  - Disruption of such key cellular compounds as DNA and ATP require neutrality.
  - The morphology of some microorganisms can be affected by pH.
  - An adverse pH makes cells much more sensitive to toxic agents.

- It appears that the internal pH of almost all cells is near neutrality.
- When microorganisms are placed in environments below or above neutrality, their ability to proliferate depends on their ability to bring the environmental pH to a more optimum value or range.
- When most microorganisms grow in acid media, their metabolic activity results in the medium or substrate becoming less acidic, whereas those that grow in high pH environments tend to effect a lowering of pH.

- The amino acid decarboxylases that have optimum activity at around pH 4.0 and almost no activity at pH 5.5 cause a spontaneous adjustment of pH toward neutrality when cells are grown in the acid range.
- When amino acids are decarboxylated, the increase in pH occurs from the resulting amines.
- Bacteria such as Clostridium acetobutylicum raise the substrate pH by reducing butyric acid to butanol, whereas Enterobacter aerogenes produces acetoin from pyruvic acid to raise the pH of its growth environment.
- When grown in the alkaline range, a group of amino acid deaminases that have optimum activity at about pH 8.0 and cause the spontaneous adjustment of pH toward neutrality as a result of the organic acids that accumulate.

2. Moisture content

- The preservation of foods by drying is a direct consequence of removal 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 (a_w) in the environment.
- Aw is defined by the ratio of the water vapor pressure of food substrate to the vapor pressure of pure water at the same temperature:

$$a_w = p/p_o$$

- Where p is the vapor pressure of the solution and p_o is the vapor pressure of the solvent (usually water).
- This concept is related to relative humidity (RH) in the following way:

$$RH = 100 \times a_w.$$

- When salt is employed to control a_w, an extremely high level is necessary to achieve a_w values below 0.80.
- **Pure water** has an a_w of 1.00
- **22% NaCl solution** (w/v) has an a_w of 0.86
- **Saturated solution of NaCl** has an a_w of 0.75.

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

	Sodium Chloride Concentration	
Water Activity	*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 3.11 *Water activities of saturated salts solution at 25 °C*

Salt	a_w
Lithium chloride	0.11
Zinc nitrate	0.31
Magnesium chloride	0.33
Potassium carbonate	0.43
Magnesium nitrate	0.52
Sodium bromide	0.57
Lithium acetate	0.68
Sodium chloride	0.75
Potassium chloride	0.86
Potassium nitrate	0.93
Pure water	1.00

- The water activity (a_w) of most fresh foods is above 0.99.

