UNIT 2 FACTORS AFFECTING GROWTH AND INHIBITION OF MICROORGANISMS IN FOOD

Structure

2.0 OBJECTIVES

After reading this unit you should be able to:

- list out the various factors that favour/inhibit the growth of microorganisms;
- explain the role played by pH in inhibition of microbial growth;
- explain the effect of water activity on microbial growth and activities;
- explain the influence of redox potential on the natural microflora of food and the type of spoilage occurring in food;
- understand the role played by nutrient composition on type of microorganisms growing in food;
- understand the role played by antimicrobial barriers in retarding microbial spoilage of food; and
- understand the role played by inhibitory substances in retarding microbial spoilage of food.

2.1 INTRODUCTION

Microorganisms use our food supply as a source of nutrients and energy. They increase their numbers by utilizing nutrients. This can result in a deterioration of the food. They produce enzymatic changes and off-flavours in food by breaking down a nutrient or synthesizing new compounds. Thus, they "spoil" our food and make it unfit for consumption. To prevent this we reduce the contact between microorganisms and our foods (prevent contamination) and also eliminate microorganisms from our foods, or adjust conditions of storage in such a way that their growth is prevented (preservation) and thus, there is no spoilage of food.

If the microorganisms involved are pathogenic, then their presence in our food will lead to outbreak of food borne diseases also. Many of our foods support the growth of pathogenic microorganisms or serve as a source of them. Here again, we attempt to prevent their entrance and growth in our foods or eliminate them by processing.

Interactions between microorganisms and our foods are also beneficial. Many of the cultured products consumed and enjoyed for example cultured buttermilk, yoghurt, sauerkraut, pickles and tofu are produced as a result of beneficial activities of microorganisms.

Food is the substrate for growth of microorganisms, so the characteristics of a food are important. Food or substrate will determine which microorganisms can or cannot grow on it so there is a need to understand the characteristics of the food or substrate. Then only one can make predictions about the microbial flora that may develop and flourish in it. This microflora will bring about the biochemical changes in food due to their activities. The types of biochemical changes will determine whether those changes are beneficial or harmful.

Knowledge of the factors that favour or inhibit the growth of microorganisms is very important. It will help us in understanding the principles of food spoilage and preservation. The chief compositional factors of food that influence microbial activity are hydrogen-ion concentration, moisture, oxidation-reduction (O-R) potential, nutrients, biological structure and presence of inhibitory substances.

2.2 HYDROGEN-ION CONCENTRATION (pH)

The acidity and alkalinity (pH) of an environment has a strong influence on the activity and stability of macromolecules such as enzymes. These enzymes play an important role during growth of microorganisms and in their metabolism. Thus, growth and metabolism of microorganisms are influenced by pH.

2.2.1 Effect on Microbial Growth

Every microorganism has a minimal, a maximal, and an optimal pH for growth. In general, bacteria grow in the pH range of 6.0–8.0, yeasts 4.5-6.0 and filamentous fungi 3.5-4.0. Molds can grow over a wider range of pH than most yeasts and bacteria, and many molds grow at acidities too high for yeasts and bacteria. Most fermentative yeasts grow well in pH range of about 4.0 to 4.5, eg. fruit juices, and film yeasts grow well on acid foods, such as sauerkraut and pickles. On the other hand, most yeast do not grow well in alkaline foods and thus do not have a significant role to play in the spoilage of food products with high pH. However, large number of yeasts grow well in near neutral pH. There are some exceptions for example some bacteria can grow in moderate acidity particularly those bacteria that produce large acids as a result of their activities like lactobacilli and acetic acid bacteria. These have pH optima between 5.0 and 6.0 and others like the proteolytic bacteria can grow in foods with a high (alkaline) pH, as found in the stored egg white. Bacteria are more sensitive to pH than molds and yeasts, with the pathogenic bacteria being the most sensitive amongst them. The pH values of some of the common foods along with the pH range for growth of some groups of microorganisms and a few of food associated pathogenic bacteria are given in Table 2.1.

Table 2.1: The pH ranges of some common food items and pH range of some common food microflora

Food	**pH range**	**Organism**	**pH range**
Citrus fruits	2-5	Molds	0-11
Soft drinks	2.5-4	Yeasts	1.5-8.5
Beer	3.5-4.5	Lactic acid bacteria	3.2-10.5
Meat	5.5-6.2	*Staphylococcus aureus*	4-9.8
Fish	6.5-7.3	*Salmonella* spp.	4.1-9
Egg white	8.6-9.6	*Escherichia coli*	4.3-9
Milk	6.5-7	*Yersinia enterocolitica*	4.5-9
Flour	6.2-7.2	*Clostridium botulinum*	4.8-8.2
Vegetables	4.8-7	*Clostridium perfringens*	5.4-8.7
Fermented shark	10-12	*Bacillus cereus*	4.7-9.3

pH minima and maxima of microorganisms also varies due to other important factors like temperature, moisture content, salt concentration, redox potential etc. For example, in the presence of 0.2 M NaCl, *Alcaligenes faecalis* can grow over a wider pH range than in the absence of NaCI or in the presence of 0.2 M sodium citrate. The pH minima of certain lactobacilli also depends upon the type of acid used, for example with citric, hydrochloric, phosphoric and tartaric acids growth can occur at lower pH than in presence of acetic or lactic acids. In general, yeast and molds are more acid-tolerant than bacteria.

When microorganisms are grown at pH either higher or lower than their optimum pH there is an increase in lag phase of the microbe. The increased lag would be of longer duration if the food has a good buffering capacity in contrast to one that has poor buffering capacity. Good buffering capacity of food would result in slower change in pH of food due to microbial activity. A respiring microbial cell is adversely affected by pH since it affects the functioning of enzymes and the transport of nutrients into the cell. In addition to the effect of pH on rate of growth of microorganisms, pH also affects rate of survival of microorganisms during storage, heating, drying and other forms of processing. Many times the initial pH may be suitable, but growth of the organism itself may alter the pH, thereby making it unfavourable. Conversely, the initial pH may be restrictive, but the growth of a limited number of microorganisms may alter the pH to a more favourable range for the growth of many other microorganisms.

The inherent pH of foods varies, although most are neutral or acidic. Materials with an alkaline pH generally have a rather unpleasant taste with some exceptions like egg white where the pH increases to around 9.2, as CO_2 is lost from the egg after laying. The pH of a product can be easily determined with a pH meter. However, this value alone is not sufficient for predicting microbial spoilages. It is also desirable, for example, to know the acid responsible for a given pH, because some acids, particularly the organic acids, are more inhibitory than others.

2.2.2 Effect on Microbial Ecology and Food Spoilage

The acidity of a product plays an important role in deciding the type microflora present in food and the rate and type of its spoilage. For example, most of the meats and seafoods have a final ultimate pH of about 5.6 and above. Thus, these products are susceptible to bacterial as well as to mold and yeast spoilage. Similarly, most vegetables have higher pH values than fruits, and thus vegetables would be more prone to bacterial than fungal spoilage since such pH values favour bacterial growth. Soft-rot producing bacteria such as *Erwinia carotovora* and pseudomonads play a significant role in their spoilage. In fruits, however, a lower pH (below 4.5) prevents bacterial growth and yeasts and molds dominate spoilage.

