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Factors Influencing the Development of Microbes in Food

S.S.Preetha
M.Tech Food Technology
College of Food and Dairy Technology (TANUVAS), Chennai, Tamil Nadu, India

Rita Narayanan
Professor, Department of Food Processing Technology
College of Food and Dairy Technology (TANUVAS), Chennai, Tamil Nadu, India

Abstract
In this review article, the different parameters, mostly intrinsic, extrinsic, implicit, and processing, that have an impact on the microbial growth in the food environment has been discussed in detail. Intrinsic factors (pH, moisture content, O/R potential, antimicrobial constituents, biological structures, and nutrient content) as well as extrinsic parameters (Relative humidity, storage temperature, gaseous environment and activities of other microbes in the environment) determines the range and population of microorganisms associated with the food at any given point throughout their production and post-harvest handling, thus influencing the rate and type of spoilage that eventually renders the food inedible.
Keywords: intrinsic, extrinsic, implicit, processing, spoilage and microorganism

Introduction

When microorganisms grow in the food, they cause varying degrees of change in the food's attributes as a result of metabolic activity. Some of these changes, like those taking place during fermentation, are desirable, while others, like those resulting in food spoilage and food poisoning, are undesirable (Siddig *et al.* 2012). The most important factors that influence microbial growth in foods can be summarized in the following categories as given in table 1:

1. Intrinsic factors - factors related to the food itself
2. Extrinsic factors - factors related to the environment in which the food is stored
3. Implicit factors - factors related to the microorganisms themselves,
4. Processing factors,
5. Interactions between factors, as mentioned above, can also affect the growth of microbes in foods in a complicated way; the combined effects of these interactions may be additive or synergistic.

Table 1 Factors influencing the development of microbes in food

Intrinsic factors	Extrinsic factors	Implicit factors	Process factors
Nutrient content	Relative humidity	Metabiotic	Physical treatments
pH	Storage temperature	Symbiotic	Use of chemicals
Oxidation–reduction potential	Gaseous atmosphere	Commensalism	Contamination
Water activity	Presence of other microorganisms	Antagonism	
Biological structure		Predation	
Antimicrobial content		Competition	

Source*:* Osman Erkmenand T.Faruk Bozoglu, 2016, Food Microbiology: Principles into Practice, First Edition.

Intrinsic Factors

pH

Each group of microorganisms has optimum, minimum, and maximum pH requirements for their growth in foods. Table 2 lists the approximate pH ranges of selected organisms relevant to food for their growth in laboratory media. Usually, pH interacts with other parameters in the food to inhibit growth. The pH can interact with the parameters such as aw, salt, temperature, redox potential, and preservatives to inhibit the growth of pathogens and other organisms.

Table 2 Approximate pH values for the growth of selected microbes in food

Microorganism	Minimum	Optimum	Maximum
Clostridium perfringens	5.5 to 5.8	7.2	8.0 to 9.0
Vibrio vulnificus	5.0	7.8	10.2
Bacillus cereus	4.9	6.0 to 7.0	8.8
Campylobacter spp.	4.9	6.5 to 7.5	9.0
Shigella spp.	4.9	9.3	
Staphylococcus aureus			
Growth	4.0	6.0 to 7.0	10.0
Toxin	4.5	7.0 to 8.0	9.6
Salmonella (most).	45 to 5.0	6.0 to 7.5	8.0 to 9.6
S. typhi	4.0 to 4.5	6.5 to 7.2	8.0 to 9.0
Escherichia coli	4.3-4.4	6.0-8.0	9.0-10.
L. acidophilus	4.0 to 4.6	5.5 to 6.0	7.0
L. plantarum	3.5	5.5 to 6.5	8.0
C. perfringens	5.0 to 5.5	6.0 to 7.6	8.5 to 9.0
Clostridium botulinum	4.8 to 5.0	6.0 to 8.0	8.5 to 8.8
Propionibacterium	4.7	6.2 to 7.0	7.5
Pseudomonas (most)	5.6	6.6 to 7.0	8.0
Leuconostoc cremoris	5.0	5.5 to 6.0	6.5
Saccharomyces cerevisiae	2.0 to 2.4	4.0 to 5.0	-
S. rouxii	1.5	3.5 to 5.5	8.5 to 10.5
Aspergillus niger	1.2	3.0 to 6.0	-
A. oryzae	1.6 to 1.8	-	9.0 to 9.3
Penicillium	1.9	4.5 to 6.7	9.3

Source: Comprehensive Reviews in Food Science and Food Safety, table 4.6 in G. J. Banwart, Basic Food Microbiology.

In general, pathogens do not grow or grow very slowly, at pH levels below 4.6; but there are few exceptions. Many pathogens can survive in foods at pH levels below their growth minimal.

Table 3 Approximate pH values for the Growth of Selected Microbes in Food

Products	pH
Vegetables	
Eggplant	4.5
Potatoes (tubers and sweet)	5.3 to 5.6
Tomatoes (whole)	4.2 to 4.3
Cabbage	5.2-6.3
Spinach	5.1-6.S
Brussels sprouts	6.3-6.6
Beets	4.9-5.S
Asparagus	5.0-6.1
Carrots	4.9 to 6.3
Fruits	
Watermelons	5.2 to 5.6
Bananas	4.5 to 4.7
Limes	1.8 to 2.0
Grapes	3.3-4.5
Cherries	3.2-4.7
Pineapple	3.2-4.1
Strawberries	3.0-4.2
Raspberries	2.9-3.7
Apples	2.9-3.5
Oranges	2.8-4.0
Dairy products	
Milk	6.3 to 6.5
Butter	6.1 to 6.4
Camembert cheese	6.1-7.0
Cottage	4.1-5.4
Gouda	4.7
Meat	
Chicken	6.2 to 6.4
Beef	5.1 to 6.2
Turkey	5.6 - 6.0
Pork	5.3 - 6.4
Dry sausages	4.4 - 5.6
Sea food	
Shrimp	6.8 - 8.2
Crab	6.8 - 8.0
Catfish	6.6 - 7.0

Source: Table 5.5 in ICMSF 1980, p. 109–110; Table 3-2 in May 2000, p 39; Table 4.7 in. J. Banwart, Basic Food Microbiology.

From **Table 3**, it can be observed that generally, fruits have lower pH values than vegetables, meat, poultry, fish, and dairy products. Hence, fruits are more susceptible to spoilage by yeasts and molds, while bacteria are the most important spoilage agents of the other groups of food (Lund *et al.,* 2000).

According to the pH values of the foods, they are generally divided into four groups:

Group 1: low-acid foods with a pH of more than 5.3 include corn, meat, fish, and milk. All types of microorganisms can grow on them, especially bacteria.

Group 2: medium-acid foods with a pH range of 5.3–4.5 include bananas, yogurt, and pumpkin. All types of microorganisms can grow on them.

Group 3: acid foods with a pH range of 4.5–3.7 include tomatoes, orange juice, sugar beet, and grapes; they are susceptible to the growth of yeasts and molds, and a few types of bacteria. Most pathogenic bacteria do not grow on these foods.

Group 4: high-acid foods with a pH below 3.7 include apples, grapefruit juice, and limes; they are susceptible to the growth of yeasts and molds. Most bacteria, particularly the pathogenic ones, do not grow on such foods.

The pH of foods can be natural, due to microbial fermentation, or result from acids added to the food. Microbial growth leads in most cases to a change in the pH of the food. When lactic acid bacteria, for example, grow in the milk, they consume its lactose and produce lactic acid, which lowers the pH of the milk. Also, the changes in pH can transform food into one that can support the growth of pathogens (ICMSF, 1980)

Different foods tend to spoil in different ways. For instance, carbohydrate-rich foods often undergo acid hydrolysis when they spoil, which usually reduces the pH and reduces the risk of pathogen growth. This principle is used in the dairy and lactic meat fermentations. On the contrary, protein-rich foods tend to increase in pH when they spoil, making them possibly less safe as the pH rise to the zone where more pathogens can grow.