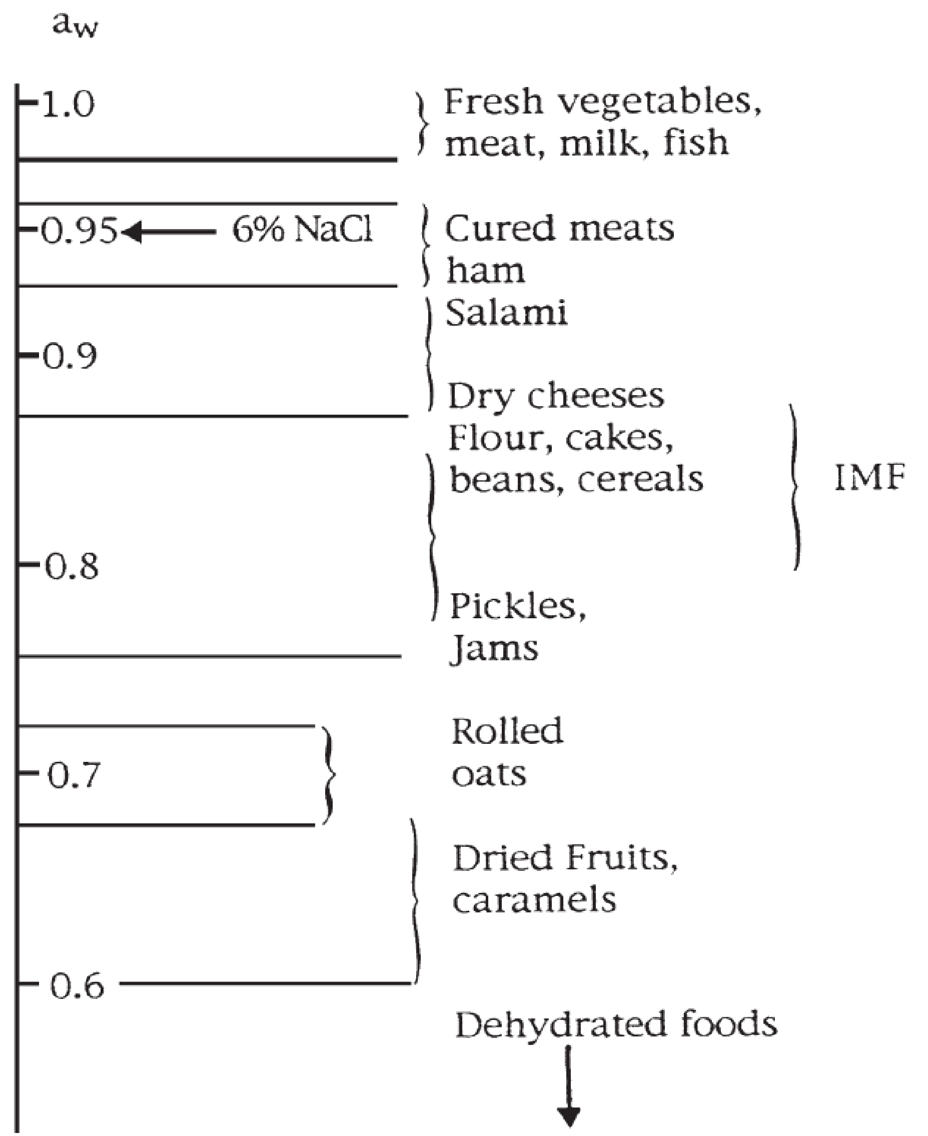

Figure 3.9 *Range of a_w values associated with a number of food commodities*

- In general, bacteria require higher values of a_w for growth than fungi
- Gram-negative bacteria having higher requirements than Gram positives.
- Just as yeasts and molds grow over a wider pH range than bacteria, the same is true for a_w.

- halophiles = “salt-loving”
- Xerophilic = “dry-loving”
- Osmophilic= “preferring high osmotic pressures”

(i) *halotolerant* – able to grow in the presence of high concentrations of salt

(ii) *osmotolerant* – able to grow in the presence of high concentrations of unionized organic compounds such as sugars

(iii) *xerotolerant* – able to grow on dry foods.

Table 3.10 *Minimum water activities at which active growth can occur*

Group of micro-organism	*Minimum* a_w
Most Gram-negative bacteria	0.97
Most Gram-positive bacteria	0.90
Most yeasts	0.88
Most filamentous fungi	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.61

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. 0.9
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

Table 3-2. Approximate a_w values for growth of selected pathogens in food.			
Organism	**Minimum**	**Optimum**	**Maximum**
Campylobacter spp.	0.98	0.99	
Clostridium botulinum type E*	0.97		
Shigella spp.	0.97		
Yersinia enterocolitica	0.97		
Vibrio vulnificus	0.96	0.98	0.99
Enterohemorrhagic *Escherichia coli*	0.95	0.99	
Salmonella spp.	0.94	0.99	>0.99
Vibrio parahaemolyticus	0.94	0.98	0.99

Pathogens are active at higher a_w.

Table 3-1. Approximate a_w values of selected food categories.