Fish is spoiled more rapidly than meat under chilled conditions. This is due to the fact that the pH of post-rigor mammalian muscle is around 5.6 and this contributes to the longer storage life of meat. On the other hand, fish have a pH between 6.2-6.5. *Shewanella* (formerly *Alteromonas*) mainly causes spoilage under chilled conditions. It is a pH-sensitive microbe and hence, plays a significant role in fish spoilage but not in normal meat (pH<6.0). Those fishes that have a naturally low pH such as halibut (pH~5.6) as a result have better keeping qualities than other fish. Thus, a food with inherently low pH would tend to be more stable microbiologically than a neutral food.

Upon the death of a well-rested meat animal, the usual 1% glycogen is converted into lactic acid, which directly causes a depression in pH values from about 7.4 to about 5.6. Most of the bacteria cannot tolerate lower pH, hence meat has a longer storage life. Meat from fatigued animals spoils faster than that from rested animals. This is because most of the glycogen present had already been used during its lifetime and hence, final pH attained upon completion of rigor mortis is not as low as that of a well-rested animal. Thus, bacteria are able to grow and spoil it.

The excellent keeping quality of certain foods is related to their restrictive pH, for example fruits, soft drinks, fermented milks, sauerkraut and pickles which have an acidic pH. Fruits, soft drinks, vinegar, and wines have an excellent keeping quality mainly due to pH, which falls far below the point at which bacteria normally grow. 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.

Some foods have a low pH because of inherent acidity; others, for example, the fermented products like sauerkraut, pickles and fermented milks have a low pH because of acidity produced due to the activity of microorganisms. This acidity is also known as biological acidity and is generally due to the accumulation of lactic acid during fermentation. Regardless of the source of acidity, the effect upon keeping quality appears to be the same. This ability of low pH to restrict microbial growth has been employed since the earliest times for preservation of foods using acetic acid and lactic acids.

2.2.3 Inhibition of Microbes by Weak Acids

With the exception of those soft drinks that contain phosphoric acid, in most other acidic foods acidity is due to the presence of weak organic acids. These do not dissociate completely into protons and conjugate base in solution but establish equilibrium:

$$HA \rightleftarrows H^{+} + A^{-}$$

The partial dissociation of weak acids, such as acetic acid, plays an important role in their ability to inhibit microbial growth. Although addition of strong acids has a more profound effect on pH but at the same pH, they are less inhibitory than weak lipophilic acids. This is because microbial inhibition by weak acids is directly related to the concentration of undissociated acid (Figure 2.1). These undissociated lipophilic acid molecules can pass freely through the membrane, in doing so they pass from an external environment of low pH where the equilibrium favours the undissociated molecule to the high pH of the cytoplasm. At this higher pH, the equilibrium shifts in favour of the dissociated molecule, so the acid ionizes producing protons. These protons tend to acidify the cytoplasm. The cell tends to maintain its internal pH by expelling protons leaking in. This process requires energy and the microbe diverts energy from growth related functions to removing protons from the cell thereby slowing its growth. The burden on the cell becomes too great. The cytoplasmic pH drops to a level where growth is no longer possible and the cell eventually dies. Strong acids on the other hand dissociate completely into protons and conjugate base in solution. These dissociated acid molecules cannot pass freely through the cell membrane. Hence there is not much change in the pH of the cytoplasm. As a result these are less inhibitory than weak acids at the same pH.

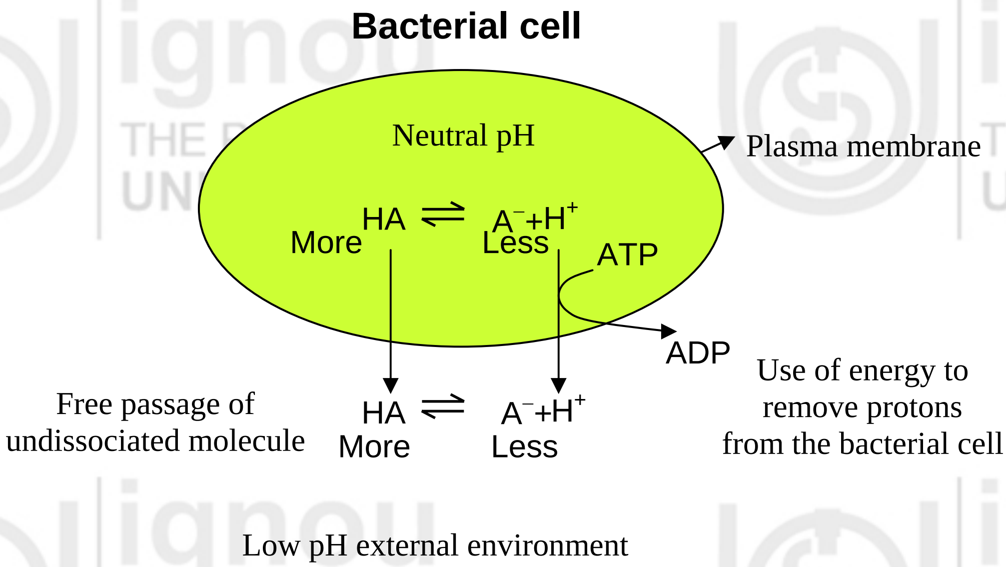

Figure 2.1: Inhibition of bacterial growth by weak acids

2.2.4 Buffers in Foods

Some foods are better able to resist changes in pH than others. These tend to resist changes in pH since these are buffered and the ability to resist changes in pH is known as buffering capacity. The buffers are the compounds present in food that resist changes in pH and thus are important. These are especially effective within a certain pH range. Buffers permit an acid (or alkaline) fermentation to go on longer with a greater yield of products and organisms than would otherwise be possible. In general, meats are more 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 by the growth of microorganisms. Hence these permit an appreciable decrease in pH with the production of small amounts of acid by the lactic acid bacteria during the early part of sauerkraut and pickle fermentations. This is desirable since it enables the lactic acid bacteria to suppress the undesirable pectin-hydrolyzing and proteolytic organisms which cause spoilage. Low buffering power makes for a more rapidly appearing succession of microorganisms during fermentation than high buffering power. Milk is fairly high in protein (a good buffer) and therefore permits considerable growth and acid production by lactic acid bacteria during the manufacture of fermented milks before growth is suppressed.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.

b) Compare your answers with those given at the end of the unit.

1. Why do fishes spoil more rapidly than meat?

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2. Why does the meat from fatigued animal spoil faster than that from a well-rested animal?

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3. What is biological acidity?

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4. Why are the weak organic acids more inhibitory to growth of microorganisms than the strong acids?

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5. How does adverse pH affect the microorganism?

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2.3 MOISTURE REQUIREMENT/WATER ACTIVITY

One of man's oldest methods of preserving foods is drying or desiccation. The preservation of foods by drying is a direct result of removal of moisture. Microorganisms need water for growth. Without water no growth can occur. The exact amount of water needed for growth of microorganisms varies. This water requirement of microorganisms is best expressed in terms of available water or water activity a_w, the vapour pressure of the solution (of solutes in water in most foods) divided by the vapour pressure of the solvent (usually water). Thus a_w, for pure water would be 1.00. The water activity depends on the number of molecules and ions present in solution, rather than their size. Thus a compound like sodium chloride, which dissociates into two ions in solution, is more effective at reducing the water activity than a compound like sucrose on mole-to-mole basis.