While the pH of many vegetables is in a range suitable for the growth of pathogenic bacteria, some, e.g., fully ripe tomatoes, are in a pH range (3.9–4.4) that prevents or retards growth. Yeasts and molds, on

the other hand, have a competitive advantage over bacteria that may access bruised tissues of acidic vegetables and many fruits because they can to grow at a lower pH range (2.2–5.0) characteristic of much of this produce. Spoilage of fruits is often caused by specific molds or groups of molds and yeasts (Larry *et al.,* 2002)

Table 4 Limiting Conditions for Pathogen Growth

Pathogen	Min aw	Min. pH	Max. pH	Min. Temp	Max. Temp	Oxygen requirement
Bacillus cereus	0.92	4.3	9.3	4^0C	55^0C	Facultative anaerobe
Campylo-bacterjejuni	0.935	4.9	9.5	30^0C	45^0C	Micro- aerophile
Clostridium botulinum, type A, and proteolytic types B and F	0.935	4.6	9	10^0C	48^0C	Anaerobe
Clostridium botulinum, type E, and non-proteolytic types B and F	0.97	5	9	3.3^0C	45^0C	Anaerobe
Clostridium perfringens	0.93	5	9	10^0C	52^0C	Anaerobe
Listeria monocyto-genes	0.92	4.4	9.4	-0.4^0C	45^0C	Facultative anaerobe
Salmonella spp.	0.94	3.7	9.5	5.2^0C	46.2^0C	Facultative anaerobe
Shigella spp.	0.96	4.8	9.3	6.1^0C	47.1^0C	Facultative anaerobe
Staphylococcus aureus growth	0.83	4	10	7^0C	50^0C	Facultative anaerobe

Source: FDA, Appendix 3 (Bacterial Pathogen Growth and Inactivation)

Table 4 provides information on the minimum water activity (a_w), minimum and maximum pH, and minimum and maximum temperatures that limit growth for the bacterial pathogens that are of greatest concern in food processing. It also provides data on the oxygen requirements for the pathogens listed.

Nutrient Content

Some basic nutrients are required by microorganisms for the growth and maintenance of metabolic functions. The type and amount of nutrients required range widely depending on the microorganism. These nutrients include water, a source of energy, nitrogen, vitamins, and minerals (Ray, 1996; Jay 2000).

Foodborne microorganisms can procure energy from carbohydrates, alcohols, and amino acids. Simple sugars such as glucose are metabolized by most of the microorganisms, while others have the potential to metabolize more complex carbohydrates, such as starch or cellulose found in plant foods or glycogen found in muscle foods. Some microbes also use fat as a source of energy, but these compounds are attacked by a relatively small number of microbes in foods.

Amino acids also act as a source of nitrogen and energy and are utilized by most microorganisms. Some microorganisms can metabolize peptides and more complex proteins. Other sources of nitrogen include, for example, urea, ammonia, creatinine, and methylamines.

Phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium are some examples of minerals required for microbial growth. In general, small amounts of these minerals are required, and a wide range of foods serves as a good source of minerals.

Many bacterial species, especially Gram-negative rods such as Pseudomonas, Acinetobacter, Moraxella, Shewanella, and Aeromonas, as well as pathogenic spore formers like Clostridium botulinum, are proteolytic and can grow well in protein-rich foods and spoil them rapidly or cause disease (ICMF, 2005).

Foods rich in carbohydrates can be spoiled by carbohydrate-fermenting microorganisms, particularly by yeasts and molds. Bacterial species of the genera Bacillus, Clostridium, Aeromonas, Pseudomonas, Leuconostoc and Enterobacter are saccharolytic and can also attack carbohydrates (Ray, 2004)

Microorganisms may require B vitamins in small quantities, and almost all-natural foods have an ample quantity for those organisms that are unable

to synthesize their essential requirements. On the whole, 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 can synthesize most or all of their requirements, and hence, these two groups of organisms may be found growing on foods low in B vitamins. All microorganisms require sources of energy and electrons for growth to take place. Microorganisms can be grouped into nutritional classes based on how they satisfy all these requirements (table 5). There are only two sources of energy available to organisms: (1) light energy, and (2) the energy derived from oxidizing organic or inorganic molecules (Prescott, 2002)

Table 5 Groups of Microorganism based on the Way they Satisfy their Energy Requirements

Sources	Description
Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source
Heterotrophs	Reduced, preformed, organic molecules from other organisms
Energy Sources	
Phototrophs	Use light as their energy source
Chemotrophs	Use energy from the oxidation of chemical compounds (either organic or inorganic)
Electron Sources	
Lithotrophs	Uses reduced inorganic molecules as their electron source
Organotrophs	Extract electrons from organic molecules
photoautotrophs /photolithoautotrophs	Utilize light energy and have CO2 as their carbon source

Source: Table 5.1 in Microbiology, 5th Edition, Lansing M. Prescott, 2002.

Moisture Content

Microorganisms require water in an available form for their growth in food products. The water requirements of microorganisms are described in terms of the water activity (aw) of the food or environment. The requisite for a meaningful term to describe the behavior of microorganisms in environments with reduced moisture helped to establish the term water activity (Breene *et al.,* 1988). Water activity has a significant effect on the growth of microorganisms.

According to Chichester *et al.,* 1963, water activity can be defined as the ratio of the water vapor pressure of the food to the vapor pressure of pure water at the same temperature.Mathematically, it can be written as

$$a_w = P/P°$$

Where, P denotes the Vapour pressure of the food; P° denotes the Vapour pressure of pure water.

The water activity of pure water is 1.00, and that of completely dehydrated food is 0.00. Aw of food on this scale from 0.00 to 1.00 is related to the equilibrium relative humidity above the food on a scale of 0 to 100%. Therefore, % Equilibrium Relative Humidity (ERH) = a_w x 100.

Labuza *et al.,* 1972 stated that reducing water activity below 0.7 prevents microbial spoilage. Even though the food would not spoil from microorganisms, other deteriorative reactions can still occur. To efficiently preserve a food product, water activity would have to be lowered to a range where the rate of deteriorative reactions is minimized.

Table 4 displays information on the minimum water activity (aw) that limit growth for the bacterial pathogens that are of greatest concern in food processing.

Microorganisms commonly have optimum and minimum levels of aw for growth depending on other growth factors in their environments.

The food is classified into three categories based on it's aw range

- High-moisture foods (aw above 0.85);
- Intermediate-moisture foods, (aw 0.60–0.85);
- Low-moisture foods (aw below 0.60).

High-moisture foods with water activity above 0.85 are highly perishable foods as they are

susceptible to the growth of spoilage and pathogenic microorganisms, especially the fast-growing bacteria. Microbial spoilage of intermediate-moisture foods is a relatively slow process, caused mainly by yeasts and molds. Low moisture foods are not spoiled by microorganisms unless their moisture content is raised by any means. Microbial growth stops at water activity below the minimal value, but the microbes don't die immediately and may remain dormant in the food for prolonged periods (Siddig, 2012).

Almost all fresh foods, such as fresh meat, vegetables, and fruits, have aw values that are close to the optimum growth level of most microorganisms (0.97-0.99). The addition of solutes such as salt or sugar, physical removal of water through drying or baking, or binding of water to various macromolecular components in the food is some of the means to manipulate aw in foods (Urmila et al., 2017). Table 6 gives a_w (app.) values for selected food at their normal moisture content and the phenomenon that takes place at that particular aw range.

