KARPAGAM ACADEMY OF HIGHER EDUCATION

Class: II M.Sc Microbiology

COURSE NAME: FOOD MICROBIOLOGY

Subject **code: 18MBP302**

S y l l a b u s

M.Sc. Microbiology **2019-2020**

Semester - III

18MBP302 **FOOD MICROBIOLOGY** **4H –4C**

Instruction Hours / Week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

COURSE OBJECTIVES

This paper adds information about the role of microorganisms in many food, beverage and pharma industries both in production and spoilage processes.

COURSE OUTCOME (CO'S)

To encode the importance of the role of microorganisms in food industries both in beneficial and harmful ways. To obtain a good understanding of industrial microbiology and become qualified as microbiologist in food and other industries.

UNIT – I

Food and microorganisms – Important microorganisms in food – Fungi, Bacteria; Intrinsic and extrinsic parameters of food affecting microbial growth – sources of contamination of food. Food sanitation – indicators of food safety – Coliform bacteria.

UNIT – II

Food preservation – principles – factors affecting preservation – food preservation using temperature – low temperature food preservation – characteristics of psychrotrophs – high temperature food preservation – characteristics of thermophiles – preservation of foods by drying chemicals and radiation – limitations – commercial application.

UNIT – III

Food borne diseases - food poisoning - food borne infection and intoxication- Food control agencies - microbiological criteria for food, microbial quality control and food laws, Hazard Analysis Critical Control Point (HACCP).

UNIT – IV

Microorganisms in Foods and methods for detection: Fresh meat, Processed meat and poultry, Culture, Microscopic, and Sampling Method for detecting microbes, Physical, Chemical methods, Whole animal assays, Immunological methods.

UNIT – V

Applications of Food Microbiology: Beneficial Uses of Microorganisms in Food, Intestinal Beneficial Bacteria-Concept of Prebiotics and Probiotics, Genetically modified foods.

Biosensors in food.

SUGGESTED READINGS

TEXT BOOKS

1. Banwart, G.J. (2004). *Basic Food Microbiology*. (2nd ed.). CBS Publishers and Distributors New Delhi.
2. Casida, L.E. Jr., (2003). *Industrial Microbiology*. New Age International Publishers, New Delhi.
3. Doyle, M.P., Beuchat, R.L., and Montuille, T.J., (2001). *Food Microbiology – Fundamentals and Frontiers*. ASM press.
4. Frazier, W.C., and Westhoff, D.C., (1995). *Food Microbiology*. Tata McGraw-Hill Publishing Company Limited, New Delhi.
5. Adams, M.R. and Moss, M.O. 2008. Food Microbiology, RSC Publishing, Cambridge, UK.
6. Blackburn C. de W. 2006, Food spoilage microorganisms, Woodhead Publishing, Cambridge, UK
7. Ray. B. 2000. Fundamental Food Microbiology. 2nd Edition. CRC Press. New York. USA.Press, New York.

REFERENCES

1. Atlas, R.N., and Bartha, R., (2000). *Microbial Ecology - Fundamental and Applications*. (3rd ed.). Redwood City CA. Benjamin/Cumming Science Publishing Co., New Delhi.
2. Gould, G.W. (1996). *New Methods of Food Preservation*. Blackie Academic and Professional, Madras.
3. Jay, J.M. (2000). *Modern Food Microbiology*. CBS Publishers and Distributors, New Delhi.

LECTURE PLAN

UNIT I

UNIT I S. No	Duration	Topic	Reference
1	1	Food and microorganisms	T1: 33-38
2	1	Important microorganisms in food	T1: 39-56,17-33
3	1	Important microorganisms in food – fungi	T1: 39-56,17-33
4	I	Important microorganisms in food bacteria	T1: 39-56,17-33
5	1	Intrinsic and extrinsic parameters of food affecting microbial growth	R4: 18-45
6	1	Sources of contamination of food	R4: 51-60
7	1	Food sanitation	T1: 479-493
8	1	Indicators of food safety; Coliform bacteria	R4: 303-305; T1: 56-57
9	1	Unit revision	
Total Hrs: 9			

T1: Frazier, WC and DC. Westhoff, 1995. Food Microbiology, Tata McGraw Hill Publishing company Ltd, New Delhi.

R4: Adams, MR and Mo. Moss, Food Microbiology, Tata Mc Graw Hill Publishing company Ltd, New Delhi.

KARPAGAM ACADEMY OF HIGHER EDUCATION

Class: II M.Sc Microbiology **COURSE NAME: FOOD MICROBIOLOGY**

Subject **code: 18MBP302 UNIT-I** **BATCH – 2018-2020**

LECTURE PLAN

UNIT II

UNIT I S. No	**Duration**	**Topic**	**Reference**
1	1	Introduction to food preservation	T1: 83-86
2	1	Food preservation – principles	T1: 86-89
3	1	Factors affecting food preservation	T1: 90-98
4	1	Food preservation using low temperature and characters of thermophiles	T1: 101-117
5	1	Food preservation using high temperature and characters of thermophiles	T1: 125-138
6	1	Food preservation by drying, and	T1: 143-160
7	1	Food preservation chemicals	T1: 143-160
8	1	Food preservation radiation	T1: 143-160
9	1	Limitations and commercial application of food preservation	T1: 161-169
10		Unit revision	
Total Hrs: 10			

T1: Frazier, WC and DC. Westhoff, 1995. Food Microbiology, Tata McGraw Hill publishing company Ltd, New Delhi.

KARPAGAM ACADEMY OF HIGHER EDUCATION

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LECTURE PLAN

UNIT III

UNIT I S. No	Duration	Topic	Reference
1	1	Food borne diseases	T1: 404-415
2	1	Food poisoning	T1: 412-426
3	1	Food borne infection and	T1: 423-456
4	1	Food intoxication	T1: 423-456
5	1	Food control agencies; HACCP	T1: 495-501; T1: 495- 501
7	1	Microbiological criteria for food	T1: 505-506
8	1	Microbial quality control	R4: 323-325
9	1	Food laws and hazard analysis	R4: 112-114, 349-358
10	1	Food intoxication	T1: 423-456
Total Hrs: 9			

T1: Frazier, WC and DC. Westhoff, 1995. Food Microbiology, Tata McGraw Hill Publishing company Ltd, New Delhi.

R4: Adams, MR and Mo. Moss, Food Microbiology, Tata Mc Graw Hill Publishing company Ltd, New Delhi.

KARPAGAM ACADEMY OF HIGHER EDUCATION

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Subject **code: 18MBP302 UNIT-I** **BATCH – 2018-2020**

LECTURE PLAN

UNIT IV

UNIT I S. No	Duration	Topic	Reference
1	1	microorganisms in food	W1
2	1	Microorganism in foods and methods for detection	W2
3	1	Processed meat	W4
4	I	Poultry	W5
5	1	Culture, microscopic and sampling methods for detecting microbes-physical	W6
6	1	Culture, microscopic and sampling methods for detecting microbes-chemical method	W7
7	1	Whole animal analysis	W8
8	1	Immunological methods	W9
9	1	Unit revision	
Total Hrs: 9			

W1. Manisa . S. How to Detect Microorganisms in Food: Methods and Techniques

W2. http://www.biotechnologynotes.com/food-biotechnology/microorganisms-in-food/how-to-detect-microorganisms-in-food-methods-and-techniques-biotechnology/14130

W3. https://link.springer.com/chapter/10.1007/978-1-4615-7476-7_4

W4. http://ecoursesonline.iasri.res.in/mod/page/view.php?id=5126

W5.Dorota Witkowska and Janina Sowińska. Identification of Microbial and Gaseous Contaminants in Poultry Farms and Developing Methods for Contamination Prevention at the Source. February 15th 2017

W6. Amélie Rouger, Odile Tresse, and Monique Zagorec. Bacterial Contaminants of Poultry Meat: Sources, Species, and Dynamics. Microorganisms. 2017 Sep; 5(3): 50.

W7. Mead GC. Microbiological quality of poultry meat: a review. Rev. Bras. Cienc. Avic. vol.6 no.3 Campinas July/Sept. 2004

W8. http://ecoursesonline.iasri.res.in/mod/page/view.php?id=5126

UNIT V

UNIT I S. No	Duration	Topic	Reference
1	1	Application of food microbiology-Introduction	W1
2	1	Application of food microbiology	W2
3	1	Beneficial uses of microorganisms in food	W3, R1
4	I	Intestinal beneficial bacteria	W1
5	1	Prebiotics and Probiotics	W2, W3
6	1	Genetically modified foods	W
7	1	Biosensors in food	W1, W3
8	1	Unit revision	
Total Hrs: 9			

W1. https://www.toppr.com/guides/biology/microorganisms/microorganisms-and-its-uses/

W2. https://loveyourgut.com/all-entries/bacteria-and-the-large-intestine/

W3. https://nccih.nih.gov/health/probiotics/introduction.htm

R1. Régine Talon and Monique Zagorec' Special Issue: Beneficial Microorganisms for Food Manufacturing—Fermented and Biopreserved Foods and Beverages. Microorganisms. 2017 Dec; 5(4): 71.

R2. Geraldine O. Canny, Beth A. McCormick. Bacteria in the Intestine, Helpful Residents or Enemies from Within?Infection and Immunity Jul 2008, 76 (8) 3360-3373.

Unit - 1

Introduction of Food Microbiology:

Both foods of plant and animal origin normally carry a microflora on the surface of their parts. The natural microflora determined by type of plant or animal and environmental conditions, every food may be contaminated from outside sources on the way from the field to the processing plant, or during storage, transport and distribution.

There are thousands of different types of micro-organisms everywhere in air, soil and water, and consequently on foods, and in the digestive tract of animals and human. Fortunately, the majority of micro-organisms perform useful functions in the environment and also in some branches of food industry, such as production of wine, beer, bakery products, dairy products etc. On the other hand unwanted spoilage of foods is generally caused by micro-organisms and contamination of food with pathogens causes food safety problems.

Growth of micro organisms in food obviously will increase number of micro organisms. Pretreatment of foods may remove or destroy some kinds of micro organisms and inactivate part or all of the food enzymes. Washing may remove organisms from the surface or may add some from the wash water. If washing is by means of an antiseptic or germicidal solution, numbers of organisms may be greatly reduced. High temperatures will kill more organisms' treatment with rays, ozone, SO2, germicidal vapors will reduce numbers.

The micro-organisms occurring on and/or in foods are from a practical point of view divided into three groups: molds, yeast and bacteria.

- Molds are generally concerned in the spoilage of foods; their use in the food industry is limited (e.g. mold ripened cheese).
- Yeasts are the most widely used micro-organisms in the food industry due to their ability to ferment sugars to ethanol and carbon-dioxide. Some types of yeast, such as baker's yeasts are grown industrially, and some may be used as protein sources, mainly in animal feed.
- Bacteria important in food microbiology may be divided into groups according to the product of fermentation, e.g. lactic acid bacteria, acetic acid bacteria, propionic acid bacteria.. Bearing in mind the food constituent attacked (used as food for microorganisms), proteolytic, saccharolytic and lipolytic.

In the framework of this article a brief overview will be given about micro-organisms that play an important role in production, storage and consumption of foods. Their occurrence, characteristics used for identification, conditions of growth and spoilage of microorganisms.

Important Microorganisms in Food Microbiology

Mold is a type of fungus that consists of small organisms found almost everywhere, that grows in the form of multicellular filaments called *hyphae*. They can be black, white, orange, green, or purple. Outdoors, molds play an important role in nature, breaking down dead leaves, plants, and trees. Molds thrive on moisture and reproduce by means of tiny, lightweight spores that travel through the air.

Generally molds are concerned in the spoilage of foods; moldy or mildewed food is considered unfit to eat. On the other hand some of molds are used in manufacture of different foods and are ingredients of some foods. Some kinds of cheese are mold-ripened (e.g. Roquefort, Camembert). Molds are grown as feed and food and are employed to produce products used in foods, such as amylases and other enzymes for bread making or citric acid used in soft drinks. Molds are major contributors in the ripening of many oriental foods. A species of *Bothrytis cinerea*, is responsible for the noble rot of grape. Molds are used for production of several antibiotics.

Morphological Characteristics

The gross appearance of a mold growing on a food is often enough to indicate its genus. Some molds are fluffy, others are compact. Some look velvety on the upper surface, some dry and powdery, and others wet or gelatinous. Pigments in the mycelium-red, purple, gray, black, etc.-are also characteristic. Some molds are restricted in size, but others seem limited only by the food container.

- *Hyphae and Mycelium:* The mold thallus consists of a mass of branching, intertwined filaments called hyphae (singular hypha), and the whole mass of these hyphae is known as the mycelium. Hyphae may be classed as vegetative or fertile based on their biological function. The vegetative hyphae or growing hyphae are concerned with the nutrition of the mold and the fertile ones with the production of reproductive parts.
- Molds are divided into two groups: septate, and non-septate hyphae. Septate, i.e., with cross walls dividing the hypha into cells; and noncoenocytic, septate with the hyphae apparently consisting of cylinders without cross walls. The non-septate hyphae have nuclei scattered throughout their length and are considered multicellular. Special, mycelial structures or parts aid in the identification of molds. Examples are the rhizoids, or "holdfasts," of *Rhizopus* and *Absidia*, the foot cell in *Aspergillus*, and the dichotomous, or Y-shaped, branching in *Geotrichum*.

Asexual reproduction in fungi

They are fission of somatic cell, Budding of somatic cell, Fragmentation or disjoining of hyphae and Asexual spore formation.

1. Fission:

- In binary fission a mature cell elongates and its nucleus divides into two daughter nuclei.
- The daughter nuclei separates, cleaves cytoplasm centripetally in the middle till it divides parent protoplasm into two daughter protoplasm.
- A double cross wall is deposited in the middle to form two daughter cell.
- Ultimately the middle layer of double cross wall degenerates and daughter cells are separated.
- Examples: Saccharomyces pobbe, Psygosaccharomyces

2. Budding:

- The cell wall bulge out and softens in the area probably by certain enzymes brought by vesicles.

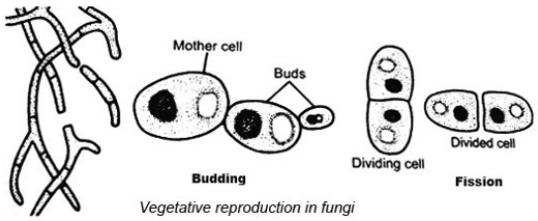
- The protoplasm also bulge out in this region as small protuberance.
- The parent nucleus also divides into two, one of the daughter nucleus migrates into bud, the cytoplasm of bud and mother remain continuous for some time
- As the bud enlarges, a septum is laid down at the joining of bud with mother cell. Then bud separates and leads independent life.
- Some time, bud starts reproducing while still attached with mother cell. This gives branching appearance.
- Budding is the typical reproductive characteristics of Ascomycetes.
- Examples: yeast

3. Fragmentation:

- In some fungi, fragmentation or disjoining of hyphae occurs and each hyphae become a new organism

4. Asexual spore of fungi:

- Spore formation is the characteristic feature of fungi.
- Different fungi forms different types of spore,



Vegetative reproduction in fungi

<u>Types of asexual spore:</u>

i. Sporangiospore:

- These asexual spore are produced in a sac like structure called sporangia (singular;saprangium).
- Sporangium are produced at the end of special aerial hyphae called sporangiophore
- Sporangium contains large numbers of haploid spores, which are released by rapture of sporangial wall
- Examples: *Rhizopus*

ii. Conidiospore:

- Conidiospore or conidia are single celled, bicelled or multicelled structure born on the tip or side of aerial hyphal structure called conidiophore
- Conidia are different from sporangiospore as these are not produced inside sporangium or any sac like structure.
- Conidia are born singly or in chain
- Examples: *Penicillium, Apergillus*

iii. Arthrospore:

- Arthrospore are very primitive type of spore formed by the breaking up of fungal mycelium
- A spore is formed by separation followed by fragmentation of hyphae

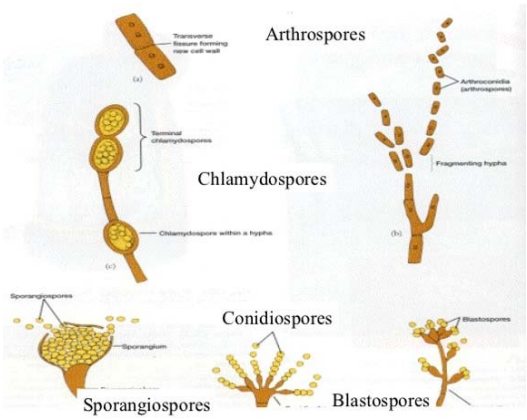
- Examples: *Trichosporium, Geotrichum, Coccididious imitis*

iv. Chlamydospore:

- These are usually formed during unfavorable condition and are thick walled single celled spore, which are highly resistant to adverse condition.
- Hyphal cell or portion of hyphae contracts, loose water, round up and develops into thick walled chalmydospore.
- When favorable condition returns, each chlamydospore give rise to a new individual fungi.
- Examples: ascomycetes, basidiomycetes, zygomycetes,
- *Histoplasma capsulatum, Candida albicans*

v. Blastospore:

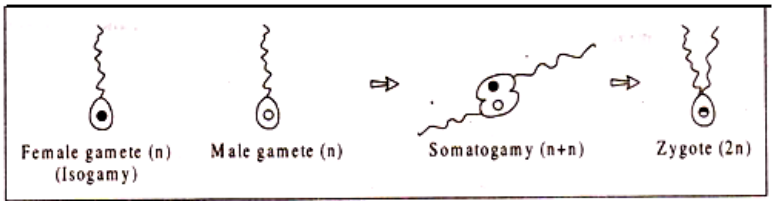
- It is a budding spores usually formed at the terminal end of hyphae.
- These spore may remains attached to hyphae and bud further to gibe branching chain of blastospores
- Examples: ascomycetes, basidiomycetes, zygomycetes

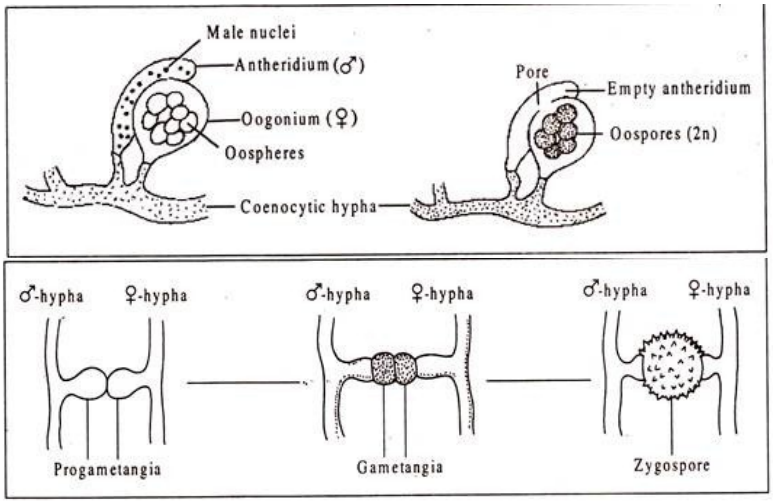


Sexual Reproduction

Sexual reproduction is carried out by diffusion of compatable nuclei from two parent at a definite state in the life cycle of fungi. The process of sexual reproduction involves three phases:

- Plasmogamy: fusion of protoplasm
- Karyogamy: fusion of nucleus
- Meiosis: reductional nuclear division





1. Gametic copulation:

- Fusion of two naked gametes, one or both of them are motile
  - Isogamous
  - Anisogamous
  - Oogamous

2. Gamete-gametangial copulation:

- Male and female gametangia comes into contact but do not fuse.
- A fertilization tube formed from where male gametangium enters the female gametangium and male gamate passes through this tube

3. Gametangial copulation;

- Two gametangia or their protoplast fuse and give rise to zygospore

4. Somatic copulation:

- Also known as somatogamy.
- In this process fusion of somatic cell occurs
- This sexual fusion of undifferentiated vegetative cell results in dikaryotic hyphae, so the process is also called dikarotization

5. Spermetization:

- It is an union of special male structure called spertatium with a female receptive structure.
- Spermatium empties its content into receptive hyphae during plasmogamy

Sexual spores of fungi-The molds which can produce sexual spores are classified on the basis of the manner of formation of these spores and the type produced.

Ascospore:

- It is usually single celled produced in a sac called ascus (plural;asci) and usually there are 4-8 ascospore in an ascus but the number may vary from species to species
- The ascospore are usually arranged in a linear order. In some case ascospores are long, narrow and are arranged in parallel order.

ii. Basidiospore:

- It is a reproductive spore produced by basidiomycetes.
- This single celled spores are born in a club shaped structure called basidium
- These basidiospore aerves as main air dispersal unit for the fungi.

iii. Zygospore:

- Zygospores are thick walled spores formed when two sexually compatible hyphae or gametangia of certain fungi fuse together.
- In suitable condition, zygospore germinates to produce a single vertical hyphae which forms a aporangium and releases its spores

iv. Oospore:

- These are formed within a special female structure called Oogonium.
- Fertilization of egg by male gamete in female sex organ give rise to oospoes.
- There are one or more oospores in each oogonium.

Classification and identification of molds

Molds are plants of the kingdom Myceteae. They have no roots, stems, or leaves and are devoid of chlorophyll. They belong to the Eumycetes, or true fungi, and are subdivided further to subdivisions, classes, orders, families, and genera.

The following criteria are used chiefly for differentiation and identification of molds:

1. Hyphae septate or non-septate
2 .Mycelium clear or dark (smoky)
3. Mycelium colored of colorless
4. Whether sexual spores are produced and the type: oospores, zygospores, or ascospores
5. Characteristics of the spore head
   a) Sporangia: size, color, shape, and location
   b) Spore heads bearing conidia: single conidia, chains, budding conidia, or masses; shape and arrangement of sterigmata or phialides; gumming together of conidia
6. Appearance of sporangiophores or conidiophores: simple or branched, and if branched the type of branching; size and shape of columella at tip of sporangiophore; whether conidiophores are single or in bundles
7. Microscopic appearances of the asexual spores, especially of conidia: shape, size, color; smooth or rough; one-, two-, or many-celled
8. Presence of special structures (or spores): stolons, rhizoids, foot cells, apo-physis, chlamydospores, sclerotia, etc

Classification of Molds and Molds of Industrial Importance.

It is beyond the scope of this article to discuss in detail the complicated system of classification of molds. In the following only genera of industrial importance will be shortly overviewed.

Genus Mucor (Mucor racemosus, Mucor rouxii). Mucors are involved in the spoilage of some foods and in the manufacture of others e.g. oriental fermented foods.

Genus Rhizopus. Rhizopus nigricans, sometimes called „bread mold", is very common and is involved in the spoilage of many foods such as berries, fruits, vegetables, bread, etc.

Genus Aspergillus. The members of this genus are very widespread. Many are involved in the spoilage of foods and some are useful in preparation of fermented foods. Many groups and hundreds of aspergillus species are known. Aspergillus niger is the leading species important for food microbiologists. Selected strains are used for commercial production of citric and gluconic acids.

Genus Penicillium. This is another widespread genus important in foods. Penicillium expansum, a green spored species, causes soft rot of fruits. Penicillium camemberti with grayish conidia, useful in the ripening of Camembert cheese, and Penicillium roqueforti, used in ripening of blue cheeses, are also well known members of this genus.

Genus Bothrytis. The species Bothrytis cinerea causes the noble rot of grape in some wine producing areas such as Tokay (Hungary).

Genus Alternaria. Molds of this genus are common causes of the spoilage of foods. Alternaria citri, Alternaria tenuis and Alternaria brassicae are the common species.

Genus Neurospora (Monilia). The species of this genus grow on various foods.

Citric Acid Production by Fungi: Since the early demonstration by Wehmer in 1893 of the presence of citric acid in culture media containing sugar and inorganic salts with species of Penicillium, a variety of fungi were screened for citric acid production. At present Aspergillus niger is most commonly used for industrial production of citric acid.

Yeasts and yeast like fungi

Like mold, the term "yeast" is commonly used but hard to define. It refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups; molasses, honey, jellies, meats, wine, beer, and other foods.

Intrinsic and Extrinsic parameter of food affecting microbial growth:

The interaction between microorganisms and other living things in the earth is natural, constant and which plays a significant role in maintaining the ecological balance and stability of biogeochemical cycling. As microorganisms are associated with living things in nature they play a significant role for survival of plants and animals.

Growth of microorganisms in food is dependent on various parameters. The factors influencing the growth of microorganisms are physical, chemical and biological in nature. The factors can be generally classified as intrinsic and extrinsic factors.

The intrinsic and extrinsic factors affecting the growth of microorganisms in food are explained below:

Intrinsic factors

- Water activity; Bacteria> Yeast> Mold

- Oxygen availability
- pH: Low acid foods, acid foods (4.5 and lower)
- Buffer capacity: change of pH
- Nutrients
- Natural antimicrobial substances
- Microflora

Extrinsic factors

- Temperature: Psychrophiles 12-15C/ Mesophiles 30-54C/ Thermophiles 55-75C
- Relative humidity
- Atmospheric condition: Aerobic/ Anaerobic/ Facultative anaerobic

Intrinsic factors

The parameters present in substrates in which the microorganisms are growing, that are internal parts of the substrate are called as intrinsic parameters.

1. pH:

pH: It is the negative logarithm of the hydrogen ion activity.

$$PH = - \log (a_H) = \log \frac{1}{(a_H)}$$

$$= \log \frac{1}{[H^{-1}]}$$

pH = Hydrogen ion activity

[ ] + H = Hydrogen ion concentration.

Every micro organism has a minimal, a maximal and an optimal pH for growth. Bacteria grow fastest in the pH range 6.0 – 8.0, yeasts 4.5 – 6.0 and filamentous fungi 3.5 – 4.0. Usually between pH 5.0 & 6.0.

Inherent acidity: Some foods have a low pH because of inherent property of the food. Ex: Fruits & vegetables.

Biological acidity:

Some foods develop acidity from the accumulation of acid daring fermentation. Ex: curd, sauerkraut, pickles etc. Molds can grow over a wide range of pH vales than the yeast and bacteria. Film yeasts grow well on acid foods such as sauerkraut and pickles. Most yeasts do not grow well in alkaline substrates. Bacteria which are acid formers are favoured by moderate acidity. Active proteolytic bacteria, can grow in media with a high pH (alkaline.) Ex: Egg white. The compounds that resist changes in pH are important not only for their buffering capacity but also for their ability to be especially effective within a certain pH range.

Vegetable juices have low buffering power, permitting an appreciable decrease in pH with the production of small amount of acid by lactic acid bacteria during the early part of

sauerkraut and pickle fermentations. This enables the lactics to suppress the undesirable pectin – hydrolyzing and proteolytic competing organisms. Low buffering power makes for a more rapidly appearing succession of micro-organisms during fermentation than high buffering power. Ex: Milk – High in protein content, act as good buffer. Lactic acid converted to pyruvic acid by glycolytic pathway. Acid again converts to lactic acid by lactic dehydrogenase enzyme. After 5-10 minutes, there will be decreased in pH. Hence the lactic acid bacteria survives and activity slows dawn. Once the acidity increase, yeasts and molds will take upper hand and all the products used by these organisms. The quantity of acid decreases and pH increases to neutral. Proteolytic bacteria acts on caesin and these proteins are broken down and gives bad smell accompanied by removal of NH3. pH increases and neutral due to deamination. Then lipolytic organisms which utilise the fat present and utilises the short chain fatty acids through hydrolysis which gives still bad smell. Egg white where the pH increases to around 9.2 as CO2 is lost from the egg after laying. Fish spoil more rapidly than meat under chill conditions. The pH of post – rigor mammalian muscle, round 5.6 and it is lower than that of fish (6.2 - 6.5) and this contributes to the longer storage life of meat.

The ability of low pH to restrict microbial growth has been employed since the earliest times in the presen ation of foods with acetic and lactic acids. Fruits are acidic than vegetables pH of milk neutral.

Water Activity or Moisture Content (a_w):

Micro organisms have an absolute demand for water. Without water, no growth can occur. The exact amant of water needed for growth of micro organisms varies. This water requirement is best expressed in terms of available water or water activity (aW).

$$a_w = \frac{Vapour\ pressure\ of\ the\ solution}{Vapour\ pressure\ of\ the\ solvent}$$

P is the vapor pressure of the solution
Po is the vapor pressure of the solvent (usually water).
The a_w content is very well related to relative humidity (RH) in the following way: RH = 100 x a_w.

Pure water has an a_w of 1.00, a 22% NaCl solution (w/v) has an a_w of 0.86, and a saturated solution of NaCl has an a_w of 0.75. The water activity (a_w) of most fresh foods is above 0.99.

Most of the food spoilage bacteria do not grow below a_w 0.91, while spoilage moulds can grow even at a_w 0.80. The aerobic food poisoning bacterium Staphylococcus aureus is found to grow at a_w as low as 0.86 while anaerobic Clostridium botulinum does not grow below a_K 0.94. Moulds differ considerably in optimal a_w for vegetative growth and spore germination.

The lowest a_w value for food borne bacteria is 0.75 for halophiles (“salt-loving”), whereas xerophilic (“dry-loving”) moulds and osmophilic (preferring high osmotic pressures)

yeasts have been reported to grow at a_w values of 0.65 and 0.61. The lowest water activity values permitting growth of spoilage microorganisms..

