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S.Y. B.Sc. (Sem. IV) (CBCS)

MICROBIOLOGY

[401]: APPLIED AND ENVIRONMENTAL MICROBIOLOGY

Unit 2
FOOD MICROBIOLOGY

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MICROBIAL FLORA OF FRESH FOOD

- The ability of microorganisms (except viruses) to grow or multiply in a food is determined by the food environment as well as the environment in which the food is stored, designated as the intrinsic and extrinsic environment of food, respectively.

- It is not possible to study the influence of any one factor on growth independently as the factors are interrelated.

- Instead, the influence of any one factor at different levels on growth is compared keeping other.

INTRINSIC FACTORS OR FOOD ENVIRONMENT

Intrinsic factors of a food include nutrients, growth factors, and inhibitors (or antimicrobial), water activity, pH, and oxidation–reduction potential. The influence of each factor on growth is discussed separately. But, as indicated previously, in a food system the factors are present together and exert effects on microbial growth in combination, either favorably or adversely.

Nutrients and Growth:

- Microbial growth is accomplished through the synthesis of cellular components and energy. The necessary nutrients for this process are derived from the immediate environment of a microbial cell and, if the cell is growing in a food, it supplies the nutrients.

- These nutrients include carbohydrates, proteins, lipids, minerals, and vita - mins. Water is not considered a nutrient, but it is essential as a medium for the biochemical reactions necessary for the synthesis of cell mass and energy.

- All foods contain these five major nutrient groups, either naturally or added, and the amount of each nutrient varies greatly with the type of food.

- In general, meat is rich in protein, lipids, minerals, and vitamins but poor in carbohydrates. Foods from plant sources are rich in carbohydrates but can be poor sources of proteins, minerals, and some vitamins.

- Some foods such as milk and many prepared foods have all five nutrient groups in sufficient amounts for microbial growth.

- Microorganisms normally present in food vary greatly in nutrient requirements, with bacteria requiring the most, followed by yeasts and molds.

- Microorganisms also differ greatly in their ability to utilize large and complex carbohydrates (e.g., starch and cellulose), large proteins (e.g., casein in milk), and lipids.

- Microorganisms capable of using these molecules do so by producing specific extracellular enzymes (or exoenzymes) and hydrolyzing the complex molecules to simpler forms outside before transporting them inside the cell.

- Molds are the most capable of doing this. However, this provides an opportunity for a species to grow in a mixed population even when it is incapable of metabolizing the complex molecules.

- Microbial cells, following death and lysis, release intracellular enzymes that can also catalyze break- down of complex food nutrients to simpler forms, which can then be utilized by other microorganisms.

Carbohydrates in Foods:

- Major carbohydrates present in different foods, either naturally or added as ingredients, can be grouped on the basis of chemical nature as follows:

Monosaccharides

- Hexoses: glucose, fructose, mannose, galactose
- Pentoses: xylose, arabinose, ribose, ribulose, xylulose

Disaccharides

- Lactose (galactose + glucose)
- Sucrose (fructose + glucose)
- Maltose (glucose + glucose)

Oligosaccharides

- Raffinose (glucose + fructose + galactose)
- Stachyose (glucose + fructose + galactose + galactose)
- Polysaccharides: Starch (glucose units)
- Glycogen (glucose units)
- Cellulose (glucose units)
- Inulin (fructose units)
- Hemicellulose (xylose, galactose, mannose units)
- Dextran (a-1, 6 glucose polymer)
- Pectin.

Gums and mucilage:

Lactose is found only in milk and thus can be present in foods made from or with milk and milk products. Glycogen is present in animal tissues, especially in liver. Pentoses, most oligosaccharides, and polysaccharides are naturally present in foods of plant origin.

- All microorganisms normally found in food metabolize glucose, but their ability to utilize other carbohydrates differs considerably.
- This is because of the inability of some microorganisms to transport the specific monosaccharides and disaccharides inside the cells and the inability to hydrolyze polysaccharides outside the cells. Molds are the most capable of using polysaccharides.
- Food carbohydrates are metabolized by microorganisms principally to supply energy through several metabolic pathways.
- Some of the metabolic products can be used to synthesize cellular components of microorganisms (e.g., to produce amino acids by amination of some keto acids).
- Microorganisms also produce metabolic by- products associated with food spoilage (CO_2 to cause gas defect) or food bioprocessing (lactic acid in fermented foods).
- Some are also metabolized to produce organic acids, such as lactic, acetic, propionic, and butyric acids, which have an antagonistic effect on the growth and survival of many bacteria.
- Microorganisms can also polymerize some monosaccharides to produce complex carbohydrates such as dextran, capsular materials, and cell wall (or outer membrane and middle membrane in Gram-negative bacteria).
- Some of these carbohydrates from pathogens may cause health hazards (forming complexes with proteins), some may cause food spoilage (such as slime defect), and some can be used in food production (such as dextran as stabilizers). Carbohydrate metabolism profiles are extensively used in the laboratory for the biochemical identification of unknown microorganisms isolated from foods.

Sr.no.	**QUESTIONS**	**ANSWER**
1	Which are the intrinsic factors affecting food?	nutrients, growth factors, and inhibitors , water activity, pH, and oxidation–reduction potential
2	Microbial growth is accomplished through which process?	the synthesis of cellular components and energy
3	Example of monosaccharide.	Glucose

4	Example of oligosaccharide.	Pectin
5	_____is present in animal liver.	Glycogen

Proteins in Foods

- The major proteinaceous components in foods are simple proteins, conjugated proteins, peptides, and non-protein nitrogenous (NPN) compounds (amino acids, urea, ammonia, creatinine, tri-methylamine).
- Proteins and peptides are polymers of different amino acids without or with other organic (e.g., a carbohydrate) or inorganic (e.g., iron) components and contain ca. 15 to 18% nitrogen.
- Simple food proteins are polymers of amino acids, such as albumins (in egg), globulins (in milk), glutelin (gluten in cereal), prolamins (zein in grains), and albuminoids (collagen in muscle).
- They differ greatly in their solubility, which determines the ability of microorganisms to utilize a specific protein.
- Many microorganisms can hydrolyze albumin, which is soluble in water.
- In contrast, collagens, which are insoluble in water or weak salt and acid solutions, are hydrolyzed only by a few microorganisms.
- As compared with simple proteins, conjugated proteins of food on hydrolysis produce metals (metalloproteins such as hemoglobin and myoglobin), carbohydrates (glycoproteins such as mucin), phosphate (phosphoproteins such as casein), and lipids (lipoproteins such as some in liver).
- Proteins are present in higher quantities in foods of animal origin than in foods of plant origin.
- But plant foods, such as nuts and legumes, are rich in proteins.
- Proteins as ingredients can also be added to foods.
- Microorganisms differ greatly in their ability to metabolize food proteins.
- Most transport amino acids and small peptides in the cells; small peptides are then hydrolyzed to amino acids inside the cells, such as in some *Lactococcus* spp.
- Microorganisms also produce extracellular proteinases and peptidases to

hydrolyze large proteins and peptides to small peptides and amino acids before they can be transported inside the cells.

- Soluble proteins are more susceptible to this hydrolytic action than are the insoluble proteins.
- Hydrolysis of food proteins can be either undesirable (texture loss in meat) or desirable (flavor in cheese).
- Microorganisms can also metabolize different NPN compounds found in foods.
- Amino acids inside microbial cells are metabolized via different pathways to synthesize cellular components, energy, and various by-products.
- Many of these by- products can be undesirable (e.g., NH3 and H2S production causes spoilage of food, and toxins and biological amines cause health hazards) or desirable (e.g., some Sulphur compounds give cheddar cheese flavor). Production of specific metabolic products is used for the laboratory identification of microbial isolates from food.
- An example of this is the ability of *Escherichia coli* to produce indole from tryptophan, which is used to differentiate this species from non-indole-producing related species (e.g., *Enterobacter* spp.).

Lipids in Foods

- Lipids in foods include compounds that can be extracted by organic solvents, some of which are free fatty acids, glycerides, phospholipids, waxes, and sterols.
- Lipids are relatively higher in foods of animal origin than in foods of plant origin, although nuts, oil seeds, coconuts, and olives have high amounts of lipids.
- Fabricated or prepared foods can also vary greatly in lipid content. Cholesterols are present in foods of animal origin or foods containing ingredients from animal sources.
- Lipids are, in general, less preferred substrates for the microbial synthesis of energy and cellular materials.
- Many microorganisms can produce extracellular lipases, which can hydrolyze glycerides to fatty acids and glycerol. Fatty acids can be transported in cells and used for energy synthesis, whereas glycerol can be metabolized separately.

- Some microorganisms also produce extracellular lipid oxidases, which can oxidize unsaturated fatty acids to produce different aldehydes and ketones.

- In general, molds are more capable of producing these enzymes. However, certain bacterial groups such as *Pseudomonas*, *Achromobacter*, and *Alcaligenes* can produce these enzymes.

- Lysis of dead microbial cells in foods causes release of intracellular lipases and oxidases, which then can carry out these reactions.

- In many foods the action of these enzymes is associated with spoilage (such as rancidity), whereas in other foods the enzymes are credited for desirable flavors (such as in mold-ripened cheeses).

- Some beneficial intestinal micro- organisms, such as *Lactobacillus acidophilus* strains, can metabolize cholesterol and are believed to be capable of reducing serum cholesterol levels in humans.

Sr. no.	QUESTION	ANSWER
1	Which are non protein nitrogenous compounds?	amino acids, urea, ammonia, creatinine, tri-methylamine
2	Full form of NPN	Non protein nitrogenous compounds
3	Fat hydrolysis is known as ___,	Rancidity
4	Hydrolysis of protein is known as ___.	Putrifraction
5	Indol produces microorganism.	E.coli

Minerals and Vitamins in Foods

- Microorganisms need several elements in small amounts, such as phosphorous, calcium, magnesium, iron, sulfur, manganese, and potassium.

- Most foods have these elements in sufficient amounts. Many microorganisms can synthesize B vitamins, and foods also contain most B vitamins.

- In general, most foods contain different carbohydrates, proteins, lipids, minerals, and vitamins in sufficient amounts to supply necessary nutrients for the growth of molds, yeasts, and bacteria, especially Gram-negative bacteria normally present in foods.

- Some foods may have limited amounts of one or a few nutrients for rapid growth of some Gram -positive bacteria, especially some fastidious *Lactobacillus* species.

- When their growth is desired, some carbohydrates, essential amino acids, and B vitamins may be added to a food. It is not possible or practical to control microbial growth in a food by restricting nutrients.

Growth Factors and Inhibitors in Food

- Foods can also have some factors that either stimulate growth or adversely affect growth of microorganisms. The exact nature of growth factors is not known, but they are naturally present in some foods.

- An example is the growth factors in tomatoes that stimulate growth of some *Lactobacillus* species. These factors can be added to raw materials during food bioprocessing or to media to isolate some fastidious bacteria from foods.

- Foods also contain many chemicals, either naturally or added, that adversely affect microbial growth. Some of the natural inhibitors are lysozyme in egg, agglutinin in milk, and eugenol in cloves. The inhibitors, depending on their mode of action, can pr event or reduce growth of and kill microorganisms.

Sr. No.	QUESTION	ANSWER
1	Microorganisms need small amount of nutrients is known as ______.	Micronutrient
2	Which are microniutrients?	phosphorous, calcium, magnesium, iron, sulfur, manganese, and potassium
3	Many microorganisms produce which vitamin?	Vitamin B
4	Growth factor present in tomato stimulate growth of which microorganisms?	Lactobacillus species
5	Natural inhibitor of egg is______.	Lysozyme

Water Activity and Growth

Principle:

- Water activity (*Aw*) is a measure of the availability of water for biological functions and relates to water present in a food in free form. In a food system, total water or moisture is present in free and bound forms.

- Bound water is the fraction used to hydrate hydrophilic molecules and to dissolve solutes, and is not available for biological functions; thus, it does not contribute to *Aw*. The *Aw* of a food can be expressed by the ratio of water vapor pressure of the food (*P*, which is <1) to that of pure water (*Po*, which is 1), i.e., *Po*/*P*.

- It ranges between 0 and 1, or more accurately >0 to <1, because no food can have a water activity of either 0 or 1. The *Aw of* a food can be determined from its equilibrium relative humidity (ERH) by dividing ERH by 100 (because ERH is expressed in percentage).

Aw of Food:

- The *Aw* of food ranges from ca. 0.1 to 0.99. The *Aw* values of some food groups are as follows: cereals, crackers, sugar, salt, dry milk, 0.10 to 0.20; noodles, honey, chocolate, dried egg, <0.60; jam, jelly, dried fruits, parmesan cheese, nuts, 0.60 to 0.85; fermented sausage, dry cured meat, sweetened condensed milk, maple syrup, 0.85 to 0.93; evaporated milk, tomato paste, bread, fruit juices, salted fish, sausage, processed cheese,0.93 to 0.98; and fresh meat, fish, fruits, vegetables, milk, eggs, 0.98 to 0.99.

- The *Aw* of foods can be reduced by removing water (desorption) and increased by the adsorption of water, and these two parameters can be used to draw a sorption isotherm graph for a food.

- The desorption process gives relatively lower *Aw* values than the adsorption process does at the same moisture content of a food. This has important implications in the control of a microorganism by reducing the *Aw* of a food. The *Aw* of a food can be reduced by several means, such as adding solutes, ions, hydrophilic colloids, and freezing and drying.