2.3.1 Effect on Microbial Growth and Activity

Bacteria require higher values of a_w for growth than fungi. Gram-negative bacteria have higher requirements than gram positives. Most spoilage bacteria do not grow below a_w 0.91, while spoilage molds can grow as low as 0.80. However, food-poisoning bacteria like *Staphylococcus aureus* can grow at a_w as low as 0.86, while *Clostridium botulinum* does not grow below 0.94. Yeasts and molds can grow over a wider a_w range than bacteria. The lowest a_w values for bacteria is 0.75 for halophilic (meaning salt-loving) bacteria, while xerophilic (dry-loving) molds and osmophilic (preferring high osmotic pressures) yeasts can grow at a_w values of 0.65 and 0.60, respectively. The limiting value of water activity for the growth of microorganisms is about 0.6 and below this value the spoilage of foods is not due to microorganisms but may be due to insect damage or chemical reaction such as oxidation. At a water activity of 0.6, corresponding to a water potential of -68MPa (Mega Pascals), the cytoplasm would need to contain very high concentrations of an appropriate compatible solute and it is probable that the macromolecules such as DNA would no longer function properly and active growth may stop.

Most bacteria grow well in a medium with a water activity a_w approaching 1.00 (at 0.995 to 0.998), i.e., they grow best in low concentrations of sugar or salt. Culture media for most bacteria contain not more than 1 per cent of sugar and 0.85 per cent of sodium chloride (physiological salt solution). As little as 3 to 4 percent sugar and 1 to 2 percent salt may inhibit some bacteria. The optimal a_w and the lower limit of a_w for growth vary with the bacterium, as well as with food, temperature, pH, and the presence of oxygen, carbon dioxide, and inhibitors. The optimal a_w and the lower limit of a_w for growth is lower for bacteria which are able to grow in high concentrations of sugar or salt. Some examples of lower limits of a_w for growth of some food bacteria are given in Table 2.2. These figures would vary depending on conditions used for growth of the microorganisms as mentioned above.

Table 2.2: Minimum a_w values for growth of microorganisms of importance in food

Organisms Groups	Water activity (a_w)	Organisms Specific organisms	Water activity (a_w)
Most spoilage bacteria	0.90	*Pseudomonas* spp.	0.97
Most spoilage yeasts	0.88	*Escherichia coli*	0.96
Most spoilage molds	0.80	*Bacillus subtilis*	0.95
Halophilic bacteria	0.75	*Enterobacter aerogenes*	0.945
Xerophilic molds	0.61	*Clostridium botulinum*	0.93
Osmophilic yeasts	0.60	*Staphylococcus aureus*	0.86

Molds differ considerably in optimal a_w and range of a_w for the germination of asexual spores. The minimal a_w for spore germination is as low as 0.62 for some molds and as high as 0.93 for others (e.g., *Mucor*, *Rhizopus*, and *Botrytis*). Each mold also has an optimal a_w and range of a_w for growth. Examples of optimal a_w are 0.98 for *Aspergillus* sp., 0.995 to 0.98 for *Rhizopus* sp., and 0.9935 for *Penicillium* sp. The a_w would have to be below 0.62 to stop all chances for mold growth, although a_w below 0.70 inhibits most molds that cause food spoilage. The reduction of the a_w below the optimum for a mold delays spore germination and reduces the rate of growth and therefore is an important factor in food preservation. Many of the molds can grow in foods with a_w approaching 1.00 (pure water).

With a reduction of water activity of food, the number of microorganisms capable of maintaining active growth in it decreases. On the other hand, there are microorganisms that grow better at reduced a_w. These microorganisms are generally associated with foods having low water activity. Since low water activities are associated with three distinct types of food, the following three terms are used to describe the microorganisms especially associated with these foods:

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 nonionized organic compounds such as sugars.

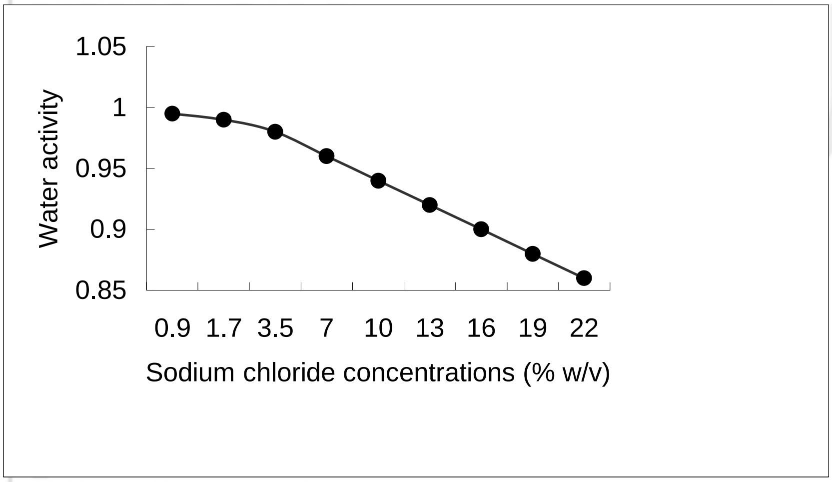
iii) **Xerotolerant** – able to grow on dry foods.

The halobacteria are obligately halophilic and cannot grow in the absence of high concentration of salt.

2.3.2 Ways of Reducing Water Activity

Water is made unavailable in various ways:

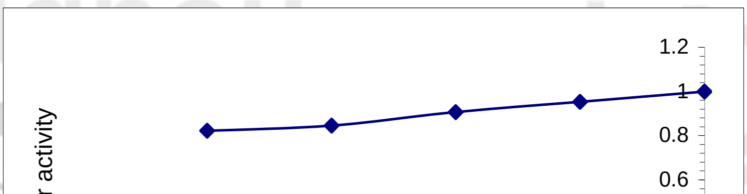
1. *Solutes and ions tie up water in solution.* Solutes lower a_w and this reduction in a_w depends on the total concentration of dissolved molecules and ions. Since these bind to water molecules, there is reduction in a_w. Therefore, an increase in the concentration of dissolved substances such as sugars and salts is in effect a drying of the material (Graph 2.1). Not only is water tied up by solutes, but also water tends to leave the microbial cells by reverse osmosis to maintain equilibrium between the concentration of solute outside and inside the cells.



Graph 2.1: Effect of sodium chloride concentration on water activity

2. *Hydrophilic colloids (gels) make water unavailable.* As little as 3 to 4 percent agar in a medium may prevent bacterial growth by leaving too little available moisture.

3. *Water of crystallization or hydration is usually unavailable to microorganisms*. Water itself, when crystallized as ice, no longer can be used by microbial cells. The a_w of water-ice mixtures (vapour pressure of ice divided by vapour pressure of water) decreases with a decrease in temperature below 0^oC (Graph 2.2). In a food, as more and more ice is formed, the concentration of solutes in the unfrozen water increases, thus lowering available water and thereby it's a_w is reduced.



Graph 2.2: Effect of temperature on water activity

The water activity a_w varies with temperature; these variations are only slight within the range of temperatures that permit microbial growth. Variations in temperature increase in importance with increasing concentrations of solutes and increasing effects on ionization of solutes.

Each microorganism has a maximal, optimal, and minimal a_w for growth. As the a_w is reduced below the optimal level, there is a lengthening of the lag period of growth, a decrease in the rate of growth and a decrease in the amount of cell substance synthesized, changes that vary with the organism and with the solute employed to reduce a_w. This range depends on a number of factors which are mentioned below.

2.3.3 Factors Affecting Water Requirement

Factors that may affect a_w requirements of microorganisms include:

1. *Kind of solute employed to reduce the a_w*: For some organisms, like molds, the lowest a_w for growth is independent of the kind of solute used. For other organisms, however, lower limiting a_w values differ from solute to solute. For example potassium chloride usually is less toxic than sodium chloride, and it in turn is less inhibitory than sodium sulphate. Thus, sodium sulphate at a lower concentration may be as effective in reducing a_w as potassium chloride at a higher concentration.