Lowest a_w values for different types of microorganisms spoiling food

Group of Microorganism	Minimal (a_w) value
Bacteria	0.91
Yeasts	0.88
Moulds	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.65
Osmophilic yeasts	0.60

Water is made unavailable in various ways:
1. Solutes and ions tie up water in solution. Therefore an increase in the concentration of dissolved substances such as sugars and salts effectively dry the material. Water tends to leave the microbial cell by osmosis.
2. Hydrophilic colloids (gels) make water unavailable.
3. Water of crystallization or hydration is usually unavailable to micro organisms. Each micro organisms has a maximal, optimal and minimal aw for growth. Low aw – decrease in the rate of growth of organisms.

Factors that may affect water activity (aw). Requirements of micro organisms include the following.
1. The kind of solute employed to reduce aw. Potassium chloride usually less toxic than NaCl. And less inhibitory than sodium sulphate.
2. The nutritive valve of the culture medium. The better the medium for growth, the lower the limiting aw.
3. Temperature: Most organisms have the greatest tolerance to low aw at about optimal temperatures.
4. Oxygen supply: Growth of aerobes takes place at lower aw in the presence of air than in its absence.
5. pH Most organisms are more tolerant of low aw at PH valves near neutrality than in acid or alkaline media.
6. Inhibitors: The presence of inhibitors narrows the range of aw for growth of micro organisms.

Methods for the control of aw are
1. Equilibrium with controlling solutions
2. Determination of the water – sorption isotherm for the food.
3. Addition of solutes.

Methods for measuring or establishing aw valves of food:

1. Freezing point determinations by Clausius – Clayperson equation.
2. Manometric techniques
3. Electrical devices.

Favorable aw for bacteria to grow in foods – 0.995 to 0.998. They grow best in low concentration of sugar or salt. 3-4% sugar and 1-2% salt may inhibit some bacteria. Molds have optimum aw of 0.98 – 0.99; Mold spores germinate at min aw of 0.62.

Some general conclusions related to water requirement of micro organisms are

1. Each organism has its own characteristic optimal aw.
2. Bacteria require more moisture than yeasts and yeasts more than molds. Minimum aw required for bacteria – 0.91

Minimum aw required for yeasts – 0.88 Minimum aw required for molds – 0.80
Minimum aw required for Halophilic bacteria – 0.75 Minimum aw required for Xerophilic fungi – 0.65 Minimum aw required for Osmophilic yests – 0.60

3. Micro organisms that can grow in high concentrations of solutes e.g. sugar and salt have low

water activity (aw). Osmophilic yeasts grow best in high concentrations of sugar.

Redox Potential (Eh):

The reducing and oxidizing power of the food will influence the type of organism and chemical changes produced in the food. The concentration of oxygen in food, chemical composition and type of microorganisms associated contribute to the oxidation-reduction (O-R) potential of food and affect growth of microorganisms in them. The O-R potential of a food may be defined as the ease with which the substrate loses or gains electrons.

The Redox potential of food is determined by characters such as:

(a) Oxygen tension of atmosphere above the food,
(b) Access of atmosphere to the food,
(c) Resistance of food to the changes occurring and
(d) O-R state of materials present in food.

The O-R potential is written as Eh and measured and expressed as millivolts (mV). If the substrate is highly oxidized would have a positive Eh and substrate is reduced is a negative Eh. Aerobic microorganisms such as bacilli, cocci, micrococci, pseudomonas, acinetobacters require and grow at positive O-R potential and anaerobe such as Clostridia and bacteriodes require negative O-R potential for their growth.

Head space in an “evacuated” can of food contain low oxygen tension compared to air.

Micro organisms are classified as aerobic, anaerobic, and facultative based on the requirement of O2. Molds – aerobic ,Yeasts – Aerobic and facultative.

Bacteria – Aerobic, anaerobic and facultative.

High O - R potential favours aerobes and facultative organisms. Low O-R potential favours anaerobic and facultative organisms.
However some aerobes grow at low O-R potential O-R potential of a system is usually written Eh and measured and expressed in terms of millivolts (mv).

Highly oxidised substrate would have a positive Eh and a reduced substrate have a negative Eh. Aerobic microorganisms require positive Eh. Ex: *Bacillus, Micrococcus, Pseudomonads, Acinetobacters*. Anaerobic micro organisms required negative Eh. Ex: *Clostridium.* Most fresh plant and animal foods have a low and well poised O – R potential in their interior because plants contain reducing substances like ascorbic acid and reducing sugars where as animal tissues contain –SH (Sulf hydryl) and other reducing groups. As long as the plant or animal cells respire and remain active, they have low level of O-R potential.

Meat could support the aerobic growth of shine forming or souring bacteria at the same time that anaerobic putrefaction was proceeding in the interior. Heating and processing may alter the reducing and oxidising substances of food. Ex: Fruit juices lost reducing substances by their removal during extraction and filtration by their removal during extraction and filtration and therefore have become more favourable for the growth of yeasts.

Composition of Nutrients:

Nutrients are one of the most important compounds for the growth and functioning of microorganism. Nutritional quality of food depends on the chemical composition, nutritive value or nutrients, their proportion and growth promoting ability to the microorganisms. The most important factors which have to be considered are the energy substances in food, nitrogen substances, growth promoting substances, accessory food substances or vitamins, minerals, and water content which all are very essential for growth or energy production of organisms.

Carbohydrates especially the sugars are commonly used as an energy source. Complex carbohydrates such as cellulose can be utilized by few organisms and starch can be hydrolysed by any a limited number of organisms. Many organisms cannot use the disaccharide lactose (Milk sugar) and therefore do not grow well in milk. Maltose is not attacked by some yeasts. Some micro organisms hydrolyze pectin of the fruits and vegetables. Limited number of micro organisms can obtain their energy from fats by producing lipases. Aerobic their energy from fats by producing lipases. Fats are hydrolysed to glycerol and fatty acids. Aerobic micro organisms are more commonly involved in the decomposition of fats than are anaerobic ones and the lipolytic organisms usually are also proteolytic. Hydrolysis products of proteins, peptides and amino acids serve as an energy source for many proteolytic organisms when a better energy source is lacking. Meats are decomposed by proteolytic sps Ex: *Pseudomonas* sps: Concentration of food in solution increases the osmotic effect and amount of available moisture. Molds & yeasts can grow in the highest concentrations of sugars. Bacteria can grow best in low concentration of sugars.

Micro organisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. Many organisms are unable to hydrolyze proteins and hence cannot get nitrogen from them. Peptides, aminoacids, urea, ammonia and other simpler nitrogenous

compounds may be available to some organisms but not to others. These compounds may be used under some environmental conditions but not under other conditions.Ex: Some lactic acid bacteria grow best with polypeptides as nitrogen foods, cannot attack casein. Some microorganisms use fermentable carbohydrates and results in acid production which suppresses the proteolytic bacteria and hence it is called sparing action on the nitrogen compounds.

Many kinds of molds are proteolytic but very few yeasts are actively proteolytic. Proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity. Carbon for growth may come partly from CO2 and also from organic compounds. Minerals required by microorganisms are always present in low level. Sometimes an essential mineral may be unavailable, lacking or present in insufficient amounts.

Ex: Milk contains insufficient iron for pigmentation of the spores of *Penicillium roqueforti*. Accessory food substances or vitamins needed by the organisms.

Extrinsic Parameters:

The extrinsic parameters are substrate independent and in this case the storage environment that affect both the food and their microorganisms.

1. Relative Humidity (RH):

The relative humidity of the storage environment is important extrinsic parameter both from the standpoint of a_w within foods and the growth of microorganisms at the surfaces. When foods with low a_w contents are placed in high RH environments, the foods takes up more moisture until equilibrium has been established.

Similarly foods with a high a_w lose moisture when placed in an environment of low RH. There is a relationship between RH and temperature that should be borne in mind in selecting proper storage environments for foods. Generally, if the temperature high then the RH low and vice versa.

Ex: Grain silos or in tanks in which concentrates and syrups are stored. Storage of fresh fruits and vegetables requires very careful control of relative humidity. It RH is too low, many vegetables will lose water and become flaccid. It is too high then condensation may occur and microbial spoilage may be initiated.

Atmospheric Gases:

Like O_2, Carbon dioxide (CO_2) is also most important atmospheric gas that is used to control microorganisms in foods. The inhibitory effect of CO2 on microbial growth is applied in modified atmosphere packing of food and is an advantage in carbonated mineral waters and soft drinks. Moulds and bacteria are sensitive to CO2 condensation. Some yeasts such as *Bettanomyces* spp how tolerance to high CO2 levels.

Growth inhibition is usually greater under aerobic conditions than anaerobic and the inhibitory effect increases with decrease of temperature, presumably due to the increased solubility of CO2 at lower temperatures. CO2 dissolves in water to produce carbonic acid which decreases PH and partially dissociates into bicarbonate anions and protons. CO2 also affects

solute transport, inhibition of key enzymes involving carboxylation, decarboxylation reactions in which CO2 is a reactant and reaction with protein amino groups causing change in their properties and activity.

Temperature:

Microorganisms can grow over a wide range of temperatures. The lowest temperature at which a microorganism has been reported to grow is -34°C; the highest is somewhere in excess of 100°C.

Thermophiles have optimum - 55-75°C
Mesophile have optimum - 30 -40°C
Psychrophiles (Obligate psychrophiles) – 12 - 15 °C
Psychotroph (facultative) – 25-30 °C

Micro organisms can be classified into several physiological groups based on their cardinal temperatures. Low temperature affects the uptake and supply of nutrients to enzyme systems within the cell. Many microgranisms responds to growth at lower temperature by increasing the amount of unsaturated fatty acids in their membrane lipids and that psychrotrophs generally have higher level of unsaturation in a fatty acid decreases its melting point so that membranes containing higher levels of unsaturated fatly acid will remain fluid and hence functional at lower temperatures. As the temperature increases above the optimum, the growth rate declines as a result of denaturation of proteins.

Source of food of contamination

Food provides an ideal nutrition source for microorganisms, during harvesting, processing, distribution, and preparation, food is contaminated with soil, air, and waterborne microorganisms. When food items are not handled or cooked safely, the disease-causing organisms such as bacteria, parasites, and viruses result in food contamination. The disease-causing parasites produce toxins that may also lead to food intoxication.

Here are a number of reasons that can lead to food contamination. However, food contamination falls under four different categories which are:

- Biological contamination
- Chemical contamination
- Physical contamination
- Cross-contamination

Biological Contamination

Biological contamination is one of the common causes of food poisoning as well as spoilage. Contamination of food items by other living organisms is known as biological food contamination. During biological contamination, the harmful bacteria spread on foods that you consume. Even a single bacterium can multiply very quickly when they find ideal growth

conditions. Not just bacteria, but also their process of multiplying can be quite harmful to humans.

The common places where you can find bacteria are:

- Dust
- Raw meat
- The air
- The human body
- Pets and pests
- Clothes of food handler
- Kitchen clothes

Physical Contamination

When harmful objects contaminate the food it leads to physical contamination. At times, food items can have both physical and biological contamination. Physical contaminants such as rats, hair, pests, glass or metals which can contaminate food and make it unhealthy.

Some of the safety tips that you can follow when handling food items to prevent food contamination are:

- Hair-Tie your hair when handling food
- Glass or Metal-Clean away cracked or broken crockery and utensils to avoid contamination
- Fingernails-Keep your fingernails short or wear clean gloves when handling food
- Dirt-Wash fruits and vegetables with KENT Vegetable and Fruit Cleaner to remove dirt
- Jewelry-Wear minimum jewelry when preparing food

Chemical Contamination

Chemical contaminants are one of the serious sources of food contamination. These contaminants can also lead to food poisoning. Pesticides present in fruits and vegetables are one of the main sources of contamination. In addition, kitchen cleaning agents, food containers made of non-safe plastic, pest control products also lead to food contamination. Though we make it a point to wash fruits and vegetables thoroughly, however, plain water can't remove all the contaminants. This is where KENT Vegetable and Fruit Cleaner can help you out. The smart kitchen appliance uses ozone disinfection technology that removes contaminants from the surface of the fruits and vegetables to make it safe for consumption.

Cross-Contamination

Many of us are not aware of cross contamination; however, this type of contamination can lead to a number of health problems. Cross-contamination takes place when pathogens are transported from any object that you use in the kitchen. Dirty kitchen clothes, unclean utensils, pests, raw food storage can lead to cross-contamination.

Here are some of the ways to avoid cross-contamination:

- Personal Hygiene- Thoroughly wash your hands and face when handling food. Coughing, sneezing or even touching your hair can lead to cross contamination
- Utensils-Use separate utensils to prepare different types of foods. Avoid using the same chopping board and knife for ready to eat foods
- Storing Food-Make sure raw foods don't come in contact with ready to eat foods. Cover and store raw foods below cooked foods to prevent cross-contamination.
- Disposing Waste- Make sure you store and seal garbage correctly to prevent cross-contamination. Clean and sanitize the waste bins to prevent infestation risk

Contamination of foods

- Micro organisms from various natural sources act as source of contamination.
- From green plants and fruits From animals
- From sewage From soil From water From air
- During handling and processing.

1. From green plants and fruits

Natural surface flora of plants varies with the plant but usually includes species of *Pseudomonas, Alcaligenes, Flavobacterium, Micrococcus*, coliforms and lactic acid bacteria. The no. of bacteria will depend on the plant and its environment and may range from a few hundred or thousand per square centimeter of surface to millions. Ex: Surface of well washed tomato contains 400-700 micro organisms per square centimeter. Outer tissue of unwashed cabbage contain 1 million to 2 million micro organisms. Inner tissues of cabbage contain fewer micro organisms.

2. From animals:

Sources of micro organisms from animals include the surface flora, the flora of the respiratory tract, and the flora of the gastro intestinal tract. Hides, hooves, and hair contain micro organisms from soil, manure, feed and water but contain spoilage organisms. Feathers, feet of poultry carry heavy contamination of micro organisms. Skin of many meat animals may contain *Micrococci, Staphylococci* and beta haemolytic *Streptococci*. Pig or beef carcasses may be contaminated with salmonellae. Meat from slaughter houses is not frequently associated with human salmonellosis. Many of these diseases have been reduced or eliminated by improvement in animal husbandry, but animal disease causing infections from foods include *Mycobacterium, Coxiella, Listeria, Salmonella* and enteropathogenic *E.Coli* and viruses.

3. From sewage:

When untreated domestic sewage is used to fertilize plant crops, there is a chance that raw plant foods will be contaminated with human pathogens especially those causing gastrointestinal diseases. The use of "night soil" as a fertilizer still persists in some parts of the world. In addition to the pathogens, coliform bacteria, anaerobes, enterococci, other intestinal bacteria and viruses

can contaminate the foods from this source. Natural water contaminated with sewage contributes their micro organisms to shell fish, fish, and other seafood.

From soil: Soil contains greatest variety of micro organisms. They are ready to contaminate the surfaces of plants growing on or in them and the surfaces of animals roaming over the land. Soil dust is whipped up by air currents and soil particles are carried by running water to get into or onto foods. Soil is an important source of heat resistant spore forming bacteria.

From water: Natural water contain not only their natural flora but also microorganisms from soil and possibly from animals or sewage. Surface waters in streams or pools and stored waters have low microbial content because self purification of quiet lakes and ponds or of running water. Ground waters from springs or wells have passed through layers of rock and soil to a definite level hence most of the bacteria, suspended material have been removed. Kinds of bacteria in natural waters are chiefly of in *Pseudomonas, Chromobacterium, Proteus, Micrococcus, Bacillus, Streptococcus, Enterobacter* and *Escherichia coli*.

From Air: Air does not contain a natural flora of micro organisms, but accidentally they are present on suspended solid material or in moisture droplets. Micro organisms get into air on dust or lint, dry soil, spray from stream, lakes or oceans, droplets of moisture from coughing, sneezing or talking and growth of sporulating molds on floors, etc. Number of microorganisms in air at any given time depend on factors like amount of movement, sunshine, humidity, location and the amount of suspended dust or spray. No. of micro organisms vary from mountains to dusty air. Less on mountains and more in dusty air. Direct rays from the sun kill micro organisms suspended in air and hence reduce numbers. Dry air contains more organisms than moist air. Number of micro organisms in air may be reduced under natural conditions by sedimentation, sunshine and washing by rain or snow. Filters in ventilating or air conditioning systems prevent the spread of organisms from one part of a plant to another.

During handling and processing: Additional contamination may come from equipment coming in contact with foods, from packaging materials and from personnel.

Food Sanitation

Sanitation is “the creation and maintenance of hygienic and healthful conditions.” It is the application of a science to provide wholesome food processed, prepared, merchandised, and sold in a clean environment by healthy workers; to prevent contamination with microorganisms that cause foodborne illness; and to minimize the proliferation of food spoilage microorganisms.

Sanitation is an applied science that incorporates the principles of design, development, implementation, maintenance, restoration, and/or improvement of hygienic practices and conditions. Sanitation applications refer to hygienic practices designed to maintain a clean and wholesome environment for food production, processing, preparation, and storage.

Why sanitation?

- Inspection is becoming more stringent because inspectors are using the Hazard Analysis Critical Control Point (HACCP) concept to establish compliance. HACCP-based inspections focus on the items critical to the safety of foods. Thus, an effective sanitation program is essential.
- Foodborne illness can be controlled when sanitation is properly implemented in all food operations. Common problems caused by poor sanitation are food spoilage through off-odor and flavor. Spoiled foods are objectionable to consumers and cause reduced sales, increased consumer complaints, and increased claims.
- An effective sanitation program can improve product quality and shelf life because the microbial population can be reduced.
- An effective sanitation program includes regular cleaning and sanitizing of all equipment in a facility including heating, air conditioning, and refrigeration equipment. Dirty, clogged coils harbor microorganisms and blowers and fans can spread flora throughout the facility. Clean and sanitized coils lower the risk of airborne contamination and can reduce energy and maintenance costs by up to 20%.
- Various, less tangible benefits of an effective sanitation program include: (a) improved product acceptability, (b) increased product shelf life, (c) satisfied and perhaps even delighted customers, (d) reduced public health risks, (e) increased trust of regulatory agencies and their inspectors, (f) decreased product waste and removal, and (g) improved employee morale.

There are three main types of hazards or contaminants that can cause unsafe food: Biological, chemical, and physical. Biological includes microorganisms; chemical includes cleaning solvents and pest control; and physical means hair, dirt, or other matter. The sanitation tips to prevent foodborne illnesses in food service and retail businesses. They are:

1. Proper personal hygiene, including frequent hand and arm washing and covering cuts;
2. Proper cleaning and sanitizing of all food contact surfaces and utensils;
3. Proper cleaning and sanitizing of food equipment;
4. Good basic housekeeping and maintenance; and
5. Food storage for the proper time and at safe temperatures.

Indicators of food safety

Microbial indicators are employed more often to assess food safety and sanitation than quality. Ideally, a food safety indicator should meet certain important criteria. It should

1. be easily and rapidly detectable
2. be easily distinguishable from other members of the food biota
3. Have a history of constant association with the pathogen of concern
4. Always be present when the pathogen of concern is present

5. Be an organism whose numbers ideally should correlate with those of the pathogen of concern
6. Possess growth requirements and a growth rate equaling those of the pathogen
7. Have a die-off rate that at least parallels that of the pathogen and ideally persists slightly longer than the pathogen of concern
8. be absent from foods that are free of the pathogen except perhaps at certain minimum numbers

Coliform bacteria

In the historical use of safety indicators, however, the pathogens of concern were assumed to be of intestinal origin, resulting from either direct or indirect fecal contamination. Sanitary indicators were used to detect fecal contamination of waters and the possible presence of intestinal pathogens.

Following the practice of employing E. coli as an indicator of fecal pollution of waters, other organisms were also suggested for food sanitary indicator

1. Coliforms
2. Enterococci
3. Bifidobacteria
4. Coliphages/Enteroviruses

Unit I; Possible Questions
Part-A (1 Mark)
Part-B (2 Mark)

1. Define the term mold.
2. What is hyphae?
3. What is mycelium?
4. Name the reproductive parts of mold.
5. What are zygospores?
6. What are basidiospores?
7. What is mucor?
8. How whiskers are caused on meat?
9. Define the term yeast.
10. What is encapsulation?
11. What is redox potential?
12. What are the inhibitory substances present in food?
13. What is water activity?
14. What is relative humidity?

Part-C (8 Marks)

1. Why is E. coli considered as indicator of pollution?
2. What are coliforms?
3. Name several species of pathogenic organisms present in contaminated foods?
4. What are the morphological and cultural characteristics of common fungal contamination present in food?
5. List out the sources of contamination of food and water.
6. Why there is a need of sterilization of food?
7. Name some of the sanitizers used in food industry.
8. What are the preventive measures to control the contamination of food?
9. List the intrinsic and extrinsic factors affecting the food.
10. Note on important characteristics of bacteria, fungi and molds.
11. Name any five natural antimicrobial substances in food that preserve the food.
12. Name the common microflora present in air, water.
13. Explain the enzymes involved in food spoilage by microbes.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB COURSE NAME: FOOD MICROBIOLOGY

COURSE CODE: 18MBP302 UNIT: I BATCH-2018-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Cross-contamination of food occurs when	Cleaning and sanitising equipment and benches	Keeping food stored in food-grade containers	Washing hands before handling food	Using food handling gloves for handling money	Using food handling gloves for handling money
2	Which of the following are allergens?	Sources of gluten and Red meat	Fruits and vegetables	Fish and fish products	None of the above	Sources of gluten and Red meat
3	The undesirable change in a food that makes it or human consumption is referred as _____	food decay	food spoilage	food loss	all of the above	food spoilage
4	_____ microorganisms reqire positive Eh values or positive mV O-R potentials	Aerobic	anaerobic	facultative	none of these	Aerobic
5	_____ acid produced by the propionibacteria in swiss cheese is inhibitory to molds	sorbic	acetic	propionic	acetic	propionic
6	Most spoilage bacteria grow at	acidic pH	alkaline pH	neutral pH	any of the pH	neutral pH
7	The microbiological examination of coliform bacteria in foods preferably use	MacConkey broth	violet Red Bile agare	eosine Methylene blue agar	all of these	all of these
8	Which of the following can cause food to be contaminated because of chemical hazards from food handlers?	Hair	Dust	Live insects	Perfume	Perfume
9	_____ is the causative organism for a bacterial pneumonia in human.	*Flavobacterium*	*Escherichia*	*Klebsiella*	*Gluconobacter*	*Klebsiella*
10	The use of indicator microorganisms began with use of *E. coli* testing in _____	soil	plants	water	all of these	water

11	To retard the contamination and other microbial growth in meat is obtained by storing at _____ temperature	10°C	0°C	100°C	-10°C	0°C
12	The percentage of relative humidity is obtained by multiplying by_____.	aw*10	aw*1000	aw*100	aw*0.1	aw*100
13	Many infectious disease agents of animals can be transmitted to people through _____	water	food	soil	juices	food
14	_____ used to fertilize plant crops will be contaminated with human pathogens	sewage	distilled water	mineralized water	none of these	sewage
15	The o-R potential of a system is measured by	mV	mM	aw.	Eh	Eh
16	Which of the following can cause food to be contaminated because of physical hazards from food handlers?	Jewellery	Dust	Rodent droppings	Incorrectly diluted chemicals	Jewellery
17	_____ bacteria oxidize ethylalcohol to acetic acid	Aeromonas	Acetobacter	Alcaligens	Alteromonas	Acetobacter
18	The endospores of _____ do not swell the rods in which they are formed	*Streptococcus*	*Brochotrix*	*Brevibacterium*	*Bacillus*	*Bacillus*
19	_____ is associated with the market disease called bacterial soft rot	*Erwinia*	*Enterobacter*	*Corynebacterium*	*Klebsiella*	*Erwinia*
20	_____ bacteria are those which grow in high concentration of sugars	Halophilic	thermophilic	osmophilic	none of these	osmophilic
21	_____ bacteria grow and cause discoloration on foods high in salt	Halobacterium	Enterobacter	Erwinia	Corynebacterium	Halobacterium
22	The different ACC's between food categories reflect the	expected level of contamination of the raw material	potential for microbial growth during storage	potential shelf life	all of the above	all of the above

23	Yeast and mould count determination requires	nutrient agar	acidified potato glucose agar	MacConkey agar	violet Red Bile agar	acidified potato glucose agar
24	The water requirement of a microorganism is expressed in terms of _____'.	Water action	Water adsorption	Water affinity	Water activity	Water activity
25	A psychrophilic halophile would be a microbe that prefers	cold temperatures and increased amounts of salt	warm temperatures and increased amounts of pressure	cold temperatures and the absence of oxygen	warm temperatures and increased amounts of acid	cold temperatures and increased amounts of salt
26	Many microorganisms cannot use the disaccharide lactose and therefore do not grow well in _____	milk	water	food	sewage	milk
27	_____ yeast is grown with dairy starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria	*Candida sp.*	*Trichosporon*	*Rhodotorula*	*Torulopsis*	*Candida sp.*
28	Which of the following acid will have higher bacteriostatic effect at a given pH?	Acetic acid	Tartaric acid	Citric acid	Maleic acid	Acetic acid
29	Water activity can act as	an intrinsic factor determining the likelihood of microbial proliferation	a processing factor	an extrinsic factor	all of the above	all of the above
30	The culture of Brevibacterium produces _____ pigmentation and helps ripening	orange-red	yellow	black	red	orange - red
31	Pectins are complex _____ that are responsible for cell wall rigidity in vegetables	Proteins	lipids	carbohydrates	vitamins	Carbohydrate

	and fruits					
32	_____ are short rods that are defined as aerobic and facultative anaerobic	*Enterobacter*	*Coliforms*	*Proteus*	*Clostridium*	*Coliforms*
33	The _____ of many meat animals may contain micrococci, Staphylococci, and beta-hemolytic Streptococci	Hair	nail	skin	all of these	skin
34	The microorganism which apparently have no mechanism to tolerate acidic pH _____.	bacteria	fungi	viruses	viruses	fungi
35	_____ is the thermoduric bacteria	*Acenetobacteria*	*Morexella*	*Bacillus*	*Flavobacterium*	*Morexella*
36	Aeromonas grows at an optimum temperature of _____	27 to 37 °C	22 to 28 °C	35 to 37 °C	40 °C	22 to 28 °C
37	The spoilage of meat by microorganism is by_____ process.	Oxidation	Reduction	Decomposition	Precipitation	Oxidation
38	_____ bacteria is found aseptically in drawn milk and cause bovine mastitis	*Corynebacterium*	*Clostridium*	*Campylobacter*	*Enterobacter*	*Corynebacterium*
39	Sugars act as preservatives due to their ability to .	Make water unavailable to organisms	Interfere with the action of proteolytic enzyme	Osmotic effect	Both a and c	Interfere with the action of proteolytic enzyme
40	Preservation affect the growth of microorganism by .	Inhibition	Retardation	Arresting	All of the above	Retardation
41	NaCl can act as	antagonist at optimal concentrations	synergistically if added in excess of optimum level	Both (a) and (b)	None of the above	Both (a) and (b)

42	Which of the bacteria can grow in alkaline pH?	*Lactobacilli*	*Vibrio cholera*	*Salmonella*	*Staphylococcus*	*Vibrio cholera*
43	_____ bacteria are able to grow at commercial refrigeration temperatures	Pschrotropic	halophilics	auttrophic	heterotrophic	Pschrotropic
44	_____ bacteria produce lipase enzyme that hydrolysis fat to fatty acids and glycerol	Saccharolytic	Pectinolytic	lipolytic	proteolytic	lipolytic
45	_____ does not contain a antural flora of microorganisms	soil	air	water	sewage	air
46	Saccharomyces are reclassified by Lodder in the year ____	1985	1978	1982	1984	1984
47	The water activity range of fresh meat and fresh fish was _____	0.93-0.98	0.98 and above	0.60-0.76	below 0.98	0.93-0.98
48	The o-R potential of a system is measured by	mV	mM	aw	Eh	mM
49	_____ has been used as starter culture in fermented sausages	*Photobacterium*	*Pediococcus*	*Propionibacterium*	*Proteus*	*Pediococcus*
50	Truly halophilic bacteria require minimal concentration of dissolved _____ for growth	NaCl2	Hcl	NaNo2	Cacl2	Nacl2
51	There are ___ aspects of water bacteriology that are interested by food microbiologist	2	5	6	4	2
52	Contamination of foods from _____ may be important for sanitary as well as economic reasons	air	soil	water	sewage	air
53	When microbes can use fat as an energy source _____.	absence of sugar molecule	presence of glucose	presence of fructose	Presence of high sugar	absence of sugar molecule
54	The approximate range of bacteria present in fresh vegetable is _____	10^9 -10^7/g	10^3 -10^9/g	10^3 -10^7/g	10^1 -10^7/g	10^3 -10^4/g
55	Cannery cooling water often contain _____	Coliforms	*Aeromonas*	*Klebsiella*	*Clostridium*	Coliforms

56	_____ causes ropiness in milk	Lactobacillus plantarum	Klebsiella pneumonia	Klebsiella oxytoca	Flavobacterium	Klebsiella oxytoca
57	_____ contain the greatest variety of microorganisms f any source of contamination of food	plants	sewage	water	soil	soil
58	Pig and beef carcasses may be contaminated with _____	*Salmonellae*	*Klebsiella*	*E. coli*	*Enterobacter*	*Salmonellae*
59	In fruit juices the growth of the fermentative yeast are favored by _____ pH	4.0-4.5	6.0-6.5	2.0-2.5	3.0-3.5	4.0-4.5
60	The water requirement of a microorganism is expressed in terms of _____	water action	water adsorption	water affinity	water activity	water activity

Unit 2

Syllabus

Food preservation – principles – factors affecting preservation – food preservation using temperature – low temperature food preservation – characteristics of psychrotrophs – high temperature food preservation – characteristics of thermophiles – preservation of foods by drying chemicals and radiation – limitations – commercial application

Food preservation

Introduction

Most foods deteriorate in quality following harvest, slaughter or manufacture, in a manner that is dependent on food type, its composition and storage conditions. The principal quality deterioration reactions of foods may be

Microbiological

The microorganisms present in a food may be contributed by its own natural microflora or from the processing conditions like in the course of harvesting/manufacturing, storage, and transport. In some cases the microflora has no discernible effect on the food quality and food safety while in others, this may affect the quality in several ways like causing food spoilage, food borne illness or food fermentations. While food fermentations are desirable transformations of food but food spoilage, food borne infections and intoxications may result into huge economic losses as in cases where a particular batch of food has been found to be involved in an outbreak of a disease or has low shelf life as desired and hence the complete batch has to be recalled back from the market and destroyed. In developing countries like India, losses due to microbial spoilage have been estimated between 10-25% in various types of foods, which adds to the problems of acute shortage of food supply in these countries.