Table 6 Approximate aw values for Selected Food at their Normal Moisture Content

a_w	Food	Phenomenon
0.95 - 1.00	Meat (fresh and cooked), fresh poultry, fresh fish, milk, eggs, fruit (fresh and canned in syrup), vegetables (fresh and canned in brine), bread, liver, sausage, pudding, fresh fruit and vegetable juices, some types of cheese	Inhibition of Pseudomonas, *Bacillus, C.perfringens,* and some yeasts
0.90 - 0.95	Cured meat products, some hard cheese, mayonnaise, low-calorie jams, baked cake, refrigerated biscuit dough	Lower limit for bacterial growth; *Salmonella, V.parahaemolyticus, C.botulinum,* and *Lactobacillus.* Some yeast can grow. Inhibition of spoilage bacteria.
0.85 - 0.90	Aged hard cheeses, dry ham, fruit juice concentrates, maple syrup (saturated sucrose solution; aw 0.86)	Inhibition of many types of yeast and molds. Inhibition of *S.aureus*
0.80 - 0.85	Sweetened condensed milk, parmesan cheese, fruit cake, high-moisture prunes, rice,beans, peas	Lower limit for most enzyme activity and growth of most fungi
0.75 - 0.80	Heavily salted fish, molasses, jams, marmalades, conserves, marzipan, glace fruits, prunes, fondants (saturated NaCl solution; aw 0.75)	Lower limit for halophilic bacteria
0.65 - 0.75	Some dried fruit (dates, figs, sultanas) and nuts, rolled oats, malt extract, chocolate candies	Lower limit for most xerotolerant molds
0.60 - 0.65	Some dried fruits, honey, caramels, toffee	Maillard reactions can occur. Lower limit for growth of Osmophilic or xerophilic yeasts and molds
Below 0.60	Dry pasta, spices, biscuits, crackers, dried milk, dried whole egg, dried vegetables, flour, instant coffee, cereals, sugar, noodles	

Source: Christian, 2000; Banwart, 2004; Montville and Matthews, 2005 and Food Microbiology: Principles into Practice, First Edition, Osman Erkmen and, T.Faruk Bozoglu, 2016.

Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, it's aw is low. Most spoilage bacteria do not grow below aw = 0.91, whereas spoilage molds can grow as low as 0.80 (James, 2000).

Staphylococcus aureus can grow as low as 0.86, whereas *Clostridium botulinum* does not grow below 0.94. Much like yeasts and molds grow over a wider pH range than bacteria, the same is true for aw. The lowest reported value for foodborne bacteria is 0.75 for halophiles (literally, "salt-loving"), whereas xerophilic ("dry-loving") molds and osmophilic (preferring high osmotic pressures) yeasts have been reported to grow at aw values of 0.65 and 0.61, respectively (Table 3-5). When salt is employed to control aw, an extremely high level is necessary to achieve a_w values below 0.80 (James, 2000).

Table 7 Minimum aw Range Required for the Growth of Various Foodborne Microbes

Organism	a_w
Groups	
Most spoilage bacteria	0.90
Most spoilage yeasts	0.88
Most spoilage molds	0.80
Halophilic bacteria	0.75
Xerophillic molds	0.61
Osmophillic yeasts	0.61
Escherichia coli	0.96
Clostridium botulinum, type E	0.97
Pseudomonas spp.	0.97
Candida utilis	0.94
Rhizopus stolonifer	0.93
Enterobacter aerogenes	0.95
Staphylococcus aureus	0.86
Bacillus	0.90- 0.99
Citrobacter	0.95-0.98
C. perfringens	0.93-0.97
Lactobacillus	0.90-0.96
Leuconostoc	0.96-0.98
Aspergillus	0.68-0.88
A. niger	0.80-0.84
Mucor	0.80-0.93
Micrococcus	0.90-0.95
Fusarium	0.80-0.92
B. cereus	0.92-0.95
Salmonella	0.93-0.96

Source: Table 3.5 in Jameys.M.Jay, Martin J.Loessner, David A.Golden, Modern Food Microbiology, 7th edition, 2005, Table 4.3 in G. J. Banwart, Basic Food Microbiology.

In general, the strategy employed by microorganisms as protection against osmotic stress is the intracellular accumulation of compatible solutes (osmolytes).

Bacteria mostly accumulate N-compounds, molds,mainly sugars/sugar alcohols. The three most common compatible solutes in most bacteria are carnitine, glycine betaine, and proline. Accumulation of Compatible Solutes and modification of membrane lipid composition are the result of lowered aw. Under salt stress, L. *monocytogenes* produces 12 proteins one of which is highly similar to the Ctc protein of B. *subtilis,* and it is involved in osmotic stress tolerance in the absence of osmoprotectants in the medium (Gardan *et al.*, 2003) An increase in the length of the lag phase of growth and a decrease in the growth rate and size of the final population is the general effect of lowering aw below optimum. This effect results from adverse influences of lowered water on all metabolic activities because all chemical reactions of cells require an aqueous environment. It must be kept in mind, however, that aw is influenced by other environmental parameters such as pH, the temperature of growth, and Eh. In their study of the effect of aw on the growth of *Enterobacter aerogenes* in culture media, *Wodzinski and Frazier* found that the lag phase and generation time were progressively lengthened until no growth occurred with a lowering of a_w.

The effect of a lowered a_w on the nutrition of microorganisms seems to be general, where cell necessities that have got to mediate through an aqueous milieu are progressively shut off. In addition to the effect on nutrients, a lowered aw undoubtedly has adverse effects on the functioning of the cell membrane, which must be kept in a fluid state. The drying of internal parts of cells occurs upon placing cells in a medium of lowered a_w to a point where the equilibrium of water between cells and substrate occurs. Although the mechanisms are not entirely clear, all microbial cells may require the same effective internal aw Those that can grow under extreme conditions of a low aw apparently do so by their ability to concentrate salts, polyols, and amino acids (and possibly other types of compounds) to internal levels sufficient not only to prevent the cells from losing water but that it may allow the cell to extract water from the water-depressed external environment.

Table 8 Nomenclature Describing Different Environmental Adaptations

Descriptive Term	Definition	Examples
Solute and Water Activity		
Osmotolerant	Ability to grow over wide ranges of water activity or osmotic concentration	*Staphylococcus aureus, Saccharomyces rouxii*

Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	*Halobacterium, Dunaliella, Ectothiorhodospira*
pH		
Acidophile	Growth optimum between pH 0 and 5.5	*Sulfolobus, Picrophilus, Ferroplasma, Acontium, Cyanidium caldarium*
Neutrophile	Growth optimum between pH 5.5 and 8.0	*Escherichia, Euglena, Paramecium*
Alkalophile	Optimum growth between pH 8.5 and 11.5	*Bacillus alcalophilus*
Temperature		
Psychrophile	Can grow well at 0°C and has an optimum growth temperature of 15°C or lower	*Bacillus psychrophilus, Chlamydomonas nivalis*
Psychrotroph	Grows at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	*Listeria monocytogenes, Pseudomonas fluorescens*
Mesophile	Growth optimum around 20–45°C	*Escherichia coli, Neisseria gonorrhoeae, Trichomonas vagi*
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	*Bacillus stearothermophilus, Thermus aquaticus, Cyanidium*
Hyperthermophile	Has an optimum between 80 and about 113°C	*Sulfolobus, Pyrococcus, Pyrodictium*
Oxygen Concentration		
Obligate aerobe	Dependent completely on atmospheric O_2 for growth.	*Micrococcus luteus, Pseudomonas, Mycobacterium; most algae, fungi, and protozoa*
Facultative anaerobe	Do not require O_2 for growth but grows better in its presence	*Escherichia, Enterococcus, Saccharomyces cerevisiae*
Aerotolerant anaerobe	Can grow equally well in the presence or absence of O_2	*Streptococcus pyogenes*
Obligate anaerobe	No tolerance to O_2 and dies in its presence	*Clostridium, Bacteroides, Methanobacterium, Trepomonas agilis*
Microaerophile	Requires O_2 levels below 2–10% for growth and is damaged by atmospheric O_2 (20%).	*Campylobacter, Spirillum volutans, Treponema pallidum*

Source: Table 6.3 in Microbiology, 5th Edition, Lansing M. Prescott, 2002

Oxidation-Reduction Potential

The oxidation-reduction or redox potential of a substance is defined as the ratio of the total oxidizing (electron accepting) power to the total reducing (electron-donating) power of the substance. Redox potential is a measurement by which a substance gains or losses electrons. Eh is measured in terms of millivolts. The Eh is dependent on the pH of the substrate; normally, the Eh is taken at pH 7.0 (Beuchat *et al.,* 2001).

Aerobic organisms need food to have a positive redox potential (an oxidized state), and anaerobes need a negative potential (a reduced state) for growth. The presence of oxygen is not an absolute requirement for oxidation-reduction reactions as other compounds can accept electrons. Each food has distinct redox potentials, and this influences the type of microbial growth typically seen in that food.