Aw and Microbial Growth

- The free water in a food is necessary for microbial growth. It is necessary to transport nutrients and remove waste materials, carry out enzymatic reactions, synthesize cellular materials, and take part in other biochemical reactions, such as hydrolysis of a polymer to monomers (proteins to amino acids).

- Each microbial species (or group) has an optimum, maximum, and minimum *Aw* level for growth. In general, the minimum *Aw* values for growth of microbial groups are as follows: most molds, 0.8, with xerophilic molds as low as 0.6; most yeasts, 0.85, with osmophilic yeasts, 0.6 to 0.7;

most Gram- positive bacteria, 0.90; and Gram-negative bacteria, 0.93.

- Some exceptions are growth of *Staphylococcus aureus* at 0.85 and halophilic bacteria at 0.75.
- The *Aw* need for spore- forming bacteria to sporulate and the spores to germinate and the toxin-producing microorganisms to produce toxins is generally higher than the minimum *Aw* for their growth.
- Also, the minimum *Aw* for growth in an ideal condition is lower than that in a nonideal condition. As an example, if Adsorption minimum *Aw* for growth of a bacterial strain at pH 6.8 is 0.91, then at pH 5.5, it can be 0.95 or more.
- When the *Aw* is reduced below the minimum level required for growth of a microorganism, the cells remain viable for a while. But if the *Aw* is reduced drastically, microbial cells in a population lose viability, generally rapidly at first and then more slowly. This information is used to control spoilage and pathogenic microorganisms in food as well as enhance the growth of desirable types in food bioprocessing (such as adding salt in processing of cured ham; and in laboratory detection of microorganisms (adding salt to media to enumerate *Staphylococcus aureus*.

Sr.no.	QUESTION	ANSWER
1	Water activity is denoted by _____.	Aw
2	What is the range of water activity?	0-1
3	The *Aw* of food ranges from____.	0.1 to 0.99
4	Gram-negative bacteria's water activity value is_____.	0.93
5	Adding salt to media is used to enumerate which microorganisms?	Staphylocollus

pH and Growth

Principle:

- pH indicates the hydrogen ion concentrations in a system and is expressed as – log [H^+], the negative logarithm of the hydrogen ion or proton concentration.
- It ranges from 0 to 14, with 7.0 being neutral pH. [H^+] concentrations can differ in a system, depending on what acid is present.

- Some strong acids used in foods, such as HCl and phosphoric acid, dissociate completely.
- Weak acids, such as acetic or lactic acids, remain in equilibrium with the dissociated and undissociated forms:

[HCl] [H^+] + [Cl^-], pH of 0.1 *N* HCl is 1.1

CH_3COOH [H^+] + [CH_3COO^-], pH of 0.1 *N* CH_3COOH 2.9

Acidity is inversely related to pH: a system with high acidity has a low pH, and vice versa.

pH of Food:

- Depending on the type, the pH of a food can varies greatly.
- On the basis of pH, foods can be grouped as high-acid foods (pH below 4.6) and low-acid foods (pH 4.6 and above).
- Most fruits, fruit juices, fermented foods (from fruits, vegetables, meat, and milk), and salad dressings are high-acid (low-pH) foods, whereas most vegetables, meat, fish, milk, and soups are low-acid (high-pH) foods.
- Tomato, however, is a high-acid vegetable (pH 4.1 to 4.4).
- The higher pH limit of most low-acid foods remains below 7.0; only in a few foods, such as clams (pH 7.1) and egg albumen (pH 8.5), does the pH exceed 7.0.
- Similarly, the low pH limit of most high-acid foods remains above 3.0, except in some citrus fruits (lemon, lime, grapefruit) and cranberry juice, in which the pH can be as low as 2.2.
- The acid in the foods can either be present naturally (as in fruits), produced during fermentation (as in fermented foods), or added during processing (as in salad dressings).
- Foods can also have compounds that have a buffering capacity. A food such as milk or meat, because of good buffering capacity, does not show pH reduction when compared with a vegetable product in the presence of the same amount of acid.

pH and Microbial Growth:

- The pH of a food has a profound effect on the growth and viability of microbial cells.
- Each species has an optimum and a range of pH for growth. In general, molds

and yeasts are able to grow at lower pH than do bacteria, and Gram-negative bacteria are more sensitive to low pH than are Gram-positive bacteria.

- The pH range of growth for molds is 1.5 to 9.0; for yeasts, 2.0 to 8.5; for Gram-positive bacteria, 4.0 to 8.5; and for Gram- negative bacteria, 4.5 to 9.0. Individual species differ greatly in lower pH limit for growth; for example, *Pediococcus acidilactici* can grow at pH 3.8 and *S.aureus* can grow at pH 4.5, but normally *Salmonella* cannot.
- The lower pH limit of growth of a species can be a little higher if the pH is adjusted with strong acid instead of a weak acid (due to its undissociated molecules).
- Acid-resistant or tolerant strains can acquire resistance to lower pH compared with the other strains of a species (e.g., acid-resistant *Salmonella*).
- When the pH in a food is reduced below the lower limit for growth of a microbial species, the cells not only stop growing but also lose viability, the rate of which depends on the extent of pH reduction. This is more apparent with weak acids, especially with those that have higher dissociation constant (pK), such as acetic acid vs. lactic acid (with pK values 4.8 and 3.8, respectively).
- This is because at the same pH, acetic acid has more undissociated molecules than lactic acid does.
- The undissociated molecules, being lipophilic, enter into the cell and dissociate to generate H^+ in the cytoplasm.
- This causes a reduction in internal pH, which ultimately destroys the proton gradient between the inside and the outside of the cells and dissipates proton motive force as well as the ability of the cells to generate energy.
- The information on the influence of pH on growth and viability of microbial cells is important to develop methods to prevent the growth of undesirable microorganisms in food (e.g., in acidified foods), used to produce some fermented foods (e.g., sequential growth of lactic acid bacteria in sauerkraut fermentation), and to selectively isolate aciduric microorganisms from food (e.g., yeasts and molds in a medium with pH 3.5.
- Acquired acid tolerance by pathogens and spoilage bacteria can impose problems in their control in low-pH foods.

Sr. no.	QUESTION	ANSWER
1	Range of pH is ____.	0-14pH
2	Acidic PH is____.	0-7pH
3	Alkaline pH is______.	7-14pH

4	pH of water is ______.	Neutral
5	Dissociation constant is denoted as_______.	PKa

Redox Potential, Oxygen, and Growth

Principle:

- The redox or oxidation–reduction (O–R) potential measures the potential difference in a system generated by a coupled reaction in which one substance is oxidized and a second substance is reduced simultaneously.
- The process involves the loss of electrons from a reduced substance (thus it is oxidized) and the gain of electrons by an oxidized substance (thus it is reduced).
- The electron donor, because it reduces an oxidized substance, is also called a reducing agent.
- Similarly, the electron recipient is called an oxidizing agent.
- The redox potential, designated as Eh, is measured in electrical units of millivolts (mV).
- In the oxidized range, it is expressed in +mV, and in reduced range in –mV.
- In biological systems, the oxidation and reduction of substances are the primary means of generating energy.
- If free oxygen is present in the system, then it can act as an electron acceptor.
- In the absence of free oxygen, oxygen bound to some other compound, such as NO3 and SO4, can accept the electron.
- In a system where no oxygen is present, other compounds can accept the electrons. Thus, presence of oxygen is not a requirement of O–R reactions.[10]

Redox Potential in Food:

- The redox potential of a food is influenced by its chemical composition, specific processing treatment given, and its storage condition (in relation to air).
- Fresh foods of plant and animal origin are in a reduced state, because of the presence of reducing substances such as ascorbic acid, reducing sugars, and –SH group of proteins.

- Following stoppage of respiration of the cells in a food, oxygen diffuses inside and changes the redox potential.
- Processing, such as heating, can increase or decrease reducing compounds and alter the Eh.
- A food stored in air will have a higher Eh (+mV) than when it is stored under vacuum or in modified gas (such as CO_2 or N_2).
- Oxygen can be present in a food in the gaseous state (on the surface, trapped inside) or in dissolved form.

Redox Potential and Microbial Growth:

- On the basis of their growth in the presence and absence of free oxygen, microorganisms have been grouped as aerobes, anaerobes, facultative anaerobes, or microaerophiles.
- Aerobes need free oxygen for energy generation, as the free oxygen acts as the final electron acceptor through aerobic respiration.
- Facultative anaerobes can generate energy if free oxygen is available, or they can use bound oxygen in compounds such as NO_3 or SO_4 as final electron acceptors through anaerobic respiration.
- If oxygen is not available, then other compounds are used to accept the electron (or hydrogen) through (anaerobic) fermentation.
- An example of this is the acceptance of hydrogen from $NADH_2$ by pyruvate to produce lactate.
- Anaerobic and facultative anaerobic microorganisms can only transfer electrons through fermentation.
- Many anaerobes (obligate or strict anaerobes) cannot grow in the presence of even small amounts of free oxygen as they lack the superoxide dismutase necessary to scavenge the toxic oxygen free radicals.
- Addition of scavengers, such as thiols (e.g., thiol glycolate), helps overcome the sensitivity to these free radicals. Microaerophiles grow better in the presence of less oxygen.
- Growth of microorganisms and their ability to generate energy by the specific metabolic reactions depend on the redox potential of foods. The Eh range at which different groups of microorganisms can grow are as follows: aerobes, +500 to +300 mV; facultative anaerobes, +300 to +100 mV; and anaerobes, +100 to –250 mV or lower.

- However, this varies greatly with concentrations of reducing components in a food and the presence of oxygen.
- Molds, yeasts, and *Bacillus, Pseudomonas*, *Moraxella*, and *Micrococcus* genera are some examples that have aerobic species.
- Some examples of facultative anaerobes are the lactic acid bacteria and those in the family *Enterobacteriaceae*.
- The most important anaerobe in food is *Clostridium*. An example of a microaerophile is *Campylobacter spp*.
- The Eh range indicates that in each group some species are stricter in their Eh need than others.
- Although most molds are strict aerobes, a few can tolerate less aerobic conditions.
- Similarly, yeasts are basically aerobic, but some can grow under low Eh (below +300 mV). Many clostridial species can grow at Eh +100 mV, but some need –150 mV or less.
- The presence or absence of oxygen and the Eh of food determine the growth capability of a particular microbial group in a food and the specific metabolic pathways used during growth to generate energy and metabolic by-products.
- This is important in microbial spoilage of a food (such as putrefaction of meat by *Clostridium* spp. under anaerobic conditions) and to produce desirable characteristics of fermented foods (such as growth of *Penicillium* species in blue cheese under aerobic conditions).
- This information is also important to isolate microorganisms of interest from foods (such as *Clostridium laramie*, a strict anaerobe from spoiled meat) in the laboratory.

Sr. no.	QUESTION	ANSWER
1	Redox potential is also known as_____.	Oxidation reduction potential
2	Redox potential is denoted by which symbol?	Eh
3	Redox potential is measured by____.	mV
4	Redox potential of clostridia is ____.	+100mV
5	Putrefaction is caused by which microorganisms?	*Clostridium*

EXTRINSIC FACTORS

- Extrinsic factors important in microbial growth in a food include the environmental conditions in which it is stored.
- These are temperature, relative humidity, and gaseous environment. The relative humidity and gaseous condition of storage, respectively, influence the *Aw* and Eh of the food.
- The influence of these two factors on microbial growth has been discussed previously.

Temperature and Growth

Principle:

- Microbial growth is accomplished through enzymatic reactions. It is well known that within a certain range, with every 10˚C rise in temperature, the catalytic rate of an enzyme doubles.
- Similarly, the enzymatic reaction rate is reduced to half by decreasing the temperature by 10˚C.
- This relationship changes beyond the growth range.
- Because temperature influences enzyme reactions, it has an important role in microbial growth in food.

Food and Temperature:

- Foods are exposed to different temperatures from the time of production until consumption.
- Depending on processing conditions, a food can be exposed to high heat, from 65˚C (roasting of meat) to more than 100˚C (in ultrahigh temperature processing).
- For long-term storage, a food can be kept at 5˚C (refrigeration) to –20˚C or below (freezing).
- Some relatively stable foods are also kept between 10 and 35˚C (cold to ambient temperature).
- Some ready-to-eat foods are kept at warm temperature (50˚ to 60˚C) for several hours (e.g., in the supermarket deli). Different temperatures are

also used to stimulate desirable microbial growth in food fermentation.

Microbial Growth and Viability:

- Microorganisms important in foods are divided into three groups on the basis of their temperature of growth, each group having an optimum temperature and a temperature range of growth:

  (1) thermophiles (grow at relatively high temperature), with optimum ca. 55˚C and range 45˚ to 70˚C;

  (2) mesophiles (grow at ambient temperature), with optimum at 35˚C and range 10˚ to 45˚C;

  (3) psychrophiles (grow at cold temperature), with optimum at 15˚C and range –5˚ to 20˚C. However, these divisions are not clear-cut and overlap each other.

- Two other terms used in food microbiology are very important with respect to microbial growth at refrigerated temperature and survival of microorganisms to low heat treatment or pasteurization, because both methods are widely used in the storage and processing of foods.

- Psychrotrophs are microorganisms that grow at refrigerated temperature (0˚ to 5˚C), irrespective of their optimum range of growth temperature. They usually grow rapidly between 10˚ and 30˚C.

- Molds; yeasts; many Gram- negative bacteria from genera *Pseudomonas, Achromobacter, Yersinia, Serratia*, and *Aeromonas*; and Gram-positive bacteria from genera *Leuconostoc, Lactobacillus, Bacillus, Clostridium*, and *Listeria* are included in this group).