Enzymatic

Enzymes native to plant and animal tissues or from microorganisms are responsible for changes in the texture, color, smell and appearance of foods e.g. microbial enzymes cause hydrolytic reactions, rancidity and browning in foods, and plant enzymes may cause over ripening of fruits and vegetables rendering them unsuitable for consumption.

Chemical

Chemical reactions like oxidative rancidity, oxidative and reductive discoloration, non enzymatic browning and destruction of nutrients contribute to the deterioration of foods if not stored in a proper environment.

Physical

Physical changes are responsible for loss of texture, flavors and structural damage. The most serious forms of quality deterioration include those due to microorganisms, following the survival and/or growth of spoilage, infectious pathogenic bacteria or the growth of toxinogenic ones. In this chapter we are going to study how these losses due to microbial spoilage of foods can be minimized and how foods are made safe for our consumption. The techniques employed to achieve these targets are called food preservation.

Principles of food preservation

The food preservation methods by which the microbial decomposition of foods can be delayed or prevented include

1. Restrict access of microorganisms to foods (packaging and aseptic packaging),
2. Removal of microorganisms (by filtration or centrifugation),
3. Slow or prevent the growth and activity of microorganisms (reduction in temperature, water activity and pH, removal of oxygen, modified atmosphere packaging and addition of preservatives) and
4. Inactivation of microorganisms (by heat, radiations, high hydrostatic pressures, ultra sound and pulsed electric fields).

These methods usually are also effective against enzymatic activity or chemical reactions in the food, responsible for its self-decomposition. Changes in the requirement of consumers in recent years have included a desire for foods which are more convenient, higher quality, fresher in flavor, texture and appearance, more natural with fewer additives and nutritionally healthier than hitherto. Food industry reactions to these changes have been to develop less severe or minimal preservation and processing technologies with less intensive heating or use of less chemical preservatives. However, minimal technologies tend to result in a reduction in the intrinsic preservation of foods, and may, therefore, also lead to a potential reduction in their microbiological safety. A major trend is to apply these techniques in new combinations, in ways that minimize the extreme use of any one of them, and so improve food product quality. This has formed the basis of hurdle technologies or combination preservation systems proposed by Leistner (2000) that have fostered the development of new routes to food preservation around the world. Thus an ideal method of food preservation has the following characteristics:

1. it improves shelf-life and safety by inactivating spoilage and pathogenic microorganisms,
2. it does not change organoleptic (smell, taste, color, texture, etc.) and nutritional attributes,
3. it does not leave residues,
4. it is cheap and convenient to apply and
5. it encounters no objection from consumers and legislators.

These methods of food preservation are being discussed under two headings:

1. physical methods of preservation and
2. chemical methods of preservation.

Physical methods of food preservation

The foods to be preserved are physically processed or treated in such a way that the metabolic activity of microorganisms and their spores either slowed down or completely arrested. These various physical methods used for the preservation of foods are as follows.

Asepsis

Keeping quality of foods can be increased by introducing as few spoilage organisms as possible i.e., by reducing the amount of contamination. In nature, there are numerous examples of asepsis or removal of microorganisms as a protective factor. The presence of a protective covering surrounding some foods e.g. shells of nuts, shells of eggs, skin of fruits and vegetables and fat on meats and fish, prevents microbial entry and decomposition until it is damaged.

In food industries, contamination is prevented by packaging foods in a wide variety of artificial coverings ranging from a loose carton or wrapping to the hermetically sealed containers of canned foods. Moreover, practicing sanitary methods during the processing and handling of foods reduces total microbial load and thus improves the keeping quality of food. Both flexible

and rigid packaging materials, alone or in combination with other preservation methods, have been developed to offer the necessary barrier, inactivation, and containment properties required for successful food packaging. Rigid packaging materials such as glass and metal packages are considered absolute barriers, preventing contamination. However, the economic and functional disadvantages of metal and glass have led to the development of flexible packaging materials made from composites of polyester, nylon, polypropylene, polyethylene and polyvinyl. The microbiology of the flexible packaged foods is influenced by the permeability of the packaging material to oxygen, carbon dioxide and water vapor. Packaging with certain additional conditions like controlled atmosphere, modified atmosphere, and vacuum packaging can produce microbiostatic effect, which is more effective with further decrease in storage temperature.

Controlled atmosphere packaging conditions are defined as the alteration of a gaseous atmosphere over a food product regardless of environmental or temperature fluctuations encountered by the product throughout its distribution. This extends the microbial lag phase, depresses microbial and product respiration, and minimizes adverse changes in sensory and textural qualities of stored fruits and vegetables, while inhibiting the growth of certain spoilage organisms.

Vacuum packaging is accomplished by evacuating all the air before sealing, either by inserting a vacuum probe into the neck of the package, or by placing the package into a chamber and evacuating. The absence of oxygen from vacuum packed foods will not only prevent oxidative transformations in both plant or animal tissues and aerobic microbes, but also control oxidative rancidity of fats. Vacuum packaging retards the growth of common aerobic spoilage bacteria such as *Pseudomonas* species, on refrigerated fresh meat, poultry and fish, reducing putrefaction and slime formation. Therefore, It has become the method for packing table-ready meat items. However, it may permit conditions suitable for the growth and toxin production by anaerobic and facultative pathogenic organisms.

Modified atmospheres are generated during packaging by the initial alteration of the gaseous environment in the immediate vicinity of the product. This is achieved by filling the headspace of the food packages by 20-60% carbon dioxide, which will further vary depending on the type of fruits and vegetables and the targeted microorganisms. Modified atmospheres slow down the respiration rate of food as well as microbial growth and reduce the enzymatic degradation. Under these conditions, a variety of spoilage organisms, including *Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp. are inhibited, yet lactic acid bacteria grow slowly.

Removal of microorganisms

The removal of microorganisms is not a very suitable and effective way of food preservation, though it may be helpful under special conditions. Removal may be accomplished by filtration, centrifugation, washing, or trimming. Filtration through a previously sterilized filter made of asbestos pads, sintered glass, diatomaceous earth or similar materials has been used successfully for fruit juices, beer, soft drinks, wine, and water. Centrifugation or sedimentation, generally is not very effective in removing all microorganisms, though is applied for the treatment of water and clarification of milk. The bacteria- removing - centrifuge called bactofuge is used to remove heat-resistant and other bacteria from the milk prior to pasteurization (Fig. 1). This includes the spores of heat resistant bacteria such as *Clostridia sp.* and *Bacillus sp.*, which can remain active in the milk after pasteurization. By using bactofuge, milk has a longer shelf life, better taste, lower bacterial cell counts and reduced impurities.

Food preservation by high temperature

• By destructive effect of heat on microorganisms
• Temperature higher than ambient temperature is applied to food
• By two methods *viz*. pasteurization and sterilization.

Pasteurization:

• Use of heat at 60~80^{0}C for a few minutes for the elimination/ destruction of all disease causing microorganisms, and reduction of potential spoilage organisms
• Commonly used in the preservation of milk, fruit juices, pickles, sauces, beer etc
• Milk Pasteurization - heating the milk at 63^{0}C for 30 min, called low temperature long time (LTLT) process;
• 72^{0}C for 15 sec, called high temperature short time (HTST) process. This process destroys most heat resistant non-spore forming pathogens (Ex. *Mycobacterium tuberculosis*), all yeasts, molds, Gram negative bacteria and most Gram positive bacteria

Organisms surviving pasteurization

• Some organisms survive pasteurization process. The surviving organisms are of two types;
1. Thermoduric microorganisms
2. Thermophilic microorganisms
• Thermoduric microorganisms - survive exposure to relatively high temperature but do not grow at these temperatures
• Example: The non-spore forming *Streptococcus* and *Lactobacillus* sp can grow and cause spoilage at normal temperature. So, milk need to be refrigerated after pasteurization to prevent spoilage.
• Thermophilic - not only survive high temperature treatment but require high temperature for their growth and metabolic activities. Example: *Bacillus, Clostridium, Alicyclobacillus, Geobacillus etc.*

Sterilization:

• Sterilization or appertization - destruction of all viable organisms in food as measured by an appropriate enumeration method
• Kills all viable pathogenic and spoilage organisms
• Survivors - non-pathogenic and unable to develop in product under normal conditions of storage
• Thus, sterilized products have long shelf life.
• Commercial sterility - canned foods to indicate the absence of viable microorganisms detectable by culture methods or the number of survivors is so low that they are of no significance under condition of canning and storage
• Foods (solid or semisolid) - packing in cans, sealing and then sterilized
• Liquid foods are sterilized, packed in suitable containers and sealed aseptically
• Temperature and time of sterilization given to a food depends on the nature (pH, physical state, nutritional type etc) of the food being processed

Heat resistance of spores

• Bacterial spores - more heat resistant than vegetative cells. High temperature in canning- spore inactivation .The heat resistance of bacterial endospore is due to their ability to maintain very low water content in the DNA containing protoplast. Presence of calcium

and dipicolonic acid in high concentration in spores helps to reduce cytoplasmic water. Higher the degree of spore dehydration greater will be its heat resistance

Factors affecting heat destruction of microorganisms

Water: Heat resistance of microorganisms increases with decrease in moisture/ water activity and humidity. This is due to faster denaturation of protein in presence of water than air.

Fat: Heat resistance increases in presence of fat due to direct effect of fat on cell moisture. Heat protective effect of long chain fatly acids is better than short chain fatly acids.

Salts: Effect depends on type of salt, concentration used, and other factors. Some salts (sodium salts) have protective effect on microorganisms and others (Ca^{2+} and Mg^{2+}) make cells more sensitive. salts (Ca and Mg) increase water activity, while others (Na^{+}) decrease water activity there by affecting heat sensitivity

Carbohydrtes: Increases heat resistance of microorganisms due to decreased a_w. Heat resistance decreases in the order of; sucrose>glucose>sorbitol>fructose>glycerol

PH: Microorganisms are most heat resistant to heat at their optimum pH for growth (about pH 7-0). Increase or decrease in pH reduces heat sensitivity. High acid foods require less heat processing than low acid foods

Proteins: Proteins have protective effect on microorganisms. High protein foods need a higher heat treatment than low protein foods to obtain similar results

Number of microorganisms: Larger the number of microorganisms, higher the degree of heat resistance due to the production of protective substance excreted by bacterial cells, and natural variations in a microbial population to heat resistance

Inhibiting compounds: Heat resistance of most microorganisms decreases in the presence of heat resistant microbial inhibitor such as antibiotic (nisin), sulphur dioxide etc. Heat and inhibiting substances together are more effective in controlling spoilage of foods than either alone

Time and temperature: The longer the heating time, greater the killing effect. But higher the temperature, greater will be the killing effect. As temperature increases, time necessary to achieve the same effect decreases. The size and composition of containers affect heat penetration **Thermal destruction of microorganisms**

• The preservative effect of high temperature treatment depends on the extent of destruction of microorganisms

• Certain basic concepts are associated with the thermal destruction of microorganisms include;

– Thermal death time (TDT)

– D- value

– Z- value

– F- value

– 12D concept

Thermal death time (TDT):

• TDT - time required to kill a given number of organisms at a specified temperature

• Temperature is kept constant and the time necessary to kill all cells is determined

• Thermal death point is the temperature necessary to kill given number of organisms in a fixed time, usually 10 min. But it is of less importance

• TDT is determined by placing a known number of bacterial cells/spores in sealed

containers, heating in a oil bath for required time and cooling quickly

• The number of survivors from each test period is determined by plating on a suitable growth media

• Death is defined as the inability of organism to form viable colonies after incubation.

D-value (Decimal reduction time):

• D-value is the time in minutes required at specified temperature to kill 90% of microorganisms thereby reducing the count by 1 log units

• Hence D – value is the measure of death rate of microorganisms

• It reflects the resistance of an organism to a specific temperature and can be used to compare the relative heat resistance among different organisms/spores

• D-value for the same organism varies depending on the food type

• D -value is lower in acid foods and higher in presence of high proteins

• Example: D o o for *B. stearothermophilus*: 4-5 min

250 F (121.1 C)
o
C. botulinum: 0.1 – 0.2 min.
» D 95 C for *B. coagulans*: 13.7 min
B. licheniformis: 5.1 min.

Z – Value:

• Z-value refers to degrees of Fahrheit required for the thermal destruction curve to drop by one log cycle

• Z value gives information on the relative resistance of an organism for different destruction temperature

• It helps to determine equivalent thermal process at different temperature

• Example: If adequate heat process is achieved at 150^{0}F for 3 min and Z -value was determined as 10 ^{0}F, which means the10 ^{0}F rise in temperature reduces microorganisms by 1 log unit

• Therefore, at 140 ^{0}F , heat process need to be for 30 min and at 160 ^{0}F for 0.3 min to ensure adequate process

F – Value:

• F- value is the better way of expressing TDT. F- is the time in minutes required to kill all spores/vegetative cells at 250 ^{0}F (1210 ^{0}C)

• It is the capacity of heat process to reduce the number of spores or vegetarian cells of an organism

• F – Value is calculated by

$F_0 = D_r$ (log a-log b)
D_r = Decimal reduction time (D value) a = initial cell numbers
b = final cell numbers

12D concept:

• 12D concept is used mainly in low acid canned foods (pH >4.6) where *C. botulinum* is a serious concern

• 12D concept refers to thermal processing requirements designed to reduce the probability of survival of the most heat resistant *C. botulinum* spores to 10^{-12}

• This helps to determine the time required at process temperature of 121^o C to reduce spores of *C. botulinum* to 1 spore in only 1of 1 billion containers (with an assumption that each container of food

containing only 1 spore of *C. botulinum)*

Protection of Foods with Low Temperatures

☐The use of low temperatures to preserve foods is based on the fact that the activities of 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 (Q10) may be generally defined as follows:

$$Q_{10} = \frac{(\text{Velocity at a given temp.} + 10^{\circ}\text{C})}{\text{Velocity at T}}$$

☐The Q10 for most biological systems is 1.5–2.5, so that for each 10◦C rise in temperature within the suitable range, there is a twofold increase in the rate of reaction. For every 10◦Cdecrease in temperature, the reverse is true.

☐**Psychrophile:** This term is now applied to organisms that grow over the range of subzero to 20◦C, with an optimum range of 10–15◦C.

☐**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 in this temperature range.

☐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.

Table 3.12 *Cardinal temperatures for microbial growth*

Group	*Temperature (°C)*		
	Minimum	*Optimum*	*Maximum*
Thermophiles	40–45	55–75	60–90
Mesophiles	5–15	30–40	40–47
Psychrophiles (obligate psychrophiles)	−5 to +5	12–15	15–20
Psychrotrophs (facultative psychrophiles)	−5 to +5	25–30	30–35

☐**Psychrotrophs include:**

➢ Eurypsychrotroph (eurys, wide or broad):

☐Typically do not form visible colonies until sometime between 6 and 10 days. Can grow well at 43◦C. Such as Enterobacter cloacae, Hafnia alvei, and Yersinia enterocolitica .

➢ Stenopsychrotroph (stenos, narrow, little, or close):

☐ Stenopsychrotrophs typically form visible colonies in about 5 days. Do not grow at 40◦C Such as *Pseudomonas fragi* and *Aeromonas hydrophila* . Psychrotrophs can be distinguished from nonpsychrotrophs by their inability to grow on a nonselective medium at 43◦C in 24 hours, whereas the latter do grow.

☐**There are three distinct temperature ranges for low-temperature stored foods:**
1) Chilling temperatures are those between the usual refrigerator (5–7◦C) and ambient temperatures, usually about 10–15◦C. These temperatures are suitable for the storage of certain vegetables and fruits such as cucumbers, potatoes, and limes.

2) Refrigerator temperatures are those between 0◦C and 7◦C (ideally no higher than 40◦F or 4.4◦C).

3) Freezer temperatures are those at or below −18◦C.

PREPARATION OF FOODS FOR FREEZING
☐Blanching is achieved either by a brief immersion of foods into hot water or by the use of steam.

☐Its primary functions are as follows:

- 1. Inactivation of enzymes that might cause undesirable changes during freezing storage
- 2. Enhancement or fixing of the green color of certain vegetables
- 3. Reduction in the numbers of microorganisms on the foods
- 4. Facilitating the packing of leafy vegetables by inducing wilting
- 5. Displacement of entrapped air in the plant tissues

➢ Although it is not the primary function of blanching to destroy microorganisms, the amount of heat necessary to effect destruction of most food enzymes is also sufficient to reduce vegetative cells significantly.

☐**FREEZING OF FOODS AND FREEZING EFFECTS**
☐The two basic ways to achieve the freezing of foods are:

☐Quick (fast) freezing :
➢ Temperature of foods is lowered to about −20◦C within 30 minutes.

➢ Form small intracellular ice crystals.

☐**Slow freezing:**

➢ Temperature of foods is lowered within 3–72 hours.

➢ This is essentially the type of freezing utilized in the home freezer.

➢ Form large extracellular ice crystals.

➢ Crystal growth is one of the factors that limit the freezer life of certain foods, because ice crystals growin size and cause cell damage by disrupting membranes, cell walls, and

internal structures to the point where the thawed product is quite unlike the original in texture and flavor.
➢ Upon thawing, foods frozen by the slow freezing method tend to lose more drip (drip for meats; leakage in the case of vegetables) than quick-frozen foods held for comparable periods of time.

□EFFECT OF FREEZING ON MICROORGANISMS
In considering the effect of freezing on those microorganisms that are unable to grow at freezing temperatures, it is well known that freezing is one means of preserving microbial cultures, with freeze drying being perhaps the best method known.
However, freezing temperatures have been shown to effect the killing of certain microorganisms of importance in foods.
□The salient facts of what happens to certain microorganisms upon freezing:
➢ 1. There is a sudden mortality immediately on freezing, varying with species.

➢ 2. The proportion of cells surviving immediately after freezing die gradually when stored in the frozen state.

➢ 3. This decline in numbers is relatively rapid at temperatures just belowthe freezing point, especially about −2◦C, but less so at lower temperatures, and it is usually slow below

−20◦C.
➢ Bacteria differ in their capacity to survive during freezing, with cocci being generally more resistant than Gram-negative rods.

➢ Of the food-poisoning bacteria, salmonellae are less resistant than Staphylococcus aureus or vegetative cells of clostridia, whereas endospores and food-poisoning toxins are apparently unaffected by low temperatures.

➢ From the strict standpoint of food preservation, freezing should not be regarded as a means of destroying foodborne microorganisms.

➢ Low freezing temperatures of about −20◦C are less harmful to microorganisms than the median range of temperatures, such as −10◦C.

➢ For example, more microorganisms are destroyed at −4◦C than at −15◦C or below.

➢ Temperatures below −24◦C seem to have no additional effect.

➢ Food constituents such as egg white, sucrose, corn syrup, fish, glycerol, and undenatured meat extracts have all been found to increase freezing viability, especially of food- poisoning bacteria, whereas acid conditions have been found to decrease cell viability.

Consider some of the events that are known to occur when cells freeze:

➢ 1. The water that freezes is the so-called free water. Upon freezing, the free water forms ice crystals. Bound water remains unfrozen. The freezing of cells depletes them of usable liquid water and thus dehydrates them

➢ 2. Freezing results in an increase in the viscosity of cellular matter, a direct consequence of water being concentrated in the form of ice crystals.

➢ 3. Freezing results in a loss of cytoplasmic gases such as O2 and CO2. A loss of O2 to aerobic cells suppresses respiratory reactions. Also, the more diffuse state of O2 may make for greater oxidative activities within the cell.

➢ 4. Freezing causes changes in pH of cellular matter. Various investigators have reported changes ranging from 0.3 to 2.0 pH units. Increases and decreases of pH upon freezing and thawing have been reported.

➢ 5. Freezing effects concentration of cellular electrolytes. This effect is also a consequence of the concentration of water in the form of ice crystals.

➢ 6. Freezing causes a general alteration of the colloidal state of cellular protoplasm. Many of the constituents of cellular protoplasm such as proteins exist in a dynamic colloidal state in living cells. A proper amount of water is necessary to the well-being of this state.

➢ 7. Freezing causes some denaturation of cellular.

➢ 8. Freezing induces temperature shock in some microorganisms. This is true more for thermophiles and mesophiles than for psychrophiles. More cells die when the temperature decline above freezing is sudden than when it is slow.

➢ 9. Freezing causes metabolic injury to some microbial cells such as certain Pseudomonas spp. Some bacteria have increased nutritional requirements upon thawing from the frozen state and as much as 40% of a culture may be affected in this way.

Effect of Thawing

➢ Repeated freezing and thawing will destroy bacteria by disrupting cell membranes.

➢ Also, the faster the thaw, the greater the number of bacterial survivors. Why this is so is not entirely

clear.

➢ It has been pointed out that thawing is inherently slower than freezing and follows a pattern that is potentially more detrimental.

Among the problems attendant on the thawing of specimens and products that transmit heat energy primarily by conduction, are the following:

➢ 1. Thawing is inherently slower than freezing when conducted under comparable temperature differentials.

➢ 2. In practice, the maximum temperature differential permissible during thawing is much less than that which is feasible during freezing.

➢ 3. The time–temperature pattern characteristic of thawing is potentially more detrimental than that of freezing. During thawing, the temperature rises rapidly to near the melting point and remains there throughout the long course of thawing, thus affording considerable opportunity for chemical reactions, recrystallization, and even microbial growth, if thawing is extremely slow.

➢ It has been stated that microorganisms die not upon freezing but, rather, during the thawing process.

➢ As to why some organisms are able to survive freezing while others do not, Luyet39 suggested that it is a question of the ability of an organism to survive dehydration and to undergo dehydration when the medium freezes.

➢ With respect to survival after freeze-drying, Luyet has stated that it may be due to the fact that bacteria do not freeze at all but merely dry up.

➢ It is fairly well established that the freeze-thaw cycle leads to:

(1) ice nucleation

(2) dehydration,

(3) oxidative damage.

➢ During thawing, an oxidative burst has been shown to occur and superoxide dismutase (SOD) provides resistance to the deleterious oxidative effects

➢ Most frozen-foods processors advise against the refreezing of foods once they have been thawed.

➢ Although the reasons are more related to the texture, flavor, and other nutritional qualities of the frozen product, the microbiology of thawed frozen foods is pertinent.

➢ Some investigators have pointed out that foods from the frozen state spoil faster than similar fresh products.

➢ organisms into deeper parts of the produce and, consequently, facilitate the spoilage process. Upon thawing, surface condensation of water is known to occur.

o There is also, at the surface, a general concentration of water-soluble substances such as amino acids, minerals, B vitamins, and, possibly, other nutrients.

o Freezing has the effect of destroying many thermophilic and some mesophilic organisms, making for less competition among the survivors upon thawing.

o It is conceivable that a greater relative number of psychrotrophs on thawed foods might increase the spoilage rate.

o Some psychrotrophic bacteria have been reported to have Q10 values in excess of 4.0 at refrigerator temperatures.

o For example, P. fragi has been reported to possess a Q10 of 4.3 at 0◦C.

o Organisms of this type are capable of doubling their growth rate with only a 4–5◦C rise in temperature.

o Although there are no known toxic effects associated with the refreezing of frozen and thawed foods, this act should be minimized in the interest of the overall nutritional quality of the products.

o One effect of freezing and thawing animal tissues is the release of lysosomal enzymes consisting of cathepsins, nucleases, phosphatases, glycosidases, and others.

o Once released, these enzymes may act to degrade macromolecules and thus make available simpler compounds that are more readily utilized by the spoilage biota.

SOME CHARACTERISTICS OF PSYCHROTROPHS AND PSYCHROPHILES

There is an increase in unsaturated fatty acid residues.

➢ It is known that an increase in the degree of unsaturation of fatty acids in lipids leads to a decrease in the lipid melting point.

➢ It has been suggested that increased synthesis of unsaturated fatty acids at low temperatures has the function of maintaining the lipid in a liquid and mobile state, thereby allowing membrane activity to continue to function.

This concept, referred to as **the lipid solidification theory Psychrotrophs** synthesize high levels of polysaccharides.

➢ From a practical standpoint, increased polysaccharide synthesis at low temperatures manifests itself in the characteristic appearance of low-temperature spoiled meats.

➢ Slime formation is characteristic of the bacterial spoilage of frankfurters, fresh poultry, and ground beef.

➢ The coalescence of surface colonies leads to the sliminess of such meats, and no doubt contributes to the increased hydration capacity that accompanies low-temperature meat spoilage.

➢ This extra polymeric material undoubtedly plays a role in biofilm formation.

☐**Pigment production is favored**.
➢ This effect appears to be confined to those organisms that synthesize phenazine and carotenoid pigments.

☐**Some strains display differential substrate utilization.**
THE EFFECT OF LOW TEMPERATURES ON MICROBIAL PHYSIOLOGIC MECHANISMS
Psychrotrophs have a slower metabolic rate.
➢ The precise reasons as to why metabolic rates are slowed at low temperatures are not fully understood.

➢ The temperature coefficients (Q10) for various substrates such as acetate and glucose have been shown by several investigators to be lower for growing psychrotrophs than for mesophiles.

➢ As noted above, psychrotrophs tend to possess in their membrane lipids that enable the membrane to be more fluid.

➢ The greater mobility of the psychrotrophic membrane may be expected to facilitate membrane transport at low temperatures.

➢ In addition, the transport permeases of psychrotrophs are apparently more operative under these conditions than are those of other mesophiles.

➢ As the temperature is decreased, the rate of protein synthesis is known to decrease, and this occurs in the absence of changes in the amount of cellular DNA.

➢ One reason may be the increase in intramolecular hydrogen bonding that occurs at low temperatures, leading to increased folding of enzymes with losses in catalytic activity.

➢ **Psychrotroph membranes transport solutes more efficiently**.
➢ It has been shown in several studies that upon lowering the growth temperature of mesophiles within the psychrotrophic range, solute uptake is decreased.

➢ **Some psychrotrophs produce larger cells**.
➢ Yeasts, molds, and bacteria have been found to produce larger cell sizes when growing under psychrotrophic conditions than under mesophilic conditions.

➢ On the other hand, psychrotrophic organisms are generally regarded as having higher levels of both RNA and proteins.

➢ **Flagella synthesis is more efficient.**
➢

➢ It has been commonly observed that plate counts on many foods are higher with incubation at low temperatures than at temperatures of 30◦C and above.

➢ The generally higher counts are due in part to the increased solubility and consequently, the availability of O2.

➢ **Some psychrotrophs display an increased requirement for organic nutrients.**
➢ In one study, the generation times for unidentified aquatic bacterial isolates in low- nutrient media were two to three times longer than in high-nutrient media.

NATURE OF THE LOW HEAT RESISTANCE OF PSYCHROTROPHS/PSYCHROPHILES
➢ The maximum growth temperatures of bacteria may bear a definite relationship to the minimum temperatures of destruction of respiratory enzymes.

➢ It has been shown that many respiratory enzymes are inactivated at the temperatures of maximal growth of various psychrotrophic types.

➢ Thus, the thermal sensitivity of certain enzymes of psychrotrophs is at least one of the factors that limit the growth of these organisms to low temperatures.

➢ Somewhat surprisingly, the proteinases of many psychrotrophic bacteria found in raw milk are heat resistant.

➢ The typical raw milk psychrotrophic pseudomonad produces a heat stable metalloproteinase with molecular weight in the 40- to 50-kDa range, which has a D value at 70◦C of 118 minutes or higher.

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Irradiation

The type of radiation of primary interest in food preservation is electromagnetic which includes microwaves, ultraviolet rays, and ionizing radiations.