Animal Products	a_w
fresh meat, poultry, fish	0.99 - 1.00
natural cheeses	0.95 - 1.00
pudding	0.97 - 0.99
eggs	0.97
cured meat	0.87 - 0.95
sweetened condensed milk	0.83
Parmesan cheese	0.68 - 0.76
honey	0.75
dried whole egg	0.40
dried whole milk	0.20

fruit juice concentrates	0.79 - 0.84
fruit cake	0.73 - 0.83
cake icing	0.76 - 0.84
flour	0.67 - 0.87
dried fruit	0.55 - 0.80
cereal	0.10 - 0.20

- Certain relationships have been shown to exist among a_w, temperature, and nutrition:
  - At any temperature, the ability of microorganisms to grow is reduced as the a_w is lowered.
  - Second, the range of a_w over which growth occurs is greatest at the optimum temperature for growth.
  - The presence of nutrients increases the range of a_w over which the organisms can survive.

- **Effects of Low a_w**
- The general effect of lowering aw below optimum is:
  - Adverse effects on the functioning of the cell membrane, which must be kept in a fluid state
  - Disruption of nutrition of microorganisms (due to disruption of nutrient transportation through an aqueous environment)
  - Increase the length of the lag phase of growth
  - Decrease the growth rate
  - Decrease size of final population

- Aw is influenced by other environmental parameters such as pH and temperature:
  - When both the pH and temperature of incubation were made unfavorable, the minimum a_w for growth is higher.

- In general, the strategy employed by microorganisms as protection against osmotic stress is the intracellular accumulation of compatible solutes.
- The three most common compatible solutes in most bacteria are:
  - Carnitine
  - Glycine betaine
  - Proline

  - **Osmophilic yeasts** accumulate **polyhydric alcohols** to a concentration commensurate with their extracellular aw.

3. Oxidation–reduction (O/R) potential ---(symbol =Eh)

- **Definition**: ease with which the substrate loses or gains electrons.
- When electrons are transferred from one compound to another, a potential difference is created between the two compounds and expressed as millivolts (mV).

- When an element or compound loses electrons, the substrate is oxidized,
- When a substrate that gains electrons becomes reduced.
- A substance that readily gives up electrons is a good reducing agent.
- A substance that readily takes up electrons is a good oxidizing agent.

- The more highly oxidized a substance, the more positive will be its electrical potential.
- The more highly reduced a substance, 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.
- **Anaerobes:** require negative Eh values (reduced) .
- **Microaerophiles**: Aerobic bacteria actually grow better under slightly reduced conditions such as lactobacilli and campylobacters.

- According to Frazier The *O/R potential of a food is determined by the following:
  - The characteristic O/R potential of the original food.
  - The poising capacity (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.

pH: (Eh tends to be more negative under progressively alkaline conditions)

Microbial activity: Microorganisms decrease the Eh of their environments during growth:

- As aerobes grow, O_2 in the medium is depleted, resulting in the lowering of Eh.
- By production of metabolic such as H_2S (lower Eh to −300 mV)

Reducing substances in some foods:

- –SH groups in meats
- Ascorbic acid and reducing sugars in fruits and vegetables

Eh of some food:

- Plant foods, especially plant juices, tend to have Eh values of from +300 to 400 mV.
- Cheeses of various types have been reported to have Eh values on the negative side, from −20 to around −200 mV.
- Solid meats have Eh values of around −200 mV; in minced meats, the Eh is generally around +200 mV.
- Cheese : -200mv
- Fruit Juices: 200-300mv

- **Change in pH of meat pre and post slaughter:**
  - Eh of muscle **after death** is +250 mV, at which time clostridia failed to multiply.
  - **At 30 hours postmortem**, the Eh had fallen to about 30 mV in the absence of bacterial growth.
  - **When bacterial growth** was allowed to occur, the Eh fell to about -250 mV.
  - Thus, anaerobic bacteria do not multiply until the onset of rigor mortis because of the high Eh in pre-rigor meat.