- Microorganisms that survive pasteurization temperature are designated as thermoduric. They include species from genera *Micrococcus, Bacillus, Clostridium, Lactobacillus, Pediococcus*, and *Enterococcus*.

- Bacterial spores are also included in this group. They have different growth temperatures and many can grow at refrigerated temperature as well as thermophilic temperature.

- When the foods are exposed to temperatures beyond the maximum and minimum temperatures of growth, microbial cells die rapidly at higher temperatures and relatively slowly at lower temperatures.

- Microbial growth and viability are important considerations in reducing food spoilage and enhancing safety against pathogens, as well as in food bioprocessing.

- Temperature of growth is also effectively used in the laboratory to enumerate and isolate microorganisms from foods.

Sr. no.	QUESTION	ANSWER
1	Which are extrinsic factor affect food?	temperature, relative humidity, and gaseous environment
2	with every which temperature rise in temperature, the catalytic rate of an enzyme doubles?	10˚C
3	Psychrotrophs can grow at which temperature?	0-5 ˚C
4	Microorganisms that survive pasteurization temperature are designated as _____.	thermoduric
5	Temperature of growth also used in which purpose in laboratory?	To enumerate and isolate microorganisms

Microbial spoilage of foods: Fresh foods & Canned foods Fruits and Vegetables

- Fruits and vegetables are generally contaminated by bacteria including species of *Bacillus, Enterobacter, Lactobacillus, Leuconostoc, Pseudomonas, Sarcina, Staphylococcus, Streptococcus* etc. Various molds and yeasts also inhabit the fruits and vegetables.

- Contamination through infection: Fruits and vegetables are normally susceptible to bacterial, fungal and viral infections. These infections invade the fruit and vegetable tissue using various stages of their development and result in the subsequent spoilage.

- Contamination through post-harvest handling: Usually, mechanical handling of fruits and vegetables during post-harvest period produces 'breaks' in them which invite microbial invasion. Since the pH of the fruits is relatively acidic (i.e., high in sugar), they are more susceptible to fungi in contrast to vegetables, which are more susceptible to bacteria because of their pH being slightly higher (5.0 to 7.0; less in sugar).

Cereals: Cereals and cereal products contain microorganisms from insects, soil and other sources *Bacillus, Lactobacillus, Micrococcus, Pseudomonas* etc. are the bacteria, which are generally found on freshly harvested grains.

Mostly bacteria such as species of *Bacillus, Lactobacillus, Micrococcus, Sarcina, Serratia, coliforms* etc. contaminate wheat flours. Molds like *Aspergillus, Penicillium* are also very common.

General types of microbial spoilage:

The most commonly occurring types of spoilage are as follows:

- **Bacterial soft rot:** Caused by *Erwinia caratovora*. These are fermenters of pectin. *Pseudomonas marginalis* and *Clostridium*, *Bacillus spp*. have also been isolated from these rots. It results in a water-soaked appearance, a soft mushy consistency and often a bad odor.
- **Gray mold rot:** *Botrytis cinerea*. *Botrytis* name derived from the gray mycelium of the mold. It is favored by high humidity and a warm temperature.
- **Rhizopus soft rot:** It is caused by *spp*. of *Rhizopus* Ex: *Rhizopus stolonifer*. This rot is often soft and mushy. The cottony growth of the mold with small, black dots of sporangia often covers masses of the foods.
- **Anthrachose:** Usually caused by *Collectotrichum lindemuthianum*, *Collectotrichum coccodes*, defect is spotting of leaves and fruits or seed pods.
- **Alternaria Rot:** Caused by *Alternaria tenuis*. Areas become greenish brown early in the growth of the mold and later turn to brown or black spots.
- **Blue mold rot:** Caused by *Penicillium digitatum*. Bluish green color spores are produced.
- **Downey mildew:** Caused by *Phytopthora bremia*.
- **Watery soft rot:** Caused chiefly by *Sclerotinia sclerotium*. Found in mostly vegetables.
- **Stem end rots:** Caused by *Diplodia, Alternaria, Phomopsis, Fusarium*.
- **Black mold rot:** Caused by *Aspergillus niger*. Dark brown to black masses of spores of the mold termed "Smut" called by layperson.
- **Black rot:** Caused by *Alternaria, Ceratostomella, Physalospora*.
- **Pink mold rot:** Caused by pink spored *Trichothecium roseum*.
- **Fusarium rots:** Caused by *Fusarium spp*.
- **Green mold rot:** Caused by *Cladosporium, Trichoderma*.
- **Brown rot:** Caused chiefly by *Sclerotinia* (*Monilinia fructicola*) *spp*.
- **Sliminess or souring:** Caused by Saprophytic bacteria in piled, wet, heating vegetables.
- Fungal spoilage of vegetables often results in water soaked, mushy areas. Fungal rots of fleshy fruits such as apples and peaches frequently shown brown or cream-colored areas in which mold mycelia are growing in the tissue below the skin and aerial hyphae and spores may appear later. Some types of fungal spoilage appear as "dry rots" where the infected area is dry and hard and often discolored. Rots of juicy fruits may result in leakage. Bacterial soft rot is present in vegetables which are not very acid.

Sr. No.	QUESTION	AMSWER
1	Brown rot caused by which organisms?	*Sclerotinia*
2	Pink mold rot caused by _________.	Trichothecium roseum

3	Black mold rot caused by which organisms?	*Aspergillus niger*
4	Green mold rot is caused by _______.	*Cladosporium, Trichoderma*
5	Rhizopus soft rot It is caused by *spp.* of ___.	*Rhizopus*

Spoilage of fruit and vegetable juices:

Molds can grow on the surface of acidic fruit juices if juices are exposed to air. High moisture content favors the faster growing of yeasts. The removal of solids from the juices by extraction and sieving raises the oxidation-reduction potential and favors the growth of yeasts. Most fruit juices are acid enough and have sufficient sugar to favor the growth of yeasts within the range of temperature that favors them from 15.6 to 35°C. The deficiency of B-vitamins discourages some bacteria.

In addition to the usual alcoholic fermentation, fruit juices may undergo other changes caused by microorganisms:

1. The lactic acid fermentation of sugars, mostly by heterofermentative lactic acid bacteria such as *Lactobacillus pastorianus*, *Lactobacillus brevis* and *Leuconostoc mesenteroides* in apple or pear juice add by homofermentative lactic acid bacteria such as *Lactobacillus arabinosus*, *Lactobacillus leichmanii* and *Microbacterium*.

2. The fermentation of organic acids of the juice by lactic acid bacteria. E.g., *Lactobacillus psatorianus*, malic acid to lactic acid and succinic acid, quinic acid to dehydroshikimic acid, and citric acid to lactic and acetic acids

3. Slime production by *Leuconostoc mesenteroides*, *Lactobacillus brevis*, and *Lactobacillus plantarum* in apple juice and by *L. plantarum* and *Streptocooci* in grape juice.

- Vegetable juices contain sugars but less acid than fruit juices and have pH around 5.0 to 5.8.
- The accessory growth substances support growth of fastidious lactic acid bacteria by acid fermentation.
- Concentrates of fruit and vegetable juices contain high sugar content. It favors the growth of yeasts like acid and sugar tolerant like *Leuconostoc* and *Lactobacillus* species.

Sr. No.	**QUESTION**	**ANSWER**
1	Where molds can grow?	Surface of acidic fruit juice if it was exposed to air

2	Vegetable juice has pH around……..	5.0 to 5.8
3	The deficiency of ……… discourages some bacteria to grew on juice	Vitamin – B
4	In fermentation process melic acid is coverted into which acids?	Lactic acid & succinic acid
5	Which bacteria are responsible for slime production in grape juice?	*L. plantarum* and *Streptocooci*

Contamination of Animal Food Products:

Meats:

- The interior portions of meat are usually free of microbial contaminations if healthy animal is properly slaughtered.
- The fresh cut meat gets immediately contaminated with microorganisms derived from globes, hands, implements used to cut the meat, hides, hairs, intestines of the animals and the air of the slaughter house.
- Each new surface of meat, resulting from a new cut, adds more microorganisms to the exposed tissue.
- The more common microorganisms occurring on fresh, meats include both bacteria and molds.
- Bacteria such as species of Bacillus, *Clostridium*, *Escherichia*, *Pseudomonas*, *Lactobacillus*, *Micrococcus*, *Streptococcus*. *Sarcina*, *Salmonella* occurs most commonly.
- Molds that contaminate fresh meat include *Cladosporium*, *Geotrichum*, *Mucor*, *and Penicillium Sporotrichum*etc. Yeasts are less commonly occurring.

Eggs:

- Clean eggs with uncracked shell normally do not contain microorganisms within. Poor sanitary and storage conditions under which it is held determine its subsequent microbial content. Bacteria and molds may enter the egg through cracks in the shell. The microbial flora recovered from the eggshells generally includes the species of bacteria *Micrococcus*, *Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Sarcina* and the molds.

Poultry:

- The surface of freshly dressed eviscerated poultry has microbial flora, which is derived from the live birds or from the manipulations during killing, defeathering and evisceration. Species of *Bacillus*, *Enterobacter*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella; and Staphylococcus* constitute the major microbial flora on the skin of freshly dressed remove the guts from poultry.

Fish:

- The microbial flora of freshly caught fish usually reflects the microbial conditions of water from where they are harvested. Fish micro flora includes bacteria like *Alcaligenes*, *Micrococcus, Pseudomonas, Serratia, and Vibrio* etc. When the fish are cleaned and cut on shipboard under poor handling conditions, they invite more microorganisms to grow on it. These microorganisms can be exemplified by the species of *Achromonobacter, Bacillus, Micrococcus, and Pseudomonas* etc.

Sr. No.	QUESTION	ANSWER
1	Give example of molds that contaminants meat	*Cladosporium, Geotrichum,* Mucor, and *Penicillium Sporotrichumetc*
2	How egg contaminants with microorganisms	Poor sanitary and storage condition
3	Major microbial flora of poultry	*Bacillus, Enterobacter, Escherichia, Proteus, Pseudomonas, Salmonella; and Staphylococcus*
4	Natural microbial flora of fish came from where?	Water
5	Due to poor handling condition which organisms grew on fish	*Achromonobacter, Bacillus, Micrococcus, and Pseudomonas* etc

Spoilage of Canned Foods:

- Canned foods may spoil either, due to biological or chemical reasons. We would discuss only the biological spoilage as it is the point at issue.

Biological spoilage of canned foods:

- Biological spoilage of canned foods occurs due to the action of various microorganisms. Spore forming bacteria, e.g., Clostridium, Bacillus represent the most important group of canned food spoiling microorganisms because of their heat resistant nature (thermophilic nature).
- In addition, there are other microorganisms, which are not heat resistant (mesophilic) but enter through the leakage of the container during cooling and spoil the food. In this way we can divide biological spoilage of canned foods, into two following categories.

Biological spoilage by thermophilic bacteria:

- Under processing of canned foods result in spoilage by thermophilic bacteria, the bacteria that grow best at temperature of 50^{O}C or higher. Five types of this spoilage can be recognized.

Flat sour spoilage:

- In this, type the spoilage can, not be detected unless the can is opened, the spoilage is caused by *Bacillus spp*., such as *B. coagulqns and S. stearothermophilus* resulting in sour, abnormal odor, sometimes cloudy liquor in the food content of the can.

Thermophilic anaerobic (TA) spoilage:

- *Clostridium thermosaccharolyticum*, an obligate thermophile, causes spoilage. The can swells and may burst due to production of CO_2 and H_2. The food becomes fermented, sour, cheesy and develops butyric odor.

Sulfide spoilage:

- *Clostridium nigricans* is involved in this spoilage. It produces H_2S gas which is absorbed by the food product which is usually blackened and gives “rotten egg” odor.

Putrefactive anaerobic spoilage:

- *Clostridium sporogenes* causes spoilage through putrefaction. The can swells and may burst. Putrefaction may result from partial digestion of the food. The latter develops typical "putrid" odor.

Aerobic sporeformers spoilage:

- *Bacillus spp*., the aerobic bacteria, causes spoilage. If the canned food is cured meat, swelling of the can is observed.

Biological spoilage by mesophilic microorganisms:

- *Bacillus spp*., *Clostridium* spp., yeasts and other fungi, which are mesophilic (an organism growing best at moderate temperature range ± 25 to 40^{O}C) are mainly responsible for this type of canned food spoilage. As stated earlier, these organisms enter through the leakage of the container during cooling.

Clostridium butyricum and *C. pasteurianum* result in butyric acid type of fermentation in acidic (tomato juice, fruits, fruit juices etc.) or medium acidic (corn, peas, spinach etc.) foods with swelling of the container due to the production of CO_2 and H_2.

Bacillus subtilis and B. mesentroides have been reported spoiling canned sea foods, meats etc. Other mesophilic bacteria, which have been reported in cans, are *Bacillus polymixa*, *B. macerans*, *Streptococcus spp*., *Pseudomonas*, *Proteus* etc. Yeasts and molds have also been found present in canned foods. Yeasts result in CO_2 production and swelling of the cans.