Microwave radiations

The microwave region of the electromagnetic spectrum occupies frequencies between the infrared (10^9 Hz) and radio frequency (10^{12} Hz) and has relatively low quantum energy. Most food research has been carried out at two frequencies; 915MHz and 2450 MHz. Microwaves are generated using a magnetron, a device first developed in the UK during research into radar during the Second World War. Although microwaves are used both commercially and domestically in domestic microwave ovens and in catering, these have been slow to find industrial applications in food processing. Microwaves have been used to defrost frozen meats before cutting, in blanching of vegetables and fruits, destruction of molds in bread, pasteurization of beer and sterilization of wine.

Microwaves act indirectly on microorganisms through the generation of heat. When food-containing water is placed in a microwave field of 950 MHz, water molecules oscillate back and forth 915 million times/sec creating an intermolecular fraction. This kinetic energy is transmitted to neighboring molecules leading to a rapid rise in temperature throughout the product. This heating effect is responsible for killing microorganisms in food exposed to microwave radiations.

UV Radiation

Ultraviolet light is a powerful bactericidal agent with the most effective wavelength being about 260 nm. It is absorbed by purine and pyrimidine bases causing the production of covalent bonds between adjacent thymine molecules giving thymine dimers. This may prevent the DNA replication in the normal way and disrupt gene functioning by creating new mutants. Although microorganisms have the capacity to repair this DNA damage, extensive damage may cross the limits of DNA repair mechanisms leading to cell death. The resistance of microorganisms to UV is largely determined by their ability to repair such damage. In addition to the repair mechanisms, some organisms such as micrococci also synthesize protective pigments. Generally, the resistance to UV irradiation follows the pattern. Gram-negative < Gram-positive < yeast < bacterial spores < mold spores < viruses. The UV D values for these groups are 3-4, 6-8, 6-10, 8-10, 20-100 and >200 ergs x 10^2 respectively.

High intensity ultraviolet radiation generated by low-pressure mercury vapor lamps is extremely effective in killing microorganisms. The poor penetrating capacity of UV light restricts its use in food applications. UV radiations are able to penetrate only to 300-500 cms in air, 30 cms in water, 0.1 cm in glass and 0.01 cm in milk. Therefore, the practical applications of UV light are limited to surface disinfections and air sterilization such as in hospital theaters, aseptic filling rooms in pharmaceutical industry, in food and dairy industry (in sterile packaging of UHT milk and in bakery to control mold spores). UV radiation is commonly used as an alternative to chlorination in the disinfections of water in water filters installed at homes and offices such as Aqua Guard, Aqua Care etc.

Ionizing Radiations

Ionizing radiations such as X-rays and gamma γ-rays generated by X-ray apparatus and radioisotopes such as cobalt 60 (^{60}Co and ^{137}Cs) respectively are highly effective in killing microorganisms. Since they destroy microorganisms without appreciably raising temperature, the process is termed "cold sterilization." Ionizing radiations can affect the cells directly by interacting with key molecules with in the microbial cell. The main site of damage in cells is the chromosome where hydroxyl radicals cause single and double strand breaks in the DNA molecule as a result of hydrogen removal from deoxyribose sugar. Further cleavage of the molecule occurs by β elimination of phosphate. Ionizing radiations also have indirect inhibitory effect on cell constituents by generating free radicals produced by the radiolysis of water. Free radicals formed from water can combine with each other or oxygen molecules to give powerful oxidizing agents that can damage cell components. Thus in the absence of water and oxygen, radiation doses 2-3 times higher are required to obtain the same lethality

Death of microorganisms caused by ionizing radiation is logarithmic, producing survivor curves that are similar to those produced by heat. In this case, the number of survivors is plotted against the radiation dose and D values are calculated as the dose required to kill 90% of the population. The radiation dose is currently measured in Gray (Gy), which is equivalent to 1 joule of energy absorbed/kg of material. Microbial resistance to radiation usually decreases in the order viruses > bacterial spores > pigmented mold spores > yeast and molds > Gram positive bacteria > Gram

negative bacteria. The most resistance organism is *Micrococcus radiodurans* which has a D value of >30 kGy.

The electromagnetic radiations (gamma rays) emitted from the excited nucleus of ^{60}Co or ^{137}Cs are the cheapest form of radiation for food preservation. Unlike UV light, gamma rays have excellent penetration power so that foods can be packaged and then irradiated to destroy contaminating microorganisms, making it potentially an ideal method of food preservation. Foods are irradiated by using gamma rays in the three following ways:

i) Radappertization

Radappertization is equivalent to radiation sterilization or "commercial sterility" of low acid foods which requires a dose of radiation capable of giving a 12D reduction in the number of spores of *Clostridium botulinum*. As the D value for *C. botulinum* is 3.5 kGy, the dose required will be 42 kGy to achieve 12D kill. The application of radappertization is restricted to only few food products such as bacon as the high doses of radiation may cause color changes and or production of off odors.

ii) Radicidation

Radicidation refers to reduction of the number of viable specific non-sporeforming bacterial pathogens such as *Salmonella* and is equivalent to pasteurization of milk. Irradiation levels of 2-5 kGy are effective in destroying non-sporeforming and non-viral pathogens. The foods such as fresh poultry, cod and red fish, and spices and condiments are preserved by irradiating at these levels.

iii) Radurization

Radurization refers to the enhancement of the keeping quality of a food by causing substantial reduction in the numbers of viable spoilage microorganisms especially gram-negative, non-sporeforming rods by low levels of radiation. Common dose levels are 0.075-2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains. The shelf life of seafood, fish and shellfish may be extended from two to six folds by radurization.

Not all foods are suitable for irradiation treatment. Softening and discoloration may occur in the case of some fruits. Milk may acquire an unpleasant taste. Certain protein foods are flavor sensitive to irradiation and may develop off-flavors. Another major limitation of irradiation processing of food is its slow acceptance by the consumers, due to a perceived association with radioactivity.

High Hydrostatic Pressures

During high hydrostatic pressure (HHP) processing, foods are subjected to pressures in the range 100 to 1000 MPa (megapascals). High pressures are known to have an antimicrobial effect which appears to be associated with the denaturation of cell proteins and damage to cell membranes. Membrane lipid bilayers have been shown to compress under pressure that alters their permeability. The application of high pressures for food processing is referred to as pascalization. Overall, HHP is very effective in inactivating vegetative cells of microorganisms, but pressure treatment alone does not achieve a substantial inactivation of spores and reduction in activity of certain enzymes. Although, vegetative bacteria, yeast and molds can be reduced by at least one log cycle by 400 MPa applied for 5 min, bacterial endospores can tolerate pressures

as high as 1200 MPa. Therefore, the commercial application of pascalization has been limited to only acid and high acid foods like fruit juices and sauces in which bacterial spores that survive processing are unable to grow. Foods preserved by this technology resemble very much to the fresh product and appears natural to the consumer with none of the negative associations of processes such as heat, irradiation and chemical preservation. Interest in using high-pressure technology to extend the shelf life of low acid foods is increasing by combining this treatment with other food preservation methods.

Relative resistance of microorganisms to HHP is as follows: Bacterial spores > Gram-positives (vegetative cells) > Gram-negatives ≈ yeast and molds.

porganisms. They do not include substances, which enhance the shelf life of foods by iting a chemical reaction such as rancidity or discoloration. The chemical preservatives may ither intentionally added to the food or may be developed during the growth of porganisms as in case of some fermentation (lactic acid, acetic acid, bacteriocins etc.). The f chemical preservatives in foods may allow products to be subjected to less severe heat ments, resulting in an improvement in product quality and consumer acceptability. While a number of chemicals have been described that show potential as food preservatives, only a vely small number are allowed in food products. This is due in large part to the strict rules fety adhered to by Food and Drug Administration (FDA).

emical preservative should have a wide range of antimicrobial activity, should be non-toxic mans or animals, should be economical, should not have an effect on flavor, taste, aroma of riginal food, should not be inactivated by food and should not encourage the development of ant strains. There are added chemical preservatives which are not defined as such by law as natural organic acids (lactic, malic, citric etc.), vinegars, sodium chloride, sugars, spices heir oils, wood smoke etc. On the other hand, there are some chemical substances, which enerally recognized as safe (GRAS) for addition to foods such as organic acids and their (Propionic, sorbic and benzoic) sodium nitrite, sulfur dioxide and metabisulfites and nisin, a eservative. Most of the common antimicrobial additives used in foods and their current rable levels are presented in Table 2.

nic Acids and their Salts

c, citric, tartaric acids are found naturally in fruits and will inhibit most bacteria. Lactic and c acids are produced naturally by microorganisms in amounts sufficient to exert an effect on and the pH of the product, thus potentiating their own action by increasing the proportion ndissociated acid present. Propionic, sorbic, benzoic acids and parabens (*para* xybenzoic acid esters) are not generally found naturally in foods or produced by porganisms. There are exceptions, e.g. propionic acid is produced in Swiss cheese by *ionibacterium spp* and benzoic acid is found in cranberries. These acids are sometime dered to be 'true' chemical preservatives.

oic Acids and Parabens

oic acids and its sodium salts are widely used as antimicrobial compounds in a large er of foods. The antimicrobial activity of benzoate is related to pH, the greatest activity at low pH values and essentially ineffective at neutral values. This indicates that the icrobial activity resides in the undissociated molecule at pH between 2.5 and 4.0. This ts in the restriction of benzoic acid and its sodium salts to high acid products such as apple , soft drinks, jams, jellies, fruit salads, pickles, tomato catsup. As used in acidic foods, oates and their sodium salts act mainly as a mold and yeast inhibitor.

ng parabens, ethyl and methyl parabens are extensively used in foods. Though, these ounds are similar to benzoic acid in their effectiveness, they have an added advantage of effective at even higher pH values. Because of the esterification of the carboxyl group, the sociated molecule is retained over a wider pH range exerting inhibitory effect even at al pH. This means that they can be used effectively in low and non-acid foods.

Maximum levels of some GRAS chemical food preservatives permitted in foods

Preservatives	Maximum concentrations allowed	Organisms affected	Foods
Benzoic acids	0.1%	Yeasts and molds	Jams, jellies, salad dressings, apple cider, soft drinks, pickles, tomato catsup
Parabens (methyl-, propyl-, and heptyl esters of p-hydroxybenzoic acid)	0.1%	Yeasts and molds	Fruit drinks and beverages, bakery products, salad dressings, apple cider, soft drinks, pickles, tomato catsup
Propionic acid Sorbic acid	0.32% 0.2%	Molds Molds and yeast	Bread, cakes, Swiss cheese Hard cheeses, baked goods, fruit cocktails, syrups, fruit juices, jams and jellies, dried fruits, margarine
SO_2 and sulfites	200-300 ppm	Insects and microorganisms	Wines, molasses, fruit juices, lemon juice, dried fruits (not to be used in meats or other foods containing thiamine)
Nitrites and nitrates	100-120 ppm	Clostridia and molds	Meat and meat products as a meat curing agent
Ethylene and propylene oxide	700 ppm	Yeasts, molds and Clostridia	Fumigants for dried fruits, dried eggs, gelatin, cereals, dried yeast and spices
Ozone	>100 ppm 0.2-0.4 ppm 5-15 ppm	Viruses *Salmonella*, *Pseudomonas* *Botrytis*	Animal sanitation Fish and Poultry Vegetables
Nisin (biopreservative)	100 ppm	Gram+ve spore formers	Processed cheeses, canned fruits and vegetables, condensed milk

In the undissociated form these compounds are soluble in the cell membrane and act apparently as proton ionophores. As such they facilitate proton leakage into the cells thereby increasing the energy output of cells to maintain their usual internal pH. With this disruption in membrane activity, amino acid transport is adversely affected. These compounds have also been found to block the oxidation of glucose and pyruvate at the acetate level. Benzoates have also been found to inhibit the outgrowth of vegetative cells during endospore germination. Maximum concentration of benzoates permitted in foods is 0.1%.

Sorbic Acid

Sorbic acids and their calcium, potassium or sodium salts are permissible in foods at levels not to exceed 0.2%. Like benzoates, they are also most effective at low pH values when present in the undissociated form. These compounds are more effective than sodium benzoate at pH values

between 4.0 and 6.0. Sorbic acid and its salts are used either as a direct antimicrobial additive in foods or as a spray, dip, or coating on packaging materials. These are widely used in cheeses, cheese products, bakery products, beverages, syrups, fruit juices, jellies, jams, pickles and salad dressings. They are active against yeasts, molds and catalase positive bacteria. Inhibition of mold growth by sorbates is due to the inhibition of the dehydrogenase enzyme system, several other Krebs cycle enzymes and the membrane function impairment affecting the cellular uptake of substrate molecules such as amino acids, phosphate and organic acids. Sorbic acid is also known to inhibit the germination and outgrowth of *C. botulinum* spores.

Propionic acid

Propionic acid and its calcium or sodium salts are permitted in breads, cakes, and certain cheeses as a mold inhibitor to maximum levels of not more than 0.32%. In bread and bread dough it prevents ropiness by inhibiting the rope forming bacilli e.g. *Bacillus subtilis or B. licheniformis*. The mode of action of these compounds on microorganisms is similar to that of benzoates and sorbates. Dissociation tendency of these compounds at high pH values makes them useful preservatives for low acid foods.

Nitrite

Sodium nitrate ($NaNO_3$) and sodium nitrite ($NaNO_2$) are used in curing formulae for meats since they stabilize red meat color, inhibit some spoilage and food poisoning organisms, and contribute to flavor development. In an acid environment nitrite ionizes to nitrous acid that further decomposes to nitric oxide. The nitric oxide co-ordinates to the haem ferrous ion in the muscle pigment myoglobin under reducing conditions converting it to the desirable red pigment nitosomyoglobin. The antibacterial effect of nitrite increases with decreasing pH suggesting that nitrous acid is the active agent. This nitrous acid, being a powerful reducing agent, causes disruption of the cell metabolism and also inhibits the germination and outgrowth of endospores. Nitrite acts as a preservative by inhibiting a wide range of bacteria; including *Clostridium* spp (*C. botulinum* is of particular interest), *Bacillus spp* and *Staphylococcus aureus*. However nitrite is not very effective against lactobacilli or members of the enterobacteriaceae including salmonellae.

Interestingly, it has been shown that the ability of nitrite to inhibit these spore formers in cured, canned, vacuum packed meats and culture media will increase about ten fold if it is added before heating the product. This increased inhibitory activity of nitrite upon heating in a medium is due to the production of a substance referred to as 'Perigo factor'.

It is this Perigo factor that results from the heat processing or smoking of certain meats and fish products containing nitrite that warrants the continued use of nitrite in such products. Nitrite levels of 100 ppm or more in the presence of 3 5% sodium chloride are sufficient to impart an adequate flavor and antibotulinal and antilisterial (against *Listeria monocytogenes*, a bacterial food pathogen) effect in meat products. The only problem with the use of nitrite is their reaction with secondary amines forming nitrosamines that are known to be carcinogenic.

Sulfur Dioxide

Sulfur dioxide (SO_2) and the sodium and potassium salts of sulfite, bisulfite, and metabisulfite have been used as disinfecting agents in wine industry particularly to sanitize wine making equipment and storage vessels and to reduce the normal flora of the grape must. It is also used as an antioxidant to inhibit enzymatic and non-enzymatic browning reactions in some food products. Sulfur dioxide has also been used, in syrups, fruits juices and to treat most light colored dehydrated fruits. The unionized forms of SO_2, which can readily penetrate the cell, have the greatest antimicrobial activity. As a reducing agent it can break disulfide linkages in proteins, and interfere with redox processes. It can also form addition compounds with pyrimidine bases in nucleic acids, sugars and several key metabolic intermediates. However, it has been found to react and destroy the vitamin thiamine present in meat and meat products prohibiting its use in these products.

Sulfu dioxide is active against bacteria, yeasts and molds. Sulfu dioxide, sulfites and metabisulfites are used at 200 300 ppm levels in most of the foods to have their bactericidal effect on all types of microorganisms.

NaCl and Sugars

Both of these preservatives are similar in their mode of action in preserving foods. These compounds tend to tie up moisture and thus exert a drying effect on both food and microorganisms. Salts are added in brine and curing solutions or applied directly to foods to slow down and prevent the activity of food spoilage and pathogenic organisms. The addition of salts has the following effects on food and microorganisms:

1. It causes high osmotic pressure and hence, plasmolysis of cells,
2. It dehydrates foods and microbial cells by drawing out and tying up moisture,
3. It ionizes to yield the chlorine ion, which is harmful to organisms,
4. It reduces the solubility of oxygen in water.
5. It sensitizes the cell against carbon dioxide and
6. It interferes with the action of proteolytic enzymes. The concentration of salt in food varies with the taste of the consumer and type of food. In the absence of refrigeration, salting may effectively preserve fish and other meats.

Sugars such as sucrose exert the same preserving effect, as salt but requires in about six times higher concentrations than salt to affect the same degree of inhibition. The most common uses of sugars as preserving agents are in the making of fruit preserves, candies, chocolates, condensed milk, cakes and pies. The shelf stability of these products is due in large part to the preserving effect of high concentrations of sugar.

Gases

Gases can be used to sterilize materials, which can not withstand the high temperatures of heat sterilization like many organic compounds, volatile food flavors and some plastic material. Gaseous sterilization offers a means for packaging heat sensitive products that only affect airborne surface bacteria but also it can attack the microbial cells after penetrating the porous materials. Some of these gases used to inactivate microorganisms are ethylene oxide, propylene oxide, methyl bromide and formaldehyde.

-*Ethylene oxide*

Ethylene oxide, cyclic ether, is the most commonly used gas for effective sterilization of packaged items, dry products etc., at room temperature because of its good penetration with little damage to materials. The microbicidal action of ethylene oxide gas is directly related to the alkylating activity of cellular enzymes and other proteins. It has been used to sterilize spices, cereals, fruits and dry fruits and dried yeast. However, it is flammable, expensive, and toxic and requires three hours or more for effective sterilization and may alter nutrients and other quality factors of foods.

Ozone

Ozone has recently gained the attention of food and agricultural industries, though it has been used effectively as a primary disinfectant for the treatment of municipal and bottled drinking water for 100 years. In 2001, the Food and Drug Administration (FDA) allowed for the use of ozone as a direct contact-sanitizing agent.

Because of its very high oxidation reduction potential, ozone acts as an oxidant of the constituent elements of cell walls before penetrating inside microorganisms and oxidizing certain essential components e.g., unsaturated lipids, enzymes, proteins, nucleic acids, etc. When a large part of the membrane barrier is destroyed causing a leakage of cell contents, the bacterial or protozoan cells lyse resulting in the destruction of the cell. Most of the pathogenic and food borne microbes are susceptible to this oxidizing effect.

In aqueous solutions, ozone can be used to disinfect equipment, process water, and some food products. It has been used to decontaminate poultry meat, salmon, apples, strawberries and cauliflower. In gaseous form it has been to preserve eggs during cold storage, fresh fruits and vegetables, and fresh fish. Ozone can also be used during the washing of produce before it is packaged and shipped to supermarkets, grocery stores, and restaurants. In food industry, much attention is given to the cleaning and sanitizing operations of food-processing equipment. Water containing low concentrations of ozone can be sprayed onto processing equipment, walls or floors to both remove and kill bacteria or other organic matter that may be present.

The concentrations of ozone, which are large enough for effective decontamination, may change the sensory qualities and colour of some food products, such as meat, milk powder and fish cake due to lipid oxidation. Additionally, microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed to the sanitizer. Ozone is a toxic gas and can cause severe illness, and even death, if inhaled in high quantity. Exposure restrictions to plant operators must be addressed with leak proof system design and process operation

Biopreservatives

Artificial chemical preservatives are employed to limit the number of microorganisms capable of growing within foods, but increasing consumer awareness of potential health risks associated with some of these substances, has led researchers to examine the possibility of using natural additives. For instance, egg white lysozyme is employed at levels in excess of 100 tones per annum in some cheeses to prevent blowing (gas production) by lysing the vegetative cells of *Clostridium tyrobutyricum*. Activation of the lactoperoxidase system has been shown to be

useful to extend the keeping quality of milk in countries like India where pasteurization is not possible immediately after milking and refrigerated transport systems are poorly developed Plant derived antimicrobials such as the extracts of herbs and spices are being commonly used in preservation of foods for controlling microorganisms. Microbial products like antibiotics and bacteriocins in particular whether produced by fermenting microorganisms or added from outside are being increasing used in cheese and canned foods. The broad -spectrum antibiotics such as chlorotetracycline (CTC) or oxytetracycline (OTC) were permitted at 5-7µg/g in fish, poultry, shrimps, etc till 1959 However due to the hazards of the development of resistant strains of pathogens, the potential of hypersensitivity of humans to the antibiotics, the presence of residual antibiotics after cooking, costs and difficulties in monitoring these aspects, the use of these antibiotics in foods was never appreciated.

Bacteriocins produced by lactic acid bacteria (LAB) are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties. The food products that have been targeted for use of bacteriocins or bacteriocin like inhibitory substances include meat and meat products, fish products, dairy products, cereals, fruits and vegetables, and beverages. The bacteriocins can effectively be used to inhibit some gram-positive bacteria, spore-forming bacteria, and food-borne pathogens. The major classes of bacteriocins produced by LAB include:

1. lantibiotics,
2. small heat stable peptides,
3. large heat labile proteins, and
4. complex proteins whose activity requires the association of carbohydrates or lipid moieties.

Out of these, first two groups have received increased attention as food biopreservatives.

The most studied member among lantibiotics is Nisin A, a 34-residue antibacterial peptide that is produced by several strains of *Lactococcus lactis* and strongly inhibits the growth of a wide range of Gram positive bacteria. This mature peptide displays several unusual features, such as the dehydrated residues dehydroalanine, dehydrobutyrine, lanthionine and β-methyl-lanthionine residues. In Gram-positive bacteria nisin has been shown to act on energized membrane vesicles to disrupt the proton motive force, inhibit uptake of amino acids, and cause release of accumulated amino acids. Nisin A is being used at the concentrations of 100-200 ppm in the preservation of, dairy products such as cheeses and milk, meat products, and fish

Microgard products are bacteriocins like inhibitory substances produced by fermenting grade A skim milk with lactic acid bacteria. It has been approved by FDA and widely used as a biopreservative for more than a decade by the Cottage cheese industry. It is antagonistic toward most gram-negative bacteria and some yeasts and molds, but not against gram-positive bacteria

Lacticin 481 produced by *L. lactis*, lactocin S produced by *Lactobacillus sake* and carnocin U149 produced by *Carnobacterium piscicola* are the other lantibiotics, which are being tried as food biopreservatives.

Class II LAB bacteriocins are small heat stable, non-lanthionine containing membrane-active peptides. Few examples of class II bacteriocins, which have been studied for their antibacterial

effect, are pediocin produced by pediococci (widely applied in the fermentation of meat and vegetables) and leucocin A produced by *Leuconostoc spp*, another LAB found in meat and vegetable fermentations. These peptides are active against broad range of gram-positive bacteria including *Listeria monocytogenes*.

Reuterin is a water-soluble non-proteinaceous product produced by *Lactobacillus reuteri*. It has been described to have antimicrobial effect against certain gram-negative and gram-positive bacteria, yeasts, fungi, and protozoa. It inhibits *Salmonella*, *Shigella*, *Clostridium*, *Staphylococcus*, *Listeria*, and *Trypanosoma*.

Bacteriocins exhibit a very narrow inhibiting spectrum, typically active against only one target microorganism. The bacteriocin activity is not stable and loss occurs when it interacts with food components by binding with food lipids and proteins or being degraded by proteolytic enzymes.

Unit-II; Possible Questions
Part- A (1 Marks)

Part- B (2 Marks)
1. What are the principles of food preservation?
2. What are the methods of food preservation?
3. What is blanching?
4. What is pasteurization?
5. Define thermal temperature.
6. What is the use of benzoic acid?
7. Name the inorganic acids and their salts for food preservation?
8. Which chemical preservative is used for fish and meat products?
9. What are antibiotics?
10. What is radiation pasteurization?
11. Write the methods for drying
12. What is sterilization?

Part- C (8 Marks)
1. List and describe the principles upon which methods of food preservation are based.
2. Compare the preservation efficiency by temperature - high/low, which is best.
3. What is the lowest temperature range at which food poisoning bacteria will grow?
4. What is the difference between pasteurization and sterilization?
5. List the chemicals used for food preservation. Name the chemical used for beer and meat preservation.
6. List the factors responsible for food preservation.
7. Outline a procedure suitable for enumeration, isolation and identification of the following groups of microbes from a sample of food: thermophilic, spore formers, coliforms and viruses.
8. What is modified atmosphere packaging?
9. Define asepsis.
10. How will you remove the microbes?
11. What are the types of filters?
12. How do spores survive at high temperature?
13. What is thermal death point?
14. Compare quick and slow freezing.
15. What are the effects of thawing?

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M.Sc MB COURSE NAME: FOOD MICROBIOLOGY

COURSE CODE: 18MBP302 UNIT: II BATCH-2018-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	The concentration of salt used in high protein containing vegetables is _____	4.3-10.3	17.5-20.0	18.6-26.5	19.2-22.2	18.6-26.5
2	_____ is a term used to label foods treated with low level ionizing radiation	Radicidation	radurization	picowaved	radappertization	Picowaved
3	Flavoring etracts such as vanilla and lemon etracts are preserved by their content of ____	sugar	salt	alcohol	ethylene	alcohol
4	Which of the following statements are true about chemical preservatives _____	microbicidal or microstatic	chemical preservatives often hazardous to humans	sodium benzoate is a widely used preservative	all these	All of these
5	The time temperature combination for HTST paterurization of 71.1°C for 15 sec is selected on the basis of	*Coxiella Burnetii*	*E. coli*	*B. subtilis*	*C. botulinum*	*Coxiella Burnetii*
6	_____ contains a large number of olatile compounds that may have bacteriostatic and bactericidal effect	spices	woodsmoke	formaldehyde	alcohol	woodsmoke
7	---------- is a storage method uses bins or boxes for equalization of moisture	sweating	springer	cooling	freezing	springer
8	_____ is used most extensively in the prevention of mold growth and rope development in baked goods	calcium propionate	calcium sorbate	monocholroacetic acid	nitrates	calcium propionate
9	_____ can be dried by a process called explosive puffing	meat	vegetables	fruits	juices	vegetables
10	_____ in 1765 preserved food by heating it in a sealed containers	Spallanzani	Ruiz-Argueso	Rodrigeuz-Navarro	Christophersen	spallanzani
11	Combination of _____ irradiation with	Ultraviolet	infra red	gamma	none of the	ultraviolet

	chilling storage helps preserve foods				above	
12	Which solvent is commonly used to determine fat content	Ethyl alcohol	Hexane	Acetone	Benzene	Hexane
13	During _____ the internal temperature of bread, cake or other bakery products approaches but neve reaches 100 °C	Heating	boiling	baking	all of these	baking
14	Pasteurization is done to kill	Selective microorganism	All the microorganism	Yeast	Yeast and its spores	Selective microorganism
15	Sanitising is_____	Applying detergent to a clean surface	Done before washing	Reducing bacteria by application of heat or chemical	Wiping all surfaces with a clean cloth	Reducing bacteria by application of heat or chemical
16	The simplest dryer is the _____	sun	air	heat	evaporator	evaporator
17	Bacteria which is present in raw or undercooked meat, eggs, sea food and unpasteurized milk is	*E.coli*	*Salmonella*	*Staphylococcus*	cyano bacteria	*salmonella*
18	Milk and curry left over can be turned into sour and spoiled at	high temperature	very low temperature	room temperature	constant temperature	room temperature
19	_____ rays are streams of electrons emitted from radioactive materials	beta	cathode	gamma	X-rays	beta
20	Increase in the concentration of dissolved substances like sugar and salt helps in _____ of the food material	drying	freezing	moistening	thawing	drying
21	Sulfur stinker spoilage of canned food is caused by	*E.coli*	*D. nigrificans*	*Bacillus*	*Clostridium*	*D. nigrificans*
22	Radiation dose in kilograys of ____ inhibits sprouting in potatoes, onions and garlic	0.05-0.15	0.01-0.14	0.05-0.07	0.05-0.11	0.05-0.15

23	Preservation affects the growth of microorganism by	inhibition	retardation	arresting	all the above	retardation
24	Souring of canned meat is caused by _____.	thermoduric cells	thermostatic cells	thermo liable cells	none of the above	thermoduric cells
25	Significant numbers of S. aureus in a food can be determined by examining the food	RNase	thermostable nuclease	protease	thermostable DNase	protease
26	To retard the contamination and other microbial growth in meat is obtained by storing at _____ temperature	10°C	0°C	100°C	-10°C	0°C
27	Gazing at ultraviolet lamps produces irritation of the ____ within few seconds	eye	ear	nose	throat	eye
28	Sugars act as preservatives due to their ability to	make water unavailable to organism's	interfere with the action of proteolytic enzyme	osmotic effect	both a and c	interfere with the action of proteolytic enzyme
29	The minimal pH for the growth of staphylococcus is about ----------	2.5	4.8	2	3.5	4.8
30	_____ alcohol is used as coagulant and enaturizer of cell proteins	methanol	ethanol	butanol	none of these	ethanol
31	The fumes of burning ____ are used to treat light colored dehydrated fruits	sulfur	ethylene	potassium	sodium	sulfur
32	_____ can be used to control bacterial and fungal growth in tapholes of maple tree	paraformaldehyde	benzaldehyde	formaldehyde	all of these	paraformaldehyde
33	Christophersen classified microroganisms on the basis of sensitivity to freezing in the year_____	1984	1989	1973	1981	1973
34	The percentage fat constituent of double	0.5	1.5	3	4.5	1.5

	toned milk is					
35	---------- is mostly used preservative to prevent mold growth	sodium propionate	springer	sorbates	acetate	sodium propionate
36	_____ solvent is poisonous and should not be added to foods	propylene	ethanol	methanol	glycerol	methanol
37	_____ drying is limited to climates with a hot sun and dry atmosphere to fruits	mechanical	solar	freeze	all of these	solar
38	Food should be cooked to which temperature?	5°C	75°C	100°C	60°C	75°C
39	The sclerotia from a species of Penicillium can survive a heat treatment of ____	70 °C	90 to100 °C	50-60 °C	37 °C	90 to100 °C
40	The sodium salt of _____ acid has been used extensively as an antimicrobial agent in foods	propionic	benzoic	sorbic	acetic	benzoic
41	Fruit juice is sterilized by _____.	filteration	freezing	cooling	heating	filteration
42	Pasteurization is a _____	low temperature treatment	steaming treatment	high temperature treatment	low and high temperature treatment	high temperature treatment
43	The reddish liquid comes out from meat on thawing process is called as	drying	wilting	bleeding	leakage	bleeding
44	The spoilage organism bring about the spoilage of meat by	purification	oxidation	decomposition	hydrolysis	decomposition
45	The minimum growth temperature of Bifidobacteria range from	43 to 45	25 to 28	29 to 32	30 to 35	43 to 45
46	_____ acid is used in soft drinks such as colas	phosphoric	benzoic	acetic	sorbic	phosphoric

47	_____ freezing usually refer to freezing in air with only natural air circulation	Sharp	slow	quick	all of these	sharp
48	Jones and Loackhead found enterotoxin forming Staphylococci in _____food	frozen corn	cheese	bread	jam	frozen corn
49	_____ from retail market contain from 0 to2 million bacteria per piece	caramels	jellies	fudges	candies	candies
50	---------- is a storage method uses bins or boxes for equalization of moist	Sweating	Springer	Cooling	Freezing	Springer
51	To retard the contamination and other microbial growth in meat is obtained by storing at _____ temperature	10 °C	0°C	100°C	-10°C	0°C
52	_____organic acid is used in syrups, drinks, jam and jellies	lactic	acetic	propionic	citric	citric
53	Food preservation involves_____	increasing shelf life of food	ensuring safety for human consumption	both a and b	none of these	both a and b
54	97 to 99 % of *E.coli* in air were killed in ____ seconds with a 15 watts lamp	40	10	50	30	10
55	_____ is used as treatment for wrappers use don butter	sodium diacetate	calcium carbonate	sodium nitrate	potassium nitrite	sodium diacetate
56	_____ temperature are more lethal	high freezing	frozen storage	freezing rate	thawing	high freezing
57	About ____ percent of the suspected samples contained viable spores	20	10	30	50	10
58	Sugars act as preservatives due to their ability to	make water unavailable to organism's	interfere with the action of proteolytic enzyme	osmotic effect	both a and c	interfere with the action of proteolytic enzyme
59	_____organic acid is used in syrups, drinks,	lactic	acetic	propionic	citric	citric

	jam and jellies					
60	Sanitising is_____	Applying detergent to a clean surface	Done before washing	Reducing bacteria by application of heat or chemical	Wiping all surfaces with a clean cloth	Reducing bacteria by application of heat or chemical

Unit 3

Syllabus

Food borne diseases - food poisoning - food borne infection and intoxication- Food control agencies - microbiological criteria for food, microbial quality control and food laws, Hazard Analysis Critical Control Point (HACCP).