2. *Nutritive value of the culture medium*: In general, the better the medium for growth, the lower the limiting a_w permitting growth of microorganism.

3. *Temperature*: Most organisms have the greatest tolerance to low a_w at about optimal temperatures.

4. *Oxygen supply*: Growth of aerobes takes place at a lower a_w in the presence of air than in its absence, and the reverse is true of anaerobes.

5. *pH*: Most organisms are more tolerant of low a_w at pH values near neutrality than in acid or alkaline media.

6. *Inhibitors*: The presence of inhibitors narrows the range of a_w for growth of microorganism.

Each organism has its own characteristic optimal a_w and its own range of a_w for growth in a given set of environmental conditions. This range of a_w permitting growth is narrowed if any of the above mentioned environmental factors are not optimal and is narrowed still more if two or more conditions are not favourable. An unfavourable a_w will result not only in a reduction in the rate of growth but will also reduce the yield of cells. The delay (lag) in initiation of growth or germination of spores will be more under more unfavourable a_w of the substrate. It is known that growth of at least some cells may occur in high numbers at reduced a_w values, but the production of certain extracellular products may be limited or these may not be produced at all. For example, reduced a_w results in the cessation of enterotoxin B production by *Staphylococcus aureus* even though high numbers of cells are produced at the same time This often is as important in food preservation as reduction in the rate of growth of the organism. Microorganisms that can grow in high concentrations of solutes, e.g., sugar and salt, obviously have a low minimal a_w. Halophilic bacteria require a certain minimal concentration of dissolved sodium chloride for growth. Osmophilic yeasts grow best in high concentrations of sugar.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.

b) Compare your answers with those given at the end of the unit.

1. List out the types of microorganisms associated with foods having low water activity.

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2. Define water activity.

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3. How water is made unavailable to microorganisms?

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4. Describe the factors that may affect a_w requirements of microorganisms.

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2.4 OXIDATION REDUCTION POTENTIAL

The tendency of a substrate to accept or donate electrons, is termed its redox potential (*E*h). The O/R potential of a substrate may be defined generally as the ease with which the substrate loses or gains electrons. When a substrate loses electrons, the substrate is oxidized while a substrate that gains electrons becomes reduced. Therefore, a substance that readily gives up electrons is a good reducing agent, while one that readily takes up electrons is a good oxidizing agent. In the equation below, this is represented in its most general form to include the many redox reactions, which also involve protons and have the overall effect of transferring hydrogen atoms.

$$[\text{Oxidant}] + H^{+} + ne \rightleftharpoons [\text{Reductant}]$$

Where *n* is the number of electrons, e, transferred.

The tendency of an atom or molecule to accept or donate electrons is expressed as its standard redox potential, *E*o'. When electrons are transferred from one compound to another, a potential difference is created between the two compounds. This difference may be measured by use of an appropriate instrument and expressed as millivolts (mv). It can be measured against an external reference by an inert metal electrode, usually platinum. The more highly oxidized a substance, the more positive will be its electrical potential, and the more highly reduced a substance, the more negative will be its electrical potential.

2.4.1 Redox Couples in Food

Pair of oxidizing and reducing agents present in food are known as redox couples. A large positive *E*o' of food indicates that the oxidized species of the couple is a strong oxidizing agent and the reduced form only weakly reducing. A large negative *E*o' of food indicates the reverse. When the concentration of oxidant and reductant is equal, a zero electrical potential exists. The relative proportions of oxidized and reduced species present will also influence the measured Eh. If the balances of the various redox couples present favours the oxidized state then there will be a tendency to accept electrons from the electrode creating a positive potential, which signifies an oxidizing-environment. If the balance is reversed, the sample will tend to donate electrons to the electrode, which will then register a negative potential – a reducing environment.

With the notable exception of oxygen, most of the couples present in foods, *e.g.* glutathione and cysteine in meats and ascorbic acid and reducing sugars in plant products, would on their own tend to establish reducing conditions. Oxygen, which is present in the air at a level of around 21 %, is usually the most influential redox couple in food systems. It has a high *E*o' and is a powerful oxidizing agent. If sufficient air is present in food, a high positive potential will result and most other redox couples present will, if allowed to equilibrate, be largely in the oxidized state. Hence, increasing the access of air to food material by chopping, grinding or mincing will increase its *E*h. Similarly, exclusion of air as in modified vacuum packing or canning will reduce the *E*h.

2.4.2 Effect of Microbial Growth on Redox Potential of Food

Microbial growth in food reduces its *E*h. This is usually because during their growth, microorganisms consume oxygen and produce reducing compounds such as hydrogen. Oxygen is the most important terminal electron accepter in the electron transport chain, especially in case of aerobes. During passage of electrons through the electron transport chain, microorganisms generate energy and thereby oxygen is depleted. As the oxygen content of the medium decreases, so the redox potential declines from a positive potential to a negative potential.

The decrease in *E*h as a result of microbial activity is the basis of some rapid tests for determination of microbial load of food, particularly dairy products. Redox dyes such as methylene blue or resazurin are used to indicate changes in *E*h, which are correlated with microbial levels. These dyes become colourless when these are reduced. The time taken for reduction of the dyes will be inversely proportional to the microbial load of food i.e. more the microorganisms in food, less is the time taken for dye to be reduced and *vice versa*. The factors influencing redox potential of foods are summarized in Table 2.3 given below:

Table: 2.3 Factors affecting redox potential of foods

1. Redox couples present
2. Ratio of oxidizing species to reducing species
3. pH
4. Poising capacity
4. Availability of oxygen
5. Microbial activity

2.4.3 Effect on Microbial Growth and Ecology

Redox potential exerts an important selective effect on the microflora of a food since it will decide the type of microorganism which can grow in that food. Microbial growth can occur over a wide spectrum of redox potential. However, individual microorganisms have their own redox ranges over which they can grow. They are classified into one of several physiological groups on the basis of the redox range over which they can grow and their response to oxygen. Based on their ability to use free oxygen, microorganisms have been classified as

1. **Aerobic** when they require free oxygen.
2. **Anaerobic** when they grow best in the absence of free oxygen.
3. **Facultative** when they grow well either aerobically or anaerobically.

Molds are aerobic, most yeast grow best aerobically and bacteria may be aerobic, anaerobic, or facultative. A high (oxidizing) potential favours aerobes but will permit the growth of facultative organisms also, and a low (reducing) potential favours anaerobic or facultative organisms. Growth of an organism may alter the O-R potential of a food enough to inhibit other organisms. Anaerobes, for example, may lower the O-R potential to a level which is inhibitory to aerobes.

Obligate aerobes are those organisms that generate their energy from oxidative phosphorylation using oxygen as the terminal electron acceptor. Consequently, they have a requirement for oxygen and a high *E*h and will predominate at food surfaces exposed to air or where air is readily available, for example, pseudomonads, such as *Pseudomonas fluorescens*, which grows at an *E*h of +100 to +500 mv, and other oxidative Gram-negative rods. These grow on meat surfaces and produce slime and off-odours. *Bacillus subtilis (E*h -100 to +135 mv) produces ropiness in the open texture of bread and *Acetobacter* species growing on the surface of alcoholic beverages oxidize ethanol to acetic acid to produce vinegar or spoil the alcoholic beverage.

Plant juices, tend to have *E*h values of +300 to +400 mv. It is not surprising to find that aerobic bacteria and molds are the common cause of spoilage of products of this type. Minced meats have *E*h values of around +200 mv while in solid meats the *E*h is generally around -200 mv. Cheeses have *E*h values on the negative side from –20 to around –200 mv.