Generally, the ranges of Eh at which different microorganisms can grow are as follows: aerobes +500 to +300 mV; facultative anaerobes +300 to -100 mV; and anaerobes +100 to less than -250 mV

Microorganisms may be grouped as aerobes, anaerobes, facultative anaerobes, and micro aerobes depending on the oxygen (O_2) requirements as given in table 8.

As aerobes grow, reduce O_2 in the medium, resulting in the lowering of Eh. The medium becomes poorer with oxidized substrates and reaches in reduced ones. An oxidation-reduction (redox) reaction occurs as the result of the transfer of electrons between atoms or molecules. The substrate receiving

the electrons is oxidized, and the substrate giving electron is reduced. In living cells, both electron and hydrogen transfer reactions are an essential feature of the metabolism and energy generation by oxidative (substrate) phosphorylation. For instance, C. botulinum is a strict anaerobe that requires an Eh of less than +60 mV for growth; however, slower growth can occur at higher Eh values. Foods of plant origin have in general positive Eh values; hence they are normally spoiled by aerobic bacteria and fungi.

Table 9 Eh Values for Different Food and Food Products

Food	Eh (mV)
Milk	+300 to +340
Cheese	-20 to -310
Fruit juices	+300 and +400
Cherries	+179
Peaches	+175
Spinach	+74
Barley	+225
Wheat germ	-470
Solid meats	-200
Minced meats	+200
Canned foods	
Sliced carrots	-18
Beef stew	-446

Source: (Jay, 2000; Banwart, 2004).

The growth of aerobic microorganisms leads to the lowering of the Eh value of food because they consume oxygen and produce reducing metabolic products such as hydrogen sulfide and carbon dioxide. When the Eh of the food reaches negative values due to the growth of aerobes, then the growth of anaerobes present as contaminants in the food may start, leading to further spoilage. The rate at which the Eh value of a food changes due to microbial growth depends on its redox-buffering capacity (also known as the poising effect), i.e., its resistance to change in redox potential. The buffering capacity, in turn, is determined by the concentration and the ratio in which the oxidizing and reducing compounds are present in the food (Morris, 2000). The most important reducing agents (antioxidants) naturally present in plant and animal tissues include reducing sugars, enzymes (catalase, superoxide dismutase, peroxidase), thiols (mercaptans), ascorbic acid, and the polyphenols (tannins, lignin, flavonoids) (Siddig *et al.,* 2012).

Antimicrobial Constituents

Antimicrobial substances are naturally present in certain foods that will exhibit an inhibitory action on the growth of micro-organisms. These could be present naturally in the food substance or formed during processing or added intentionally to the food. The various types of antimicrobial effects include:

1. Compounds damaging structure of the cell or its function (cell wall, cytoplasmatic membrane, ribosome)
2. Compounds affecting microbial enzymes (oxidative agents, chelating agents, heavy metals, antimetabolites)
3. Compounds reacting with DNA (chemical mutagenes –alkylating or deaminating agents, cytostatics)

Antimicrobials Present Naturally in Food

Certain plant species are known to contain essential oils that possess antimicrobial activity. Those substances 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 (Shelef., 1983).

Eggs comprise lysozyme, as in milk, and this enzyme, along with conalbumin, provides fresh eggs with a fairly efficient antimicrobial system (table 10). 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 (James, 2005)

Table 10 Inhibitor Substances in Egg and their Effect on Microbes

Inhibitor	Effect on Microorganisms	Egg White Solids (%)
Lysozyme	Lysis of cell walls (Gram-positive bacteria)	3.5
Ovomucoid	Enzyme inhibitor	11.0

Conalbumin (ovotransferrin)	Chelates metal, ions, especially ferric ions	13.0
Ovoflavoprotein	Sequesters riboflavin	
Avidin	Binds biotin	0.05
pH	Alkaline conditions restrict growth, accentuate iron chelation	

Source: G. J. Banwart, Basic Food Microbiology

Phytoalexins and the lectins are some other plant-derived antimicrobial constituents. Lectins are proteins that will specifically bind to a range of polysaccharides, including the glycoproteins of cell surfaces (Mossel *et al.,* 1995). Through this binding, lectins can exert a slight antimicrobial effect.

There exist in milk several natural anti-microbial defense mechanisms. These include:

- lysozyme is an enzyme that hydrolyzes glycosidic bonds in gram-positive cell walls yet, its effect as a bacteriostatic mechanism in milk is probably negligible.
- Lactoferrin is an iron-binding protein that sequesters iron from microorganisms, thus removing one of their growth factors and its effect as a bacteriostatic mechanism in milk is also probably negligible.
- Lactoperoxidase is the most significant and is an enzyme naturally present in raw milk that catalyzes the conversion of hydrogen peroxide to water. On addition of hydrogen peroxide and thiocyanate to raw milk, the thiocyanate is oxidized by the enzyme/ hydrogen peroxide complex producing bacteriostatic compounds that inhibit Gram-negative bacteria, *E. coli, Salmonella* spp., and streptococci
- There are three distinct components in the lactoperoxidase system in bovine milk that are required for its antimicrobial action: lactoperoxidase, thiocyanate, and hydrogen peroxide. Gram (–) psychotrophs such as the pseudomonads are very sensitive to the lactoperoxidase system.

Certain types of fermentations can result in the natural production of antimicrobial substances, including bacteriocins, antibiotics, and other related inhibitors. Bacteriocins are those proteins or peptides that are produced by certain strains of bacteria that inactivate other, usually closely related bacteria (Lück *et al.,* 1997). Bacteriocins produced by the lactic acid bacteria are the most commonly characterized bacteriocins. The antibiotic nisin made by certain strains of Lactococcus lactis is one of the best characterized of the bacteriocins. In over 50 countries around the world, Nisin has been approved for food applications (Jay, 2000). Nisin's first food application was to prevent late blowing in Swiss cheese by Clostridium butyricum. Nisin is a polypeptide that is effective against most Gram (+) bacteria but is ineffective against Gram (–) organisms and fungi.

Antimicrobials formed During Processing

The deposition of antimicrobial substances such as formaldehyde, phenols, and cresols onto the product surface due to smoking of fish, meat, poultry, and cheese has also been observed (Ray, 2004) and these chemicals act as bactericidal and bacteriostatic agents. Maillard compounds such as melanoidins formed during the heating of certain foods as a result of reactions between sugars and amino acids or peptides in the food are found to have antimicrobial activities against some Gram-positive and Gram-negative foodborne pathogens like Staphylococcus aureus and Escherichia coli (Rufian *et al.*, 2007).

Antimicrobials added to Foods

Many organic and inorganic chemicals are added to foods as preservatives (Jay, 2000; Ray, 2004). Benzoic acid is an effective antimicrobial in high-acid foods, fruit drinks, cider, carbonated beverages, and pickles. Potassium sorbate inhibits the growth of mold, yeast, and some bacteria. Parabens are more effective against molds and yeast than against bacteria, and more active against gram-positive than gram-negative bacteria. Freese *et al.,* 1973 found that the parabens inhibited serine uptake as well as the oxidation of α-glycerol phosphate and NADH (nicotinamide adenine dinucleotide) in membrane vesicles of Bacillus subtilis. Sorbic acid and

other sorbates are effective against yeasts and molds. Inhibition of bacterial spore germination (Sofos *et al.,* 1979d) by sorbate is believed to occur at the stage of germination.

Biological Structures

Most raw foods derived from plant or animal origins normally have one or another type of natural biological structure that may hinder microorganisms from entry into the cells and tissues of the food (Frazier *et al.,* 1988; Jay, 2000). 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.

The skin does not favor microbial growth because it has low water activity, is deficient in readily available nutrients, and, often, contains antimicrobial compounds such as short-chain fatty acids in the case of animal skins or essential oils in the case of plant coatings (Bohra, 2006).

According to Halloin (1983), the seed coat is the most important component in the resistance of seeds to deterioration. In 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 (James, 2005).