FOOD POISONING:

Food poisoning syndrome results from ingestion of water and wide variety of food contaminated with pathogenic microorganisms (bacteria, viruses, protozoa, fungi), their toxins and chemicals. Food poisoning must be suspected when an acute illness with gastrointestinal or neurological manifestation affect two or more persons, who have shared a meal during the previous 72 hours. The term as generally used encompasses both food-related infection and food-related intoxication.

Some microbiologists consider microbial food poisoning to be different from food-borne infections. In microbial food poisoning, the microbes multiply readily in the food prior to consumption, whereas in food-borne infection, food is merely the vector for microbes that do not grow on their transient substrate. Others consider food poisoning as intoxication of food by chemicals or toxins from bacteria or fungi. Consumption of poisonous mushroom leads to mycetism, while consumption of food contaminated with toxin producing fungi leads to mycotoxicosis. Some microorganisms can use our food as a source of nutrients for their own growth. By growing in the food, metabolizing them and producing by-products, they not only render the food inedible but also pose health problems upon consumption. Many of our foods will support the growth of pathogenic microorganisms or at least serve as a vector for their transmission. Food can get contaminated from plant surfaces, animals, water, sewage, air, soil, or from food handlers during handling and processing.

Classification Of Food Poisoning:

I. Based on symptoms and duration of onset

a. Nausea and vomiting within six hours (*Staphylococcus aureus, Bacillus cereus*)

b. Abdominal cramps and diarrhoea within 8-16 hours (*Clostridium perfringens, Bacillus cereus*)

c. Fever, abdominal cramps and diarrhoea within 16-48 hours (*Salmonella, Shigella, Vibrio parahemolyticus, Enteroinvasive E.coli, Campylobacter jejuni*)

d. Abdominal cramps and watery diarrhoea within 16-72 hours (*Enterotoxigenic E.coli, Vibrio cholerae*

O1, O139, Vibrio parahemolyticus, NAG vibrios, Norwalk virus)

e. Fever and abdominal cramps within 16-48 hours (*Yersinia enterocolitica*)

f. Bloody diarrhoea without fever within 72-120 hours (*Enterohemorrhagic E.coli O157:H7*)

g. Nausea, vomiting, diarrhoea and paralysis within 18-36 hours (*Clostridium botulinum*)

II. Based on pathogenesis

a. Food intoxications resulting from the ingestion of preformed bacterial toxins. (*Staphylococcus aureus, Bacillus cereus, Clostridium botulinum, Clostridium perfringens*)

b. Food intoxications caused by noninvasive bacteria that secrete toxins while adhering to the intestinal wall (*Enterotoxigenic E.coli, Vibrio cholerae, Campylobacter jejuni*)

c. Food intoxications that follow an intracellular invasion of the intestinal epithelial cells. (*Shigella, Salmonella)*

d. Diseases caused by bacteria that enter the blood stream via the intestinal tract. (*Salmonella typhi, Listeria monocytogenes)*

Some of the guidelines prevalent in India are listed below:

☐Food Safety and Standards Act, 2006 (FSSA)

☐Edible Oils Packaging (Regulation) Order, 1998

☐Environment (Protection) Act, 1986

☐Fruit Products Order, 1955 (FPO)

☐Meat Food Products Order, 1973 (MFPO)

☐Milk and Milk Product Order, 1992 (MMPO)

☐Solvent Extracted Oil, De-oiled Meal and Edible Flour (Control) Order, 1967

☐Standards of Weights and Measures Act, 1976

☐The Essential Commodities Act, 1955

☐The Export (Quality Control and Inspection) Act, 1963

☐The Insecticides Act, 1968

☐Vegetables Oil Products (Control) Order, 1998

☐Prevention of Food Adulteration Act & Rules (PFA Act), 1954

A few of the Food Laws which can be declared voluntarily by the manufacturers of finished products are as follows:

☐Agmark Standards (AGMARK)

☐Codex Alimentarius Standards

☐BIS Standards and Specifications

☐Consumer Protection Act, 1986

Food laws and Regulations

☐To meet a country's sanitary and phytosanitary requirements, food must comply with the local laws and regulations to gain market access.

☐These laws ensure the safety and suitability of food for consumers.

☐ whether a country adopts international norms developed by the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations and the World Health Organization or a country may also has its own suite of food regulations.

☐Each country regulates food differently and has its own food regulatory framework.

Food laws in our country

The Indian Parliament has recently passed the *Food Safety and Standards Act, 200*6 that overrides all other food related laws. Such as;

☐Prevention of Food Adulteration Act, 1954 Fruit Products Order,1955

☐Meat Food Products Order ,1973;

☐Vegetable Oil Products (Control) Order, 1947 Edible Oils Packaging (Regulation) Order 1988

☐Solvent Extracted Oil, De- Oiled Meal and Edible Flour (Control) Order, 1967,

☐Milk and Milk Products Order, 1992 etc are repealed after commencement of FSS Act, 2006.

Food Safety and Standards Authority of India (FSSAI)

The Food Safety and Standards Authority of India (FSSAI) has been established under Food Safety and Standards Act, 2006 which consolidates various acts & orders that have hitherto handled food related issues in various Ministries and Departments.

FSSAI has been created for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption.

Functions performed by FSSAI

- Framing of Regulations to lay down the Standards and guidelines in relation to articles of food and specifying appropriate system of enforcing various standards.

• Laying down mechanisms and guidelines for accreditation of certification bodies engaged in certification of food safety management system for food businesses.

• Laying down procedure and guidelines for accreditation of laboratories and notification of the accredited laboratories.

• To provide scientific advice and technical support to Central Government and State Governments in the matters of framing the policy and rules in areas which have a direct or indirect bearing of food safety and nutrition .

• Collect and collate data regarding food consumption, incidence and prevalence of biological risk, contaminants in food, residues of various, contaminants in foods products, identification of emerging risks and introduction of rapid alert system.

• Creating an information network across the country so that the public, consumers, Panchayats etc receive rapid, reliable and objective information about food safety and issues of concern.

• Provide training programmes for persons who are involved or intend to get involved in food businesses.

• Contribute to the development of international technical standards for food, sanitary and phyto-sanitary standards.

• Promote general awareness about food safety and food standards

Bureau of Indian Standards (BIS)

☐The Bureau of Indian Standards (BIS), the National Standards Body of India, resolves to be the leader in all matters concerning Standardization, Certification and Quality.

Main Activities

☐Harmonious development of standardization, marking and quality certification

☐To provide new thrust to standardization and quality control.

☐To evolve a national strategy for according recognition to standards and integrating them with growth and development of production and exports.

☐Certification of Product

☐Hallmarking of Gold Jewellery.

☐Quality Management System

☐Environmental Management Systems

☐Occupational Health and Safety Management System

☐Food Safety Management System

☐Hazard Analysis and Critical Control Points

☐Imported Products

☐Laboratory Management

☐International Activities

☐Training Services

AGMARK

☐ The Directorate of Marketing and Inspection enforces the Agricultural Produce (Grading and Marketing) Act, 1937. Under this Act Grade standards are prescribed for agricultural and allied.

☐It ensures quality and purity of a product.

☐It acts as a Third Party Guarantee to Quality Certified.

☐Quality standards for agricultural commodities are framed based on their intrinsic quality.

☐Food safety factors are being incorporated in the standards to complete in World Trade.

☐ Standards are being harmonized with international standards keeping in view the WTO

requirements. Certification of agricultural commodities is carried out for the benefit of producer/manufacturer and consumer.

☐Products available under AGMARK are as follows:-

☐Whole spices & ground spices

☐Vegetable oils

☐Wheat Products

☐Milk products.

☐Other products such as Honey, Compounded asafetida, Rice, Tapioca Sago, Seedless tamarind, Besan (Gram flour)

Fruit Product Order (FPO), 1955

☐ The main objective is lay down quality standards to manufacture fruit & vegetable products maintaining sanitary and hygienic conditions in the premises.

☐ It is mandatory for all manufacturers of fruit and vegetable products including some non fruit products like non fruit vinegar, syrup and sweetened aerated water to obtain a license under this Order.

☐ Following minimum requirements are laid down in the Fruit Product Order for hygienic production and quality standards:

☐Location and surroundings of the factory

☐Sanitary and hygienic conditions of premises

☐Personnel hygiene

☐Portability of water

☐Machinery & Equipment with installed capacity

☐Quality control facility & Technical staff

☐Product Standards

- ☐Limits for preservatives & other additives
- o Fruit product means any of the following articles, namely
- ☐Non fruit beverages, syrups and sherbets
- ☐Vinegar, whether brewed or non-fruit
- ☐Pickles

☐Dehydrated fruits and vegetables

☐ Squashes, crushes cordials, barley water, barreled juice, and ready to serve beverages, fruit nectars or any other beverages containing fruit juices or fruit pulp

☐Jams, jellies and marmalades

☐Tomato products, ketchup and sauces

☐Preserves, candied and crystallized fruit and peel

☐Chutneys

☐Canned and bottled fruits, juices and pulps

☐Canned and bottled vegetables

☐Frozen fruits and vegetables

☐Sweetened aerated water and without fruit juice pr fruit pulp

☐Fruit cereal flakes

☐ All unspecified fruit and vegetable products which are considered microbiologically safe and

contains only permitted additives within permissible limits.

☐Each container in which any fruit product is packed shall specify a code number indicating the lot or the date of manufacture of such fruit product.

☐No person can carry on the business of a manufacturer except under and in accordance with the terms of an effective license granted to him under this Order in Form B and shall not use the License number on labels of non-fruit products.FPO mark should be printed on the label with license number.

Meat Food Products Order (MFPO)

Objectives :

☐The main objective is to regulate production and sale of meat food products through licensing of manufacturers, enforce sanitary and hygienic conditions prescribed for production of wholesome meat food products, exercise strict quality control at all stages of production of meat food products, fish products including chilled poultry etc.

☐Meat & Meat Products are highly perishable in nature and can transmit diseases from animals to human-beings.

☐Processing of meat products is licensed under Meat Food Products Order,(MFPO) 1973 which was hitherto being implemented by Ministry of food Processing industries

☐Under the provision of MFPO all manufacturers of meat food products engaged in the business of manufacturing, packing, repacking, relabeling meat food products meant for sale are licensed but excluding those manufacturers who manufactures such products for consumption on the spot like a restaurant, hotel, boarding house, snack bar, eating house or any other similar establishment.

Milk and Milk Product order (MMPO)

☐The objective of the order is to maintain and increase the supply of liquid milk of desired quality in the interest of the general public and also for regulating the production, processing and

distribution of milk and milk products.

☐As per the provisions of this order, any person/dairy plant handling more than 10,000 liters per day of milk or 500 MT of milk solids per annum needs to be registered with the Registering Authority appointed by the Central Government.

☐In every case where the milk or milk product is packed by the holder of a registration certificate in a tin, barrel, carton or any other container, the registration number shall either be exhibited prominently on the side label of such container or be embossed, punched or printed prominently thereon.

Prevention of Food Adulteration Act, 1954

• The Act was promulgated by Parliament in 1954 to make provision for the prevention of adulteration of food. Broadly, the PFA Act covers food standards, general procedures for sampling, analysis of food, powers of authorized officers, nature of penalties and other parameters related to food.

• It deals with parameters relating to food additives, preservative, colouring matters, packing & labelling of foods, prohibition & regulations of sales etc. The provisions of PFA Act and Rules are implemented by State Government and local bodies as provided in the rules.

• In every case where the milk or milk product is packed

Prevention of Food Adulteration Act, 1954 is repealed from 05.08.2011by the Central Government as per the Food Safety and Standards Act,2006.

• The act clearly defines “What is meant by Food Adulteration and what is the punishment given to person/manufacturer involved in the crime?

o The food is considered adulterated if it fulfills any of the below -

o If food is sub-standard rotten, decomposed or obtained from diseased animal or is insect-infested or is otherwise unfit for human consumption.

o If food contains any other substance which affects, or if the article is so processed as to affect, injuriously the nature, substance or quality thereof

o if the article has been prepared, packed or kept under insanitary conditions whereby it has become contaminated or injurious to health;

o if any constituent of the article has been wholly or in part abstracted so as to affect injuriously the nature, substance or quality thereof.

o if the article contains any poisonous or other ingredient which renders it injurious to health

o if any colouring matter other than that prescribed in respect thereof is present in the article, or if the amounts of the prescribed colouring matter which is present in the article are not within the prescribed limits of variability

o if the article contains any prohibited preservative or permitted preservative in excess of the prescribed limits;

o if the quality or purity of the article fall below the prescribed standard or its constituents are present in.

A few definitions as given by FAO / WHO:

☐ **Codex Alimentarius Commission:** The Codex Alimentarius Commission is a subsidiary body of the Food and Agriculture Organization of the United Nations and the World Health Organization. The Commission is entrusted with the elaboration of international standards of food to protect the health of consumers and to ensure fair practices in the food trade.

☐ **Codex Committees:** These subsidiary bodies of the Codex Alimentarius Commission include nine general subject committees, fifteen specific commodity committees, six regional coordinating committees and time-limited ad-hoc Intergovernmental Task Forces on specific subjects.

Critical Control Point: A step at which control is essential to prevent or eliminate a food safety

hazard or reduce it to an acceptable level.

Food Contaminant: Any biological or chemical agent, foreign matter, or other substance not intentionally added to food which may compromise food safety or suitability.

Food Control: A mandatory regulatory activity of enforcement by national or local authorities to provide consumer protection and ensure that all foods during production, handling, storage, processing and distribution are safe, wholesome and fit for human consumption; conform to quality and safety requirements; and are honestly and accurately labelled as prescribed by law.

☐ **Food Hygiene:** All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

☐ **Food Inspection:** The examination, by an agency empowered to perform regulatory and/or enforcement functions, of food products or systems for the control of raw materials, processing, and distribution. This includes in-process and finished product testing to verify that they conform to regulatory requirements.

Good Agricultural Practices (GAP): Practices of primary food producers (such as farmers and fishermen) that are necessary to produce safe and wholesome agricultural food products conforming to food laws and regulations.

☐ **Good Manufacturing Practices (GMP):** Conformance with codes of practice, industry standards, regulations and laws concerning production, processing, handling, labelling and sale of foods decreed by industry, local, state, national and international bodies with the intention of protecting the public from illness, product adulteration and fraud.

☐ **HACCP Plan**: A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration.

HACCP System: The hazard analysis critical control point system (HACCP) is a scientific and systematic way of enhancing the safety of foods from primary production to final consumption through the identification and evaluation of specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that

focus on prevention rather than relying mainly on end-product testing.

Some establishments may use **Good Manufacturing Practices (GMP)** to reduce the likelihood of certain hazards. GMPs are minimum sanitary and processing requirements. GMPs are fairly broad and general, for example, *"Training: All employees should receive training in personal hygiene."* GMPs are usually not designed to control specific hazards, but are intended to provide guidelines to help establishments produce safe and wholesome products.

☐ **Standard Operating Procedures (SOP)** are step-by-step directions for completing important procedures and are usually very specific. SOP may be used to address a specific hazard, for instance, an establishment may have specific preventive maintenance procedures for its processing equipment, which prevent the hazard of metal fragments.

☐ **Sanitation SOP (SSOP)** may be considered by establishments to reduce the likelihood of occurrence of some food safety hazards. For example, the SSOP may address washing and sanitizing of knife and hands between carcasses to reduce potential contamination with pathogens.

Product specific GMPs

☐thermally processed low-acid canned foods

☐acidified foods

☐bottled drinking water

GMPs Regulations

21CFR Part 110

o *Subpart A - General Provisions*

o *Subpart B - Building and Facilities*

o *Subpart C - Equipment*

o *Subpart D - [Reserved]*

o *Subpart E - Production and Process Controls*

o *Subpart F - [Reserved]*

o *Subpart G - Defect Action Levels*

GMPs - General Provisions

o provides definitions necessary for *important in understanding implications and applications*

☐ Buildings and Facilities. Buildings must be designed and constructed to facilitate *effective maintenance and sanitation.The* results specified rather than method for achieving detailed expectations in sanitation of operations**.**

☐ The equipment and utensils are *designed and constructed to be easily and properly cleaned*, temperature is measured and recorded by refrigerators and freezers. Also the critical parameters are measured.

☐Production and Process Controls-

o The end results emphasizes *ensuring that no adulterated food enters marketplace.The terms used subject to variation in interpretation.*

o *The* raw materials and ingredients properly *inspected, analyzed, segregated, stored and handled.*

o manufacturing operations must be monitored

o *pH, water activity, temperatures*

o *elimination of metal from product*

o personnel should be trained and aware of GMP requirements

☐Defect Action Levels

o natural or unavoidable defects may be in food

o *not harmful at levels present*

o *present even with GMPs*

o FDA establishes DALs when necessary and possible

o defect level may not be reduced by blending

Thus GMPs are Intended to prevent adulteration. Opportunity for considerable judgment in defining and interpreting regulations. *"spirit" of GMPs is to do what is reasonable and necessary to ensure safe and unadulterated food supply.*

Specific GMPs:

Low acid canned foods

☐*Life threatening risk if improperly processed*

☐Regulations quite detailed for equipment design and operation

☐Extensive record keeping requirements

Acidified foods:

☐*Aw greater than 0.85*

☐*acid added to lower pH to 4.6 or lower*

☐Product examples

☐*includes beans, cucumbers, cabbage*

☐Personnel trained under approved program

Bottled Drinking Water:

☐*All water sealed in bottles, packages for human consumption*

☐Regulations are general and similar to umbrella GMPs

☐Source of water must be approved

☐Sanitation, equipment designed, personnel emphasized Extensive record keeping

What is HACCP?

☐ The National Advisory Committee on Microbiological Criteria for Food (NACMCF) working group created guidelines and redefined the seven basic principles of HACCP as an effective and

rational means of assuring food safety from harvest to consumption.

☐ The working group published the HACCP principles and application guideline document in August 1997.

☐The hazard analysis critical control point system (HACCP) is a scientific and systematic way of enhancing the safety of foods from primary production to final consumption through the identification and evaluation of specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing.

☐ Under the HACCP regulatory system, establishments assume full responsibility for producing products that are safe for consumers.

History of HACCP

☐Developed by Pillsbury in 1959 as a nontesting approach to assure the safety level required by NASA for foods produced for the space program

☐NASA's major concerns • Food crumbs • Foodborne illness

☐Testing materials

☐ National Research Council – 1985 • An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients

☐Microbiological hazards not controlled by testing

☐Recommended using HACCP for food safety assurance

PRINCIPLES OF THE HACCP SYSTEM

The seven principles of HACCP, which encompass a systematic approach to the identification, prevention, and control of food safety hazards include:

PRINCIPLE 1 Conduct a hazard analysis.

PRINCIPLE 2 Determine the Critical Control Points (CCPs).

PRINCIPLE 3 Establish critical limit(s).

PRINCIPLE 4 Establish a system to monitor control of the CCP.

PRINCIPLE 5 Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

PRINCIPLE 6 Establish procedures for verification to confirm that the HACCP system is working effectively.

PRINCIPLE 7 Establish documentation concerning all procedures and records appropriate to these principles and their application.

APPLICATION

The application of HACCP principles consists of the following tasks as identified in the Logic Sequence

for Application of HACCP (Diagram 1).

1. Assemble HACCP team

The food operation should assure that the appropriate product specific knowledge and expertise is available for the development of an effective HACCP plan. Optimally, this may be accomplished by assembling a multidisciplinary team. Where such expertise is not available on site, expert advice should be obtained from other sources, such as, trade and industry.

associations, independent experts, regulatory authorities, HACCP literature and HACCP guidance (including sector-specific HACCP guides).

It may be possible that a well-trained individual with access to such guidance is able to implement HACCP in house.

The scope of the HACCP plan should be identified. The scope should describe which segment

of the food chain is involved and the general classes of hazards to be addressed (e.g. does it cover all classes of hazards or only selected classes).

2. Describe product

A full description of the product should be drawn up, including relevant safety information such as:

composition, physical/chemical structure (including Aw, pH, etc), microcidal/static treatments (heattreatment, freezing, brining, smoking, etc), packaging, durability and storage conditions and method of distribution. Within businesses with multiple products, for example, catering operations, it may be effective to group products with similar characteristics or processing steps, for the purpose of development of the HACCP plan.

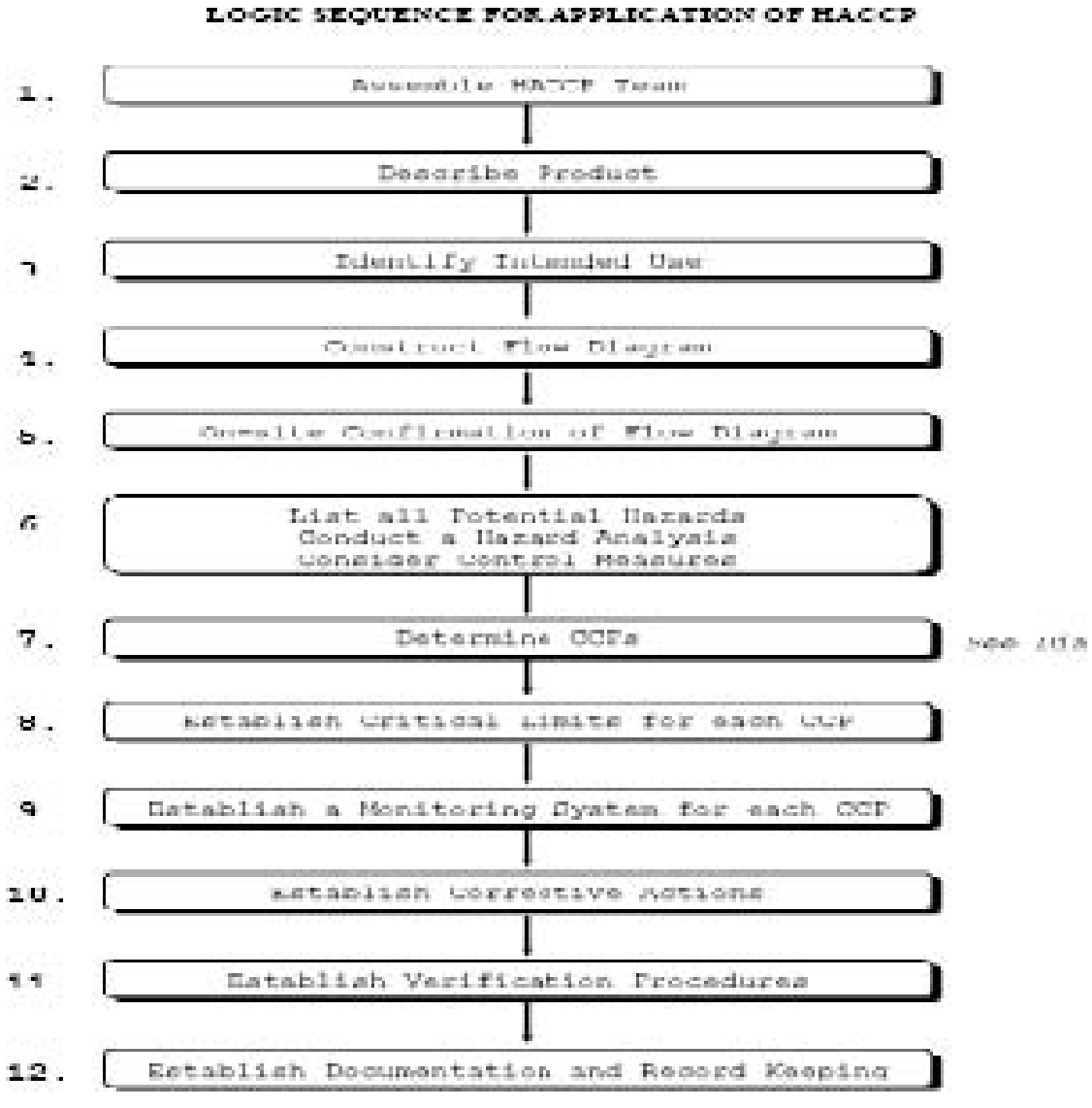
3. Identify intended use

The intended use should be based on the expected uses of the product by the end user or consumer. In

specific cases, vulnerable groups of the population, e.g. institutional feeding, may have to be considered.

4. Construct flow diagram

The flow diagram should be constructed by the HACCP team (see also paragraph 1 above). The flow diagram should cover all steps in the operation for a specific product. The same flow diagram may be used for a number of products that are manufactured using similar processing steps. When applying HACCP to a given operation, consideration should be given to steps preceding and following the specified operation.



5. On-site confirmation of flow diagram

Steps must be taken to confirm the processing operation against the flow diagram during all stages and hours of operation and amend the flow diagram where appropriate. The confirmation of the flow diagram should be performed by a person or persons with sufficient knowledge of the processing operation.

6. List all potential hazards associated with each step, conduct a hazard analysis, and consider

any measures to control identified hazards

The HACCP team should list all of the hazards that may be reasonably expected to occur at each step according to the scope from primary production, processing, manufacture, and distribution until the point of consumption.

The HACCP team should next conduct a hazard analysis to identify for the HACCP plan, which hazards are of such a nature that their elimination or reduction to acceptable levels is essential to the production of a safe food.

In conducting the hazard analysis, wherever possible the following should be included:

• the likely occurrence of hazards and severity of their adverse health effects;

• the qualitative and/or quantitative evaluation of the presence of hazards;

• survival or multiplication of micro-organisms of concern;

• production or persistence in foods of toxins, chemicals or physical agents; and,

• conditions leading to the above.

Consideration should be given to what control measures, if any exist, can be applied to each hazard. More than one control measure may be required to control a specific hazard(s) and more than one hazard may be controlled by a specified control measure.