Sr. no.	QUESTION	ANSWER
1	How canned foods are spoiled?	Due to chemical and biological reason

2	Bacillus spp. Are responsible for which type of spoilage?	Flate sour spoilage
3	What types of changes are seen in food due to TA spoilage?	Food becomes fermented, sour , cheese, and developed butyric acid.
4	Moderate temperature range of mesophilic organisms	±25 to40 ˚C
5	Yeast responsible for………..	Co_2 production and swelling of the cans

Food Borne infection & intoxication: Role of *S. aureus, C. botulinum* &*Salmonella spp*. in food poisoning:

Role of *S. aureus:*

This is the most common type of food-poisoning caused due to the food contaminated with a potent toxin namely, enterotoxin. This toxin is produced by certain strains of *Staphylococcus aureus.* A sudden onset of illness starts usually within 3 to 6 hours after ingestion of the contaminated food.

Source:

These bacteria are commonly present on the skin, nose and other parts of human body. People who handle foods carelessly usually transfer them to the food. Foods most commonly contaminated involve those which are eaten cold, e.g., cold meat, poultry, salads, bakery products etc.

Symptoms:

As said earlier, the disease starts within 3 to 6 hours after ingestion of the contaminated food and is manifested by nausea, vomiting, abdominal pain and diarrhea within 24 to 48 hours. If the case becomes severe, dehydration and collapse may follow. However, in usual conditions death is rare.

Control:

The disease can be controlled by preventing the entry of the bacteria to food. It is important that all susceptible foods are kept under refrigeration to restrict the growth of the bacteria; and also, by the destruction of the bacteria.

Clostridium botulinum:

Botulism

Botulism is caused by the ingestion of food containing the neurotoxin (toxin that affects the nervous system) produced by *Clostridium botulinum,* an anaerobic spore forming bacterium. Sixty to seventy percent-cases of botulism die. There are 7 types (type A, B, C, D, E, F, G) of these neurotoxins recognized on the basis of serological specificity. The neurotoxin of *C. botulinum* is a protein. It has been purified and crystallized and is so powerful that only a does as low as 0.01 mg is said to be fatal to human being. The toxin is absorbed mostly in the small intestine and paralyzes the involuntary muscles of the body.

Serological Types of Botulism Neurotoxins:

Type A: It commonly causes human botulism in the western part of the United States and is more toxic than type B.
Type B: This type occurs more often than type A in most soils of the world end is less toxic to human being.
Type C: So far as is known, this type causes botulism of cattle, fowls, and other animals but not of human beings.
Typed D: This type has been reported causing forage poisoning of cattle in South African Countries.
Type E: This type ties been obtained chiefly from fish and fish products and is toxic for human being.
Type F: This type has been isolated in Denmark end causes human botulism.
Type G: It has recently been isolated from the soil in Argentina. It does not concern with human botulism.

Source:

The main sources of botulism are canned meat, fish, string beans, sweet corn, beets and other low medium acid foods. The foods implicated are generally those of a type that have undergone some treatment intended for the preservation of the product such as canning, pickling or smoking, but one which failed to destroy the spores of this bacterium. When the intended preservative treatment is inadequate and is followed by storage conditions which permit the germination and growth of the microorganisms, one of the most lethal toxins known to humanity is produced, the toxin has been known to persist in foods for long periods, especially when storage has been at low temperatures. It is unstable at pH value above 6.8.

Temperature is considered to be the most important factor in determining whether toxin production will take place and what the rate of production will be. Various strains of *C. botulinum* types A and B vary in their temperature requirements; a few strains grow at 10 to 11°C. However, the lowest temperature for germination of spores of most of the strains is 15°C and maximum of 48°C.

Symptoms

Symptoms generally occur within 12 to 36 hours after consumption of the spoiled food. Early symptoms are digestive disturbances followed by nausea, vomiting, diarrhea together with dizziness and headache. Double vision may occur early and there may be difficulty in speaking. Mouth may become dry, throat constricted, tongue may get swollen, and coated. Involuntary Muscles become paralyzed and paralysis spreads to the respiratory system and to the heart. Death normally results from respiratory failure.

Prevention

Canned food should be properly processed by using approved heat processes. Avoiding food that has been cooked but not well heated. Raw foods, frozen foods thawed and held at room temperature should be avoided. Gassy and spoiled canned foods should be rejected. Boiling of suspected food for at least 15 minutes. Treatment: Successful treatment is by the administration of polyvalent antitoxin in the early stages of infection. Once the symptoms appear the fails to prove useful.

Sr. no.	QUESTION	ANSWER
1	……… toxin produced by S. *aureus*	enterotoxin
2	Source of S. aureus in food	Skin , nose and other parts of human biody
3	Botulism is caused by………	C. botulinum
4	……. Toxin produced by C. botulinum	neurotoxin
5	……. dose of neurotoxin is fetal to human begins	0.01 mg

Salmonella:

Source:

- *Salmonella* is widely dispersed in nature. It can colonize the intestinal tracts of vertebrates, including livestock, wildlife, domestic pets, and humans, and may also living in environments such as pond-water sediment.
- It is spread through the fecal-oral route and through contact with contaminated water. (Certain protozoa may act as a reservoir for the organism). It may, for example, contaminate meat, farm-irrigation water (thus contaminating produce in

the field), soil and insects, factory equipment, hands, and kitchen surfaces and utensils.

- Since *S. typhi and S. paratyphi A* are found only in human hosts, the usual sources of these organisms in the environment are drinking and/or irrigation water contaminated by untreated sewage. It is highly recommended that only potable water and cooked vegetables be consumed in areas where these organisms are endemic.

- **Symptoms:**

High fever, from 103° to 104°F; lethargy; gastrointestinal symptoms, including abdominal pains and diarrhea or constipation; headache; achiness; loss of appetite. A rash of flat, rose colored spots sometimes occurs.

Sr. no.	QUESTION	ANSWER
1	How salmonella is spread?	through the fecal-oral route and through contact with contaminated water.
2	Salmonella is colonized with whom…….	intestinal tracts of vertebrates, including livestock, wildlife, domestic pets, and humans,
3	………… and ……… are found in human host	*S. typhi and S. paratyphi A*
4	Source of these organisms in environment are	drinking and/or irrigation water contaminated by untreated sewage.
5	Salmonella spared through	the fecal-oral route and through contact with contaminated water.

GENERAL PRINCIPLES & METHODS OF FOOD PRESERVATION

Principles of Food Preservation:

A good method of food preservation is one that slows down or prevents altogether the action of the agents of spoilage. Also, during the process of food preservation, the food should not be damaged. In order to achieve this, certain basic methods were applied on different types of foods. For example, in earlier days, in very cold weather condition, ice was used to preserve foods. Thus, very low temperature became an efficient method for preventing food spoilage. Let us now list the principles of food preservation.

Removal of micro-organisms or inactivating them: This is done by removing air, water (moisture), lowering or increasing temperature, increasing the concentration of salt or sugar or acid in foods. If you want to preserve green leafy vegetables, you have to remove the water from the leaves so that microorganisms cannot survive. You do this by drying the green leaves till all the moisture evaporates.

Inactivating enzymes: Enzymes found in foods can be inactivated by changing their conditions such as temperature and moisture, when you preserve peas, one of the methods of preservations is to put them for a few minutes in boiling water. This method also known as blanching inactivates enzymes and thus, helps in preserving the food.

Removal of insects, worms and rats: By storing foods in dry, air tight containers the insects, worms or rats are prevented from destroying it.

CONTROL OF MICROORGANISMS

- Heat
- Cold
- Drying
- Acids
- Sugar and salt
- Oxygen concentration
- Smoke
- Radiation
- Chemicals (preservatives)

CONTROL OF ENZYMES

- Heat
- Oxygen removal
- Acids
- Chemicals (antioxidants)

CONTROL OF OTHER FACTORS

- Protective packaging
- Sanitation

Sr. no.	QUESTION	ANSWER
1	How food is preserved in earlier in cold temperature	Ice was used to preserve food
2	How	by removing air, water (moisture), lowering or

	microorganisms is remove or inactive in food	increasing temperature, increasing the concentration of salt or sugar or acid in foods.
3	How peas is preserved?	By Put them for a few minutes in boiling water
4	How insects worms and rats removed?	storing foods in dry, air tight containers
5	Any two methods for control of microorganism	Heat and radiation

PRESERVATION METHODS

Thermal processing:

- Application of heat
- Inactivate enzymes
- Kill microorganisms. Most bacteria are killed in the range 82-93°c. Spores are not destroyed even by boiling water at 100°c for 30 min.
- To ensure sterility (total microbial destruction, including spores), a temperature of 121°c must be maintained for 15 min or longer.

Various methods are -

- Blanching
- Pasteurization
- Sterilization
- Boiling
- Steam under pressure

Removal of heat (cold processing):

- Lowering temperature of food
- Decreases the rate of enzymatic, chemical and microbial reactions in food
- Storage life is extended

Various methods are -

Refrigeration
Freezing

CONTROL OF WATER CONTENT (DRYING)

Microorganisms require free water

- Free water is removed from the food and therefore, is unavailable to microbial cells.
- Multiplication will stop
- Water unavailable for chemical/biochemical reactions.

Storage life extended Various methods are -

- Freezing
- Physical removal of water from food (dehydration)
- Removal of some of the water from food (concentration)
- Addition of substances that bind water in food, making it unavailable (sugar, salts)

Radiation

- Ionizing radiation
- Inactivate microorganisms in food
- Destroy storage pests
- Inactivate enzymes Various methods are -
  a. Infrared radiation
  b. Ultraviolet radiation

Atmosphere composition

- Removal of oxygen
- Inhibits O_2 dependent enzymatic and chemical reactions Inhibits growth of aerobic microorganisms' Various methods are

  a. Paraffin wax
  b. Nitrogen backflushed bags (potato chips)
  c. Controlled atmosphere storage
  d. Vacuum packaging of fresh food (cured meats)

Fermentation:

- Specific microorganisms are used (starter cultures)
- Facilitate desirable chemical changes
- Longer storage life
- Produce acids, alcohol that will prevent growth of undesirable microorganisms
- Produce antimicrobial substances

Addition of chemicals Various chemicals used are -

a. Acids (inhibit microbial growth and enzymatic reactions)
b. Organic acids (acetic, citric, tartaric acids)
c. Inorganic acids (hydrochloric, phosphoric acids)
d. Food grade, comply with regulations
e. Antioxidants (to delay oxidative rancidity)
f. Antimicrobial agents:
   - sodium propionate (mold inhibitor)
   - sodium benzoate (antibacterial)
   - sugar and salt (high concentrations)

Smoke

- Contains preservative chemicals (e.g., formaldehyde) from the burning wood
- Heat also helps destroy microorganisms
- Heat dries the food

Curing (Salt and Sugar)

- Salt binds with water molecules and thus acts as a dehydrating agent in foods.
- Impair the conditions under which pathogens cannot survive.
- Curing is used with certain fruits and vegetables. (Sauerkraut, pickles),
- Meats can be submerged in a salt solution known as brine

Sr. no.	QUESTION	ANSWER
1	Most bacteria are killed in the range of ……..˚C	82-93
2	To ensure sterility…….. temperature to be maintained	121˚C
3	…….. rediations are used to inactivate enzymes	Infrared and ultraviolate
4	Name of mold inhibitor	Sodium propionate
5	How salts act as dehydrating agent	Salt binds with water molecule

PRESERVATION BY USING CHEMICALS

A preservative is defined as only substance which is capable of inhibiting, retarding or arresting the growth of microorganisms. Microbial spoilage of food products is also controlled by using chemical preservatives. The inhibitory action of preservatives is due to their interfering with the mechanism of cell division, permeability of cell membrane and activity of enzymes.

Pasteurized squashes, cordials and crushes have a cooked flavor. After the container is opened, they ferment and spoil within a short period, particularly in a tropical climate. To avoid this, it is necessary to use chemical preservatives. Chemically preserved squashes and crushes can be kept for a fairly long time even after opening the seal of the bottle. It is however, essential that the use of chemicals is properly controlled, as their indiscriminate use is likely to be harmful. The preservative used should not be injurious to health and should be non-irritant. It should be easy to detect and estimate.

Two important chemical preservatives are permitted to beverages according to the FPO (1955).

1. Sulphur dioxide
2. Benzoic acid

SULPHUR DIOXIDE

It is widely used throughout the world in the preservation of juice, pulp, nectar, squash, crush, cordial and other products. It has good preserving action against bacteria and molds and inhibits enzymes, etc. In addition, it acts as an antioxidant and bleaching agent. These properties help in the retention of ascorbic acid, carotene and other oxidizable compounds. It also retards the development of nonenzymatic browning or discoloration of the product. It is generally used in the form of its salts such as sulphite, bisulphate and metabisulphite.

`Potassium metabisulphite (K_2O $2SO_2$ (or) $K_2S_2O_5$) is commonly used as a stable source of SO_2. Being a solid, it is easier to use than liquid (or) gaseous SO_2. It is fairly stable in neutral (or) alkaline media but decomposed by weak acids like carbonic, citric, tartaric acid and malic acids. When added to fruit juice (or) squash it reacts with the acid in the juice forming the potassium salt and SO_2, which is liberated and forms sulphureous acid with the water of the juice.

$$SO_2 + H_2O \rightarrow H_2SO_3 \text{ (Sulphureous acid)}$$

SO_2 has a better preservative action than sodium benzoate against bacteria and molds. It also retards the development of yeasts in juice, but cannot arrest their multiplication, once their number has reached a high value.

It is well known that fruit juices with high acidity do not undergo fermentation readily. The preservative action of the fruit acid its due to is hydrogen ion concentration. The pH for the growth of molds ranges from 1.5 to 8.5, that of yeasts from 2.5-8.0, and of bacteria from 4.0 to 7.5. As fruit beverage like citrus squashes and cordials have generally a pH of 2.5 to 3.5, the growth of molds and yeasts in them cannot be prevented by acidity alone. Bacteria, however, cannot grow. The pH is therefore, of great importance in the preservation of food product and by regulating it, one or more kinds of microorganisms in the beverage can be eliminated.