Obligate anaerobes grow only at low or negative redox potentials and require absence of oxygen. Anaerobic metabolism gives the organism a lower yield of utilizable energy than aerobic respiration. A reducing environment minimizes the loss of reducing power from the microbial cell and thus, is favoured. Hence, presence of oxygen, which provides an oxidizing environment to the microbes is not favoured. However, for many anaerobes, oxygen itself exerts a specific toxic effect. For example, *Clostridium acetobutylicum* can grow at an *E*h as high as +370 mv maintained by ferricyanide, but would not grow at +110mv in an aerated culture. This effect is due to the inability of obligate anaerobes to scavenge and destroy toxic products of molecular oxygen such as hydrogen peroxide and superoxide anion radical (0_2^-) produced by one electron reduction of molecular oxygen. They lack the enzymes catalase and superoxide dismutase, which catalyse the breakdown of these radicals.

Thus, in a highly oxidized food, there will be a predominance of aerobic organisms especially at food surfaces exposed to air. Whereas, in food with negative *E*h, the anaerobic microflora requiring reduced conditions will be favoured. For example, anaerobic bacteria do not multiply until the onset of rigor mortis (stiffening of body after death) of muscles of horse because of the high *E*h (+250 mv) in prerigor meat. At 30 h postmortem (after death), the *E*h falls to about -130 mv in the absence of bacterial growth and this low *E*h values favour the growth of obligate anaerobes like *Clostridium.* Obligate anaerobes, such as clostridia, have the potential to grow wherever conditions are anaerobic such as deep in meat tissues and stews, in vacuum packs and canned foods causing spoilage and C. *botulinum* is of major public health concern, since it causes botulism.

Aerotolerant anaerobes are incapable of aerobic respiration, but can nevertheless grow in the presence of air. Many lactic acid bacteria fall into this category. They can only generate energy by fermentation and lack both catalase and superoxide dismutase, but are able to grow in the presence of oxygen because they have a mechanism for destroying superoxide.

Microorganisms affect the *E*h of their environment during growth. This is true especially of aerobes, which can lower the *E*h of their environment while anaerobes cannot. As aerobes grow, oxygen in the medium is depleted, resulting in the lowering of *E*h. Growth is not slowed, however, due to the ability of cells to make use of oxygen donating or hydrogen-accepting substances in the medium. The result of this is that the medium becomes poorer in oxidizing and richer in reducing substances. Microorganisms can reduce the *E*h of a medium by their production of certain metabolic by-products such as hydrogen sulphide, which has the capacity to lower *E*h to –300 mv. Since hydrogen sulphide reacts readily with oxygen, it will accumulate only in anaerobic environments.

2.4.4 Poising Capacity of Food

As redox conditions change, there will be some resistance to change in a food's redox potential. This is known as poising capacity of food. This capacity is dependent on the concentration of the redox couple. Poising is greatest when the two components of a redox couple are present in equal amounts.

Most fresh plant or animal foods have a low and well-poised O-R potential in their interior: the plants because of reducing substances such as ascorbic acid and reducing sugars and the animal tissues because of SH (sulfhydryl) and other reducing groups. As long as the plant or animal cells respire and remain active, they tend to poise the O-R system at a low level, resisting the effect of oxygen diffusing from the outside. Therefore, a piece of fresh meat or a fresh whole fruit would have aerobic conditions only at and near the surface. The meat could support aerobic growth of slime-forming or souring bacteria at the surface at the same time as anaerobic putrefaction could be proceeding in the interior.

Processing procedures may alter this situation. For example, heating may reduce the poising power of the food by destroying or altering the reducing and oxidizing substances present and also allow more rapid diffusion of oxygen inward, either because of the destruction of poising substances or because of changes in the physical structure of the food. Processing also may remove oxidizing or reducing substances. For example, clear fruit juices loose reducing substances by their removal during extraction and filtration and therefore become more favourable to the growth of yeasts than the original juice containing the pulp.

In the presence of limited amounts of oxygen the same aerobic or facultative organisms may produce incompletely oxidized products, such as organic acids, from carbohydrates, while with plenty of oxygen available, complete oxidation to carbon dioxide and water might result. Protein decomposition under anaerobic conditions may result in putrefaction, whereas under aerobic conditions, the products are likely to be less obnoxious. Thus, the redox potential of the food would decide the course of spoilage and the type of end products being produced due to microbial activities.

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Define redox potential.

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2. Enlist the physiological groups of microorganisms based on their oxygen requirement.

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3. Define the poising capacity of food.

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4. How is the poising capacity of food destroyed?

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5. Enlist the factors which affect the redox potential of food.

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2.5 NUTRIENT CONTENT

Microorganisms use foods as a source of nutrients and energy. Each kind of microorganism has a definite range of food requirements. For some species that range is wide and growth takes place in a variety of substrates e.g. coliform bacteria; but others, e.g., many of the pathogens, being fastidious in their nutrient requirements can grow in limited kinds of substrates. The better the medium for an organism, the wider the ranges of temperature, pH, and a_w over which growth can take place.

The food based on their nutrient composition can be classified as (1) foods for energy, (2) foods for growth, and (3) accessory food substances, or vitamins, which may be necessary for energy or growth.

2.5.1 Foods for Energy

The carbohydrates, especially the sugars, are most commonly used as an energy source, but other carbon compounds may also serve the purpose, e.g., esters, alcohols, peptides, amino acids, organic acids and their salts. Comparatively few organisms can utilize complex carbohydrates, e.g., cellulose and starch. Microorganisms differ even in their ability to use some of the simpler soluble sugars. Many organisms cannot use the disaccharide lactose (milk sugar) and therefore do not grow well in milk. Some yeast do not attack maltose. Most organisms, if they utilize sugars at all, can use glucose. The ability of microorganisms to hydrolyze pectin, which is characteristic of some kinds of bacteria and many molds, is important in the softening or rotting of fruits and vegetables or fermented products got from them. The ability to synthesize amylolytic (starch degrading) enzymes will favour the growth of an organism on cereals and other starchy products. The addition of fruits containing sucrose and other sugars to yoghurt increases the range of carbohydrates available and allows the development of a more diverse spoilage microflora of yeasts.

Bacteria differ in their ability to utilize different foods as a source of energy. Some can use a variety of carbohydrates, e.g., the coliform bacteria and *Clostridium* spp., and others only one or two. Some can use other carbon compounds like organic acids and their salts, alcohols, and esters *(Pseudomonas* spp.). Some can hydrolyze complex carbohydrates, although others cannot.

A limited number of microorganisms can obtain their energy from fats but do so only if a more readily usable energy food, such as sugar, is absent. First, the fat must be hydrolyzed with the aid of lipase to glycerol and fatty acids, which then can serve as energy source for the hydrolyzing organism or others microbes that grow on products of lipid hydrolysis. Aerobic microorganisms are more commonly involved in the decomposition of fats than anaerobic ones, and the lipolytic organisms usually are also proteolytic.

Split products of proteins, for example, peptides and amino acids, serve as an energy source for many proteolytic organisms when a better energy source is lacking. These also serve as source of energy for some non-proteolytic organisms. Meats for example, may be low in carbohydrate and therefore will be decomposed by proteolytic species, e.g., *Pseudomonas* spp.

Molds in general can utilize many kinds of foods as energy source, ranging from simple to complex. Most of the common molds possess a variety of hydrolytic enzymes and some are grown for their amylases, pectinases, proteinases, and lipases.