Extrinsic Factors

Storage Temperature

Microorganisms grow over a wide range of temperatures, and each microorganism has a defined temperature range in which they grow, with a minimum, maximum, and optimum. The temperature has a drastic impact on both the generation time of an organism and its lag period. Micro-organisms can be grouped into one of four groups depending on their optimum growth.

Table 11 Temperature Ranges for the Four Groups of Microorganism

Group	Minimum Temperature (^{0}C)	Optimum Temperature (^{0}C)	Maximum Temperature (^{0}C)
Thermophiles	40 to 45	55 to 75	60 to 90
Mesophiles	5 to 15	30 to 45	35 to 47
Psychrophiles	–5 to +5	12 to 15	15 to 20
Psychrotrophs	–5 to +5	25 to 30	30 to 35

Source: Table 1.1 in ICMSF 1980, p 4

Table gives data for approximate minimum and maximum temperature values in ^{0}C, permitting the growth of selected pathogens relevant to food.

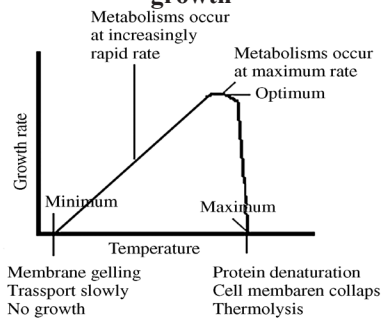
As the temperature increases above the optimum, the growth rate declines much more sharply as a result of the irreversible denaturation of enzymes and proteins, and breakdown of the cytoplasmic membrane. At the temperatures above the maximum, these changes are sufficient to kill the microorganism.

As the temperature decreases from the optimum, growth rates lows due to the slowing enzyme reactions within the cell. Many microorganisms grow at lower temperatures by increasing the quantity of unsaturated fatty acids in their membrane lipids, and psychrophiles usually have higher levels of unsaturated fatty acids than mesophiles. Increasing the degree of unsaturation in fatty acids decreases its melting point. Membranes containing higher levels of unsaturated fatty acid will remain fluid and functional at lower temperatures. However, the cytoplasmic membrane fluidity is reduced at low temperature in mesophile, and thermoduric microorganisms and nutrient transport through membrane are reduced.

The expression of virulence genes in certain foodborne pathogens is regulated by growth temperature (Montville, 2001). For example, the expression of proteins governed by the Yersinia enterocolitica virulence plasmid is high at 37°C (99°F), low at 22°C (72°F), and not detectable at 4°C (39°F). Growth temperature also impacts an organism's thermal sensitivity. Listeria monocytogenes, when held at 48°C (118°F) in inoculated sausages, has an increase of 2.4-fold in its D value at 64°C (147°F). The lag period and rate of growth of a microorganism are influenced not only

by temperature but by other intrinsic and extrinsic factors as well. For instance, the growth rate of Clostridium perfringens is significantly lower at pH 5.8 versus pH 7.2 across a wide range of temperatures (ICMSF, 1980). In general, the production of toxins below about 20°C is slow. For example, in laboratory media at pH 7, the time to produce detectable levels of enterotoxin ranged from 78 to 98 h at 19°C (66°F) to 14 to 16 h at 26°C (79°F) (ICMSF, 1980). Less favorable conditions, such as reduced pH, slowed enterotoxin production even further.

Figure 1 Effect of temperature on microbial growth



Source: Figure 5.1 Food Microbiology: Principles into Practice, First Edition. Osman Erkmenand T. Faruk Bozoglu. © 2016

Heat causes membrane damage, loss of nutrients and ions, ribosome aggregation, DNA strand breaks, inactivation of essential enzymes, and protein coagulation. Almost every cellular structure is somewhat affected by elevated temperatures. Each microorganism has a lipid fatty acyl composition that is adapted for its particular growth temperature range. As the temperature falls, membrane fluidity will decrease, and membrane-associated metabolic processes mediated by enzymes, cytochromes, and permeases will slow down. These events will trigger compensatory changes in fatty acyl composition to make the membrane more fluid. As long as the temperature change is within the normal growth temperature range, the fatty acyl changes will more or less compensate for the kinetic loss of activity. If the temperature is shifted to or just beyond the lower or upper limit, then cold shock or heat shock respectively will occur. The effect of low temperature on the microbes includes the block in the initiation of protein synthesis, disruption of ribosome structure, the formation of secondary structures, and increased negative supercoiling of DNA to each of which the microbe has a cellular response.

Effect of Concentration of Gases in the Storage Environment

Gases inhibit microorganisms by two mechanisms. The first mechanism involves a direct toxic effect that can inhibit growth and proliferation. Carbon dioxide (CO_2), ozone (O_3), and oxygen (O_2) are gases that directly exhibit toxicity to certain microorganisms. This inhibitory mechanism depends upon the chemical and physical properties of the gas and its interaction with the aqueous and lipid phases of the food. O_3 and O_2 generate oxidizing radicals that are highly toxic to anaerobic bacteria and can have an inhibitory effect on aerobes depending on their concentration. Carbon dioxide is very effective against obligate aerobes and, at high levels, will deter other microorganisms.

A second inhibitory mechanism is accomplished by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment. The various technologies used to modify the gas composition in the storage environment of food are Modified Atmospheric Packaging (MAP), Controlled Atmospheric Packaging (CAP), Controlled Atmospheric Storage (CAS and vacuum packaging. Altering the atmosphere also alters the competitive environment, and the atmospheres that exert a negative effect on the growth of one particular microorganism may promote the growth of another. This result could have positive or negative consequences relying upon the native pathogenic microflora and their substrate. Nitrogen replacement of oxygen gas is an example of this indirect antimicrobial activity (Loss *et al.,* 2002).

The various groups of microorganisms based on their oxygen requirements are given in table 8.

Effect of CO_2

High-pressure CO_2 has a severe lethal effect on microorganisms. It has been reported that living cell numbers reduced remarkably within 24 h at CO2 partial pressures ≥1000 kPa (Schulz *et al*., 2012). Chen *et al.,* 2017 demonstrated that the cell counts of E. coli were decreased by N3 log(10) (CFU/ml)

after 15 min high-pressure CO_2 treatment. Using Pseudomonas aeruginosa and Bacillus subtilis as model species, Spilimbergo *et al.,* 2002 investigated the anti-microbial potential of high-pressure CO_2 against Gram-negative and Gram-positive bacteria and found that it reduced the survival ratio of bacteria by about seven orders of magnitude.

Spilimbergo *et al.*,2002 found that CO2 could be dissolved in the phospholipids of a model cell membrane. When CO_2 is dissolved in water, it first dissolves into the liquid phase of cells, after which it is absorbed as carbonic acid in the undissociated form (Daniels *et al.,* 1985). As the inner cell membrane layer is hydrophilic, gaseous CO_2 can accumulate in the cell membrane after it has diffused into it (Isenschmid *et al.,* 1995). The accumulated CO_2 in the lipid phase will cause the order loss of lipid chains, which will increase membrane fluidity and permeability (Damar *et al.,* 2006). The increase of the CO_2 concentration would destroy the integrity of the microbial cell membrane and cause the leakage of the intracellular substances and interfere with the intracellular and extracellular material transfer, which may be one of the reasons that CO_2 inhibits the growth of microbes. The destruction of cell membrane integrity by CO_2 would result in the loss of cell protection function, thereby damaging microbial cells and even causing cell lysis and death (McNeil *et al.,* 1997).