☐A hazard is defined by NACMCF as a biological, chemical or physical agent that is **reasonably likely to occur**, and will **cause illness or injury in the absence of its control**. Establishments must consider all **three types of hazards – biological, chemical, and physical**

– at each step of the production process.

7. Determine Critical Control Points

☐ A ***critical control point*** is defined as a point, step, or procedure in a food process at which control can be applied, and, as a result, a food safety hazard can be prevented, eliminated, or reduced to acceptable levels. Critical control points are locations in a process at which some aspect

of control can be applied to control food safety hazards that have been determined reasonably likely to occur.

Examples of CCPs include product temperature, certification of incoming product, microbiological testing, testing for foreign objects such as metal contamination, the chemical concentration of a carcass rinse or spray, and other such parameters.

There may be more than one CCP at which control is applied to address the same hazard. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree, which indicates a logic reasoning approach. Application of a decision tree should be flexible, given whether the operation is for production, slaughter, processing, storage, distribution or other. It should be used for guidance when determining CCPs. This example of a decision tree may not be applicable to all situations. Other approaches may be used. Training in the application of the decision tree is recommended.

If a hazard has been identified at a step where control is necessary for safety, and no control measure exists at that step, or any other, then the product or process should be modified at that step, or at any earlier or later stage, to include a control measure.

8. Establish critical limits for each CCP

Critical limits (CL) are the parameters that indicate whether the control measure at the CCP is in or out of control. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) states that a CL is **a maximum or minimum value** to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard.

Critical limits must be specified and validated for each Critical Control Point. In some cases more than one critical limit will be elaborated at a particular step. Criteria often used include measurements of temperature, time, moisture level, pH, Aw, available chlorine, and sensory parameters such as visual appearance and texture.

Where HACCP guidance developed by experts has been used to establish the critical limits, care

should be taken to ensure that these limits fully apply to the specific operation, product or groups of products under consideration. These critical limits should be measurable.

9. Establish a monitoring system for each CCP

Monitoring is the scheduled measurement or observation of a CCP relative to its critical limits. The monitoring procedures must be able to detect loss of control at the CCP. Further, monitoring should ideally provide this information in time to make adjustments to ensure control of the process to prevent violating the critical limits. Where possible, process adjustments should be made when monitoring results indicate a trend towards loss of control at a CCP. The adjustments should be taken before a deviation occurs. Data derived from monitoring must be evaluated by a designated person with knowledge and authority to carry out corrective actions when indicated. If monitoring is not continuous, then the amount or frequency of monitoring must be sufficient to guarantee the CCP is in control. Most monitoring procedures for CCPs will need to be done rapidly because they relate to online processes and there will not be time for lengthy analytical testing. Physical and chemical measurements are often preferred to microbiological testing because they may be done rapidly and can often indicate the microbiological control of the product. All records and documents associated with monitoring CCPs must be signed by the person(s) doing the monitoring and by a responsible reviewing official(s) of the company.

10. Establish corrective actions

Specific corrective actions must be developed for each CCP in the HACCP system in order to deal with deviations when they occur.

The actions must ensure that the CCP has been brought under control. Actions taken must also include proper disposition of the affected product. Deviation and product disposition procedures must be documented in the HACCP record keeping.

The corrective actions consist of:

☐Identifying and eliminating the cause of the deviation,

☐Ensuring that the CCP is under control after the corrective action is taken,

☐Ensuring that measures are established to prevent recurrence, and

☐Ensuring that no product affected by the deviation is shipped.

11. Establish verification procedures

Establish procedures for verification. Verification and auditing methods, procedures and tests, including random sampling and analysis, can be used to determine if the HACCP system is working correctly. The frequency of verification should be sufficient to confirm that the HACCP system is working effectively.

Verification should be carried out by someone other than the person who is responsible for performing the monitoring and corrective actions. Where certain verification activities cannot be performed in house, verification should be performed on behalf of the business by external experts or qualified third parties.

Examples of verification activities include:

• Review of the HACCP system and plan and its records;

• Review of deviations and product dispositions;

• Confirmation that CCPs are kept under control.

Where possible, validation activities should include actions to confirm the efficacy of all elements of the

HACCP system.

12. Establish Documentation and Record Keeping

Efficient and accurate record keeping is essential to the application of a HACCP system. HACCP procedures should be documented. Documentation and record keeping should be appropriate to the nature and size of the operation and sufficient to assist the business to verify that the HACCP controls are in place and being maintained. Expertly developed HACCP guidance materials (e.g. sector- specific HACCP guides) may be utilised as part of the documentation, provided that those materials reflect the specific food operations of the business.

Documentation examples are:

Hazard analysis; CCP determination;

Critical limit determination.

Record examples are:

• CCP monitoring activities;

• Deviations and associated corrective actions;

• Verification procedures performed;

• Modifications to the HACCP plan;

A simple record-keeping system can be effective and easily communicated to employees. It may be integrated into existing operations and may use existing paperwork, such as delivery invoices and checklists to record, for example, product temperatures.

Benefits of HACCP

Although the adoption of HACCP systems worldwide is due primarily to the added food safety protection provided to consumers, there are other benefits to the food industry that can be realized by implementing a successful HACCP system.

a. Formally incorporates food safety principles as integral steps of production processes

HACCP recognition status cannot be completed without a firm commitment by senior management to formally support food safety control measures throughout the production process. The implementation and maintenance of those control measures play a critical role in raising awareness of front line production management and staff of the presence and importance of specific food safety procedures within their process.

b. Increased employees' ownership of the production of safe food

As a sign of this commitment, it is the responsibility of senior management to foster the idea within the facility that food safety is the responsibility of everyone. Through the process of developing and implementing a HACCP system, employees become more aware of food safety

and their role in contributing to food safety. This increased knowledge leads to ownership of and pride in the production of a safe food product.

c. Increased buyer and consumer confidence

Establishments that have implemented a HACCP system provide buyers and consumers with a greater degree of confidence that the facility is producing a safe food product. Establishments can demonstrate by showing documents and records that food safety is under control.

d. Maintaining or increasing market access

Market forces continue to drive HACCP implementation throughout the food industry. In many cases, buyer demands and foreign governments require HACCP implementation to maintain market share and/or gain access to previously inaccessible markets. As HACCP systems are accepted worldwide, FSEP helps the Canadian industry to maintain and expand its international markets.

e. Reduced waste

The preventative nature of HACCP allows a company to control costs by minimizing the amount of product requiring rejection or recall, and by focusing resources on areas that have been identified as critical in the manufacture of a safe food product. With the regular monitoring inherent in a HACCP system, establishments become aware of problems earlier and the costs of waste are reduced.

Unit-III; Possible Questions

Part-A (1 Mark)

Part-B (2 Mark)

1. What is food poisoning?
2. What are the two major food-poisoning?
3. What are the seven types of neurotoxins?
4. What is toxin?
5. What are the main sources of botulism?
6. What are the symptoms of botulism?
7. What is enterotoxin?
8. What is Asiatic cholera?
9. Difference between infection and intoxication.
10. Write the food laws in our country.
11. What is food safety?
12. Give expansion for FSSAI.
13. What is AGMARK?
14. What is HACCP?
15. What is bottled drinking water?

Part-C (8 Mark)

1. What are the physiological types of bacteria are most likely to be present when canned food spoils?
2. List the types of microbes involved in spoilage of refrigerated foods with those incriminated in spoilage of canned foods.
3. List several types of food spoilage and name the organism responsible for each instance.

4. Why is milk an excellent bacteriological culture medium?

5. Describe the various types of changes brought about by the microorganisms in foods and name the organism.

6. What are the indicators of food spoilage?

7. List the symptoms of food poisoning.

8. Name the pathogens that cause food poisoning.

9. Differentiate between food poisoning and food intoxication.

10. Give the food laws prevalent in India.

11. Importance of BIS and AGMARK.

12. Functions preferred by FSSAI.

13. Write down the seven principles of HACCP.

14. Benefits of HACCP.

15. Comment on food quality and its importance.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB COURSE NAME: FOOD MICROBIOLOGY

COURSE CODE: 18MBP302 UNIT: III BATCH-2018-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Which of the following toxin causing botulism is less toxic to human beings?	Type A	Type B	Type C	None of these	Type B
2	Which of the following statements are true regarding *Staphylococcus* food poisoning_____	is an enterotoxin	causes gastroenteritis	is produced by Staphylococcus aureus	All of these	All of these
3	Aflatoxin is produced by _____	*Aspergillus sp.*	*Salmonella sp.*	*Fusarium sp.*	*Streptococcal sp.*	*Aspergillus sp.*
4	Which of the following statements are regarding botulinal toxin _____	is a neurotoxin	water soluble exotoxin	is produced by Clostridium botulinum	All of these	is produced by Clostridium botulinum
5	The sore and throat symptom caused by _____ etiologic agent	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Bacillus anthrax*	*E.coli*	*Streptococcus pyogenes*
6	Botulism is caused by the presence of toxin developed by	*Clostridium tyrobutyricum*	*Clostridium sporogenes*	*Clostridium botulinum*	*none of these*	*Clostridium botulinum*
7	The control measure of foods that cause disease by *Vibrio parahaemolyticus* infection is to _____	reheat left over	sanitize equipment	control files	pastuerization	sanitize equipment
8	Salmonellois involves _____	an enterotoxin and exotoxin	an enterotoxin and cytotoxin	is produced by Staphylococcus aureus	All of these	an enterotoxin and cytotoxin
9	The term heat tolerant is a misnomer and refers to growth at ___ temperature	37 °C	40 °C	42 °C	25 °C	42 °C
10	The mold Penicillium islandicum produces _____ toxin	Luteoskyrin	aflatoxin	penicillic acid	roquefortine	Luteoskyrin
11	The major carrier of Salmonellosis are _____	meat and eggs	meat and fish	eggs and fish	eggs and fruits	meat and egs

12	Yersinia enterocolitica is a small _____ shaped bacteria	cocci	chain	rod	bacilli	rod
13	The staphylococcal intoxication refers to presence of	an enterotoxin	neurotoxin	mycotoxin	All of these	an enterotoxin
14	The FDA and USDA cooperative is a _____ surveillance program for dry milk products	*Pseudomonas*	*E. coli*	*Salmonella*	*Vibrio*	*Salmonella*
15	The application of Gamma rays destroys botulism toxin. The dose of gamma rays required for this purpose is	73 Gy	73 Rad	7.3 Mrad	173 Rad	7.3 Mrad
16	The Bacillus cereus causes gasteroenteritis by the production of an exoenterotoxin which is released in food as a result of	cell growth	cell autolysis	cell permeation	cell damage	cell autolysis
17	Nursery epidemics diarrheal disease in infants was implicated in the year _____	1950	1940	1962	1980	1940
18	Botulism is caused by _____	*Clostridium botulism*	*All Clostridium species*	*Clostridium tetanai*	*Clostridium subtilis*	*Clostridium botulinum*
19	The toxin patulin is produced by _____fungi	*Penicillium expansum*	*Fusarium*	*Aspergillus flavus*	*Mucor*	*Penicillium expansum*
20	Miller and Kolurger examined forty environmental isolates of P. shigelloides in the year _____	1987	1982	1980	1986	1986
21	Which of the following is a food infection?	Salmonellois	Botulism	Staphylococcal intoxication	None of these	Salmonellois
22	The symptoms such as nausea and dehydration is caused by _____	*Shigella sonnei*	*Yersinia*	*Arizona*	*E.coli*	*Shigella sonnei*
23	Staphylococcal intoxication is caused by	*Staphylococcus*	*S. cerevisiae*	*S. thermophillus*	none of these	*Staphylococcus*

	the toxin in the food from	*aureus*				*aureus*
24	The etiologic agent of diarrheal syndrome is ____	*Shigellosis*	*Yersiniosis*	*Bacillus cereus*	*Vibrio*	*Bacillus cereus*
25	_____ involves the identification of ingredients and products that have effect on food safety	Hazard analysis	critical control points	fishery service	research and development service	Hazard analysis
26	The term ___ is used to distinguish strains of different antigenetic complements	biovars	serovar	herbivore	none of these	serovar
27	A bacterial food intoxication refers to	illness caused by presence of pathogens	food borne illness caused by the presence of a bacterial toxin formed in food	both (a) and (b)	none of the above	food borne illness caused by the presence of a bacterial toxin formed in food
28	Salmonellois is caused by the	enterotoxin of Salmonella spp	endotoxin of Salmonella spp	neurotoxin of Salmonella spp	exoenterotoxin of Salmonella spp	endotoxin of Salmonella spp
29	Group I *C. botulinum* strains generally includes in	all types of strains (proteolytic)A, B and F	all types of strains (non-proteolytic) E and F	all types of strains (proteolytic)C, D and F	none of the above	all types of strains (proteolytic)A, B and F
30	A_____ refers to food borne illnesses caused by the entrance of bacteria into the body through ingestion of comtaminated food	Food infection	food poisoning	food intoxication	all of these	food infection
31	_____ organism can be isolated from seafoods and sea water	*Vibrio cholerae*	*Vibrio vulnificus*	*Vibrio parahaemolyticus*	All of these	*Vibrio vulnificus*
32	Botulism prevention involves_____	Proper heat sterilization before	addition of chemical	Proper low temperature	All of these	Proper heat sterilization before

		food canning	preservatives	treatment before food canning		food canning
33	Entheropathogenic Escherischia coli infection is involved in ____ foods	vegetables	apple cider	ice creams	cheese	cheese
34	The etiological agent of Arizona infection is ____	*Vibrio*	*E. coli*	*Arizona*	*Streptococcus*	*Arizona*
35	Aeroonas hydrophillia is a gram negative motile rods which are ubiquitous in ____	air	soil	water	land	water
36	The term ___ is used to distinguish strains of different antigenetic complements	biovars	serovar	herbivore	none of these	serovar
37	The method of successful treatment of botulism prior to appearance of botulism symptoms involve administration of	antibiotic	analgesic	antitoxin	antipyretic	antitoxin
38	_____ organism can be isolated from seafoods and sea water	*Vibrio cholerae*	*Vibrio vulnificus*	*Vibrio parahaemolyticus*	All of these	*Vibrio vulnificus*
39	The optimal temperature for growth of Shigellosis is _____	27 ℃	37 ℃	40 ℃	50 ℃	37 ℃
40	The FDA and USDA cooperative is a _____ surveillance program for dry milk products	*Pseudomonas*	*E. coli*	*Salmonella*	*Vibrio*	*Salmonella*
41	_____ is associated with warm blooded animals	*C. jejuni*	*C. botulinum*	*C. perferigens*	*E. coli*	*C. jejuni*
42	Human beings and animals are directly or indirectly the source of the contamination of food with _____	*Salmonella*	*Staphylococcus*	*Bacillus*	*E. coli*	*Salmonella*
43	The food and Drug Administration act was amende in the year ____	1983	1980	1989	1988	1980

44	The _____ virus enters a person through oral route in the fecal contamination of food	Poliomyelitis	Hepatitis	Adeno	Herpes	Hepatitis
45	The mode of transmission of poliomyelitis is _____	food	air	contaminated water	all of these	contaminated water
46	Clostridium perfingens poisoning is associated with_____	meat products	vegetables	canned foods	fish products	meat products
47	Clostridium perfingens poison is an _____	exotoxin	enterotoxin produced during sporulation	endotoxin	enterotoxin produced during vegetative phase	enterotoxin produced during sporulation
48	The pH near ____ favors *C. botulinum*	neutrality	alkalinity	acidic	both b and c	neutrality
49	In the early _____ numerous surveys have been conducted on the detection aflatoxins in foods	1980s	1940s	1950s	1960s	1960s
50	The optimal pH for enteropathogenic E. coli is _____	4.0 to 5.0	7.0 to 7.5	3.0 to 4.0	8.0 to 9.0	7.0 to 7.5
51	The disease gastroenterities caused by C. perfringens was first reported in the year___-	1952	1961	1978	1945	1945
52	Depending on the food and the serotype the _____ values from 0.06 to 11.3 min	D50 C	D40 c	D60 c	D30 c	D60 c
53	Pathogenecity involves the release of a _____ endotoxin which affects the intestinal mucosa	lipopolysaccharides	monosaccharides	polysaccharides	peptidoglycon	lipopolysaccharides
54	Common food poisoning microbes are_____	*Clostridium and Salmonella*	*Clostridium and E. coli*	*E. coli and Salmonella*	*Clostridium and Streptococcus*	*Clostridium and Salmonella*
55	Typhoid fever is caused by _____	*Salmonell*	*Salmonella*	*Salmonella typhi*	*Salmonella*	*Salmonella typhi*

		enteritidis	*infantis*		*typhimurium*	
56	The incubation period of Vibrio parahaemolyticus infection is _____	2-48 hrs	5-24 hrs	40 hrs	37 hrs	2-48 hrs
57	The incubation period of Streptococcus faecalis is _____	5 to 10	2 to 10	2 to 18	8 to 12	2 to 18
58	The growth of Staphylococcus aureus on solid media is usually ____ in color	red	brown	pink	yellow	yellow
59	A_____ refers to food borne illnesses caused by the entrance of bacteria into the body through ingestion of comtaminated food	Food infection	food poisoning	food intoxication	all of these	food infection
60	What is the main type of micro-organism responsible for food poisoning?	Bacteria	Mould	Virus	Parasite	Bacteria

Unit IV:

Food serves as excellent substrate for the growth of different kinds of microorganisms. Microorganisms enter into food and grow as contaminants or intended additions. Growth of microorganisms in food may spoil food quality and consumption of such food creates hazardous health effects in human and animal. Food is assessed for their quality in terms of physical, chemical, sensory and microbiological characteristics. Microbiological characteristics are assessed in terms of the microorganisms- moulds, yeast, bacteria, protozoa and virus- present in food, their characters, ability to change the quality, their influence on health of consumer. It is necessary for food microbiologists to become acquainted with the microorganisms important in food at least to the extent that will enable them to identify the main types with their characteristics. Knowledge of general characters and primary identification methods is necessary for the people working with food science and technology.

The most important methods for detecting the microorganisms from food are:

1. Microscopic studies-morphology and staining reactions
2. Motility studies
3. Cultural characteristics
4. Biochemical tests
5. Chemical and molecular biology techniques, and
6. Immunological techniques.

Culture, Microscopic and Sampling Methods:

The examination of food for the presence, types, and numbers of microorganisms and their products is the fundamental procedure in food microbiology. The detection of microorganisms mainly looks for the total count of a particular type of microorganisms in a particular gram weight of food.

Direct Microscopic Count (DMC):

This method is very simple and rapid method for the initial morphological identification and count of bacteria and moulds. Morphologically bacteria are classified into different groups.

The most important standard microbiological methods used for the detection of total number of microorganism in food are: 1. Direct Microscopic Count (DMC) 2. Aerobic Plate Counts (APC) or Standard Plate Counts (SPC) 3. Most Probable Numbers (MPN) 4. Microscope Colony Counts 5. Agar Droplets 6. Dry Film 7. Dye Reduction – MBRT Test 8. Roll Tubes

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1. Breed Count:

It is developed by R.S. Breed (Breed count). The sample is spread (about 0.0 1 ml) over I cm^2 of a microscope slide. Milk smear is dried and stained with Newman Lampert stain; methylene blue milk smear stain. This stain fixes the smear, dissolves fat globules and

stains bacteria with methylene blue. Slide is then observed under several oil immersion microscopic fields.

The number of organisms in milk is determined based the following calculation, are made as follows:

Area of one microscope field = 0.02 mm^2.

Area over which milk sample is spread on the slide = 1 cm^2 or 100 mm^2

Then, number of fields possible under the lens = 100 mm^2/0.02 mm^2= 5000 fields

This number of field is for 0.01 ml of milk. However, final count is expressed as bacterial number per ml of sample. Thus, factor is to be multiplied by 100, i.e. 5000 x 100 = 500,000

This is the microscope factor.

2. Slide Method Using INT:

A slide method to detect and enumerate viable cells has been developed. The method employs the use of the tetrazolium salt (p-iodophenyl-3-p-nitrophenyl)-5-phenyl tetrazolium chloride (INT). In this method cells are exposed to filter-sterilized INT for 10 minutes at 37°C in water bath followed by filtration on 0.45-µm membranes. Following drying of membranes for 10 minutes at 50°C, the special membranes are mounted in cottonseed oil and viewed with cover slip in place. The method was found to be effective for pure cultures of bacteria and yeasts.

3. Howard Mould Counts:

This is a microscope slide method developed by B.J. Howard in 1911 primarily for the purpose of monitoring tomato products. This method involves the detection of fungi especially moulds. The method requires the use of a special chamber (slide) designed to enumerate mould mycelia. By this method we can identify almost all moulds which are responsible for the spoilage of fruits and vegetables

Merits and Demerits of Direct Microscopic Count:

The advantages of DMC are it is rapid and simple, cell morphology can be assessed and it lends itself to fluorescent probes for improved efficiency.

DMS has got so many disadvantages also. It is a microscopic method and therefore fatiguing to the analyst, both viable and nonviable cells are enumerated, food particles are not always distinguishable from microorganisms, microbial cells are not uniformly distributed relative to single cells and clumps, some cells do not take the stain well and may not be counted and DMC counts are invariably higher than counts by SPC.

2. Aerobic Plate Counts (APC) or Standard Plate Counts (SPC):

In this method portions of food samples are blended or homogenized, serially diluted in appropriate diluents, plated in or onto a suitable agar medium and incubated at an appropriate temperature for a given time, after which all visible colonies are counted by use of a colony counter. The SPC is the most important method for determining the number of viable cells or colony-forming units (CFU) in a food product.

SPC determines the following factors:

(i) The method of sampling and plating.
(ii) Nature and type, distribution of microorganisms in the food sample.
(iii) Nature and type of food material.
(iv) The pre-evaluation results of the food product.
(v) Nutritional contents of plating medium.
(vi) Temperature of incubation and time.
(vii) pH, water activity (a_w), and oxidation-reduction potential (Eh) of the plating medium.
(viii) Type of diluents used for the preparation of food samples.
(ix) Relative number of organisms in food sample.
(x) Presence and competition between microorganisms in food.

SPC can be performed by two methods:

(i) Spread plate and
(ii) Pour plate

(i) Spread Plate Method:

In spread plate method pre poured and hardened agar plates with dry surfaces are used. The food samples are serially diluted and 0.1 mm inoculum is taken using sterile pipette and evenly distributed over the agar surface. The inoculum is distributed over the agar surface with the help of bent glass rod.

The dispersed cells develop into isolated colonies called Colony Forming Units (CFU). Because the number of colonies should equal the number of viable organisms in the food sample and spread plates can be used to count the microbial population in food sample.

(ii) Pour Plate Method:

The original food sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating. Then small volumes, around one ml of several diluted samples are mixed with liquid agar that has been cooled to about 45°C, and the mixtures are poured immediately into sterile culture dishes.

After the agar has hardened, each cell is fixed in place and forms an individual colony (CFU). Plates containing between 30 and 300 colonies are counted. The total number of colonies equals the number of viable microorganisms in the diluted sample.

Preparation of Food Sample for SPC:

The primary steps of food sample preparation for the detection of microorganisms are homogenization.

The most important types of food homogenization equipments are:

(i) Colwell Stomacher:

this device is one of the best methods for homogenizing food for plating and enumeration. The Stomacher is a simple device which homogenizes specimens in a special plastic bag by

the vigorous pounding of two paddles. The pounding effects the shearing of food specimens releases microorganisms into the diluents.

(ii) High-Speed Blender:

It helps to homogenize the food material effectively by high speed-blender and the microorganisms are released from food.

(iii) Shaker:

It is the common method for homogenizing the food. Shaking of food samples releases the microorganisms in to diluents.

Membrane Filter Techniques:

Membranes with a pore size that will retain bacteria (generally 0.45 and μm) but allow water or diluent to pass are used.

The most important types are explained below:

a. Direct Epifluorescent Filter Technique:

The Direct Epifluorescent Filter Technique (DEFT) employs fluorescent dyes and fluorescent microscopy and it is a rapid method to enumerate microorganisms in food. In this method, the food sample is first homogenized, diluted and filtered through a 5-μm nylon filter; the filtrate is then collected and treated with 2 ml of Triton X-100 and 0.5 ml of trypsin.

The trypsin will breake somatic cells and to prevent clogging of filters.

b. Microcolony-DEFT:

Microcolony-DEFT is a variation of DEFT that allows one to determine viable cells only. The food homogenates are filtered through DEFT membranes, placed on the surface of appropriate culture media and incubated for microcolony development.

c. Hydrophobic Grid Membrane Filter (HGMF):

The Hydrophobic Grid Membrane Filter (HGMF) technique was developed by Sharpe and Michaud and it used to enumerate microorganisms from a variety of food products. The method employs a special type of filter that consists of 1600 wax grids on a single membrane filter that restricts growth and colony size to individual grids. Using one filter 10 to 9 x 10^4 cells can be enumerated by an MPN procedure and enumeration can be automated.

It can be used to enumerate all CFUs or specific groups such as indicator organisms, other types of bacteria and fungi. In a typical application, 1 ml of a 1:10 homogenized food sample is filtered through a membrane and the membrane is placed on suitable agar medium for overnight incubation for colony development.

3. Most Probable Numbers (MPN):

Monitoring and detection of indicator and disease-causing microorganisms are a major part of sanitary microbiology. A wide range of viral, bacterial and protozoan diseases result from the contamination of water with human fecal wastes.

4. Microscope Colony Counts:

It is a simple method to detect the microbial count in variety of food that involves the counting of colonies that developed over a microslide consists of a thin layer of culture medium. A 0.1 ml of milk-agar mixture is spreaded over a 4-cm^2 area on a glass slide.
Following incubation, drying, and staining, microcolonies are counted with the aid of a microscope. In another method, 2 ml of melted agar are mixed with 2 ml of warmed milk and 0.1 ml of the inoculated agar is spread over a 4-cm^2 area. The slide is then viewed with the 16-mm objective of a microscope after staining.

5. Agar Droplets:
The food homogenate is diluted in tubes of melted agar. For each food sample, three tubes of agar are used, the first tube being inoculated with 1 ml of food homogenate. After proper mixing transfer a line of 5 x 0.1-ml droplets to the bottom of an empty petri dish by sterile capillary pipette.
With the same capillary pipette, three drops (0.1 ml) from the first 9-ml tube are transferred to the second tube, after mixing; another line of 5 x 0.1-ml droplets is placed next to the first. This step is repeated for the third tube of agar. Petri plates containing the agar droplets are incubated for 24 hours and colonies are enumerated.

6. Dry Film:
A dry film method involves the use of two plastic films attached together on one side and coated with culture medium ingredients and a cold-water-soluble jelling agent to designated petrifilm. For use, 1ml of diluent is placed between the two films and spread over the nutrient area by pressing with a special flat-surface device.

7. Dye Reduction – MBRT Test:
Dye reduction test is a common technique used to detect the microorganisms from food. Two dyes are commonly employed in this procedure to estimate the number of viable organisms in suitable products: methylene blue and resazurin. To conduct a dye-reduction test, the supernatant of food is prepared and added to standard solutions of either dye for reduction from blue to white for methylene blue; and from slate blue to pink or white for resazurin. The time for dye reduction to occur is inversely proportional to the number of organisms in the sample.

8. Roll Tubes:
Screw-capped tubes or bottles of varying sizes are used in this method. A known amount of the melted and inoculated agar is added to the tube and it is solidified as a thin layer inside the vessel. Following appropriate incubation, colonies are counted by rotating the vessel. It has been found to be an excellent method for enumerating fastidious anaerobes.

Chemical and Molecular Biology Techniques:
1. Radiometry:
The radiometric detection of microorganisms is based on the incorporation of a ^{14}C-labeled metabolite in a growth medium so that when the organisms utilize this metabolite, $^{14}CO_2$ is released and measured by use of a radioactivity.

2. Adenosine Triphosphate (ATP) Measurement:

Adenosine triphosphate (ATP) is the primary source of energy in all living cells. It disappears within 2 hours after cell death, and the amount per cell is generally constant, with values of 10^{-18} to 10^{-17} moles per bacterial cell. The complete extraction and accurate measurement of cellular ATP can be equated to individual groups of microorganisms in the same general way as endotoxins for gram-negative bacteria.

3. DNA Amplification (PCR):

PCR has been used to detect enterotoxigenic E. coli, Vibrio, Clostridium, etc. and this method is an elegant technique to determine the pathogen by amplifying their DNA using specific primer.

4. Nucleic Acid (DNA) Probe:

A DNA probe consists of the DNA sequence of an organism of interest that can be used to detect homologous DNA or RNA sequence. The probe DNA must hybridize with that of the strain.

5. Immunologic Methods:

Serological reactions are effective method for detecting the pathogenic microorganisms or their toxin.

The most commonly used serological methods are discussed below:

i. Fluorescent Antibody:

An antibody to a given antigen is made fluorescent by coupling it to a fluorescent compound and when the antibody reacts with its antigen, the antigen-antibody complex emits fluorescence and can be detected by the use of a fluorescence microscope. The fluorescent markers used are rhodamine B, fluorescein isocyanate, and fluorescein isothiocyanate with the last being the most widely used.