Not only is the kind of energy food important but also its concentration in solution and hence it's osmotic effect and the amount of available moisture, which will determine its growth rate. For a given percentage of sugar in solution, the osmotic pressure will vary with the weight of the sugar molecule, Therefore, a 10% solution of glucose has about twice the osmotic pressure of a 10% solution of sucrose or maltose; i.e., it ties up twice as much moisture. Molds can grow in the highest concentrations of sugars and yeasts in fairly high concentrations but most bacteria grow best in fairly low concentrations. There are, of course, some exceptions to this generalization: osmophilic yeasts grow in as high concentrations of sugar as molds and some bacteria can grow in fairly high concentrations of sugar.

An adequate supply of foods for growth will favour utilization of the foods for energy. More carbohydrate will be used if a good nitrogen food is present in sufficient quantity than if the nitrogen is in poor supply. Organisms requiring special accessory growth substances might be prevented from growing if one or more of these vitamins were lacking, and thus the whole course of decomposition might be altered due to a change in the microflora.

2.5.2 Foods for Growth

Microorganisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids. A large number of other nitrogenous compounds may serve this function for example, nucleotides, free amino acids, peptides and proteins. Simple compounds such as amino acids will be utilized by most of the organisms before they utilize complex compounds such as high molecular weight proteins. The nitrogen requirements of some bacteria such as *Pseudomonas* spp. may be satisfied by simple compounds like ammonia or nitrates whereas for others like lactics, more complex compounds like amino acids, peptides, or proteins may be utilized or even required.

Many molds are proteolytic, but comparatively few bacteria and very few yeast are actively proteolytic. Proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity. Only exceptions are the acid-proteolytic bacteria that hydrolyze protein while producing acid. Carbon for growth for most of the microorganisms is derived from organic compounds but some can use carbon dioxide also.

The minerals required by microorganisms are nearly always present at the low levels required.

2.5.3 Accessory Food Substances or Vitamins

Bacteria also vary in their need for vitamins or accessory growth factors. Some microorganisms are unable to synthesize some or all of the vitamins needed for their growth. For example, *Staphylococcus aureus* synthesizes part while *Pseudomonas* or *Escherichia coli* all of the factors needed. The lactics and many pathogens must have all of the vitamins furnished. Most natural plant and animal foodstuffs contain an array of these vitamins, but some may be low in amount or lacking. For example, meats are high in B vitamins and fruits are low, but fruits are high in ascorbic acid.

Microorganisms may require B vitamins in low quantities and most of the natural foods have an abundant quantity of these. Gram positive bacteria are the least synthetic and must, therefore, be supplied with one or more of these compounds before they will grow. Gram negative bacteria and molds are able to synthesize most of their requirements. Consequently, these two groups of organisms may be found growing on foods low in B vitamins. Fruits tend to be lower in B vitamins than meats. Thus, the usual spoilage of fruits is by molds rather than bacteria since fruits also have a low pH and positive *E*h, which favour mold growth.

Egg white contains biotin but also contains avidin, which ties it up, making it unavailable to microorganisms and thus eliminate spoilage of eggs through biotin requiring organisms. The processing of foods often reduces the vitamin content. For example, thiamine, pantothenic acid, folic acid and ascorbic acid (in air) are heat-labile. Drying causes a loss in vitamins such as thiamine and ascorbic acid. Even storage of foods for long periods, especially if the storage temperature is elevated, may result in a decrease in the level of some of these growth factors.

Check Your Progress Exercise 4

Note: a) Use the space below for your answer.

b) Compare your answers with those given at the end of the unit.

1. What is the effect of sugar concentration on microbial growth?

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2.6 BIOLOGICAL STRUCTURE

The plants and animals that serve as food sources have all evolved mechanisms of defense against the invasion and proliferation of microorganisms. By taking these natural phenomena into account, one can make effective use of these in preventing the microbial spoilage of the products.

2.6.1 Antimicrobial Barriers

The inner parts of whole, healthy tissues of living plants and animals are either sterile or low in microbial content. Therefore, unless opportunity has been given for their penetration, spoilage organisms within raw food may be few or lacking.

The first barrier is the integument: a physical barrier to protect the food, e.g., the shell on eggs, the skin on poultry, the shell on nuts and the rind or skin on fruits and vegetables, or these may be surrounded by natural wax. It is usually composed of macromolecules relatively resistant to degradation and provides an inhospitable environment for microorganisms either with a low water activity or nutrients deficiency or antimicrobial compounds e.g. short chain fatty acids on animal skin, essential oils on plant surfaces etc. This physical protection to the food may not only help in its preservation but may also determine the kind, rate and course of spoilage. Layers of fat over meat may protect that part of the flesh, or scales may protect the outer part of the fish.

2.6.2 Effect of Destruction of Microbial Barriers

Physical damage to the integument allows microbial invasion of the underlying nutrient-rich tissues and it is a common observation that damaged fruits and vegetables deteriorate more rapidly than entire products and that this process is initiated at the site of injury. Consequently, it is important that during harvesting and transport these barriers are maintained intact as far as possible.

An increase in exposed surface, brought about by peeling, skinning and chopping may serve not only to distribute spoilage organisms but also to release juices containing food materials for the microorganisms. The disintegration of tissues by freezing may accomplish a similar result. In meat the growth of spoilage bacteria takes place mostly in the fluid between the small meat fibers and it is only after rigor mortis that much of this food material is released from the fibers to become available to spoilage organisms.

Check Your Progress Exercise 5

Note: a) Use the space below for your answer.

b) Compare your answers with those given at the end of the unit.

1. What is the role of antimicrobial barriers in preventing food spoilage by microorganisms?

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2. What happens when integument is physically damaged?

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2.7 INHIBITORY SUBSTANCES

These may be originally present in the food, added purposely or accidentally, or developed there by growth of microorganisms or by processing methods. These may prevent growth of all microorganisms or, more often, may deter certain microorganisms. The mechanism of action for nearly all antimicrobials can be classified into one or more of the following groups: (1) reaction with the cell membrane, (2) inactivation of essential enzymes, or (3) destruction or functional inactivation of genetic material.

2.7.1 Biological Inhibitory Substances Originally Present in Food

The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances e.g. plants such as mustard, horseradish, watercress, cabbage and other brassicas produce antimicrobial isothiocyanates (mustard oils) (Fig. 2.2) and in *Allium* species (garlic, onions and leeks) thiosulfinates such as allicin. Antimicrobials collectively known as phytoalexins are produced by many plants in response to microbial invasion, for example phaseollin an antifungal compound is produced in green beans.

Many natural constituents of plant tissues such as pigments, alkaloids and resins also have antimicrobial properties. Benzoic and sorbic acids found in cranberries and mountain ash berries respectively are commonly used in their pure forms as food preservatives. The anthocyanins are a group of water-soluble pigments, which occur naturally in fruits. The aglycone portion of these compounds, the anthocyanidins, has antimicrobial powers against several bacterial spp.