Table 3.6 *Redox potentials of some food materials*

	E (mV)	*pH*
Raw meat (post-rigor)	–200	5.7
Raw minced meat	+225	5.9
Cooked sausages and canned meats	–20 to –150	*Ca*. 6.5
Wheat (whole grain)	–320 to –360	6.0
Barley (ground grain)	+225	7.0
Potato tuber	*Ca*. –150	*Ca*. 6.0
Spinach	+74	6.2
Pear	+436	4.2
Grape	+409	3.9
Lemon	+383	2.2

4. Nutrient content

- Microorganisms to grow and function normally require the following:

1. Water

2. Energy

3. Nitrogen

4. Vitamins

5. Minerals

- The importance of water to the growth and welfare of microorganisms is obvious.
- With respect to the other four groups of substances requirement:

  Molds < Gram-negative bacteria < Yeasts < Gram-positive bacteria.

- As sources of energy, microorganisms may utilize:

➢ Sugars

➢ Alcohols

➢ Amino acids (Also as nitrogen source)

- Complex carbohydrates <u>such as starches and cellulose</u> are also used by some microorganisms as sources of energy by <u>first degrading these compounds to simple sugars</u>
- Fats are also used by few microorganisms as sources of energy.

- 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.

- Microorganisms may **require B vitamins** in low quantities, and almost all natural foods 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.
- Thus, **Gram-negative bacteria and molds** may be found growing on foods low in B vitamins.

- **Whys fruits spoilage by molds more than bacteria?**

1) Fruits have lower B vitamins (not suitable for most bacteria)
2) Fruits have lower pH (not suitable for most bacteria)
3) Fruits have positive Eh (not suitable for most bacteria)

5. Antimicrobial constituents

- Some plant species are known to contain essential oils that possess antimicrobial activity. Among these are:
  - Eugenol in cloves
  - cinnamic aldehyde and eugenol in cinnamon,
  - Allicin in garlic
  - Allyl isothiocyanate in mustard
  - Antilisterial compounds in carrot (not exactly determined yet but probably: phytoalexin 6-methoxy-mullein)
  - eugenol and thymol in sage, and carvacrol isothymol and thymol in oregano.
  - phytoalexins and the lectins. Lectins are proteins that can specifically bind to a variety of polysaccharides, including the glycoproteins of cell surfaces. Through this binding, lectins can exert a slight antimicrobial effect.

- Cow's milk contains several antimicrobial substances, including:
  - Lactoferrin (an iron-binding glycoprotein)
  - Conglutinin
  - A rotavirus inhibitor in raw milk (susceptible to pasteurization)
  - Casein
  - Free fatty acids
  - Lysozyme
  - Lactoperoxidase system.

- **Lactoperoxidase system in milk**
- This is an inhibitory system that occurs naturally in bovine milk, and it consists of three components:
  1) Lactoperoxidase
  2) Thiocyanate (SCN^-)
  3) H_2O_2
- All three components are required for antimicrobial effects.

- **Activation of Lactoperoxidase system:**
- The quantity of lactoperoxidase in bovine milk is enough.
- But the quantity of H_2O_2 and thiocyanate maybe lower than required.
- So, lactoperoxidase system in raw milk is activated by adding thiocyanate and H_2O_2 and finally hypothiocyanate be generated.
- Then, shelf life was extended to 5 days compared to 48 hours for controls.

- Gram-negative psychrotrophs such as the pseudomonads are sensitive.
- The system was more effective at 30◦C than at 4◦C.
- The lactoperoxidase system can be used to preserve raw milk in countries where refrigeration is uncommon.