Effect of O_2

All microorganisms are sensitive to molecular oxygen at some partial pressure (Haugaard, 1968; Morris, 1975). The low solubility of oxygen in aqueous media ensures that many environments are inhabited by predominantly anaerobic organisms. It is possible that oxygen itself is a toxic agent, perhaps by acting as an alternative electron acceptor depriving the cell of reductant for biosynthetic reactions. It may oxidize labile thiol groups, for example, those present in low-potential carriers such as ferredoxin, or inactivate specific enzymes, such as nitrogenase in nitrogen-fixing bacteria. Oxygen may be toxic as one of its metabolites; these include singlet oxygen, the superoxide anion, hydrogen, and the hydroxyl free radical. The absence of the enzyme superoxide dismutase accounted for the sensitivity of anaerobes to oxygen (McCord et al., 1971. Generally, increased oxygen concentrations promote higher leakage of reactive oxygen species (superoxide and H_2O_2) from the respiratory chain affecting metalloenzymes and DNA that, in turn, cause impaired growth and elevated mutagenesis. The microorganisms and cells respond by activating antioxidant defenses and repair systems to prevent potential damage. On exposing cells to high extracellular oxygen concentration, oxygen diffuses through the membranes and abstract electrons from reduced flavoenzymes to partly provide reduced oxygen species like superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Since the ROS production-rate is proportional to the collision frequency of oxygen and redox enzymes, the rate of O_2^- and H_2O_2 formation inside the cells depends directly on the oxygen concentration in the extracellular environment. The main source of endogenous superoxide (O_2^-) in bacteria was found to be the respiratory chain. It was stated that at hyperoxia conditions, the main ROS accumulated in the mitochondrial matrix is H_2O_2. The stated steps for its accumulation are the following: when cells are exposed to an increased oxygen concentration, there is higher leakage of electrons from complex I and III of the respiratory chain that leads to an increase in superoxide production. This superoxide is instantaneously converted to H_2O_2 by the mitochondrial superoxide dismutase. At lower oxygen concentrations, catalases and glutathione peroxidase systems minimize the accumulation of H_2O_2, but at higher oxygen concentrations, these antioxidant defenses are overwhelmed resulting in accumulation of H_2O_2 which can diffuse freely from the mitochondria reaching targets that can be damaged such as dehydratases and DNA and eventually cause cell death (Microb cell Fact, Vol 13, 2014).

Effect of Ozone

Ozone (O_3) is the other atmospheric gas that has antimicrobial properties, and it has been tried over several decades as an agent to extend the shelf life of certain foods. It is 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.

The cell target for O_3 is the membrane, where it disrupts permeability functions. Ozone was tested

against *Escherichia coli* 0157: H7 in culture media, and at 3 to 18 ppm, the bacterium was destroyed in 20 to 50 minutes. Ozone is effective in reducing pathogens on several food products, and its effect on apple surfaces and the stem and calyx where *E. coli* 0157: H7 was killed much more efficiently on apple surfaces than on stem/calyx (Kim, 1999). A typical concentration used is 0.1–0.5 ppm, which is effective against Gram-positive and Gram-negative bacteria as well as viruses and protozoa.

Relative Humidity

The RH of the storage environment is important for the growth of microorganisms on the surface area of food. The relative humidity in which a food is held will influence on the water activity of that product and influence on the growth of microorganisms on the surface of a product. When foods with low aw values are placed in an environment of high RH, foods pickup moisture until equilibrium has been established. Likewise, food with a high aw loses moisture when placed in an environment of low RH. If food has low aw, it will need a storage condition of low RH to maintain that low aw at the surface of the product.

There is a relationship between RH and temperature that ought to be borne in mind in choosing proper storage environments for foods. In general, the higher the temperature, the lower the RH, and vice versa. At high humidity, the growth of bacteria is expected to be higher than at lower humidity. Also, the temperature can affect humidity by altering the amount of liquid water present. This is expected since humid conditions can provide ideal environments for microbial growth.

Foods that bear surface spoilage from molds, yeasts, and certain bacteria should be stored under conditions of low RH. Improperly wrapped meats like whole chickens and beef cuts suffer a lot of surface spoilage in the refrigerator before deep spoilage happens, due to the generally high RH of the refrigerator and also the undeniable fact that the meat-spoilage biota is essentially aerobic. Though it is possible to lessen the chances of surface spoilage in certain foods by storing under low conditions of RH, it should be remembered that the food itself will lose moisture to the atmosphere underneath such conditions and thereby become undesirable. In choosing the right environmental conditions of RH, consideration must be given to both the possibility of surface growth and the desirable quality to be maintained in the foods in question. By altering the gaseous atmosphere, it is possible to retard surface spoilage without lowering the RH.

Presence and Activities of Other Microorganisms

Some food borne organisms generate substances that are either inhibitory or fatal to others, and these embrace antibiotics, bacteriocins, hydrogen peroxide, and organic acids.

Antibiotics are secondary metabolites produced by microorganisms that inhibit or kill a wide spectrum of other microorganisms. Most of the useful ones are produced by molds and bacteria of the genus *Streptomyces,* and a few by *Bacillus and Paenibacillus* spp. Many of the clinically useful agents now in use are synthetic products.

Monensin

It inhibits Gram-positive bacteria, and thus its long-term use has the potential of shifting the gastrointestinal tract bacterial biota from one that is normally Gram-positive to one that is more Gram-negative. Like nisin, monensin is also an ionophore that destroys the selective permeability of cell membranes.

Natamycin

This antibiotic (also known as pimaricin, tennecetin, and myprozine) is a polyene that is quite effective against yeasts and molds but not bacteria. Natamycin is the international nonproprietary name, as it was isolated from *Streptomyces natalensis*. In controlling fungi on salami, the spraying of fresh salami with a 0.25% solution was found to be effective (Hechelman *et al.,* 1969). Natamycin appears to act in the same manner as other polyene antibiotics—by binding to membrane sterols and inducing distortion of selective membrane permeability (Hamilton *et al.,* 1974). Because bacteria do not possess membrane sterols, their lack of sensitivity to this agent is thus explained.

Subtilin

Subtilin is produced by some strains of *Bacillus subtilis*. Like nisin, it is effective against Gram-positive bacteria are stable to acid and possesses enough heat resistance to withstand destruction at 121◦C for 30–60 minutes. Subtilin is effective in canned foods at levels of 5–20 ppm in preventing the outgrowth of germinating endospores, and its site of action is the same as for nisin

Tylosin

This antibiotic is a nonpolyene macrolide, more inhibitory than nisin or subtilin. As a macrolide, it is most effective against Gram-positive bacteria. It inhibits protein synthesis by associating with the 50S ribosomal subunit and shows at least partial cross-resistance with erythromycin.

Nisin

Nisin is produced by some strains of *Lactococcus lactis,* and it is a lantibiotic (contains the rare amino acids, *meso*-lanthionine, and 3-methyl-lanthione). It is the prototype of foodborne bacteriocins. The compound is effective against Gram-positive bacterium, primarily spore formers, and is ineffective against fungi and Gram-negative bacterium. *Enterococcus faecalis* is one of the most resistant Gram-positives. The cell target for these agents is the cytoplasmic membrane, where they depolarize energized bacterial membranes (reduce transmembrane potential) and form voltage-dependent multistate pores. 1,166 The result of a pore formation is the loss of accumulated amino acids and the inhibition of amino acid transport. The first use of nisin as the food was shown by Hurst 88 to prevent the spoilage of Swiss cheese by *Clostridium butyricum.* Unlike antibiotics, bacteriocins generally inhibit only closely related species and strains of Gram-positive bacteria. They consist of small proteins, and most are plasmid-mediated. It appears that some species and strains of all genera of lactic acid bacteria possess the capacity to produce bacteriocins or bacteriocin-like compounds.

Two strains of *Carnobacterium piscicola* were added to cold-smoked salmon stored at 5◦C, and one was effective in reducing L. *monocytogenes* from 103 to <10 CFU/ml after 32 days (Nilsson *et al.,*1999). This strain was antilisterial by agar diffusion assay, and the non-bacteriocin producer prevented the pathogen from growing on salmon. Another strain of *C. piscicola* was tested in cold-smoked salmon against L. *monocytogenes,* and it was also found to be bactericidal to the pathogen within 21 and 12 days at 4 and 12◦C, respectively (Yamazaki, K., 2003).