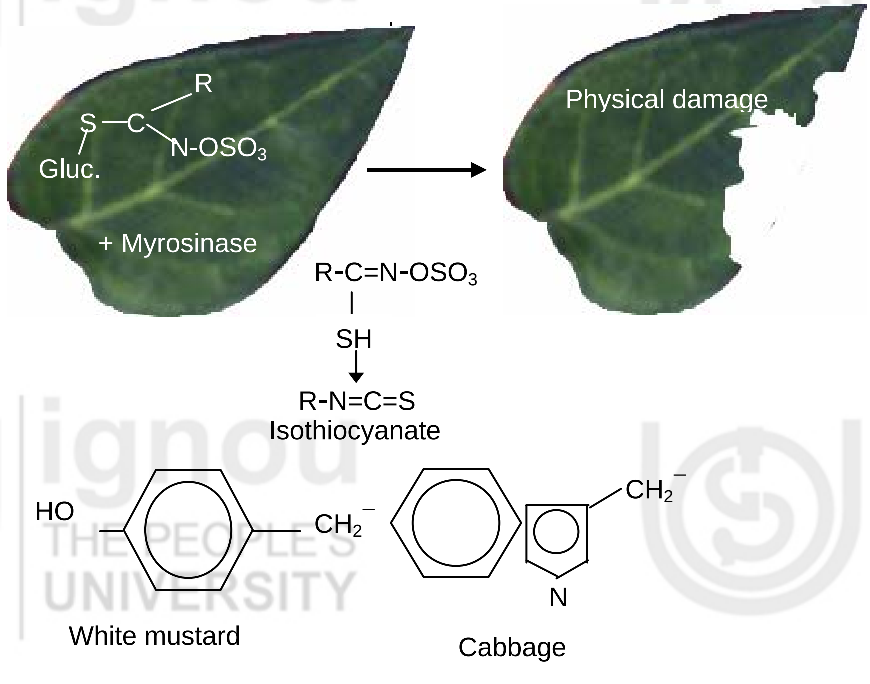

Figure 2.2: Production of plant antimicrobials as a result of physical damage

Some spices are known to contain essential oils that possess antimicrobial activity e.g. eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allspice (pimento) and cloves, allyl isothiocyanate in mustard, eugenol and thymol in sage and carvacrol (isothymol) and thymol in oregano etc. As a consequence, herbs and spices may contribute to the microbiological stability of foods in which they are used. It has, for example, been claimed that inclusion of cinnamon in raisin bread retards mould spoilage. However, in some cases, they can be a source of microbial contamination leading to spoilage or public health problems. Outbreaks of botulism associated with crushed garlic in oil and home canned peppers demonstrate that even in relatively high concentrations plant antimicrobials are not a complete guarantee of safety.

These antimicrobial components differ in their spectrum of activity and potency. They are present at varying concentrations in the natural product and are frequently at levels too low to have any effect. Humulones contained in the hop resin impart the characteristic bitterness of the product but have also been shown to possess activity against the common beer spoilage organisms, lactic acid bacteria.

Antimicrobial oleuropein from green olives and its aglycone are also inhibitory to lactic acid bacteria and if not removed at this early stage, they would prevent the necessary fermentation occurring subsequently. The hydroxycinnamic acid derivatives (coumaric, ferulic, caffeic and chlorogenic acids) found in fruits, vegetables, tea, molasses and other plant sources all show antibacterial and some antifungal activity.

Animal products too, have a range of non-specific antimicrobial constituents, for example egg white or albumen possesses a variety of inhibitory components (Table 2.4). Similar factors can also be found in milk, however, in lower concentrations e.g. enzyme lysozyme which catalyses the hydrolysis of glycosidic linkages in peptidoglycan. Destruction or weakening of this layer causes the cell to rupture (lyse) under osmotic pressure. Lysozyme is most active against gram- positive bacteria, where the peptidoglycan is more readily accessible, but it can also kill gram- negatives if their protective outer membrane is damaged in some way.

Table 2.4: Antimicrobial substances in egg and milk

Egg	Milk
Ovotransferrin (conalbumin)	Lactoferrin
Lysozyme	Lysozyme
Avidin	-
Ovoflavoprotein	-
Ovomucoid and ovoinhibitor	-
-	Lactoperoxidase
-	Immunoglobulin

Other components limit microbial growth by restricting the availability of key nutrients. Ovotransferrin and conalbumin in egg white and lactoferrin in milk are proteins that scavenge iron from the medium. Iron is an essential nutrient for all bacteria. Infact, lysozyme with conalbumin provides fresh eggs with a fairly efficient antimicrobial system. In addition, egg white has powerful cofactor-binding proteins such as avidin and ovoflavoprotein, which remove biotin and riboflavin restricting the growth of those bacteria for which they are essential nutrients.

Cows' milk contains several other antimicrobial substances including conglutinin, lactenins, anticoliform factor and the lactoperoxidase system.

Casein as well as some free fatty acids that occur in milk have also been shown to be antimicrobial.

2.7.2 Inhibitory Substances Developed/ Destroyed in Food due to the Activity of Microorganisms

Microorganism growing in food may produce one or more substances inhibitory to other organisms, products such as acids, alcohols, peroxides, or even antibiotics. Propionic acid produced by the propionibacteria in Swiss cheese is inhibitory to molds; alcohol formed in quantity by wine yeasts inhibits competitors; and nisin a polypeptide produced by *Streptococcus lactis* may be useful in inhibiting lactate fermenting, gas-forming clostridia during curing of cheese. These may however, be undesirable during the manufacturing process since these would slow down some of the essential lactic acid streptococci. *Streptococcus cremoris* produces an inhibitor named diplococcin. The most pathogenic member of genus - *S. pyogenes* forms an inhibitor, streptococcin A-FF22. Streptococcin A-PF22 had many properties in common with nisin.

Gram-negative organisms and molds are insensitive to nisin. However, its effectiveness against sensitive gram-positive organisms depends on the bacterial load. As the number of organisms increases, the inhibitory effectiveness of nisin decreases. Nisin can be used along with heat processing since heat treated spores become more nisin sensitive. Thus, sterility might be attained with less heat treatment than presently used thereby decreasing the fuel consumption.

In addition to inhibitory polypeptides and bacteriocins, lactic streptococci produce acids and peroxides. These add up to a formidable array of substances designed to hinder and suppress other microbes. Hence, lactic acid bacteria are excellent competitors in foods. *S. diacetylactis* produces inhibitor inhibiting a broad-spectrum of gram-positive and gram-negative organisms. The undissociated molecule is the toxic component.

There is also the possibility of the destruction of inhibitory compounds in foods by microorganisms. Certain molds and bacteria are able to destroy some of the phenolic compounds that are added to meat or fish by smoking or benzoic acid added to foods; yeasts resistant to it destroy sulfur dioxide; and lactobacilli can inactivate nisin.

2.7.3 Inhibitory Substances Developed During Processing of Food

Heating foods may result in the formation of inhibitory substances e.g. heating lipids may hasten autoxidation and make them inhibitory and browning concentrated sugar syrups may result in production of furfural and hydroxymethylfurfural, which are inhibitory to fermenting organisms. Milk also has the capacity to generate antimicrobials in the presence of hydrogen peroxide. The milk enzyme lactoperoxidase will catalyse the oxidation of thiocyanate by hydrogen peroxide to produce *inter alia* hypothiocyanate. This can kill gram-negative bacteria and inhibit gram-positives, possibly by damaging the bacterial cytoplasmic membrane.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Enlist the various mechanisms of action of inhibitory substances.

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2. Elaborate on the inhibitory substances naturally present in plants with suitable examples.

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3. Enlist all the antimicrobial constituents present in egg and milk.

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4. What is the mode of action of lysozyme?

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5. Give examples of inhibitory substances developed in food due to the activity of microorganisms.

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2.8 LET US SUM UP

Food is the substrate for growth of microorganisms. Since our foods are of plant and animal origin, all the characters of plant and animal tissues that affect the growth of microorganisms are important. In other words, food will dictate what grows or does not grow on it. Thus, knowledge about the factors that favour or inhibit growth of microorganisms is a must to make predictions about the microflora that may develop. The main factors, which influence microbial activity are pH, water activity, redox potential, nutrient composition, biological structure and presence of inhibitory substances in foods.