The concentration of SO_2 required preventing the growth of mirgroorganism at different pH levels are as under.

Ph	*S. ellipsoideus* (yeasts)	*Mucor* (mold)	*Penicillium* (mold)	Mixed bacteria
2.5	200	200	300	100
3.5	800	600	600	300
7.0	Above 5000	Above 5000	Above 5000	Above 1000

The toxicity of SO_2 increases at high temperature. Hence its effectiveness depends on the acidity, pH, temperature and substances present in fruit juice.

According to FPO, the maximum amount of SO_2 allowed in fruit juice is 700 ppm, in squash, crush and cordial 350 ppm and in RTS and nectar 100 ppm.

ADVANTAGES OF USING SO_2 ARE:

- It has a better preserving action than sodium benzoate against bacterial fermentation.
- It helps to retain the color of the beverage for a longer time than sodium benzoate.
- being a gas, it helps in preserving the surface layer of juices also
- being highly soluble in juices and squashes, it ensures better mixing and hence their preservation and any excess of SO2 present can be removed either by heating the juice to about 71oC or by-passing air through it or by subjecting the juice to vacuum. This causes some loss of the flavoring materials due to volatilization, which can be compensated by adding flavors.

DISADVANTAGES (OR) LIMITATIONS

- It cannot be used in the case of some naturally colored juices like those of jamun, pomegranate, strawberry, colored grapes, plum etc. on account of its bleaching action.
- It cannot also be used for juices which are to be packed in tin containers because it not only corrodes the tin causing pinholes, but also forms H_2S which has a disagreeable smell and reacts with the iron of the tin container to form a black compound, both of which are highly undesirable and
- SO_2 gives a slight taste and color to freshly prepared beverages but these are not serious defects if the beverage is diluted before drinking.

Benzoic acid

- It is only partially soluble in H_2O hence its salt, sodium benzoate is used. One part of sodium benzoate is soluble in 1.8 parts of water at ordinary temperature, whereas only 0.34 parts of benzoic acid is soluble in 100 parts of water.
- Sodium benzoate is thus nearly 170 times as soluble as benzoic acid, pure sodium benzoate is tasteless and odorless.
- The antibacterial action of benzoic acid is increased in the presence of CO_2 and acid e.g., *Bacillus subtilis* cannot survive in benzoic acid solution in the presence of CO_2. Benzoic acid is more effective against yeasts than against molds. It does not stop lactic acid and acetic acid fermentation.
- The quantity of benzoic acid required depends on the nature of the product to be preserved, particularly its acidity.
- In case of juices having a pH of 3.5-4.0, which is the range of a majority of fruit juices, addition of 0.06 to 0.10% of sodium benzoate has been found to be sufficient.
- In case of less acid juices such as grape juice at least 0.3% is necessary. The action of benzoic acid is reduced considerably at pH 5.0. Sodium benzoate is excess of 0.1% may produce a disagreeable burning taste.
- According to FPO its permitted level in RTS and nectar is 100 ppm and in squash, crush and cordial 600 ppm.
- In the long run benzoic acid may darken the product.
- It is, therefore, mostly used in colored products of tomato, jamun,

pomegranate, plum, watermelon, strawberry, colored grapes etc.

Sr. no.	QUESTION	ANSWER
1	Name of chemical preservatives are permitted to beverages	Sulphur dioxide and benzoic acid
2	One part of sodium benzoate is soluble in …….. parts of water	1.8
3	Antibacterial action of benzoic acid is increase in present of………	Co_2 and acid
4	……….. is commonly used as a stable source of So_2	Potassium metabisulphite
5	pH for growth of molds	1.5 to 8.5

PRESERVATION BY USING RADIATION

- Radiation may be defined as the emission and propagation of energy through space or through a material medium. The type of radiation of primary interest in food preservation is electromagnetic.
- Initially, the destruction of microorganisms in foods by ionizing radiation was referred to by terminology brought over from heat and chemical destruction of microorganisms.
- Although microorganisms can indeed be destroyed by chemicals, heat, and radiation, there is nevertheless, a lack of precision in the use of this terminology for radiation-treated foods. Consequently, in 1964 an international group of microbiologists suggested the following terminology for radiation treatment of foods.

Radappertization is equivalent to radiation sterilization or "commercial sterility" as it is understood in the canning industry. Typical levels of irradiation are 3(MK) kGy.

Radicidation is equivalent to pasteurization of milk, for example. Specifically, it refers to the reduction of the number of viable specific non-spore-forming pathogens, other than viruses, so that none is detectable by any standard method. Typical levels to achieve this process are 2.5-10 kGy.

Radurization may be considered equivalent to pasteurization. It refers to the enhancement of the keeping quality of a food by causing substantial reduction in the numbers of viable specific spoilage microbes by radiation. Common dose levels are 0.75-2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains.

Radappertization

Radappertization of any foods may be achieved by application of the proper dose of radiation under the proper conditions.

Sr. no.	QUESTION	ANSWER
1	Typical levels of irradiation are……. In radapprtization	3(MK)kGY
2	Typical levels to achieve redicidation are	2.5-10 kGy.
3	What is radappertization?	application of the proper dose of radiation under the proper conditions.
4	Common dose of radurization is……..	75-2.5kGy

PRESERVATION BY USING HIGH TEMPERATURE

The use of high temperatures to preserve food is based on their destructive effects on microorganisms.

By high temperatures are meant any and all temperatures above ambient. With respect to food preservation, there are two temperature categories in common use: pasteurization and sterilization.

Pasteurization by use of heat implies either the destruction of all disease-producing organisms (for example, pasteurization of milk) or the destruction or reduction in the number of spoilage organisms in certain foods, as in the pasteurization of vinegar. The pasteurization of milk is achieved by heating as follows:

- 145°F (63°C) for 30 minutes (low temperature, long time [LTLT]), 161°F (72°C) for 15 seconds (primary high temperature, short time [HTST] method), 191°F (89 °C) for 1.0 second, 194°F (90°C) for 0.5 second, 201°F (94°C) for 0.1 second, 212°F (100°C) for 0.01 second.

- These treatments are equivalent and are sufficient to destroy the most heat resistant of the non-spore-forming pathogenic organisms *Mycobacterium tuberculosis* and *Coxiella burnetii.*

- When six different strains of *M. paratuberculosis* were added to milk at levels from 40 to 100,000 colony- forming units (CFU)/m L followed by pasteurization by LTLT or HTST, no survivors were detected on suitable culture media incubated for 4 months.

- Milk pasteurization temperatures are sufficient to destroy, in addition, all yeasts, molds, gram negative bacteria, and many gram positives. The two groups of organisms that survive milk pasteurization are placed into one of two groups: thermoduric and thermophiles.

- Thermoduric organisms are those that can survive exposure to relatively high temperatures but do not necessarily grow at these temperatures. The non-spore-forming organisms that survive milk pasteurization generally belong to the genera *Streptococcus* and *Lactobacillus*, and sometimes to other genera.

- Thermophilic organisms are those that not only survive relatively high temperatures but require high temperatures for their growth and metabolic activities. The genera *Bacillus* and *Clostridium* contain the thermophiles of greatest importance in foods.

- Pasteurization (to destroy spoilage biota) of beers in the brewing industry is carried out usually for 8-15 minutes at 60°C.

Sterilization:

- means the destruction of all viable organisms as may be measured by an appropriate plating or enumerating technique. Canned foods are sometimes called "commercially sterile" to indicate that no viable organisms can be detected by the usual cultural methods employed or that the number of survivors is so low as to be of no significance under the conditions of canning and storage.

- Also, microorganisms may be present in canned foods that cannot grow in the product by reason of undesirable pH, oxidation-reduction potential (Eh), or temperature of storage.

Sr. no.	QUESTION	ANSWER
1	Range of LTLT	145˚F for 15 second
2	Range of HTST in primary high temperature	161 ˚F for 15 second
3	Pasteurization temperature of beers in brewing industry	60 ˚C
4	Which two groups are survived during milk pasteurization	Thermoduric & thermophiles
5	Full form of CFU	Colony forming unit

PRESERVATION BY USING LOW TEMPERATURE

The use of low temperatures to preserve foods is based on the fact that the activities of food borne microorganisms can be slowed at temperatures above freezing and generally stopped at subfreezing temperatures.

The reason is that all metabolic reactions of microorganisms are enzyme catalyzed and that the rate of enzyme catalyzed reactions is dependent on temperature.

With a rise in temperature, there is an increase in reaction rate. The temperature coefficient *(Qi0)* may be generally defined as follows:

The *Qi0* for most biological systems is 1.5-2.5, so that for each 1°C rise in temperature within the suitable range, there is a twofold increase in the rate of reaction. For every 10°C decrease in temperature, the reverse is true.

The term *psychrophile* was coined by Schmidt- Nielsen in 1902 for microorganisms that grow at 0°C. This term is now applied to organisms that grow over the range of sub-zero to 20°C, with an optimum range of 10-15°C. Around 1960, the term *psychrotroph* *(psychros*, cold, and *trephein*, to nourish or to develop) was suggested for organisms able to grow at 5°C or below.

It is now widely accepted among food microbiologists that a *psychrotroph* is an organism that can grow at temperatures between 0°C and 7°C and produce visible colonies (or turbidity) within 7-10 days. Because some *psychrotrophs* can grow at temperatures at least as high as 43°C, they are, in fact, *mesophiles*.

By these definitions, psychrophiles would be expected to occur only on products from oceanic waters or from extremely cold climes. The organisms that cause the spoilage of meats, poultry, and vegetables in the 0-5°C range would be expected to be *psychrotrophs*.

Methods of freezing

There are various methods of freezing

- **Sharp Freezing (Slow freezing):**

This technique, first used in 1861, involves freezing by circulation of air, either naturally or with the aid of fans. The temperature may vary from –15 to –29°C and freezing may take from 3 to 72 hours. The ice crystals formed one large and rupture the cells. The thawed tissue cannot regain its original water content. The first products to be sharp frozen were meat and butter. Now-a-days freezer rooms are maintained at –23 to –29°C or even lower, in contrast to the earlier temperature of –18°C.

- **Quick freezing:**

In this process the food attains the temperature of maximum ice crystal formation (0 to – 4°C) in 30 min or less. Such a speed results in formation of very small ice crystals and hence minimum disturbance of cell structure. Most foods are quick frozen by one of the following three methods:

a) By direct immersion

Since liquids are good heat conductors, food can be frozen rapidly by direct immersion in a liquid such as brine or sugar solution at low temperature. Berries in sugar solution packed fruit juices and concentrates are frozen in this manner. The refrigeration medium must be edible and capable of remaining unfrozen at –18°C and slightly below. Direct immersion equipment such as ottenson Brine freezer, Zarotschenzeff 'Fog' freezer, T.V.A. freezer, Bartlett freezer etc. of commercial importance earlier are not used today.

b)By air blast

In this method, refrigerated air at –18 to –34°C is blown across the material to be frozen. The advantages claimed for quick freezing over slow freezing (sharp freezing) are

(1) smaller (size) ice crystals are formed, hence there is less mechanical destruction of intact cells of the food

(2) period for ice formation is shorter, therefore, there is less time for diffusion of soluble material and for separation of ice

(3) more rapid preservation of microbial growth

(4) more rapid slowing down of enzyme action.

c) By indirect contact with refrigerant

Indirect freeing may be defined as freezing by contact of the product with a metal surface which is itself cooled by freezing brine or other refrigerating media. This is an old method of freezing in which the food or package is kept in contact with the passage through the refrigerant at –18 to -46°C flows.

ADVANTAGES

- There is perfect contact between the refrigerating medium and the product, hence the rate of heat transfer is very high.
- Fruits are frozen with a coating of syrup which preserves the color and flavor during storage.
- The frozen product is not a solid block because each piece is separate.

DISADVANTAGES

- Brine is a good refrigerating medium but it cannot be used for fruits.
- It is difficult to make a syrup that will not become viscous at low temperature.
- The refrigeration temperature must be carefully controlled, as at high temperature the medium will enter the product by osmosis and at low temperature the medium may freeze solid.
- It is very difficult to maintain the medium at a definite concentration and also to keep it free from dirt and contamination.

Cryogenic freezing:

Although most foods retain their quality when quick frozen by the above methods, a few require ultrafast freezing. Such materials are subjected to cryogenic freezing which is defined as freezing at very low temperature (below –60°C). The refrigerant used at present in cryogenic freeing are liquid nitrogen and liquid CO_2. In the former case, freezing may be achieved by immersion in the liquid, spraying of liquid or circulation of its vapor over the product to be frozen.

Dehydro-freezing:

This is a process where freezing is proceeded by partial dehydration. In case of some fruits and vegetables about 50% of the moisture is removed by dehydration prior to freezing. This has been found to improve the quality of the food. Dehydration does not cause deterioration and dehydro frozen foods are relatively more stable.

Freeze drying

In this process food is first frozen at –18°C on trays in the lower chamber of a freeze drier and the frozen material dried (initially at 30°C for 24 hrs. and then at 20°C). Under high vacuum (0.1 mm Hg) in the upper chamber. Direct sublimation of the ice takes place without passing through the intermediate liquid stage. The product is highly hygroscopic, excellent in taste and flavor and can be reconstituted readily. Mango pulp, orange juice concentrate, passion fruit juice and guava pulp are dehydrated by this method.