$$SCN^- + H_2O_2 \xrightarrow{\text{Lactoperoxidase}} OSCN^- + H_2O$$

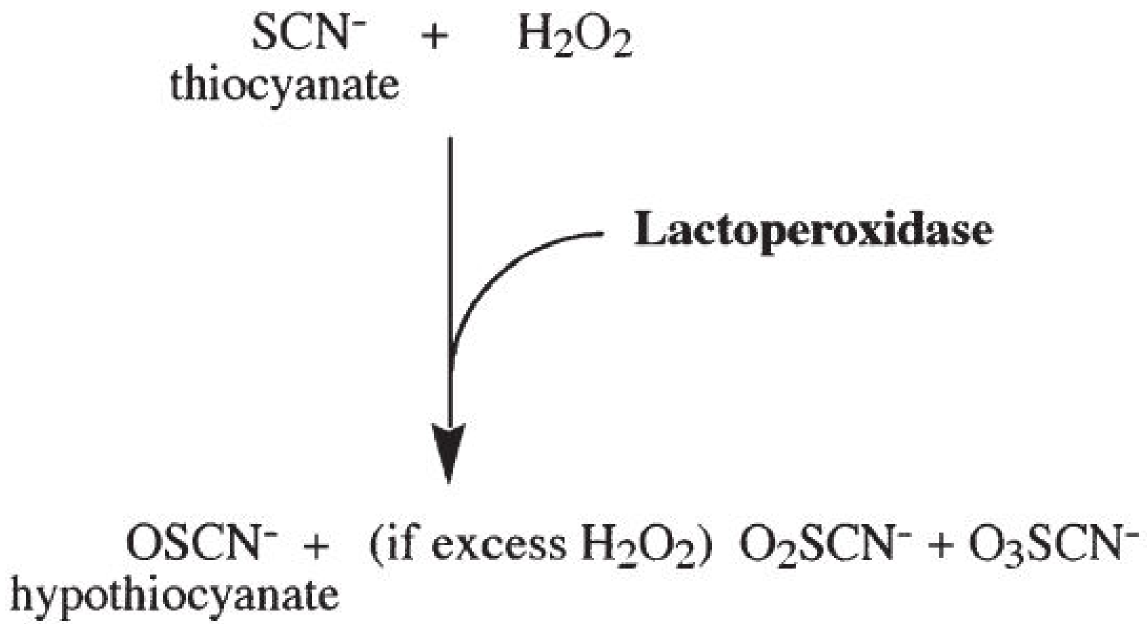

Figure 3.5 *The lactoperoxidase system*

Lacto peroxidase system

Add about 10 parts per million (PPM) of thiocyanate increases the overall level to 15 PPM (5 PPM naturally present).

The solution is thoroughly mixed for 30 seconds and then an equimolar amount (8.5 PPM) of hydrogen peroxide is added (generally in the form of a granulated sodium carbonate peroxyhydrate).

This treatment extends the shelf life of raw milk under tropical conditions for a further 7 to 8 hrs.

- **Eggs**:
  - Lysozyme
  - Conalbumin (appears to be the inhibitory substance in raw egg white that inhibits Salmonella enteritidis)

- Fruits, vegetables, tea, molasses:
  - The hydroxy-cinnamic acid derivatives (p-coumaric, ferulic, caffeic, and chlorogenic acids)

Table 3.8 *Antimicrobials in hen's egg albumen and milk*

Albumen	*Milk*
Nutrient Status	
High pH	Moderate pH
Low levels of available nitrogen	High levels of protein, carbohydrate and fat
Antimicrobials	
Ovotransferrin (conalbumin) (12% of solids)	Lactoferrin
Lysozyme (3.5% of solids)	Lysozyme
Avidin – (0.05% of solids)	–
Ovoflavoprotein –(0.8% of solids)	–
Ovomucoid & ovoinhibitor – (protease inhibitors) (11% of solids)	–
	Lactoperoxidase (30 $mg\,l^{-1}$)
	Immunoglobulin (300 $mg\,l^{-1}$)

6. **Biological structures**

- The natural covering of some foods provides excellent protection against the entry and subsequent damage by spoilage organisms such as:
  - Outer covering of fruits
  - Shell of nuts
  - Hide of animals
  - Shells of eggs
  - Skin covering of fish