The fluorescent antibody (FA) technique can be carried out by use of either of two basic methods. The direct method employs antigen and specific antibody to which is coupled the fluorescent compound (antigen coated by specific antibody with fluorescent label).

ii. Enrichment Serology:

The use of Enrichment Serology (ES) is a more rapid method for recovering salmonellae from foods than the conventional culture method. It is carried out in four steps: pre-enrichment in a nonselective medium for 18 hours; selective enrichment in selenite-cystine and/or tetrathionate broth for 24 hours; elective enrichment in M broth for either 6-8 hours or 24 hours; and agglutination with polyvalent H antisera at 50°C for 1 hour.

The Oxoid Salmonella Rapid Test (OSRT) is a variation of ES. It consists of a culture vessel containing two tubes, each of which contains dehydrated enrichment media in the lower compartments and dehydrated selective media in the upper compartments.

iii. Salmonella 1-2 Test:

Salmonella 1-2 Test employs the use of a semisolid phase. The test is conducted in a specially designed plastic device that has two chambers, one for selective broth and the other for a nonselective motility medium. In addition to selective ingredients, the nonselective medium contains the amino acid L-Serine, which is selective for salmonellae.

iv. Radioimmunoassay:

This technique consists of adding a radioactive label to an antigen, allowing the labeled antigen to react with its specific antibody, and measuring the amount of antigen that combined with the antibody by the use of a counter to measure radioactivity. Solid- phase radioimmunoassay (RIA) refers to methods that employ solid materials or surfaces onto which a monolayer of antibody molecules binds electrostatically.

v. ELISA:

The enzyme-linked immunosorbent assay (ELISA, enzyme immunoassay, or EIA) is an immunological method similar to RIA but employing an enzyme coupled to either an antigen or an antibody. A typical ELISA is performed with a solid-phase (polystyrene) coated with antigen and incubated with antiserum. Following incubation and washing, an enzyme-labeled preparation of anti-immunoglobulin is added.

After gentle washing, the enzyme remaining in the tube or microtiter well is assayed to determine the amount of specific antibodies in the initial serum. A commonly used enzyme is horseradish peroxidase and its presence is measured by the addition of peroxidase substrate.

vi. Gel Diffusion:

Gel diffusion methods have been widely used for the detection and quantitation of bacterial toxins and enterotoxins. The four most widely used methods are the single-diffusion tube (Oudin), microslide double diffusion, micro-ouchterlony slide and electroimmunodiffusion. They have been employed to measure enterotoxins of staphylococci and C. perfringens; and the toxins of C. botulinum.

vii. Hemagglutination:

Gel diffusion methods generally require at least 24 hours for results but this method yield results in 2-4 hours. There are two types of heme agglutination: hemagglutination-inhibition (HI) and reverse passive hemagglutination (RPH). Unlike the gel diffusion methods, antigens are not required to be in precipitable form for these two tests.

In the HI test, specific antibody is kept constant and enterotoxin (antigen) is diluted out. Following incubation for about 20 minutes, treated sheep red blood cells (SRBCs) are added. Hemagglutination (HA) occurs only when antibody is not bound by antigen. HA is prevented (inhibited) where toxin is present in optimal proportions with antibody. In

RPH antitoxin globulin is attached directly to SRBCs and used to detect toxin. When diluted toxin preparations are added, the test is read for HA after incubation for 2 hours.

HA occurs only where optimal antigen antibody levels occur. No HA occurs if no toxin or enterotoxin is present.

Fresh Meat:

It is generally agreed that the internal tissues of healthy slaughter animals are free of bacteria at the time of slaughter, assuming that the animals are not in a state of exhaustion. When one examines fresh meat and poultry at the retail level, varying numbers and types of microorganisms are found. The following are the primary sources and routes of microorganisms to fresh meats with particular emphasis on red meat.

• The stick knife. After being stunned and hoisted up by the hind legs, animals such as steers are exsanguinated by slitting the jugular vein with what is referred to as a "stick knife." If the knife is not sterile, organisms are swept into the bloodstream where they may be deposited throughout the carcass.
• Animal hide. Organisms from the hide are among those that enter the carcass via the stick knife. Others from the hide may be deposited onto the dehaired carcass or onto freshly cut surfaces. Some hide biota becomes airborne and can contaminate dressed out carcasses as noted below. See the section on carcass sanitizing and washing.
• Gastrointestinal tract. By way of punctures, intestinal contents along with the usual heavy load of microorganisms may be deposited onto the surface of freshly dressed carcasses. Especially important in this regard is the paunch or rumen of ruminant animals, which typically contains - 1010 bacteria/g.
• Hands of handlers. this is a source of human pathogens to freshly slaughtered meats. Even when gloves are worn, organisms from one carcass can be passed on to other carcasses.
• Containers. Meat cuts that are placed in non sterile containers may be expected to become contaminated with the organisms in the container. This tends to be a primary source of microorganisms to ground or minced meats.
• Handling and storage environment. Circulating air is not an insignificant source of organisms to the surfaces of all slaughtered animals
• Lymph nodes. In the case of red meats, lymph nodes that are usually embedded in fat often contain large numbers of organisms, especially bacteria. If they are cut through or added to portions that are ground, one may expect this biota to become prominent.

Biochemical events that lead to rigor mortis: In general, the most significant of the above are nonsterile containers. When several thousand animals are slaughtered and handled in a single day in the same abattoir, there is a tendency for the external carcass biota to become normalized among carcasses although a few days may be required. The practical effect of this is the predictability of the biota of such products at the retail level.

Upon the slaughter of a well-rested beef animal, a series of events take place that lead to the production of meat.

1. Its circulation ceases: the ability to resynthesize ATP (adenosine triphosphate) is lost; lack of ATP causes actin and myosin to combine to form actomyosin, which leads to a stiffening of muscles.
2. The oxygen supply falls, resulting in a reduction ofthe aIR (oxidation-reduction) potential.
3. The supply of vitamins and antioxidants ceases, resulting in a slow development of rancidity. 4. Nervous and hormonal regulations cease, thereby causing the temperature of the animal to fall and fat to solidify.
5. Respiration ceases, which stops ATP synthesis.
6. Glycolysis begins, resulting in the conversion of most glycogen to lactic acid, which depresses pH from about 7.4 to its ultimate level of about 5.6. This pH depression also initiates protein denaturation, liberates and activates cathepsins, and completes rigor mortis. Protein denaturation is accompanied by an exchange of divalent and monovalent cations on the muscle proteins.
7. The reticuloendothelial system ceases to scavenge, thus allowing microorganisms to grow unchecked.
8. Various metabolites accumulate that also aid protein denaturation. These events require between 24 and 36 h at the usual temperatures of holding freshly slaughtered beef (2°-5°C).

These events require between 24 and 36 h at the usual temperatures of holding freshly slaughtered beef (2°-5°C). Meanwhile, part of the normal flora of this meat has come from the animal's own lymph nodes (65), the stick knife used for exsanguination, the hide of the animal, intestinal tract, dust, hands of handlers, cutting knives, storage bins, and the like. Upon prolonged storage at refrigerator temperatures, microbial spoilage begins. In the event that the internal temperatures are not reduced to the refrigerator range, the spoilage that is likely to occur is caused by bacteria of internal sources.

In contrast to fruits and vegetables, meats are composed mainly of protein and fats rather than carbohydrates. Water content is 71–76% and therefore moisture is not an issue except for spoilage microbes on cured meats. Muscles of healthy animals do not contain any bacteria or fungi but as soon as animals are slaughtered, meat is exposed to

contaminants and good sanitation practices are essential to produce high quality meats. The number of spoilage organisms on meat just after slaughter is a critical factor in determining shelf life. The surface of beef carcasses may contain anywhere from 10^1 to 10^7 cfu/cm2, most of which are psychrotrophic bacteria. Chopping and grinding of meats can increase the microbial load as more surface area is exposed and more water and nutrients become available. A large variety of microbes are commonly found on fresh meat, but different microbes become dominant during spoilage of different meats depending on pH, composition and texture of processed meats, temperature and packaging atmosphere. *Pseudomonas* spp. is the predominant spoilage bacteria in aerobically stored raw meat and poultry. Once the initial low levels of glucose are depleted by various microbes, *Pseudomonas*has an advantage because it can catabolize gluconates and amino acids more readily than other microbes. Break down of these compounds results in production of malodorous sulfides, ammonia, and amines, including the biogenic amines putrescine and cadaverine. Dark, firm and dry meat with a relatively high pH of 6.0 spoils more rapidly because deamination of amino acids starts earlier. *Shewanella putrefaciens* does not grow on meat at pH<6.0 but can produce sulfides and ammonia even when glucose is still available. These sulfides not only smell bad but also cause color changes in meat, and therefore Shewanella has a high spoilage potential on fresh, high pH meats stored aerobically even when it is not a dominant microbe. *Brochothrix thermosphacta* is often a significant spoilage organism on fresh meat stored aerobically at refrigeration temperatures.*Enterobacteriaceae*, particularly species of *Serratia*, *Enterobacter*, and *Hafnia*, are major causes of spoilage in vacuum-packed, high pH fresh meats. These organisms are facultative anaerobes that produce organic acids, hydrogen sulfide and greening of meats.

Lactic acid bacteria (LAB) grow on meat and poultry packaged under vacuum and modified atmospheres, producing organic acids from glucose by fermentation. This gives rise to aciduric off-odors which may be accompanied by gas and slime formation and greening of meat. However, LAB are weakly proteolytic and so do not produce large amounts of amines or sulfides, and spoilage of meat by LAB is not as offensive. Psychrophilic, anaerobic *Clostridium* spp. are associated with spoilage of vacuum-packaged meats. "Blown pack" meat spoilage is characterized by excessive gas formation with off odors due to formation of butyric acid, butanol and sulfurous compounds. Yeasts and molds grow relatively slowly on fresh meat and do not compete well with bacteria. Therefore, they are a minor component of spoilage flora.

Microorganisms Associated with Meat During Processing

Meat spoilages indicate (a) color changes (b) textural changes and (c) development of off-flavour or off-odor or slime as a result of microbial growth. *Salmonella* is the primary microbial challenge for poultry. The primary microbial to the beef industry is *Escherichia coli* O157: H7. *Listeria*, which is an adulterant with zero tolerance, is the major problem for

ready to eat meat products. Treatment with organic acids, hot water steam carcass pasteurization and steam carcass vacuuming, trisodium phosphate, acidified sodium chlorite, chlorine dioxide, lactoferrins, peroxyacetic acid, sodium lactate, sodium acetate and sodium diacetate, ozone and radiation have been used as microbial decontaminants during meat processing operations. Carcass washing with hot water of 80°C for 10 seconds can reduce microbial loads by 2 logs. Current regulatory policies and inspection in the meat industry include the HACCP (Hazard Analysis Critical Control Point) food safety system with an objective to provide safe food for consumption and prevent chemical, physical and biological hazards.

Spoilage under aerobic condition

1.) Surface slime, caused by *Pseudomonas acinetobacter, Moraxella alcaligenes Streptococcus, Leuconostuoc, Bacillus*and *Micrococcus.*

2.) Change in colour of meat pigment. The red colour of meat may be changed to shades of green, brown or grey by*Lactobacillus* and *Leconostocs* spp.

3.) Changes in fat. The unsaturated fat in meat gets oxidized by lypolitic bacteria which produce off odours due to hydrolysis of fats and production of aldehydes and acids. This type of spoilage is caused by lypolitic *Pseudomonas,* Achromobacter and yeast.

4.) Surface color change. The red pigment producing bacteria, *Serratia marcescens*, caused red spots on meat. Blue color surface is caused by *Pseudomonas syncyanea* and yellow color is caused by *Micrococcus* species.

5.) Off odor and off taste. Volatile acid like formic, acetic, butyric and propionic acid produce sour odor and*Actinomycetes* produce musty or earthy flavor. Yeast also cause sliminess discoloration and off odor and taste defects.

6.) Aerobic mold also cause spoilage in meat. These are stickiness, whiskers, black-spot, white-spot, green patches off odor and off taste.

7.) Spoialage under anerobic condition.

i) Souring is caused by production of formic, acetic, butyric, lactic, succinic and propionic acid.

ii) Putrefaction. It is caused by decomposition of proteins under anaerobic condition by *Clostridium* species. The foul smell is due to production of hydrogen sulphide, mercaptans, indol, scatol, ammonia and amines.

Spoilage of egg

Breaks or cracks in egg shell taking place due to transportation or mechanical damage may allow microorganisms to enter in to the egg yolk and cause spoilage on storage. Eggs on storage may lose moisture and, therefore, weight. The white of the egg becomes thinner and more watery on storage. The major changes in the egg take place due

to spoilage organisms. In general the spoilage of eggs is caused by bacteria as compared to molds and can be described as green rot due to the growth of*Pseudomonas fluorescens,* colourless rot due to the growth of *Pseudomonas, Acinetobacter* and other species; black rots due to *P roteus, Pseudomonas;* red rots due to *Serratia* spp. and custrad rots due to *Proteus vulgaris* and Pseudomonas*intermedium.* Growth of *Aeromonas* in the egg yolk turns it to black colour and also there is strong putrid odour due to the formation of hydrogen sulphide (H_2S). Storage of eggs in high humid atmosphere may help in growth of several molds on the surface of the egg shell. Molds causing spoilage of eggs include species *of Pencillium, Mmucor, Alterneria* , etc.

Poultry Meat

Poultry meat like meat of other animals is also susceptible to contamination by various sources. Contamination of skin and lining of the body cavity take place during various processing operations. The organisms of great importance in poultry are*Salmonella* spp. and *Campylobacter jejuni.* Several Gram negative psychrotropic bacteria *viz., Pseudomonas, Acenitobacter*and *Flavobacterium* have also been isolated from poultry carasses. Ground turkey also may carry fecal streptococci. It is important to freeze the poultry fast in order to keep it in good condition for several months. Freezing further reduces the number of microorganisms in the poultry meat provided the temperature is maintained quite low (-18 ° C or below).

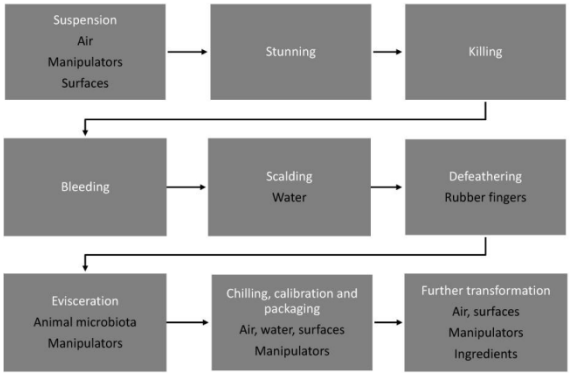
Gaseous contaminations of poultry farms

The composition of air in poultry houses significantly differs from atmospheric air. In addition to basic gaseous components (N_2-nitrogen, O_2-oxygen, Ar-argon, and CO_2-carbon dioxide), the air inside poultry houses also contains compounds that are not normally found in atmospheric air. Birds, their excrements, feed, and process equipment are the main sources of volatile chemical compounds in poultry houses. Ammonia and carbon dioxide are most frequently encountered in farm buildings, and they contribute to the risk of disease if present in excessive concentrations. For this reason, ammonia and carbon dioxide are regarded as the most toxic gases in poultry houses.

Carbon dioxide (CO_2) is a natural component of air, and its concentrations generally do not exceed 300 ppm (0.03%). Carbon dioxide is responsible for breathing control in the respiratory system. In densely stocked poultry houses, carbon dioxide concentrations are significantly higher than in atmospheric air, but they should not exceed 2000 ppm. At higher concentrations in poultry houses, CO_2 weakens respiratory defense mechanisms and increases susceptibility to respiratory diseases. Carbon dioxide poses a serious hazard to

health and life at concentrations higher than 10 000 ppm. Carbon dioxide levels in poultry houses are a robust indicator of ventilation efficiency. Its concentrations increase rapidly in poorly ventilated buildings.

In poultry houses, ammonia (NH_3) is released from excreta which contain nitrogen in the form of uric acid. Ammonia is produced in the process of microbial fermentation. Ammonia production increases in conditions that support microbial proliferation, including high temperature, high humidity, high pH, and presence of organic matter. In poultry houses, ammonia concentrations should not exceed 13 ppm for adult birds and 10 ppm for chicks. At higher concentrations, NH_3 can compromise growth, whereas exposure to more than 30 ppm of ammonia can lead to respiratory dysfunctions such as intensified mucus secretion, shallow breathing, and bronchoconstriction. High levels of ammonia can impair immunity and increase susceptibility to respiratory infections and ocular abnormalities in poultry.



Meat Borne Diseases

Food borne microbial hazards have a devastating impact on human suffering. Microbial pathogens of current concern that need to be controlled in the fresh meat include *Salmonella, Campylobacter*, enterohaemorrhagic *Escherichia colli* including serotype O157:H7 and other enteric pathogens. Infection due to *Listeria monocytogenes* following consumption of ready to eat meat and poultry products is a major

problem in the recent years. Also there are food borne infections caused by *Yersinia enterocolitica, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens* and *Bacillus cereus*. Prevalence of some food borne pathogens recognized since 1970's include *Vibrio cholerae, Vibrio vulnificus*, Noro virus,, *Enterobacter sakazakii*, prions and resistant bacteria. In recent years the food borne pathogens associate with animal health pandemics include Avian Influenza (AI) and Foot and Mouth Disease (FMD) viruses. Avian influenza is not of concern to poultry meat safety, because it is inactivated by proper cooking with a temperature of 70°C and more. Also the oral route of transmission is less important than the non food borne route. Presently there is continuous adaptation and development of resistance by pathogenic microorganisms to antibiotics and potentially to traditional food preservation barriers, like low pH, heat, cold temperatures, dryness, low water activity and chemical additives. Development of antibiotic resistance in food borne pathogens is very important from public health view point in present days and in the future.

Control of meat borne pathogens

Effective control of meat borne pathogens and enhancement of meat safety can be achieved by control of latent infections among livestock, animal welfare and humane treatment, application of antimicrobial interventions at the farm, during harvesting, dressing and product processing, improvement in process food hygiene and potential application of new or novel processing and preservation technologies. Animal stress can damage meat quality and lead to more contamination and increased pathogen shedding. Antimicrobial intervention technologies can be used effectively to improve the microbiological quality of meats. These technologies include reduction of contamination on the raw product, minimization of microbial access to the products, reduction of contamination that has gained access to the product, inactivation of the microbes on the product without cross contamination and prevention and control of the growth of non-inactivated microbes, which have gained access to the meat. An effective pathogen control at pre-harvest, postharvest, processing, storage, distribution, merchandizing, preparation, food-service and consumption of meat include activities employed during pre-harvest or in the field, during post harvest or processing in the plant, at retail and food service and at home. Pre-harvest pathogen control interventions include diet manipulation, use of food additives, antibiotic, bacteriophage therapy, and immunization of the animals, complete exclusion, probiotics and proper animal management practices like pen management, clean feed, clean and chlorinated water, and clean and unstressful transportation. Antimicrobial intervention activities during harvest and post harvesting should be designed to minimize introduction of microbial contaminants and reduce existing contaminant levels through implementation of decontamination and sanitization interventions, processing treatments for partial or complete destruction of contaminants and antimicrobial procedures for inhibition or retardation of microbial growth. Certain inspection regulations should be followed in meat and poultry plants, such as establishment of sanitation standard operating system, operations under the HACCP system and performing HACCP verifications to meet microbiological standards, establishment of good manufacturing practices (GMP) and good hygiene practices (GHP). Antimicrobial interventions used to control pathogens in further

processed meat products include physical hurdles (low and high temperature, non thermal process like irradiation and high pressure and packaging treatments), physiochemical interventions (low pH, reduced water activity, modification of oxidation reduction potential through packaging and application of antimicrobial additives), and biological interventions (microbial competitors, such as lactic acid bacteria and antimicrobial products, such as bacteriocins). Events of the most food borne illness happen due to mishandling of foods in various ways. So, there should be provisions to educate the food handlers and consumers particularly on culinary tips.

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POSSIBLE QUESTION

2marks

1. Write short notes on purification.
2. What is the special feature of molecular biology technique
3. Write the short notes ATP measurement
4. What is the role of hemagglutination
5. Write note on breed count
6. State the motility study of microorganism.
7. Write short notes on sampling method?
8. Give short notes on direct microscopic count of microbial count.
9. Comment on important microbes in fresh meat?
10. Write short notes on microorganisms in food.

8 marks

1. Elaborate the physical method for detecting microbes?
2. Explain the chemical and molecular method for detecting microbes?
3. Examine the microorganism in fresh meat?
4. Determined the microbes in poultry meat?
5. Give a brief notes serological and Immunological method detection of microbes?
6. Describe the detection of microbes in poultry.
7. Brief notes on microorganism associated with meat during processing?

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M. Sc MICROBIOLOGY **COURSE NAME: FOOD MICROBIOLOGY**

COURSE CODE: 18MBP302 **BATCH-2018-2020**

UNIT: 4

S. no.	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Which of the following is a technique applied to the processing of fresh meat?	Chopping	Protein Extraction	None of the mentioned	Chopping & Protein Extraction	Chopping & Protein Extraction
2	Statement 1: A lot of changes take place in meat on storing at a chilled temperature. These change muscle to meat. Statement 2: The above process is called ageing or conditioning.	True, False	True, True	False, False	False, True	True, True
3	Statement 1: Fermentation of meat is done at a certain temperature and then brought done to certain moisture content. Statement 2: The bacteria, during fermentation, produce lactic acid which lowers the pH of the meat and helps it stay longer	True, False	True, True	False, False	False, True	True, True
4	Oil/Lemon/Vinegar + Spices applied to meat is called _____	Marinating	Marinating	Fermenting	Coating	Marinating
5	Certain bacteria are added to minced meat products. This activity is followed by dehydration. What is this activity called?	Coating	Freezing	Curing	Fermentation	Fermentation
6	Coated meat products require _____	Breading	Pre-dusting	Battering	All three are methods of making Coated meat products	All three are methods of making Coated meat products
7	Statement 1: Sausages are minced. A lot of spices are added to it. Statement 2: The Sausage is then stuffed with stuffing. It is cooled and refrigerated. They're then packed.	True, False	True, True	False, False	False, True	False, True

8	Statement 1: Ham is from pork bellies. Bacon is from pork thighs. Statement 2: Corned Beef is strips of beef cooked and cured in cans. It is the major export product of India.	True, False	True, True	False, False	False, True	False, True
9	Statement 1: Dry preserved meat is smoked to give it flavour and increase its shelf life. Statement 2: The above makes the meat hard.	True, False	True, True	False, False	False, True	True, True
10	Statement 1: Pasteurized products are kept at room temperature and Sterilized products are kept in refrigeration.Statement 2: Canning is a process in which steam is sent in to sterilize the products.Which of the following holds true for statement 1 and statement 2 respectively?	True, False	True, True	False, False	False, True	False, False
11	Statement 1: Canned, Frozen, Dry- preserved and cured meats are types of meat present. Statement 2: Sausages, Prepared dinner meats, fermented and Poultry meats are the types of meat present.	True, False	True, True	False, False	False, True	True, True
12	Bacteria which is present in raw or undercooked meat, eggs, sea food and unpasteurized milk is	E.coli	salmonella	staphylococcus	cyano bacteria	salmonella
13	Myoglobin, when combined with oxygen, as in a freshly-cut piece of red meat, will be	pink	brown	bright red	dark red	bright red

14	Which one of the following groups of foods is most likely to contain some food-poisoning bacteria when purchased?	breakfast cereals	pasteurised milk	fresh meat and poultry	jams and marmalades	fresh meat and poultry
15	Which one of the following frozen foods often produces 'drip' on thawing?	chips	peas	fish fingers	meat	fish fingers
16	The technique first described to determine the incipient spoilage in meat was	homogenate extract volume	agar Plate Count	extract Release Volume	none of the above	extract Release Volume
17	Vacuum packaged meats are spoiled by	thermosphacta	Lactobacilli	Both (a) and (b)	none of these	Both (a) and (b)
18	The growth of Streptomyces on straw or elsewhere near the egg may produce	musty or earthy	flavors hay odour	fishy flavor	cabbage-water flavor	musty or earthy
19	Beef hams are made spongy by species of	Rhodotorula	Bacillus	Pseudomonas	red Bacillus	Bacillus
20	The red color of meat, called its bloom, may be changed to shades of green, brown or grey as a result of production of oxidizing compounds by bacteria. Which of the following species are reported to cause the greening of sausage?	Lactobacillus	Leuconostoc	Pseudomonas	Both (a) and (b)	Both (a) and (b)
21	Yellow discolorations in meat are caused by bacteria with yellow pigments, usually species of	Micrococcus	Flavobacterium	both (a) and (b)	Pseudomonas syncyanea	both (a) and (b)
22	Which of the following (s) is/are responsible for the green patches on the surface of meats under aerobic conditions?	P. expansum	P. asperulum	P. oxalicum	All of these	All of these
23	Which of the following microorganisms grow in beef at a temperature of 15°C and above?	Micrococci	Pseudomonas	Both (a) and (b)	Lactobacillus	Both (a) and (b)
24	Green rots in eggs is chiefly caused by	Pseudomonas fluorescens	Micrococcus or Bacillus species	Molds or yeasts	all of the above	Pseudomonas fluorescens

25	Black rots in eggs is most commonly caused by	species of Proteus	species of Micrococcus or Bacillus	molds or yeasts	all of these	species of Proteus
26	Which of the following microorganism spoil poultry in polyethylene bags?	Pseudomonas-achromobacter	Alcaligenes	hetero-fermentative species	catalase negative bacteria	Pseudomonas-achromobacter
27	Black spot in meat are produced mainly by	Cladosporium	Thamnidium	Mucor	Rhizopus	Cladosporium
28	Lactic acid bacteria in meats may be responsible for	slime formation at the surface or within especially in presence of sucrose	production of green discoloration	souring	all of the above	all of the above
29	Which of the following is recommended for the improvement in flavor during ageing of beef under controlled conditions?	Thamnidium	Rhizopus	T. elegans	M. mucedo	Thamnidium
30	White spot in meat is formed due to presence of	P. expansum	Sporotrichum carnis	both (a) and (b)	P. oxalicum	Sporotrichum carnis
31	_____ animal intestines	Marinating tends to make meat more		Mahi mahi and tuna are examples of	Sausages are encased in	Sausages are encased in
32	165°F	Poultry should be cooked until it is well done at a temperature of	Which method for cooking poultry retains the most nutrients?	The tough membrane on meat is called	When poultry is done cooking, its juices should be	Poultry should be cooked until it is well done at a temperature of
33	In methylene blue reduction test for testing the quality of semen, a good semen should reduce the color in	10 sc	1min	10-20min d	60min	1min

34	More spoilage of eggs is caused by	bacteria than molds	molds then bacteria	synergiisticall y	non of these	bacteria than molds
35	Which one of the following actions do the food laws require you to take should you have reason to believe that you are suffering from food poisoning?	Inform your employer of the problem	Inform your workmates of the problem	Continue work but wash your hands after handling raw meat	Visit your doctor as soon as possible	Inform your employer of the problem
36	In 1993, an outbreak of _____ poisoning from undercooked hamburger meat resulted in many cases of illness and some deaths in the Washington state area	Clostridium botulinum	Salmonella	E coli 0157:H7	Listeria	E coli 0157:H7
37	Which of the following is NOT a good food safety practice	Keeping ground beef refrigerated for no more than 1-2 days before cooking	When in doubt, throw it out	Keeping hot foods hot; cold foods cold	Using the same knife to cut raw meat and vegetables	Using the same knife to cut raw meat and vegetables
38	Which of the following deals with how food is adjudged by the consumer?	Food microbiology	Product Development	Sensory Analysis	Food physics	Sensory Analysis
39	Storage requirements and stability, product attributes conductive to product sale etc. The above activities refer to which step of the food industry?	Production	Manufacture	Distribution	Marketing	Marketing
40	Which of the following is a technique applied to the processing of fresh meat?	Chopping	Protein Extraction	None of the mentioned	Chopping & Protein Extraction	Chopping & Protein Extraction
41	Ice crystals in frozen meat should be formed by rapid crystallization	True	FALSE	None of this	May be	TRUE

42	Frozen prepared chicken dinner comes under which of the following categories of processes?	Single component, single-product process	Multi component blended products	Multi component products with add-ons	Multi component, multi process assembly operations	Multi component, multi process assembly operations
43	Statement 1: Meat is more perishable than fish. Statement 2: At low temperatures, sometimes, the inside of the egg shell might solidify and expansion of the shell might lead to causing of the shell to crack.	True, False	True, True	False, False	False, True	False, True
44	Statistics show that -1 deg C is the best temperature to store eggs. With respect to that result, the room temperature can be brought down to a lower value when needed for better results of storage	true	false	none of this	may be	false
45	Shell thickness is directly proportional to the effect of storage of eggs.	True, False	True, True	False, False	False, True	True, True
46	what are poultry diseases?	Fowl cholera	Fowl typhoid	Fowl bacillus	Avian Influenza	Avian Influenza
47	For which of the following organisms is there no known animal reservoir?	Francisella tularensis	Pasteurella multocida	Bordetella pertussis	Brucella melitensis	Bordetella pertussis
48	which type of influenza virus does cause avian influenza?	type a	type b	type c	type a and b	type a
49	Histopathoglogy is an important diagnostic tool for the diagnosis of which of the following viral diseases?	infectious bursal disease	avian influenza	infectious bronchitis	Newcastle disease	infectious bursal disease

50	in chickens affected with infectious bronchitis, the virus can en isolated from which of the following organs/tissues?	tracheas	trachea and lungs	tracheas and cecal tonsils	tracheas, lungs, and cecal tonsils	tracheas, lungs, and cecal tonsils
51	Which of the following tests is used to confirm that an isolated virus is type A influenza virus?	hemagglutination test	hemagglutination-inhibition test	agar-gel immunodiffusion test	virus-neutralization test	agar-gel immunodiffusion test
52	Staphylococcus was isolated from swollen joints of turkeys. Which of the following biochemical tests would confirm that the isolate is not Staphylococcus epidermidis	catalase test	oxidase test	coagulase test	indole test	coagulase test
53	which of the following ingredients are used in the culture media for the isolation and propagation of avian mycoplasmas (to inhibit growth of bacterial and fungus)?	chlorhexidine and thalium acetate	chlorhexidine and penicillin	penicillin and thallium acetate	chlorhexidine and spectimomycin	penicillin and thallium acetate
54	in chronic stages of mycoplasma synoviae infection, it has been indicated that which of the following organs/tissues may be more reliable for the isolation of the mycoplasma?	foot pads	tracheas	ovaries	oviducts	tracheas
55	which of the following viruses is not egg-transmitted?	avian encephalomyelitis virus	chicken infectious anemia virus	viral arthritis virus	non of the above	non of the above
56	which of the following toxigenic groups of clostridium botulinum has primarily caused botulism in birds?	type a	type b	type c	type d	type c
57	which of the following lesions is frequently in checks affected with salmonellosis?	panophthalmitis	yolk sac infection and pericarditis	pericarditis, hemorrhagic enteritis and arthritis	bilaterial fibrinopurulent pleutopneumonia and panophthalmitis	panophthalmitis

58	how many cryptosporidium spp has been recognized in chickens?	1	2	3	4	1
59	Which of the following has its antiviral action attributed to interference of protein synthesis?	Amantadine	Interferons	Acycloguanosine	5'-iododeoxyuridine	Interferons
60	The cogulase test is used to differentiate	Staphylococcus epidermidis from Neisseria meningitidis	Staphylococcus epidermidis from Neisseria meningitidis	Streptococcus pyogens from Staphylococcus aureus	Streptococcus pyogens from Enterococcus faecalis	Staphylococcus epidermidis from Neisseria meningitidis

Unit V

Microorganisms are essential for the production of foods such as cheese, yogurt, bread, beer, wine and other fermented foods. The term 'microorganisms' include bacteria, fungi, viruses and protozoa. We almost always presume they are harmful to us. So this is because we read about how they cause diseases to both plants and animals including humans. But, it is a fact that microorganisms are useful to us in many ways. Microorganisms help in the production of many food items, making medicines, keeping the environment clean, in manufacturing and in research. Let us learn about microorganisms and its uses.