Sr. no.	QUESTION	ANSWER
1	Enzymes catalyzed reaction is depended on ……..	temperature
2	The QiQ for most biological system is ……..	1.5-2.5
3	The term psychrophile was coined by…….	Schmidt- Nielsen
4	The first products to be sharp frozen were…….	Meat & butter
5	In cryogenic freezing which refrigerant is used	Liquid nitrogen and Co_2

DETECTION OF MICROORGANISMS IN FOOD AND FOOD ENVIRONMENT

METHODS USED:

- The methods used for the microbiological evaluation or detection of foods, food ingredients, and environment are broadly grouped as quantitative and qualitative methods.
- Quantitative methods are designed to enumerate or estimate directly or indirectly the microbial load in a test material. None of the quantitative methods used now enumerate or estimate "total microbes," "total bacteria" or "total viable population" (terms used by many) present in a food, although it can be obtained for a pure culture of a microbial strain growing in a sterile liquid or a solid medium.
- Rather, each method enumerates or estimates a specific group among the total microbial population normally present in a food and that grows or multiplies preferentially under the conditions or methods of testing. These include composition of an enumeration medium, temperature and time of incubation and oxygen availability during incubation, pH, and treatments given to a sample before enumeration and estimation.
- Examples of some of the quantitative methods used are aerobic plate

counts (APCs, or standard plate counts, SPCs, for dairy products), anaerobic counts, psychrotrophic counts, thermoduric counts, coliform counts, *Staphylococcus aureus* counts, and yeasts and molds count.

- In contrast, qualitative methods are designed to determine whether a representative amount (a sample) of a food or a certain number of samples in a batch of a food contain a specific microbial species among the total microbial population.

- These methods are used to detect the possible presence of certain foodborne pathogens, especially those capable of inflicting high fatality rates among consumers. *Salmonella, Clostridium botulinum, Escherichia coli* O157:H7, and probably *Listeria monocytogenes* in ready-to-eat food, are some that fall into this group.

STANDARD AND RECOMMENDED METHODS:

- A food microbiology course invariably contains a laboratory component. The methods described in one or more of these books for the microbiological examination of food, food ingredients, and environment can be used in the laboratory.

- This will help the students or interested individuals become familiar with the standard and recommended methods approved by regulatory agencies in the U.S. Some of the methods are briefly discussed here. Details of these methods are available in the books listed previously and references at the end of the appendix.

SAMPLING FOR MICROBIAL ANALYSIS

Sample and Sampling Plan:

- The microbiological quality of a batch or a lot of a food, which could be liquid, solid, or semisolid, is determined by testing a small portion of the total amount.

- This portion tested is called a sample and it must be the true representative of the total mass. It is very important to develop and implement an effective sampling plan; otherwise, even the most sensitive testing method will not provide information about the microbiological quality of a food.

Sampling Procedure:

- The individual collecting the samples should know why the samples are being collected, i.e., what types of testing will be conducted, such as for APC, coliforms, or a specific pathogen.

- The person should also have information about the product if it has been implicated in a foodborne outbreak and the nature of the product (liquid, solid, or semisolid; frozen; bulk or single units).

- The sample should be collected by using proper sanitary measures to prevent any contamination. Following collection and until tested, the samples should not be handled to avoid growth or death of microorganisms. If the product is frozen, samples should be kept frozen until analyzed. Otherwise, they can be stored at 0 to 4rC.

- Each sample should be labeled to identify date, time, nature of sample, and types of analysis to be conducted, and the persons who collected the sample. The samples should be transported to the laboratory under conditions that avoid microbial contamination, growth, or death.

Sr. no.	**QUESTION**	**ANSWER**
1	The methods used for the detection of microorganisms are grouped as a……..	Qualitative method & quantitative method
2	The micribiological quaity of food is determine by	Testing a small portion of the total amount of food
3	Frozen sample is stored at ………	0- 4 ˚c
4	Example of quantitative methods	APCs, or standard plate counts, SPCs,
5	How quantitative method is designed	to enumerate or estimate directly or indirectly the microbial load in a test material

Quantitative methods for microbial enumeration in foods

Direct Enumeration

Microscopic Counts

Either stained cells under a bright field or live cells under a phase-contrast micro-scope can be counted and, using an appropriate microscopic factor, these counts can be expressed as microscopic counts per milliliter or gram food sample.

However, viable and dead cells cannot be differentiated by this method. In addition, a sample must have large numbers (10^6/ml, /g) of microorganisms for effective use of this method. Foods that have particles cannot be effectively tested for enumeration microscopically.

Colony-Forming Units (CFUs) in Nonselective Agar Media

- Aliquots from selected dilutions of a serially diluted sample (generally based on the number expected in a food) are either pour plated or surface plated by using nonselective media such as plate count agar (PCA), tryptic soy agar, or nutrient agar.

- Different media can give different results. However, PCA is recommended for CFU determination of several groups. The temperature and time of plate incubation required for the colonies to develop differ with the microbial groups being enumerated. For SPCs, they are 32rC for 48 h; APCs, 35rC for 48 h; and for psychrotrophic counts, 7rC for 10 d or 10rC for 7 d. The same

- procedure with specific modifications can be used to determine thermophilic, thermoduric, and anaerobic groups present in a food sample. The specific groups to be tested depend on their relative importance in a food. For a vacuum-packaged refrigerated food, the most important groups will be psychrotrophic, anaerobic, and facultative anaerobic groups.

CFUs in Nonselective Differential Media

- A nonselective medium is supplemented with an agent capable of differentiating the colonies produced by specific groups of microorganisms that differ in metabolic or physiological characteristics from one another in the population.

- pH indicators or O – R indicators are often used in the medium. Thus, the colonies of cells capable of metabolizing lactose to lactic acid are differentiated from those that do not ferment lactose by growing them in an agar medium supplemented with lactose as a C source and a pH indicator such as bromocresol purple.

- The lactose-fermentating colonies will be yellow and the others that cannot ferment lactose yet grow will be white against a purple background. Differential methods are also used in specific media enumerating proteolytic, lipolytic, and pectinolytic microbial groups in a food.

CFUs in Selective Agar Media

- A medium can be supplemented with one or more selective or inhibitory agents and used by pour or surface plating of serially diluted samples. In the presence of such an agent, only the microorganisms resistant to it can grow. Incubation conditions to stimulate colony formation differ with the organisms being studied.

- Enumeration of aciduric bacteria in a medium at pH 5.0, yeasts and molds in a medium at pH 3.5, and *Clo. perfringens* in the presence of cycloserine are examples of selective enumeration of specific groups present in a food. Halophilic and osmophilic microorganisms can also be enumerated similarly by specific selective procedures.

- **CFUs in Selective-Differential Agar Media**

- In this method, a medium is supplemented with one or more selective agents to allow selective growth of specific resistant microbial groups while inhibiting growth of other sensitive associative microorganisms. In addition to selective agents, a medium is also supplemented with agents that enable each type among the selective microbial groups to produce colonies that differ in characteristics from one another.

- Violet red bile agar for coliforms, KF-azide agar for *Enterococcus* spp., V-J agar or Baird-Parker agar for *Sta. aureus*, and media recommended for the enumeration of some pathogens (e.g., *Yersinia enterocolitica, Campylobacter jejuni, Salmonella* spp., *Lis. monocytogenes, Clo. perfringens, Aeromonas hydrophila*) are selective as well as differential agar media.

- Because of the presence of one or more selective compounds, they allow selective growth and colony formation of several closely related species. The differential agents then help differentiate these species or groups from one another by their specific colony characteristics.

Indirect Estimation

Dilution to Extinction in Nonselective Broths

- The method consists of serially diluting a sample and transfer of an aliquot (usually 1.0 ml or 0.1 ml) to a final 5 or 10 ml of a nonselective broth, such as tryptic soy broth.

- This is followed by incubating the tubes at a specific temperature for a specific period of time, which depends on the specific microbial group being investigated. The tubes are then examined for the presence and absence of growth (from the turbidity of the broth).

- With the highest sample dilution that gave growth and assuming that this tube had one to nine viable cells of the group of interest, microbial numbers per milliliter or gram sample are estimated. The estimated numbers, however, can vary widely from the actual numbers. This method is not used much for the microbiological estimation in food.

Most Probable Number (MPN) in Selective Broth

- In this method, aliquots from a serially diluted sample are inoculated in a broth (in tubes) having one or more selective agents that facilitate growth of selected microbial groups present in a food.

- Generally, three or five broth tubes in each dilution and a minimum of three consecutive dilutions are used. After incubating at recommended temperature and time, the broth tubes in each dilution are scored for the presence and absence of growth.

Dye Reduction Test

- The method is based on the principle that some dyes such as methylene blue and resazurin are colored in oxidized states but colorless under reduced conditions.

- This change can occur because of microbial metabolism and growth. It is assumed that the rate of reduction during incubation of a specific concentration of methylene blue added to a food is directly proportional to the initial microbial load in the food.

- **Enumeration of Injured Microbial Groups by Selective Media**

- Sub lethally injured coliforms and pathogenic bacterial cells, when enumerated by selective agar media, may die because of their developed sensitivity to selective agents in the media and thus cannot be detected. They are first allowed to repair the injury in nonselective media (broth or agar) for a short period, which enables the cells to regain resistance to the selective agents.

- **Dilution Scheme, Plating, Incubation, Selection of Plates for Counting CFUs, and Reporting Results**

- The standard and recommended method books listed above have detailed descriptions on these steps. They should be followed as accurately as possible. The average of CFUs from duplicate or triplicate plates from a selected dilution should be used to report the counts of the specific microbial group per ml or g, or cm^2 of the food sample. This data should be used in the proper interpretation of microbiological quality of the food.

Sr. no.	QUESTION	ANSWER
1	For the microscopic count the sample must have ……….numbers of cells	10^6/ml/g
2	Colony formoing unit for SPCs	32rc for 48h
3	In non selective media which indigetors are used	pH and O-R indicator
4	At which pH aciduric bacteria is	5.0

	enumeration	
5	Which dyes used in dye reduction test	Methelyne blue and resazurin

Qualitative methods to isolate microorganisms in foods

Isolation of Pathogens

The main objective of this method is to determine whether a sample contains viable cells or spores of a specific pathogen. Foods are tested for several pathogens, such as *Salmonella*, *E. coli*, *L. monocytogenes*, *Vibrio cholerae*, and *Shigella spp*., by the specific isolation procedure when necessary. For other pathogens, such as enteropathogenic *E. coli*, *Y. enterocolitica*, and *Cam. jejuni*, isolation procedures are not generally used; instead, enumeration procedures are used.

Test for bacterial toxins in foods

S. aureus and *C. botulinum* strains and several others (e.g., *Bac. cereus*, *Vib. parahaemolyticus*), while growing in foods, can produce toxins and cause intoxication or food poisoning among consumers. Specific methods have been developed to test the presence of toxins in the suspected foods.

Rapid methods and automation

- The traditional or conventional methods used for the quantitative or qualitative detection of microorganisms in foods and toxins take a relatively long time and are labor intensive.
- To overcome these difficulties, different rapid methods, many of which are automated, have been developed to detect microbial loads, foodborne pathogens, and their toxins. Some of these methods have been approved by regulatory agencies.
- In addition to being rapid, they are quite specific, sensitive, relatively accurate, and less labor intensive. Immunoassays for Rapid Detection of Pathogens Several rapid and automated methods have been developed that rely on the specific antigen–antibody reaction and production of agglutination, color formation from chromogenic substrate, formation of an immune band, or fluorescence.
    - *Immunofluorescence Method*
    - *RPLA Method*
    - *Enzyme Immunoassay (EIA) or ELISA Methods*
    - *Magnetic Immunobeads Method*

Nucleic Acid Probe for Detection of Pathogens

Hybridization Method

Measurement of Microbial Level:

Several indirect methods to estimate microbial levels or loads in a food have been developed. They are relatively more rapid than the traditional CFU enumeration method and some are also automated.

Sr. no.	QUESTION	ANSWER
1	Main objective for qualitative methods	to determine whether a sample contains viable cells or spores of a specific pathogen
2	Which method is used to detect toxin in food	Rapid method and automation
3	Which methods used for the quantitative or qualitative detection of microorganisms in foods?	The traditional or conventional methods.
4	Full form of EIA	Enzyme immunoassay
5	List of two methods for nucleic acid probe detection of pathogen	Hybridization method & polymerase chain reaction

AGMark

AGMark is a certification mark employed on agricultural products in India, assuring that they conform to a set of standards approved by the *Directorate of Marketing and Inspection*, an agency of the Government of India.

The *AGMARK* is legally enforced in India by the *Agricultural Produce (Grading and Marking) Act of 1937 (and amended in 1986)*. The present *AGMARK* standards cover quality guidelines for 205 different commodities spanning a variety of Pulses, Cereals, Essential Oils, vegetable oils, Fruits & Vegetables, and semi-processed products like Vermicelli.

The term AGMARK was coined by joining the words 'Ag' to mean agriculture and 'mark' for a certification mark. This term was introduced originally in the bill presented in the parliament of India for the *Agricultural Produce (Grading and Marking) Act.*

The entire system of AGMARK, including the name, was created by Archibald Macdonald Livingstone, Agricultural and Marketing Advisor to the Government of India, from 1934 to 1941.

He was supported by a staff of several hundred. The system was designed to benefit local growers throughout India who were, in the absence of a certification as to quality, exposed to receiving less for their produce from dealers than its true worth.