Upon the maturation of newly formed bacteriophages inside their host bacterial cells, they affect their release by the consecutive use of two small hydrophobic proteins. *Holins* disrupt the cell membrane and form holes through which endolysins can pass (Young, R. *et al.,* 1995). Endolysins target bonds in the peptidoglycan, and upon the destruction of this cell barrier, the phage progeny is released (Wang, I.-N. *et al.,* 2000). In addition to their lysis of bacterial cells from within, endolysins from Gram-positive bacteria also lyse bacteria exogenously (M., N. Vukov, *et al.,* 2002). The production and use of phage endolysins have been used to control some foodborne bacterial pathogens. Endolysins from L. *monocytogenes* phages have been introduced into a lactic starter culture, enabling the phage enzyme to reduce or eliminate the pathogen during cheese ripening. To optimize the release of the intracellularly synthesized endolysin from the bacterial cells onto the cheese surface, the endolysin encoding gene was modified to carry a signal peptide. When this construct was introduced into a dairy starter culture of *Lactococcus lactis,* a clone was identified, which expressed a strong lytic activity that was quantitatively exported from the lactococcal cells into the surrounding medium where it caused rapid lysis of L. *monocytogenes* cells. The vector was also introduced into lactose utilizing strain of L. lactis, where a functional enzyme was produced, and the vector was shown to be compatible with native lactococcal plasmids (Gaeng, S. et al., 2002). These recombinants were also used in preliminary dairy fermentation experiments to control L. *monocytogenes,* and a 95% reduction of the pathogen at the end of a Camembert cheese ripening period was demonstrated (unpublished results: M.J. Loessner *et al.*).

Phages have been shown to reduce numbers of foodborne pathogens such as L. *monocytogenes* on surface-ripened cheeses as well as *E. coli* 0157: H7

and salmonellae on fresh poultry. Coliphages are very common in fresh poultry, where they reduce the numbers of viable E. coli (Kennedy *et al.,* 1984).

Implicit Factors

Antagonisms

In antagonism, the growth of one organism inhibits or suppresses the growth of the second organism, and most of the associations between microorganisms are antagonistic. Many microorganisms generate organic acids and alcohols that are inhibitory to some of their competitors. Some produce antibiotics or bacteriocins, which possess highly specific antimicrobial activity, often against closely related species. Some microbes can gain a competitive advantage by exploitation or hoarding an essential mineral or vitamin that is needed by its competitors. Some pseudomonad produces siderophores, an iron-chelating compound, thereby preventing the growth of competitors that require iron (Gram *et al.,* 2002).

Synergisms

A synergistic association exists when two or additional microorganisms grow together, producing an impact that none of the individual microbes may produce alone. Few genuine synergisms have been documented. It has been known for a long time that Pseudomonas syncyanea and Lactococcus lactis will produce a blue color in milk only when growing together (Frazier, 1958).

In a mixed started (containing Streptococcus lactis and Leuconostoc spp. Like Leuco.dextranicum, L.citrovorum), the production of characteristic flavor due to the conversion of citrate to volatile compounds by Leuconostocs is possible only at low pH. The lowering of pH due to lactic acid production is brought about by S.lactis.

The presence of lactic acid bacteria is required for 'yeast cream' defect caused by yeasts (Candida pseudotropicalis, Torulopsis sphaerica) in cream. This is because the coagulation of milk due to lactic acid bacteria is necessary to produce the characteristic foaming due to subsequent gas production by yeasts.

Metabiosis

Metabiotic associations are essentially "sequential synergisms," in which the growth of one microorganism produces environmental conditions favorable for the growth of a second microorganism, which in turn can create favorable conditions for a third microorganism, and so on. Raw milk provides a superb example of extended metabiosis. Lactococcus lactis and a few coliforms are the first microorganisms to grow in raw milk. They turn out lactic acid, which creates a favorable environment for aciduric lactobacilli. When the accumulated acidity of the milk stops the proliferation of lactobacilli, oxidative yeasts and molds begin to grow and oxidize the lactic acid, thereby raising the pH of the milk and allowing the proteolytic bacteria growth (Frazier, 1958).

The production of sauerkraut is additionally a splendid example of metabiosis.

The growth of aerobic, oxidative microorganisms can remove oxygen and reduce the O/R potential of food, thereby creating anaerobic conditions that favor the growth of vastly different microbes. Even a straightforward spoilage pathway will exemplify a metabiotic association. The amino acid arginine can be metabolized by lactic acid bacteria to ornithine, which in turn is metabolized by enteric bacteria to the foul-smelling amine putrescine (Edwards *et al.,* 1985).

In Swiss cheese-making, the lactic acid bacteria convert lactose to lactic acid, which in turn is utilized by propionibacteria to produce propionic acid (the compound responsible for characteristic flavor production in Swiss cheese).

Table 12 The Inhibitory Substances Produced by One Microbe that Acts against the Other Microbes

Inhibitory organism	Substance produced	Organism inhibited	Reference
Serratia marcescens	Prodigiosin	*E. coli, B. subtilis, Enterobacter aerogenes, S. aureus, P.) aeruginosa*	Kalesperis, Prahlad, and Lynch(1975
P. aeruginosa	Aminoacid antimetabolite	*Bacillus sp.*	Scannell et al. (1972)

Saccharomyces cerevisiae	Glycoprotein	*Torulopsis glabrata*	Bussey and Skipper(1976)
Lactobacilli	Hydrogen peroxide	*Pseudomonas sp.* *Bacillus sp.* *Proteus sp.*	Price and Lee(1970)
C. perfringens	Unknown	*C. botulinum*	Smith (1975)
S.lactis	Nisin	*C. botulinum*	Scott and Taylor (1981)

Source: Table 4.13 G. J. Banwart, *Basic Food Microbiology*

Commensalism

Commensalism benefits the symbiont but has neutral effects for the host (Timothy D. Schowalter, 2016). Commensalism is that the interaction between populations during which one gains from the interaction, and the other is unaffected. In the microbial world, commensalism is mostly related to nutrition that is when metabolic products of one microbial population can be used by other microbial populations with no specific gain to the first population. Examples include the production of reduced redox-sensitive species, which, when oxidized, can fuel the growth of other microbial populations. Thus, methanogens produce methane, which can be oxidized by methanotrophs, and sulfate reducers produce sulfide, which can be oxidized by a variety of sulfide-oxidizing organisms (Canfield *et al.*, 2005)

Table 13 Microbial Interactions in Nature that Affect Microbial Growth

Type of Interaction	Effect of Population 1	Effect of Population 2
Synergism	+	+
Competition	-	-
Predation	+	-
Commensalism	0	+
Amensalism	0	-
Neutralism	0	0
Parasitism	+	-

Table 13 gives the effect of each of the implicit factors on the population of microbes present in that particular environment.

Predation

A predator is an organism that feeds on different organisms, and plenty of varieties of protozoans are the principal predators of prokaryotes in nature. Protists actively engulf prokaryotes (or other food particles) in a process known as phagocytosis, in which a food particle (e.g., prokaryote) is ‘’consumed’’ in special feeding organelles located at the cell surface. Prokaryotes are delivered to the protist by filter-feeding, direct interception, or passive diffusion (Fenchel, 1987). Filter feeding is accomplished by the active transport of water through a filter of cilia, or rigid tentacles on the surface of the protist, which strain small cells (and other food particles) from the environment. In direct interception, fluid flow within the medium carries particles to the surface of the feeding protist. When feeding by passive diffusion, food particles migrate to the protist, either by Brownian motion or through the prokaryote’s motility. Once ingested, food particles form a vacuole, which fuses to membrane-bound enzyme sacs called lysosomes, accomplishing the digestion of the particle (Canfield *et al.,* 2005). Numerous different prokaryotic parasites are disease-causing agents in plants and animals, and some prokaryotes can parasitize other prokaryotic organisms. For example, the Gram-negative bacteria Bdellovibrio actively hunt and kill its prey to accomplish its parasitic lifestyle. In the attack phase, it is a flagellated non-reproductive cell that enters the periplasmic space of other gram-negative host cells. There it loses its flagellum and grows, feeding off the host, into a reproductive septate filament. After growth ceases, the filament separates, lysing the host and releasing many individual attack cells, ready to repeat the cycle (Dworkin, 1992).