1. Production of dairy products:
acteria are the key players here. Bacteria help in fermentation which helps in making different forms of dairy products from milk like curd, buttermilk, butter, cheese. *Streptococcus is t*he most common genus of bacteria that are used in the commercial production of this product.

2. Bread Baking:
A species of *Streptococcus* is added to the dough before making bread to bring about the required fermentation.

3. Alcoholic Drinks:
Alcoholic drinks are prepared or manufactured by the process of fermentation. Each drink is derived from a different starting product such as potato and grapes. Then it is fermented, distilled and alcohol is prepared. The commonly used microorganism here is different types of fungus like yeast. Some even use bacteria and fungus. Alcoholic drinks include wine, rum, vodka etc.

4. Organic acids:
Organic acids are commercially prepared using fungi. Acetobacter, Rhizopus, Penicillium are a few fungi that are used to ferment substrates such as fruits and sugar-containing syrups. Examples of acids that are derived and manufactured on a large scale using fungi are acetic acid, citric acid, gluconic acid, fumaric acid and lactic acid.

5. Enzymes:
Many microbes are used in the derivation of enzymes such as lipase, lactase, protease, peptidase to name a few.

6. Steroid production:
Some bacterial and fungal species are used in the preparation of steroids that are then injected into the human body for different purposes.

7. Help in sewage treatment:
Not only are microorganisms helpful to our body, they are also helpful to the environment. They help in the secondary treatment stage of sewage treatment.

8. Used as insecticides:
Certain bacterial and fungal species are used to keep certain insects and pests away from crops.
9. Fertility of soil:
Microorganisms play a very important role in maintaining the fertility of the soil. They help in the composting process which forms manure. Also, microorganisms present in the soil help aerate it and enrich the soil with nitrates and other nutrients. These nutrients are needed by the crops for an abundant harvest.

10. Production of vitamins:
An essential vitamin that people need for proper digestion is Vitamin B 12. Fungi are responsible for manufacturing B12.

11. Production of antibiotics and antivirals:
Bacteria and viruses are isolated and their antigens and enzymes are extracted. These antigens help in the development of antibiotics and antivirals.

12. Biotechnology and research:
So many labs use bacteria, fungi and especially viruses for research studies. Non- virulent forms of these microorganisms are injected into subjects going through clinical trials. This in future helps in the development of medicines, vaccinations and cure for diseases. And DNA and RNA studies also make use of them.

It is important for us to know about microorganisms and its uses as they are both beneficial as well as harmful to other life forms. They play a crucial role in the ecosystem. Maintaining a balance between the ‘good’ and ‘bad’ microorganisms is the key to coexisting with them.

Intestinal Beneficial bacteria

Thus, intestinal bacteria represent a complex and incompletely understood microbiome. Since certain organisms are thought to play a role in the onset of inflammatory diseases of the bowel, whereas other organisms are considered protective, this review explores the relationship between bacteria that reside in the gastrointestinal tract and the host. There is an emphasis on bacterial interactions with epithelial cells, as well as on the role of bacteria in the development of inflammation and in defense mechanisms deployed by the host to counter such attacks. Selected homeostatic processes and mediators that may maintain the intestine in a state of “controlled inflammation” are also discussed.

The microflora of the intestinal microenvironment as a unit has important protective, metabolic, and trophic functions. Resident bacteria serve a central line of resistance to colonization by exogenous microbes and thus assist in preventing the potential invasion of the intestinal mucosa by an incoming pathogen. This protective function is known as the barrier effect or colonization resistance, and the bacteria have a number of important roles. One role is that adherent nonpathogenic bacteria can often prevent attachment and subsequent entry of suspected pathogens into epithelial cells. Another role is that commensal bacteria compete for available nutrients in ecological niches and, in doing so, maintain the

collective microenvironment by administering and consuming all resources. This mutual and beneficial relationship helps dampen unwanted overproduction of nutrients, which could potentially support intrusion of microbial competitors that could have a pathogenic outcome for the host.

Prebiotics

Prebiotics are compounds in food that induce the growth or activity of beneficial microorganisms such as bacteria and fungi.[1] The most common example is in the gastrointestinal tract, where prebiotics can alter the composition of organisms in the gut microbiome.

Prebiotics are a component of some foods that the body cannot digest. They serve as food for bacteria and other beneficial organisms in the gut. The benefits of prebiotics have links to the benefits of probiotics. Prebiotics may support a healthy gut, offering better digestive health, fewer antibiotic-related health problems, and other benefits.

Some research suggests that prebiotics may benefit the body by:

- improving calcium absorption
- changing how quickly the body can process carbohydrates
- supporting the probiotic growth of gut bacteria, potentially enhancing digestion and metabolism

Good bacteria play a significant role in regulating your immune system, inhibiting the growth of pathogens (disease causing bacteria) and digesting food. Galactooligosaccharides (GOS) are the most advanced form of prebiotics which belong to a group of particular nutrient fibres that feed and encourage the growth of good bacteria in the gut.

Prebiotics were first defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health” [2]. This definition was later refined to include other areas that may benefit from selective targeting of particular microorganisms [3]: “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confer benefits.” Lactobacilli and bifidobacteria are the usual target genera for prebiotics; changes in bifidobacteria are more likely to be seen compared to lactobacilli. This may be due to the fact that more bifidobacteria usually reside in the human colon than lactobacilli, and they exhibit a preference for oligosaccharides. Although all prebiotics are fiber, not all fiber is prebiotic. Classification of a food ingredient as a prebiotic requires scientific demonstration that the ingredient.

- Resists gastric acidity, hydrolysis by mammalian enzymes, and absorption in the upper gastrointestinal tract;
- Is fermented by the intestinal microflora;

- Selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being.

Prebiotics occur naturally in many foods, so there is no need for people to take prebiotic supplements. There is currently no evidence that taking prebiotics and probiotics together is harmful. However, people who have chronic diseases or serious illnesses should avoid probiotic or prebiotic supplements unless a doctor advises otherwise. Research on the side effects of prebiotics is also in its infancy and requires further investigation.

There are also many probiotic foods that naturally contain helpful bacteria, such as yogurt.
A high-quality, plain yogurt with live cultures can be a fantastic addition to your diet if you want to add beneficial bacteria.
Fermented foods are another great option, as they contain beneficial bacteria that thrive on the naturally occurring sugar or fiber in the food.

Examples of fermented foods include:

- Sauerkraut.
- Kimchi.
- Kombucha tea.
- Kefir (dairy and non-dairy).
- Some types of pickles (non-pasteurized).
- Other pickled vegetables (non-pasteurized).

If you are going to eat fermented foods for their probiotic benefits, make sure they are not pasteurized, as this process kills the bacteria.
Some of those foods can also be considered synbiotic, because they contain **both** beneficial bacteria and a prebiotic source of fiber for the bacteria to feed on.
One example of a synbiotic food is sauerkraut.

What about Probiotic Supplements?
Probiotic supplements are pills, capsules or liquids that contain live beneficial bacteria.
They are very popular and easy to find, yet not all of them are worth your money. They do not all have the same types of bacteria, or the same concentrations.
They also usually do not come with fibrous food sources for the bacteria to eat.
Some probiotic supplements are designed to carry the bacteria all the way to your large intestine for better effects, while others probably don't make it past your stomach acid.
There are some individuals who should not take a probiotic, or who may experience worsened symptoms if they do, such as people with small intestinal bacterial overgrowth (SIBO) or people sensitive to ingredients in the supplement.
However, the right strains of probiotics can be incredibly beneficial for some people.
As with all supplements, you may want to consult with a healthcare professional who is knowledgeable about probiotics.

What the Science Says About the Effectiveness of Probiotics

Researchers have studied probiotics to find out whether they might help prevent or treat a variety of health problems, including:

- Digestive disorders such as diarrhea caused by infections, antibiotic-associated diarrhea, irritable bowel syndrome, and inflammatory bowel disease
- Allergic disorders such as atopic dermatitis (eczema) and allergic rhinitis (hay fever)
- Tooth decay, periodontal disease, and other oral health problems
- Colic in infants
- Liver disease
- The common cold
- Prevention of necrotizing enterocolitis in very low birth weight infants.

There's preliminary evidence that some probiotics are helpful in preventing diarrhea caused by infections and antibiotics and in improving symptoms of irritable bowel syndrome, but more needs to be learned. We still don't know which probiotics are helpful and which are not. We also don't know how much of the probiotic people would have to take or who would most likely benefit from taking probiotics. Even for the conditions that have been studied the most, researchers are still working toward finding the answers to these questions. Probiotics are not all alike. For example, if a specific kind of *Lactobacillus* helps prevent an illness, that doesn't necessarily mean that another kind of *Lactobacillus*would have the same effect or that any of the *Bifidobacterium* probiotics would do the same thing. Although some probiotics have shown promise in research studies, strong scientific evidence to support specific uses of probiotics for most health conditions is lacking. The U.S. Food and Drug Administration (FDA) has not approved any probiotics for preventing or treating any health problem. Some experts have cautioned that the rapid growth in marketing and use of probiotics may have outpaced scientific research for many of their proposed uses and benefits.

What medical conditions are probiotics used for?

Probiotic supplements may be useful in treating and preventing inflammatory digestive tract conditions such as pouchitis (which affects people who have had their colons removed), inflammatory bowel diseases (such as ulcerative colitis and Crohn's disease), and chronic (long-term) stomach inflammation and ulcers caused by the *Helicobacter pylori*bacterium.

Probiotics may also be helpful in treating constipation, irritable bowel syndrome, acid reflux, and spastic colon; shortening the duration of infectious diarrhea; and reducing the recurrence (return) of bladder and colorectal cancer. Probiotics are also being studied as a method of boosting the immune system,

Some studies suggest that yogurt is helpful in preventing diarrhea (a common side effect of treatment with antibiotics). It has also been shown to prevent or treat urinary tract infections and vaginal yeast infections in women.

Other conditions and situations for which probiotics are being studied include:

- Skin infections, such as eczema (atopic dermatitis) in children

- Mental illness
- Childhood stomach and respiratory infections, allergies and asthma
- Sleeping problems
- Joint stiffness
- Lactose intolerance
- https://my.clevelandclinic.org/health/drugs/14598-probiotics

Benefits of probiotics

Research suggests that probiotics may help manage gastrointestinal conditions, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).
Many people use probiotics to help treat episodes of diarrhea and constipation.
There is also evidence that probiotics may help with:

- eczema
- obesity
- insulin resistance
- type 2 diabetes
- nonalcoholic fatty liver disease
- improving immune system function

Genetically modified foods

Genetically modified foods (**GM foods**), also known as **genetically engineered foods** (**GE foods**), or **bioengineered foods** are foods produced from organisms that have had changes introduced into their DNA using the methods of genetic engineering. Genetic engineering techniques allow for the introduction of new traits as well as greater control over traits when compared to previous methods, such as selective breeding and mutation breeding.

1. What are genetically modified (GM) organisms and GM foods?

Genetically modified organisms (GMOs) can be defined as organisms (i.e. plants, animals or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination. The technology is often called “modern biotechnology” or “gene technology”, sometimes also “recombinant DNA technology” or “genetic engineering”. It allows selected individual genes to be transferred from one organism into another, also between nonrelated species. Foods produced from or using GM organisms are often referred to as GM foods.

2. Why are GM foods produced?

GM foods are developed – and marketed – because there is some perceived advantage either to the producer or consumer of these foods. This is meant to translate into a product with a lower price, greater benefit (in terms of durability or nutritional value) or both. Initially GM seed developers wanted their products to be accepted by producers and have concentrated on innovations that bring direct benefit to farmers (and the food industry generally).
One of the objectives for developing plants based on GM organisms is to improve crop protection. The GM crops currently on the market are mainly aimed at an increased level of

crop protection through the introduction of resistance against plant diseases caused by insects or viruses or through increased tolerance towards herbicides.

Resistance against insects is achieved by incorporating into the food plant the gene for toxin production from the bacterium Bacillus thuringiensis (Bt). This toxin is currently used as a conventional insecticide in agriculture and is safe for human consumption. GM crops that inherently produce this toxin have been shown to require lower quantities of insecticides in specific situations, e.g. where pest pressure is high. Virus resistance is achieved through the introduction of a gene from certain viruses which cause disease in plants. Virus resistance makes plants less susceptible to diseases caused by such viruses, resulting in higher crop yields.

Herbicide tolerance is achieved through the introduction of a gene from a bacterium conveying resistance to some herbicides. In situations where weed pressure is high, the use of such crops has resulted in a reduction in the quantity of the herbicides used.

3. Is the safety of GM foods assessed differently from conventional foods?

Generally consumers consider that conventional foods (that have an established record of safe consumption over the history) are safe. Whenever novel varieties of organisms for food use are developed using the traditional breeding methods that had existed before the introduction of gene technology, some of the characteristics of organisms may be altered, either in a positive or a negative way. National food authorities may be called upon to examine the safety of such conventional foods obtained from novel varieties of organisms, but this is not always the case.

In contrast, most national authorities consider that specific assessments are necessary for GM foods. Specific systems have been set up for the rigorous evaluation of GM organisms and GM foods relative to both human health and the environment. Similar evaluations are generally not performed for conventional foods. Hence there currently exists a significant difference in the evaluation process prior to marketing for these two groups of food.

The WHO Department of Food Safety and Zoonoses aims at assisting national authorities in the identification of foods that should be subject to risk assessment and to recommend appropriate approaches to safety assessment. Should national authorities decide to conduct safety assessment of GM organisms, WHO recommends the use of Codex Alimentarius guidelines

Present there are several GM crops used as food sources. As of now there are no GM animals approved for use as food, but a GM salmon has been proposed for FDA approval. In instances, the product is directly consumed as food, but in most of the cases, crops that have been genetically modified are sold as commodities, which are further processed into food ingredients

Biosensors in Food

Biosensors have extensive applications in the food and agriculture industries. The devices contain a transducer and a biological element, which may be an enzyme, antibody, microbe,

or organelle. The biological element (bioelement) interacts with the analyte being tested and the biological response is converted into an electrical signal by the transducer.

Some of the uses of biosensors in the agricultural and food industry include:

- Enzyme biosensors based on the inhibition of cholinesterase enzymes are used to detect traces of organophosphates and carbamates from pesticides that may be present as poisonous and harmful residues on farm produce.
- Some microbial sensors are selective and sensitive in the detection of ammonia and methane.
- Biological oxygen demand (BOD) analyzers use a bacteria such as Rhodococcus erythropolis immobilized in collagen or polyacrylamide. These devices are widely used to test the quality of waste water. BOD biosensors can analyze 2 to 20 samples every hour.
- Biosensors may be used to measure carbohydrates, alcohols, and acids in fermented foods. The devices are mainly used for quality control processes in food production. The devices, however, need to be kept sterile, frequently calibrated and require analyte dilution. Enzyme-based biosensors can be used in food quality control to measure amino acids, amides, amines, carbohydrates, heterocyclic compounds, carboxylic acids, gases, inorganic ions, cofactors, alcohols and phenols. Biosensors can also be used in the assessment and analysis of produce such as wine, beer and yoghurt.
- In food quality assessment, antibodies or immunosensors may be used in assays to detect small molecules such as water-soluble vitamins and chemical contaminants. They may also be used to detect any pathogenic organisms present in meat, poultry, eggs, and fish.

Reference

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- Geraldine O. Canny, Beth A. McCormick. Bacteria in the Intestine, Helpful Residents or Enemies from Within?Infection and Immunity Jul 2008, 76 (8) 3360-3373.
- https://loveyourgut.com/all-entries/bacteria-and-the-large-intestine/
- https://nccih.nih.gov/health/probiotics/introduction.htm

POSSIBLE QUESTION

2marks

1. Write short notes on application of food microbes.
2. Define the beneficial uses of microbes in food
3. Write the short notes on intestinal beneficial bacteria?
4. What is the role of probiotic
5. Write note on prebiotics
6. Define the genetical modified food.
7. Write short notes on biosensors in food?
8. Give short notes on dairy product.
9. Comment on important microbes in industrial application?
10. Write short notes on fermented food.

8 marks

1. Elaborate the beneficial uses of microbes in food?
2. Explain the industrial application microbes and food?
3. Examine the microorganism in intestinal beneficial bacteria?
4. Give a brief notes genetic modified foods?
5. Describe the biosensors in food.
6. Brief notes on fermented foods?

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M. Sc MICROBIOLOGY **COURSE NAME: FOOD MICROBIOLOGY**
COURSE CODE: 18MBP302 **BATCH-2018-2020**

UNIT: 5

S. no.	Unit 1 Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Examination of the presence / absence of organisms refers to	incubation of a food suspension in an enrichment medium followed by inoculation on to a suitable selective medium	Incubation of a food suspension in an enrichment medium followed by inoculation on to a non-selective medium.	incubation of a food suspension in an enrichment medium	none of the above	incubation of a food suspension in an enrichment medium followed by inoculation on to a suitable selective medium
2	the vitamin whose content increases following the conversion of milk into curd by lactic acid bacteria is	vitamin c	vitamin d	vitamin b12	vitamin	vitamin b12
3	Which one of the following alcoholic drinks is produced without distillation?	wine	whisky	rum	brandy	wine
4	Big holes in swiss cheese are made by a	a machine	a bacterium that produces methane gas	a bacterium producing a large amount of carbon dioxide	a fungus that releases a lot of gases during its metabolic activites	a bacterium producing a large amount of carbon dioxide
5	Which of the following microbe is used in the production of blue cheese?	Streptococcus thermophilus	Lactobacillus bulgaricus	Penicillium roqueforti	Rhizopus stolonifer	Penicillium roqueforti
6	Pickled cucumber is made from fermented salt-stock pickles	true	false	may be	none of this	false
7	cell grown on hydrocarbon wastes from the petroleum industry are a source of _____	carbohydrates	proteins	vitamins	fats	proteins

8	Yeast-cell crops harvested from the vats is used to produce which of the following compounds?	alcoholic beverages	enzymes	antibiotics	organic acids	alcoholic beverages
9	How many tons of protein can be produced by algae grown in pond in a year?	1000	10	20	50	20
10	What is the range of protein content in yeast cells?	69%	12-15%	20-40%	40-50%	40-50%
11	Which of the following microorganism have a high vitamin content?	bacteria	yeast	algae	protozoa	yeast
12	principal microorganism for yogurt is _____	Streptococcus thermophilus	Leuconostoc citrovorum	Lactobacillus acidophilus	Streptococcus lactis	Streptococcus thermophilus
13	Which of the following products have higher acidity and lacks aroma?	Cultured buttermilk	Cultured sour cream	Cultured sour cream	Acidophilus milk	Cultured sour cream
14	Shredded cabbage is the starting product for which of the following fermented food?	Sauerkraut	Pickles	Green olives	Sausage	Sauerkraut
15	Microorganisms also help in production of food like	Bread	fruits and seeds	Vegetables	Pulses	Bread
16	antibiotics mostly obtained from	algae	fungi	antinomycetes	both b and c	both b and c
17	from witch microorganism streptomycin is prepared?	ptomyces ramous	Sterptomyces antibitics	Sterptomyces nodosus	Sterptomyces griseus	Sterptomyces griseus
18	The most abundant prokaryotes helpful to humans in making curd from milk and in production of antibiotics are the ones categorized as	Chemosynthetic autotrophs	Heterotrophic bacteria	Cyanobacteria	Archaebacteria	Heterotrophic bacteria
19	Monascus purpureus is a yeast used commercially in the production of	Citric acid	Blood cholesterol lowering statins	Ethanol	Streptokinase for removing clots from the blood vessels	Blood cholesterol lowering statins

20	Yeast is used in the production of	Bread and beer	Cheese and butter	Cheese and butter	Lipase and pectinase	Bread and beer
21	Which of the following microbes is used for commercial production of ethanol?	Trichoderma polysporum	Streptococcus	Clostridium butylicum	Saccharomyces cerevisiae	Saccharomyces cerevisiae
22	Human insulin is being commercially produced from a transgenic species of	Saccharomyces	Escherichia	Mycobacterium	Rhizobium	Escherichia
23	Which of the following helps in making curd from milk?	Lactobacillus	Plasmodium	Yeast	Salmonella	Lactobacillus
24	Which foods use genetically modified organisms in their production to the largest extent?	Cheese	Vegetables	Meat	milk	Cheese
25	What are the current benefits of having foods made from genetically modified crops?	They improve farm profitability and make some farmers' jobs easier	They allow farmers to greatly increase the amount of crops produced	They improve convenience for consumers, e.g. by creating foods with longer shelf lives	They improve the nutritional quality of foods	They improve farm profitability and make some farmers' jobs easier
26	the foods we eat, how much contains the genetic material DNA?	Less than 5 percent	20 percent	50 percent	80 percent	50 percent
27	effect does eating genetically modified foods have on your genes	It could cause your own genes to mutate	It could cause your own genes to absorb the excess genes	It has no effect on your genes	The effects on human genetics aren't known	It has no effect on your genes

28	Are foods derived from genetically modified crops nutritionally superior?	Yes, they offer substantial health advantages over foods produced from conventional crops	Yes, they offer some health advantages over foods produced from conventional crops	No, they are neither better nor worse than foods from conventional crops	No, they are slightly less healthful than foods from conventional crops	No, they are neither better nor worse than foods from conventional crops
29	Element which allows easy visualization of genetic modification products is known as	green fluorescent protein	blue fluorescent protein	white fluorescent protein	red fluorescent protein	green fluorescent protein
30	What is GM crops?	Genetically Modified Crops	Genetically poor crops	Gene Pool	Nomadic Crops	Genetically Modified Crops
31	Which toxic is used to protect plants from insects?	Blue Green Bacteria	Bacillus thuringiensis	Acidobacteria	Proteobacteria	Bacillus thuringiensis
32	This set of Analytical Instrumentation Multiple Choice Questions & Answers (MCQs) focuses on "Biosensors". Which of the following is not a characteristic of the immobilized enzymes?	They cannot be re-used	It produces reproducible results	Stability exists	Same catalytic activity is present for number of analysis	They cannot be re-used
33	They cannot be re-used	Enzymes	Anti-bodies	Transducer	Cells or tissues	Transducer
34	An example of biosensor, urea electrode makes use of which of the following electrodes?	Carbon dioxide electrode	Ammonia electrode	Fluoride electrode	Ammonium electrode	Fluoride electrode
35	Most foods derived from genetically modified crops contain	Most foods derived from genetically modified crops contain	The same number of genes as foods produced from hybrid crops	One or two additional genes	Hundreds of additional genes	Hundreds of additional genes

36	In glucose electrode, glucose oxidase has been coupled to an electrode by which of the following materials?	Ferrocene derivatives	Urease	Polyacrylamide	Biochips	Ferrocene derivatives
37	Biosensors measure glucose concentrations between which of the following ranges?	10^{-1} to 10^{-2} M	10^{-2} to 10^{-4} M	10^{-1} to 10^{-4} M	10^{-1} to 10^{-7} M	10^{-1} to 10^{-7} M
38	Transducers employed in the bulk of enzyme electrodes use which of the following principles?	Amperometric	Optical	Magnetic	Colorimetric	Amperometric
39	Which of these biosensors use the principle of heat released or absorbed by a reaction?	Potentiometric biosensor	Optical biosensors	Piezo-electric biosensors	Calorimetric biosensors	Calorimetric biosensors
40	Which of the following biosensors use the movement of electrons produced during redox reactions?	Amperometric biosensor	Potentiometric biosensors	Piezo-electric biosensors	Optical biosensors	Amperometric biosensor
41	Nanoscopic optical biosensors have fast response time but the sensitivity is reduced	true	false	may be sensitivity	none of this	false
42	Given below is the diagram of biosensor. Identify the unmarked component. Biosensor - ? – Amplifier – Processing - display	Microprocessor	Filter	Transducer	A/D converter	Transducer
43	In glucose sensor, a measure of change in oxygen value is a measure of the glucose value	true	false	may be value	ill	true
44	constructing the glucose sensor, which of the following is used as a gel?	Urea	Urease	Acrylamide	Polyacrylamide	Polyacrylamide
45	Fluoroptic temperature sensors work on the principle of _____	thermistor	thermocouple	optical fiber	rtd	optical fiber
46	Monopolar needle electrode have a coating of which material over the stainless steel wires which are bare only at the tips?	carbon	calcium	sodium	teflon	teflon

47	_____ converts biochemical events into measurable signals	amplifier	opamp	rectifier	transducer	transducer
48	The biological response of the biosensor is determined by _____	biocatalytic membrane	physio-chemical membrane	chemical membrane	artificial membrane	biocatalytic membrane
49	Home blood glucose sensor works on which principle?	electro-physiological	electrochemical	physio-chemical	chemical	electrochemical
50	The inosine and hypoxanthine can be determined simultaneously by using	inosine sensor	inosine and hypoxanthine sensor	amorphous silicon ISFET	urea sensor	inosine sensor
51	Microarry analysis involves biological assays based on	gel	filters	purification colums	small gass chips	small gass chips
52	Which of the following is correct about micro biosensors?	Implantation in human body and are suitable for in-vivo measurements	Can be integrated on one chip and are useful for measuring various substrates in a small amount of sample solution simultaneously	It is possible to develop disposable transducers for biosensors through mass production	All of these	All of these
53	Microbiosensors are based on	ions effect	ionsensitive field effect transistor	piezoelectric effect	magnetic effect	ionsensitive field effect transistor
54	To qualify as a micro array the analytical device must be	ordered	microscopic	planer and specific	all of these	all of these
55	Microarrays are also known as	biochips	DNA chips	gene chips	all of these	all of these

56	A micro array is an ordered array of microscopic elements on a planer substrate that allows the specific binding of	gene or gene products	whole genome	both (a) and (b)	none of these	gene or gene products
57	Biochips are made up of	semi-conducting molecules inserted into the protein frame work	conducting molecules inserted into the protein frame work	non-conducting molecules inserted into the protein frame work	non-conducting molecules inserted into the protein frame work	semi-conducting molecules inserted into the protein frame work
58	Hypoxanthine can be measured by	hypoxanthine sensor	amorphous silicon ISFET	urea sensor	alcohol sensor	hypoxanthine sensor
59	What effect does eating genetically modified foods have on your genes?	It could cause your own genes to mutate	It could cause your own genes to absorb the excess genes	It has no effect on your genes	The effects on human genetics aren't known	It has no effect on your genes
60	Which of the following disease is best diagnosed by serologic means?	Pulmonary tuberculosis	Gonorrhea	Actinomycosis	Q Fever	Q Fever