AGMARK Laboratories

The AGMARK certification is employed through fully state-owned AGMARK laboratories located across the nation which act as testing and certifying centers. In addition to the *Central AGMARK Laboratory (CAL)* in Nagpur, there are *Regional AGMARK Laboratories (RALs)* in 11 nodal cities (Mumbai, New Delhi, Chennai, Kolkata, Kanpur, Kochi, Guntur, Amritsar, Jaipur, Rajkot, Bhopal). Each of the regional laboratories is equipped with and specializes in the testing of products of regional significance. Hence the product range that could be tested varies across the centers.

Commodities and tests

The testing done across these laboratories include chemical analysis, microbiologic al analysis, pesticide residue, and aflatoxin analysis on whole spices, ground spices, ghee, butter, vegetable oils, mustard oil, honey, food grains (wheat), wheat products (atta, suji, and Maida), gram flour, soyabean seed, Bengal gram, ginger, oil cake, essential oil, oils and fats, animal casings, meat and food products.

Sr. no.	**QUESTION**	**ANSWER**
1	The AGMark is legally enforced in india by whom?	Agricultural produce act of 1937
2	The present AGMark cover……… different comodities	205
3	The term AGMark was coined by joining the words ……..	Ag to mean agriculture and mark for a certification mark
4	The entire system of AGMark is created by ……….	Macdonald livingstone
5	Full form of RALs	Regional AGMARK laboratories

BRIEF INTRODUCTION ABOUT FERMENTED FOODS: PICKLES, SAUERKRAUT, SILAGE, SAUSAGES & BREAD

Bread

- Bread is one of the most ancient of human foods, and is produced with the help of microorganisms. The use of yeasts to leaven bread is carefully depicted in paintings from ancient Egypt.
- A bakery at the Giza Pyramid area, from the year 2575 B.C., has been excavated. It is estimated that 30,000 people a day were provided with bread from this bakery.
- In bread making, yeast growth is carried out under aerobic conditions. This results in increased CO_2 production and minimum alcohol accumulation. The fermentation of bread involves several steps: alpha- and beta-amylases present in the moistened dough release maltose and sucrose from starch.
- Then a baker's strain of the yeast *Saccharomyces cerevisiae*, which produces maltase, invertase, and zymase enzymes, is added. The CO_2 produced by the yeast results in the light texture of many breads, and traces of fermentation products contribute to the final flavor.
- Usually, bakers add sufficient yeast to allow the bread to rise within 2 hours—the longer the rising time, the more additional growth by contaminating bacteria and fungi can occur, making the product less desirable. By using more complex assemblages of microorganisms, bakers can produce special breads such as sour doughs.
- The yeast *Saccharomyces exiguus*, together with a *Lactobacillus* species, produces the characteristic acidic flavor and aroma of such breads. Bread products can be spoiled by *Bacillus* species that produce ropiness. If the dough is baked after these organisms have grown, stringy and ropy bread will result, leading to decreased consumer acceptance.
- Acid fermentation by lactic and coliform bacteria that is normal in flour pastes or dough may be too extensive if too much time is permitted resulting that the dough and bread made from it may be too 'sour'. Excessive growth of proteolytic bacteria during this period may destroy some of the gas holding capacity so essential during the rising of the dough and produce a sticky dough.

Sticky doughs – due to the result of over mixing or gluten breakdown by reducing agents Ex: glutathione. Production of undesirable flavors other than sourness.

Chief types of microbial spoilage of baked bread have been moldiness and ropiness, usually termed as mold and rope.

Mold: Molds are most common and most important cause of the spoilage of bread and most bakery products. Temperatures attained in the baking procedure usually are high to kill mold spores in and on the loaf, so that molds must reach the outer surface or penetrate after baking. Contamination may come from air during cooling, handling, from wrappers and initiate growth in the crease of load and between the slices. Chief molds involved in the spoilage of bread are

Bread mold – *Rhizopus stolonifera* or *Rhizopus nigricans* bread mold contains white cottony mycelium and black dots of sporangia.

Green spores are produced by *Penicillium expansum*, *Penicillium stoloniferum*, *Aspergillus niger*. Greenish or purplish-brown to black conidial heads and yellow pigment diffusing into the bread. *Monilia sitophila* – produces pink conidia give a pink or reddish color to its growth. Species of *Mucor* or *Geotrichum* also contaminate the bread and produces spores.

Mold spoilage is favored by

- Heavy contamination after baking due to air heavily laden with mold spores, a long cooling time, considerable air circulation or a contaminated slicing machine.
- Slicing in that more air is introduced into the loaf.
- Wrapping, especially if the bread is warm when wrapped.
- Storage in a warm & humid place.

Little contamination occurs, if the relative humidity below 90%. Bread with 6% of milk solids retains moisture somewhat better than does milk free bread and hence there is less moisture between loaf and wrapper and hence less molding. Various methods are employed to prevent moldiness of bread:

Prevention of contamination of bread with mold spores insofar as practicable. Air in the bread has low spores. Filtration and washing of air to the room and irradiation or the room and more especially the air by means of U.V. rays cut down contamination.

Prompt and adequate cooling of the loaves before wrapping to reduce condensation of moisture beneath the wrapper. U.V. irradiation of the surface of the loaf and of slicing knives.

Destruction of molds on the surface by electronic heating. Keeping the bead cool to slow mold growth or freezing and storage in the frozen condition to prevent growth entirely.

Incorporation of mycostatic material in the bread dough. Ex: Sodium or calcium propionate 0.1-0.3%, Sodium acid – 0.1% Sodium diacetate – 0.32%, addition of vinegar or acetate to the dough or treatment of the exterior of the loaf with vinegar.

Rope: Ropiness of bread is fairly common in-home baked bread, especially during weather.

Ropiness is uncommon in commercially baked bread, because of the preventive measures now employed. Ropiness caused by mucoid variant of *Bacillus subtilis, Bacillus licheniform is*, (*B. mesentericus*), spores of these bacteria can withstand the temperature of the bread during baking, which does not exceed 100°C. Ropy condition due to capsulation of bacillus, together with hydrolysis of the flour proteins (gluten) by proteinases of the organism and starch by amylase.

Area of ropiness is yellow to brown in color and is soft and sticky to the touch. Sometimes slimy material can be drawn out into long threads, when the bread is broken and pulled apart. Unpleasant odor described as that of decomposed or overripe melons.

First odor is evident, then discoloration and finally softening of the crumb, with stickiness and stringiness.

Red Bread

Red bread or bloody bread is due to the occurrence of red color from the growth of pigmented bacteria *Serratia marcescens*. In ancient times, appearance of red color was considered miraculous.

Moist conditions favor growth. Molds such as *Monilia sitophila* may impart red color to bread. Red color in the crumb of dark bread has been caused by *Geotrichum aurantiacum* (syn, *Oidium aurantiacum*).

Chalky bread:

Chalky bread is due to white, chalk like spots. It is due to the yeast like fungi *Endomycopsis fibuligera* and *Trichosporon variable*.

Sr. no.	QUESTION	ANSWER
1	Which type of yeast strain is used in bread making process	Baker's strain
2	What is the reason behind sticky dough	due to the result of over mixing or gluten breakdown by reducing agents
3	Give name of bread mold	Rhizopus stolonifera or Rhizopus nigricans
4	Hoe red bread is occur	Due to the occurrence of red color from the growth of pigmented bacteria *Serratia marcescens*.
5	Chalky bread is due to……..	White, chalk like spots

Pickles

Sauerkraut

Characteristics

Sauerkraut is produced by fermenting shredded cabbage. The product has a sour taste with a clean acid flavor.

Processing

1. Cabbage cleaned, trimmed, and shredded fine and uniform.
2. Packaged tight to exclude air in vat, and layered with salt (2.25%).
3. Top covered to exclude air, and fermented at 18°C (65°F) for 2 months.

Fine shredding helps the sugars (3 to 6%) come out of cabbage cells. Tight packaging helps create an anaerobic condition, thus preventing the growth of aerobes. Salt stimulates growth of some lactic acid bacteria, and discourages the growth of some undesirable bacteria and pectinase (in cabbage) action. The top is covered to exclude air

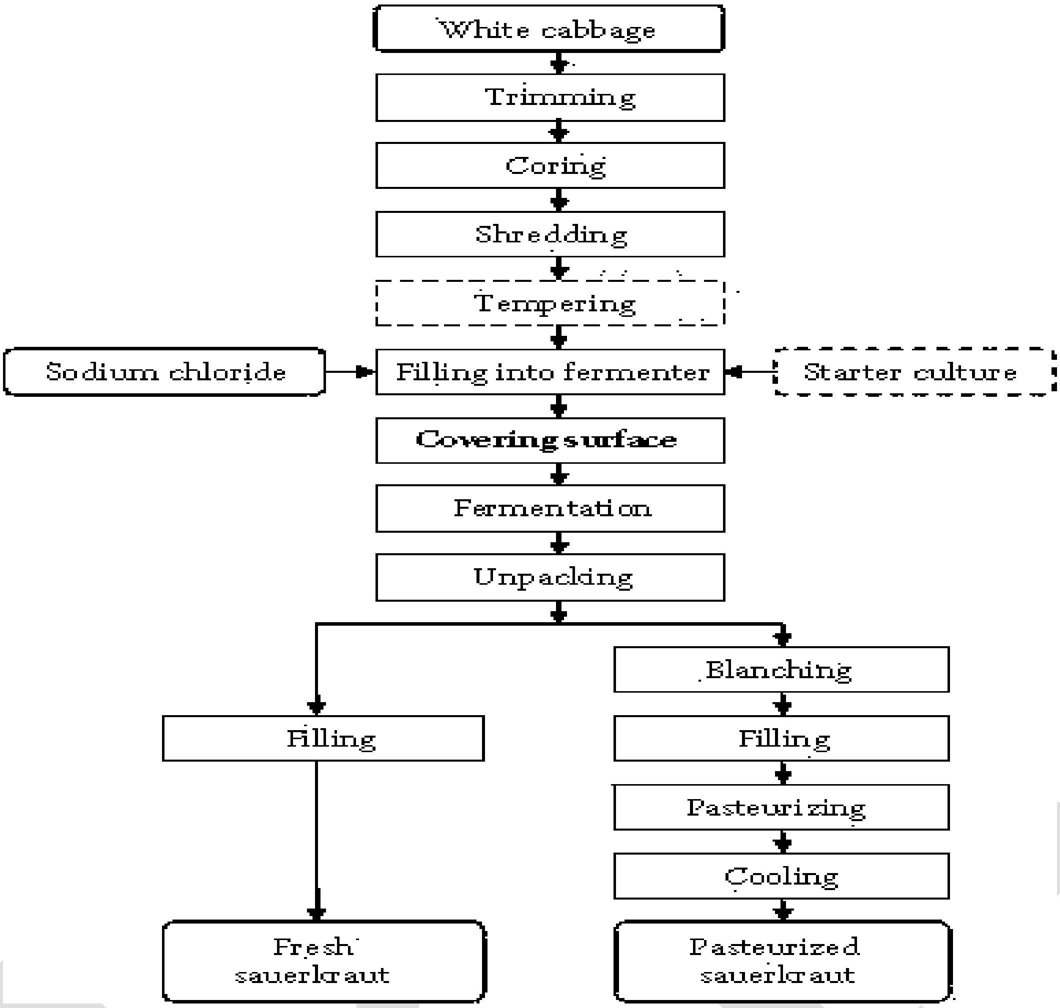
and prevent growth of some aerobes. Fermentation at 18< C (65< F) discourages the rapid growth of some undesirable bacteria (facultative anaerobic or anaerobic), but encourages the growth of desirable lactic acid bacteria. Natural inhibitors in cabbage also discourage the growth of undesirable Gram-negative and Gram-positive bacteria.

Leuconostoc spp. metabolize sucrose, hexoses, and some pentoses in the raw material

to lactate, acetate, ethanol, CO_2, and diacetyl. *Lab. brevis* (obligatory heterofermentative, such as *Leuconostoc* spp.) ferments sucrose, hexoses, and pentoses to products similar to those by *Leuconostoc spp*. *Ped. pentosaceus* metabolizes hexoses to form mainly lactic acid and some pentoses to lactic acid, acetate, and ethanol. *Lab. plantarum* also produces products from sucrose, hexoses, and pentoses similar to those by *Ped. pentosaceus*. *Leuconostoc* spp. produces D (–) lactate, whereas the other three species produce DL- lactate. The characteristic flavor of sauerkraut is the result of the combined effects of lactate, acetate, ethanol, CO_2, and diacetyl in proper amounts.

Microbial Problems

Off-flavor, soft texture, and discoloration of sauerkraut can occur by growth of molds and yeasts when air is not completely excluded. A slimy texture of sauerkraut can occur due to overgrowth of *Leuconostoc* spp. in the presence of sucrose; they metabolize fructose but synthesize dextran from glucose.




Sr. no.	QUESTION	ANSWER
1	Sauerkrute is produced from……..	Shredded cabbage
2	Give processing for sauerkrute	Cabbage cleaning, trimming, shredding and

		packaging
3	Which condition promoting growth of arobic micro?	Tight packging
4	Off flavor and soft texture can occure by…….	Yeast and molds
5	Salt stimulate the growth of…….. in saurkrute	Lactic acid bacteria

Silage

Silage is fermented, high-moisture stored fodder which can be fed to ruminants (cud- chewing animals such as cattle and sheep) or used as a biofuel feedstock for anaerobic digesters. It is fermented and stored in a process called *ensilage*, *ensiling* or *silaging*, and is usually made from grass crops, including maize, sorghum or other cereals, using the entire green plant (not just the grain). Silage can be made from many field crops, and special terms may be used depending on type (*oatlage* for oats, *haylage* for alfalfa – but see below for the different British use of the term *haylage*).