Amensalism

Amensalism defines a relationship in which the activity of one population is harmful to another. For microbes, this typically results when the

products of one type of metabolism are detrimental to another. Examples include the production of oxygen by cyanobacteria, inhibiting anaerobic organisms, or the production of inhibitory organic compounds as metabolic by products. For example, ethanol, a fermentation product, is inhibitory to many microorganisms, particularly at higher concentrations. The production of acid during sulfide oxidation, particularly in surface sediments where the pH can be driven very low, creates an extreme environment inhibitory to a wide variety of microorganisms (Canfield *et al.,* 2005). Amensalism is the opposite of commensalism, where one organism is harmed while the other is unaffected. A good example of this interaction is when one organism produces an associate antibiotic against another organism. Such an interaction is usually the premise of biological control. For example, some isolates of the microorganism *Pseudomonas fluorescens* will suppress the fungal pathogen *Gaeumannomyces graminis,* chargeable for 'take-all' in wheat (*Triticum aestivum*) (P G Hartel, 2005).

Processing Factors

The various processing factors that influence microbial growth include treatments such as heating, cooling, and drying that affect the composition of the food and also affect the types and numbers of microorganisms that stay within the food after treatment.

Microbial cell death has been associated with either structural damage or physiological dysfunctions. Among structural damage, disruption of the envelopes, DNA conformational changes, ribosome alterations, or protein aggregation are the most frequently described. Also, physiological disorders, such as membrane selective permeability alterations or loss of function of key enzymes, have been proposed as events leading to cell death (Gould, 1989). Perhaps the most important difficulties that researchers encounter in this area are that several of these lesions may occur simultaneously when the cells are subjected to an agent, and it is, difficult to attribute loss of viability of the cell to a single event.

Heat causes membrane damage, loss of nutrients and ions, ribosome aggregation, DNA strand breaks, inactivation of essential enzymes, protein coagulation, etc. (Gould, 1989). In other words, almost every cellular structure is somehow affected by elevated temperatures, and it is very difficult to discern which events are leading to cell death. It is well established that the critical target for irradiation is the chromosome (Moseley, 1989). The effects of ionizing irradiation on bacterial cells are classified as direct and indirect. Direct actions comprise the events caused by the absorption of radiation energy by the target molecules, whereas indirect actions are those derived from the interaction between the reactive species formed by the radiolysis of water, such as the hydroxyl radical, and the target molecules. The hydroxyl radical OH• reacts with the sugar-phosphate backbone of the DNA chain giving rise to the elimination of hydrogen atoms from the sugar. This causes the cutting of the phosphate ester bonds and the subsequent appearance of single-strand breaks. Double strand breaks occur once two single-strand breaks take place in every chain of the double helix at an adjacent distance.

The mechanism of inactivation of bacterial cells by ultrasound under pressure has also been described. Most authors agree that the cavitation phenomenon is responsible for the lethal effects of ultrasound (Kinsloe *et al.,* 1954; Raso *et al.,*. 1998a). When bubbles implode under an intense ultrasonic field, very high pressures and temperatures are generated, and consequently, strong mechanical forces and free radicals are formed (Suslick, 1990). Free radicals could therefore inactivate bacterial cells in a similar mode as that described for IR.

The degree of cell disruption evaluated by Raso. *et al.,* 1998b through phase-contrast microscopy, and they observed that whereas heat-treated cells maintained full cellular integrity, MS treated cells were completely broken. MTS treated cells showed a medium degree of disruption, and these outputs confirmed that ultrasound inactivates microorganism cells through envelope breakdown in an "all or nothing" sort phenomenon. It has also been found that MS treatments sensitize spores of Bacillus subtilis to lysozyme (Raso *et al.* 1998b). Therefore, it has been instructed that ultrasonic waves may injure the external layers of the spore, facilitating its rehydration and consequently reducing its extreme heat resistance.

The high pressure causes tighter packing of the acyl chains within the phospholipid bilayer of membranes and promotes membrane transition from liquid crystalline to gel section, in a very similar approach as a temperature downshift (MacDonald, 1993). Although phase transition of membrane lipids is not necessarily lethal to bacteria, it has been demonstrated that the composition and state of the bacterial cell membrane before pressure treatment affect bacterial resistance to HHP (Casadei *et al.,* 2002). Cells with a more fluid membrane, i.e., with a higher degree of unsaturation, are more barotolerant (Casadei *et al.,* 2002). It is not clear how a more fluid membrane renders a more resistant cell to HHP. The pressure at which phase transition occurs would be higher in cells with a more fluid membrane, but it is not known in which circumstances, if any, cell damage is linked to phase transition.

Damage to the cytoplasmic membrane after pressurization has also been repeatedly reported, through the loss of osmotic responsiveness (Paga´n and Mackey, 2000), loss of intracellular material, and formations of buds and vesicles of lipidic origin. In conclusion, HHP inactivation seems to be multitarget in nature. The membrane is a key target, but in some cases, additional damaging events such as extensive solute loss during pressurization, protein coagulation, key enzyme inactivation, and ribosome conformational changes, together with impaired recovery mechanisms, seem also needed to kill bacteria. Bacterial spores are extremely resistant to HHP, being able to withstand up to 1000 MPa for long treatment times unless they are in the germinated state (Cheftel, 1995).

Membrane structural or functional alteration is usually accepted as the reason behind cell death by PEF. Free charges tend to accumulate within the inner and outer surface of the membrane generating a transmembrane potential of about ten mV. When an external electric field is applied, as in PEF treatment, a higher amount of free charges of opposite charge accumulate at both membrane surfaces, resulting in compression of the membrane. When the external electric field exceeds a critical value or threshold, the membrane is unable to withstand the electrocompression, and pores are formed. The size and amount of pores depend on the electric field strength and the duration of the treatment (Zimmermann *et al.,* 1974).

Alternative theories propose that permeabilization is the consequence of the dipolar reorientation of the membrane phospholipids under an electric field (Tsong, 1991). Tsong (1991) has also suggested that the formation of hydrophilic pores would lead to a localized Joule heating phenomenon that could be responsible for the denaturation of proteins and phase changes in the membrane. Sublethal or repairable injury has been detected for irradiated, pressurized, and PEF-treated cells. For each agent, the mechanism of inactivation is different, and so is the nature and the magnitude of the sublethal injury. The various parameters of each of the processing factors are described in Table 14

Table 14 Factors Affecting Microbial Inactivation by Irradiation (IR), Manosonication (MS), High Hydrostatic Pressure (HHP) and Pulsed Electric Field

Factors	Irradiation	Manosonication	High Hydrostatic Pressure	Pulsed Electric Field
Process parameters		Treatment time (min) Amplitude (60–150 lm)Pressure (0–300 KPa)Temperature	Treatment time (min) Pressure (100–600 MPa)Temperature	Treatment time: number of pulses. pulse width (ls) Electric field strength (9–50 kV cm)1) Pulse specific energy (J ml)1) Temperature
Microbial characteristics Resistance	S > G+ > G) > Y & M	S > G+ > G)	S > G+ > G), Y & M	S > G+ > G) > Y & M
Spore inactivation	At high dose	At high intensity	Cyclic treatments	Not possible

Intraspecies variation	Medium	Low	Large	Medium
Product parameters	Oxygen Composition Water activity	Water activity	Composition Water activity pH (recovery) Preservatives	Composition Water activity pH (treatment/recovery) Preservatives

V-viruses; S- spores; Y & M- yeasts and moulds; G+- Gram-positive vegetative cells; G- Gram-negative cells.

Source: Table 2 in P. Manas and R. Pagan Microbial inactivation by new technologies of food preservation [a] 2005 The Society for Applied Microbiology, Journal of Applied Microbiology, 98, 1387–1399.

Conclusion

Thus, all the above-discussed factors (intrinsic, extrinsic, implicit, and processing) influence the growth of the microorganism to a different extent in some way or the other, and hence control of these factors is very much essential in the growth of microorganism.

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Author Details

S.S.Preetha, *M.Tech Food Technology, College of Food and Dairy Technology (TANUVAS), Chennai, Tamil Nadu, India,* ***Email ID:*** *preethatech14@gmail.com.*

Rita Narayanan, *Professor, Department of Food Processing Technology, College of Food and Dairy Technology (TANUVAS), Chennai, Tamil Nadu, India.*