Each microorganism has a minimal, maximal and optimal range for pH, water activity and redox potential at which they can grow. These in turn would determine the microflora of food. Buffers in food and poising capacity of food also play an important role in deciding the succession of microorganisms and the extent of spoilage occurring in food.

Different microorganisms have different nutritional requirements. Some have a wide range while others are very fastidious in their nutritional requirements. The inability of an organism to utilize a component of food limits its growth and others with not so stringent requirements gain a competitive edge over it and predominate, thereby typifying the natural microflora of that food.

Plants and animals have also evolved mechanism of defense against invasion and proliferation of microorganisms. Antimicrobial barriers allow food to remain relatively free from microorganisms and the other inhibitory substances present in food/ produced during invasion by microorganisms tend to maintain low microbial counts in food.

These six parameters represent nature’s way of preserving plant and animal tissue from microorganisms. All these parameters have an important role to play in determining microbial ecology of food. These in turn decide the type of microbial activities likely to occur and the type of spoilage occurring. All these factors are interlinked and the changes in one factor may affect microbial requirements. By taking these natural phenomena into account, one can make effective use of each or all in preventing or retarding microbial spoilage of the products that are derived from them.

2.9 KEY WORDS

Aerobic : microorganisms are those, which require free oxygen for growth.

Anaerobic	:	microorganisms are those, which grow best in the absence of free oxygen.
Antibiotics	:	are substances produced by microorganisms which inhibit the growth of other microorganisms.
Antimicrobials	:	are substances which inhibit the growth of microorganisms.
Bacteriocins	:	are substances produced by a strain of bacterial spp. which inhibit growth of other strains of that bacterial spp.
Buffers	:	are the compounds that resist changes in pH.
Facultative	:	microorganisms are those, which grow well either aerobically or anaerobically.
Halotolerant	:	are those microorganisms, which are able to grow in the presence of high concentrations of salt.
Osmotolerant	:	are those microorganisms, which are able to grow in the presence of high concentrations of unionized organic compounds such as sugars.
Oxidising agent	:	is a substance that readily takes up electrons.
Phytoalexins	:	are a class of antimicrobials which are produced by many plants in response to microbial invasion.
Poising capacity	:	is the ability of food to resist change in a food's redox potential.
Proteolytic microorganisms	:	are those, which are able to hydrolyze proteins.
Redox potential	:	of a substrate may be defined generally as the ease with which the substrate loses or gains electrons.
Reducing agent	:	is a substance that readily gives up electrons.
Standard redox potential, *E*o'	:	is the tendency of an atom or molecule to accept or donate electrons.
Water requirement or water activity a_w	:	is the vapour pressure of the solution (of solutes in water in most foods) divided by the vapour pressure of the solvent (usually water).
Xerotolerant	:	are those microorganisms which are able to grow on dry foods.

2.10 ANSWERS TO CHECK YOUR PROGRESS EXCERCISES

Check Your Progress Exercise 1

1. Fish is spoilt more rapidly than meat under chilled conditions. The pH of post-rigor mammalian muscle, around 5.6, is lower than that of fish (6.2-6.5) and this contributes to the longer storage life of meat. Those fishes that have a naturally low pH such as halibut (pH~5.6) have better keeping qualities than other fishes.

2. Your answer should include the following points:

   - pH attained upon rigor mortis of well rested animals
   - not much change in pH change in case of fatigued animal

3. Biological acidity is the acidity developed in food due to the activity of microorganisms growing in it. This generally occurs due to accumulation of lactic acid during fermentation.

4. Your answer should include the following points:

   - partial dissociation of weak acids
   - free passage of undissociated lipophilic acids into the cytoplasm
   - dissociation of lipophilic acids in the microbial cell
   - maintenance of internal pH by expulsion of protons

5. When microorganisms are grown on either side of their optimum pH range, an increased lag phase results. Adverse pH affects at least two aspects of a respiring microbial cell: the functioning of its enzymes and the transport of nutrients into the cell.

Check Your Progress Exercise 2

1. Your answer should include the Halotolerant, Xerotolerant and Osmotolerant microorganisms.

2. Water activity a_w, is the vapour pressure of the solution (of solutes in water in most foods) divided by the vapour pressure of the solvent (usually water).

3. Your answer should include the following points:

   - Solutes and ions tie up water in solution
   - Hydrophilic colloids (gels) make water unavailable
   - Water of crystallization or hydration is usually unavailable to microorganisms

4. Your answer should include the following points:

   - Kind of solute employed to reduce the a_w
   - Nutritive value of the culture medium
   - Temperature
   - Oxygen supply
   - pH
   - Inhibitors

Check Your Progress Exercise 3

1. The tendency of a substrate to accept or donate electrons, to oxidize or reduce, is termed its redox potential (*E*h).

2. Your answer should include aerobic, anaerobic and facultative microorganisms.

3. Poising capacity is the resistance to change in a food's redox potential with change in the redox conditions.

4. Your answer should include the following points:

   - Heating destroys/ alters reducing and oxidizing substances
   - Processing removes reducing and oxidizing substances

5. Factors affecting redox potential of foods are:

   - Redox couples present
   - Ratio of oxidant to reductant
   - pH
   - Poising capacity
   - Availability of oxygen
   - Microbial activity

Check Your Progress Exercise 4

1. Your answer should include the following points:

   - Concentration of sugars in food and their osmotic effect
   - Molds grow at high concentration of sugars
   - Osmophilic bacteria and yeasts grow at high concentration of sugars

Check Your Progress Exercise 5

1. Your answer should include the following points:

   - Type of physical barriers about food
   - These barriers provide inhospitable environment for the microbe
   - They determine type and rate of spoilage

2. Your answer should include the following points:

   - Physical damage allows microbial invasion into tissues
   - It distributes spoilage microbes
   - It releases juices from plant and animal tissues for microbial growth

Check Your Progress Exercise 6

1. The mechanism of action for nearly all antimicrobials can be classified into one or more of the following groups:

   a) Reaction with the cell membrane,

   b) Inactivation of essential enzymes, or

c) Destruction or functional inactivation of genetic material.

2. Your answer should include the following points:
   - Isothiocyanates – mustard oil
   - Thiosulfinates – in garlic, onions and leeks e.g. allium

3. Your answer should include the following points:
   - Antimicrobial substances present in egg
   - Antimicrobial substances present in milk

4. Your answer should include the following points:
   - Lysozyme catalyses the hydrolysis of glycosidic linkages in peptidoglycan
   - Destruction leads to cell lysis
   - Very active against gram positive bacteria

5. Your answer should include the following points:
   - Various types of inhibitory substances produced by microbes
   - Inhibitory substances produced by propionibacteria
   - Inhibitory substances produced by lactic streptococci

2.11 SOME USEFUL BOOKS

1. Adams, M.R. and Moss, M.O. (1996) Food Microbiology. New Age International (P) Ltd., Publishers, New Delhi.
2. ICMSF (1980) 'Microbial Ecology of Foods. Volume I. Factors affecting life and death of microorganisms', academic Press, New York, 332pp.
3. McMeekin, J.N., Olley, T. Ross and Ratkowsky, D.A. (1993) 'Predictive Microbiology: Theory and Application', Research Studies Press Ltd., Tauton, England, 340pp.
4. Stanier, R.Y., Adelberg, E.A. and Ingraham, J. (1976) The Microbial World. Prentice-HaIl, Inc., Englewood Cliffs, N.J.