Silage is made either by placing cut green vegetation in a silo, by piling it in a large heap covered with plastic sheet, or by wrapping large bales in plastic film.

Silage undergoes anaerobic fermentation, which starts about 48 hours after the silo is filled, and converts sugars to acids.

Fermentation is essentially complete after about two weeks.

Before anaerobic fermentation starts, there is an aerobic phase in which the trapped oxygen is consumed.

The closeness with which the fodder is packed, determines the nature of the resulting silage by regulating the chemical reactions that occur in the stack.

When closely packed, the supply of oxygen is limited; and the attendant acid fermentation brings about the decomposition of the carbohydrates present into acetic, butyric and lactic acids.

This product is named sour silage. If, on the other hand, the fodder is unchaffed and loosely packed, or the silo is built gradually, oxidation proceeds more rapidly and the temperature rises; if the mass is compressed when the temperature is 140 to 160 Fahrenheit, the action ceases and sweet silage results.

The nitrogenous ingredients of the fodder also suffer change: in making sour silage as much as one-third of the albuminoids may be converted into amino and ammonium compounds; while in making sweet silage, a smaller proportion is changed, but they become less digestible.

If the fermentation process is poorly managed, sour silage acquires an unpleasant odor due to excess production of ammonia or butyric acid (the latter is responsible for the smell of rancid butter).

In the past, the fermentation was conducted by indigenous microorganisms, but, today, some bulk silage is inoculated with specific microorganisms to speed fermentation or improve the resulting silage. Silage inoculants contain one or more strains of lactic acid bacteria, and the most common is *Lactobacillus plantarum*.

Other bacteria used in inoculants include *Lactobacillus buchneri*, *Enterococcus faecium* and *Pediococcus* species.

Silage must be firmly packed to minimize the oxygen content, or it will spoil. Silage goes through four major stages in a silo:

- Presealing, which, after the first few days after filling a silo, enables some respiration and some dry matter (DM) loss.
- Fermentation, which occurs over a few weeks; pH drops; there is more DM loss, but hemicellulose is broken down; aerobic respiration stops.
- Infiltration, which enables some oxygen infiltration, allowing for limited microbial respiration; available carbohydrates (CHOs) are lost as heat and gas.
- Emptying, which exposes surface, causing additional loss; rate of loss increases.


Sr. no.	QUESTION	ANSWER
1	What is silage?	Silage is fermented, high-moisture stored fodder which can be fed to ruminants or used as a biofuel feedstock for anaerobic digesters
2	Fermentation of silage complete after….. weeks	2
3	How fermentation of silage was	by indigenous

	done in past?	microorganisms ,
4	Most common bacteria in silage fermentation	L. *plantarum*
5	How oxygen is minimized in silage	By firmly packing

Sausages

1. Meat, salts, glucose, cure, spices, and starter mixed uniformly.
2. Stuffed in casings, fermented at 85 to 110< F (29.4 to 43.3°C) with 80 to 90% relative humidity.
3. Incubated until the pH drops to ca. 5.2 to 4.6, cooked to 140< F (60°C) internal temperature, and cooled to 50< F.
4. Stored at 40 to 50< F (4.4 to 10°C) for 3 to 4 d, vacuum-packaged, and consumed directly.

- Cures contain nitrite to give a final concentration of ca. 100 ppm. Fermentation can be carried out in a smokehouse. Fermentation time is usually 8 to 12 h, during which the pH is dropped to desired level.
- *Starters (Controlled or Natural Fermentation)*
- In controlled fermentation, frozen or dried concentrates are used directly at 10^{6-7} cells/g mix. Starters should not be mixed with salt, cure, or spices as it can kill injured cells. Instead, they should be thawed and immediately put into the meat.
- Starters vary, depending on the fermentation temperature and final pH of the product desired. For high temperature and low pH, *Pediococcus acidilactici* strains are preferred; for low temperature and high pH, *Lab. plantarum* strains are preferred. *Ped. pentosaceus* strains can be used under both conditions. Some starters can have both *Pediococcus* and *Lactobacillus* species.
- In addition, selected *Micrococcus* spp. or *Sta. carnosus* strains are added as secondary flora for their beneficial effects on desired product color.
- Slow acid production can be a serious problem if the starters used are not metabolically active or have lower numbers of viable cells or due to other factors such as low glucose and high salts in the mix. Sour or no flavor can occur if the starter, especially *Ped.*
- *acidilactici*, grows very rapidly and reduces the pH to below 4.5. Gas formation can occur because of growth of *Leuconostoc* spp. during fermentation and during storage in vacuum packages. *Leuconostoc* spp. are usually present in raw meat.
- Pathogens, when present in meat, can grow if acid production is slow during

fermentation. Acid-resistant pathogens can also survive in the products and cause health hazards. During long storage or curing, biogenic amines can form. Also, mycotoxin-producing molds can grow on the product surface during curing.


- Single cell proteins develop when microbes ferment waste materials (including wood, straw, cannery and food processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta.

- The problem with extracting single cell proteins from the wastes is the dilution and cost. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation and filtration, or the use of semi-permeable membranes.

- The single cell protein needs to be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels, and de-watering process require equipment that is expensive and not always suitable for small-scale operations.

- It is economically prudent to feed the product locally and shortly after it is produced.

Biomass and Single Cell Protein:

- Microbial cells are produced for two main applications, as a source of protein for animal or human food, (Single Cell Protein) or (b) for use as a commercial inoculum in food fermentations and for agriculture and waste treatment. As a commodity, SCP must be competitive with commercial animal and plant proteins, in terms of price and nutritional value and must conform to human and animal food safety requirements. Productivity, yield and selling price are the major factors affecting the economics of SCP production.

- Microbial inoculants, which are used as a process aid, generally have a higher value. In this case, the objective of the production process is to optimize yield of viable cells of defined biological activity with good shelf-life characteristics.

- *Saccharomyces cerevisiae,* is categorized primarily as a microbial inoculant. Inactive dried brewers or baker's yeast is also used as a dietary source of vitamins and trace minerals in specific medical conditions. Considerable amounts of yeast extract are produced from baker's yeast as a source of flavor and vitamins.

- Yeast, fungi, bacteria, and algae are grown on hydrocarbon wastes, and cells are harvested as sources of protein. It has been calculated that 100 lbs. of yeast will produce *250* tons of proteins in 24 hours, whereas a 1000 lbs. steer will synthesize only llb of protein 24 hours and this after consuming 12 to 20 lbs.

of plant proteins. Similar, algae grown in ponds can produce 20 tons (dry weight) of protein, per acre, per year.

- Single cell protein basically comprises proteins, fats carbohydrates, ash ingredients, water, and other elements such as phosphorus and Potassium. The composition depends upon the organism and the substrate which it grows.
- Some typical compositions which are compared with soymeal and fish meal. If SCP is to be used successfully, there are five main criteria to be satisfied;

- The SCP must be safe to eat.
- The nutritional value dependent on the amino acid composition must be high.
- It must be acceptable to the general public.
- It must have the functionality, i.e., characteristics, which are found in common staple foods.
- The economic viability of the SCP process is extremely complex and is yet to be demonstrated.

ADVANTAGES OF SCP

However, in many countries, use of microbial biomass as a supplement to diet has met with scepticism because of certain psychological barriers. But even in these countries, it will play a major role via SCP feeding to animals which will be then consumed by the humans. As compared with traditional methods of producing proteins for food or feed, large scale production of microbial biomass has the following advantages:

- Microorganisms in general have a high rate of multiplication.
- Microbes have high protein content.
- They can utilize a large number of different carbon sources, some of which are waste
- Strains with high yield and good composition can be selected or produced relatively easily.
- Microbial biomass production is independent of seasonal and climatic variation. Products.

DISADVANTAGES OF SCP

However, there are certain disadvantages also, of using microbial biomass as supplement to diet. Many microorganisms produce toxic substances and it has to be made sure that the biomass does not contain any such substance.

Sometimes the microorganisms when taken in, lead to indigestion and allergenic reactions. The high nucleic acid in many microbial biomass products is also undesirable There are several limitations in the use of SCP for human consumption and they are due to

- ➢ high nucleic acid contents of many microorganisms that would result in kidney stone formation or gout
- ➢ poor digestibility, gastrointestinal problems and skin reactions and
- ➢ the possible presence of toxic or carcinogenic compounds from residues of substrates. The high capital costs and the need for sterility controls render SCP expensive in developing countries where food shortages are common. As animal feed, SCP grown on agricultural residues should certainly found a place in the future economy of developing nations. It is however noteworthy that Dabur in India is manufacturing and selling spirulina for human consumption.

Sr. no.	QUESTION	ANSWER
1	Full form of SCP	Single cell protein
2	Yeast will produced ……… tons of protein in hydrocarbon waste	250
3	Incubation time for sausage	3-4 days
4	Which microorganisms can be used as scp	Yeast, fungi, bacteria and algae
5	SCP is basically is……..	Comprises protein

Mushrooms

Edible mushrooms are the fleshy and edible fruit bodies of several species of macro fungi (fungi which bear fruiting structures that are large enough to be seen with the naked eye). They can appear either below ground (hypogenous) or above ground (epigeous) where they may be picked by hand. Edibility may be defined by criteria that include absence of poisonous effects on humans and desirable taste and aroma.

Edible mushrooms are consumed by humans as comestibles for their nutritional value and they are occasionally consumed for their supposed medicinal value. Mushrooms consumed by those practicing folk medicine are known as medicinal mushrooms.

While hallucinogenic mushrooms (e.g., psilocybin mushrooms) are occasionally consumed for recreational or religious purposes, they can produce severe nausea and disorientation, and are therefore not commonly considered edible mushrooms.

Edible mushrooms include many fungal species that are either harvested wild or cultivated. Easily cultivatable and common wild mushrooms are often available in markets, and those that are more difficult to obtain (such as the prized truffle and matsutake) may be collected on a smaller scale by private gatherers.

Some preparations may render certain poisonous mushrooms fit for consumption.

Before assuming that any wild mushroom is edible, it should be identified. Accurate determination and proper identification of a species is the only safe way to ensure edibility, and the only safeguard against possible accident.

Some mushrooms that are edible for most people can cause allergic reactions in some individuals, and old or improperly stored specimens can cause food poisoning. Great care should therefore be taken when eating any fungus for the first time, and only small quantities should be consumed in case of individual allergies.

Deadly poisonous mushrooms that are frequently confused with edible mushrooms and responsible for many fatal poisonings include several species of the *Amanita* genus, in particular, *Amanitaphalloides*, the *death cap*. It is therefore better to eat only a few, easily recognizable, species, then to experiment indiscriminately.

Moreover, even species of mushrooms that are normally edible may be dangerous, as mushrooms growing in polluted locations can accumulate pollutants such as heavy metals.

- *Agaricus bisporus*, also known as champignon and the button mushroom. This species also includes the portobello and cremini mushrooms.
- *Auricularia polytricha* or *Auricularia auricula-judae* (Tree ear fungus), two closely

related species of jelly fungi that are commonly used in Chinese cuisine.

- *Flammulina velutipes*, the "winter mushroom", also known as *enokitake*in Japan
- *Hypsizygus tessulatus* (also *Hypsizygus marmoreus*), called *shimeji* in Japanese, it is a common variety of mushroom available in most markets in Japan. Known as "Beech mushroom" in Europe.
- *Lentinus edodes*, also known as shiitake, oak mushroom. *Lentinus edodes* is largely produced in Japan, China and South Korea. *Lentinus edodes* accounts for 10% of world production of cultivated mushrooms. Common in Japan, China, Australia and North America.
- *Pleurotus* species are the second most important mushrooms in production in the world, accounting for 25% of total world production. *Pleurotus* mushrooms are cultivated worldwide; China is the major producer. Several species can be grown on carbonaceous matter such as straw or newspaper. In the wild they are usually found growing on wood.

Substrates

Mushroom production converts the raw natural ingredients into mushroom tissue, most notably the carbohydrate chitin.

An ideal substrate will contain enough nitrogen and carbohydrate for rapid mushroom growth. Common bulk substrates include several of the following ingredients:

- Wood chips or sawdust
- Mulched straw (usually wheat, but also rice and other straws)
- Straw bedded horse or poultry manure
- Corncobs
- Waste or recycled paper
- coffee pulp or grounds
- Nut and seed hulls
- Cottonseed hulls
- Cocoa bean hulls
- Cottonseed meal
- Soybean meal
- Brewer's grain
- Ammonium nitrate
- Urea

Mushrooms metabolize complex carbohydrates in their substrate into glucose, which is then transported through the mycelium as needed for growth and energy. While it is used as a main energy source, its concentration in the growth medium should not exceed 2%. For ideal fruiting, closer to 1% is ideal.

The six steps of growing mushrooms include the following:

1. Compost preparation
2. Compost readiness
3. Spawning the mushrooms

4. Casing
5. Pinning
6. Cropping

Sr. no.	QUESTION	ANSWER
1	What is mushrooms	It is edible body of several species of macro fungi
2	Mushrooms can apper in……	Hypogenous& epigeous
3	Give name of two substance for the growth of mushrooms	Corncobs& wood chips
4	Concentration of urea for mushroom cultivation	Not exceed 2%
5	Steps for mushrooms cultivation	Compost preparation, Compost readiness, Spawning the mushrooms, Casing, Pinning and Cropping