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FACULTY OF AGRICULTURAL SCIENCES

Department of Agricultural Economics and Extension

Programme: Hospitality and Tourism Management

Course Code: **FST 302**

Course Title: **FOOD MICROBIOLOGY**

Credit Unit: **3 credit units**

FST 302

FOOD MICROBIOLOGY

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FST302 COURSE GUIDE

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Introduction

The course, FOOD MICROBIOLOGY is a core course, which carries three (3) credit units. It is prepared and made available to all degree course students offering Hospitality and Tourism related Programme in the Faculty of Agricultural Sciences**,** Department Economics and Extension at the Nation Open University of Nigeria. Food microbiology is the application of principles of food spoilage and preservation to the operations of tourism and hospitality industry. This course material is useful in your academic pursuit as well as in your workplace as managers and administrators.

What You will Learn in this Course

This course consists of six modules which are sub-divided into 27 units. This course guide tells you what the course is all about. What course materials you will be using and also suggests some general guidelines for the amount of time you are likely to spend on each unit of the course in order to complete it on schedule. It also gives you guidance in respect of your Self- Assessment Exercises (SAEs) which will be made available in the assignment file. Please attend those tutorial sessions. The course will introduce you to the rudiments of research methods.

Course Aim

The main aim of this course is to arm you with adequate information on the concept of food microbiology in hospitality and tourism management. The course also aims at making you have a greater understanding of the fundamentals of food microbiology as applicable to hospitality and tourism management. This will prepare the student for a future career in hospitality and related disciplines.

Course Objectives

To achieve the aim set out, the course has a set of objectives which are set out as intended learners' outcome under each unit. You should read these objectives before you study the unit. After going through this course, you should be able to:

- Discuss the natural flora of different foods
- Analyse the importance of natural flora in foods
- Write the predominant microorganisms of each food
- Discuss the classes of Microorganisms
- Analyse the behavours of natural flora in foods
- Discuss the Microbiology of fermented foods
- Discuss the importance of Bacteria in food industry
- Discuss the Intrinsic Factors or Food Environment
- Discuss the spoilage Yeasts and Moulds
- Discuss the extrinsic and intrinsic factors of food that affect deterioration
- Write the microorganisms that contaminate water

- Discuss food poisoning and foodborne infection
- Discuss Temperature-Based Methods of controlling food poisoning Microbes
- Discuss the Microbiological standards and criteria.
- Discuss the microorganisms involved in fermentation processes
- Discuss the functions of microbial enzymes in food
- Discuss the Flavour Compounds and Flavour Enhancers
- Discuss genetically modified foods
- Discuss the importance of GMF

Working through the Course

This course involves that you devote a lot of time to read and study the contents. Each unit contains self-assessment exercises for this course and at certain points in the course you would be required to submit assignments for assessment purposes. At the end of this course, there is a final examination. I would therefore advice that you attend the tutorial sessions where you would have the opportunity of comparing knowledge with your colleagues.

Course Materials

You will be provided with the following materials

· Course guide

· Study units

· References

· Assignments

· Presentation schedule

STUDY UNITS

There are six modules of 27 units in this course, which should be studied carefully.

Assessment

There are two components of assessment for this course:

· The Tutor Marked Assignment (TMA)

· The end of course examination.

Tutor-Marked Assignment

The TMA is the continuous assessment component of your course. It accounts for 30% of the total score. You will be given four TMA's by your facilitator to answer before you can sit for the final examination.

Final Examination and Grading

This examination concludes the assessment for the course. The examination will account for 70% of total score. You will be informed of the time for the examination.

Summary

This course intends to provide you with underlying knowledge of food microbiology principles for the study of Hospitality Management and Tourism.

MAIN CONTENT

MODULE 1

Unit 3 Uses of natural flora in food industry

Unit 4 Factors influencing the growth of microorganisms in food

UNIT 1 NATURAL FLORA OF IMPORTANCE IN FOODS

CONTENTS

1.1 Introduction

Many types of microorganisms are present in nature but under normal conditions, a food harbors only a few types. These types include those that are naturally present in raw foods and those that enter from outside sources to which the foods are exposed from the time of production till consumption. The relative numbers of a specific type of microorganism initially present in a food depend on the intrinsic and extrinsic factotrs the food is exposed to. If growth occurs, the predominant types will be the ones for which the optimum growth condition is present in the food. This unit aims to develop an understanding of the microbial types and their levels where possible that can be expected under normal conditions in different food groups. It has to be recognized that microbial load in a food results from initial contamination from different sources and growth of the contaminants before testing.

1.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the natural flora of different foods
- Analyse the importance of natural flora in foods
- Write the predominant microorganisms of each food

1.3 Foods and their Natural Flora

1.3.1 Raw and Ready-To-Eat Meat Products

Following slaughter and dressing, the carcasses of animals and birds contain many types of microorganisms, predominantly bacteria, coming from the skin, hair, feathers, gastrointestinal tract, etc.; the environment of the feedlot and pasture (feed, water, soil, and manure); and the environment at the slaughtering facilities (equipment, air, water, and humans). Normally,

carcasses contain an average of $10^{1–3}$ bacterial cells/in. Different enteric pathogens, *Salmonella serovars, Yersinia enterocolitica, Campylobacter jejuni, Escherichia coli, Clostridium perfringens,* and *Staphylococcus aureus*, both from animals or birds and humans, can be present, but normally at a low level. Carcasses of birds, as compared with those of animals, generally have a higher incidence of *Salmonella* contamination coming from fecal matter. Following boning, chilled raw meat and ground meat contain microorganisms coming from the carcasses as well as from different equipment used during processing, personnel, air, and water. Some of the equipment used can be important sources of microorganisms, such as conveyors, grinders, slicers, and similar types that can be difficult to clean. Chilled meat has mesophiles, such as *Micrococcus, Enterococcus, Staphylococcus, Bacillus, Clostridium, Lactobacillus, coliforms,* and other *Enterobacteriaceae*, including enteric pathogens. However, because the meats are stored at low temperature (–1 to 5^{o}C), the psychrotrophs constitute major problems. The predominant psychrotrophs in raw meats are some lactobacilli and leuconostocs, Brochothrix thermosphacta, *C. laramie*, some coliforms, *Serratia*, *Pseudomonas*, *Alteromonas*, *Achromobacter*, *Alcaligenes*, *Acinetobacter*, *Morexella*, *Aeromonas*, and *Proteus*. Psychrotrophic pathogens include *Listeria monocytogenes* and *Y. enterocolitica*. The microbial load of fresh meat varies greatly, with bacteria predominating. Ground meat can have $10^{4–5}$ microorganisms/g; *Salmonella* can be present at ca. 1 cell/25g. The frequency of the presence of *Salmonella* is higher in chicken than in red meats. If the products are kept under aerobic conditions, psychrotrophic aerobes will grow rapidly, especially Gramnegative rods, such as *Pseudomonas, Alteromonas, Proteus,* and *Alcaligenes*, as well as yeasts. Under anaerobic packaging, growth of psychrotrophic facultative anaerobes and anaerobes such as *Lactobacillus, Leuconostoc, Brochothrix, Serratia*, some coliforms, and *Clostiridium* predominates. The pH of

the meat (which is low in beef, ca. 5.6, but high in birds, ca. 6.0), high protein content, and low carbohydrate level, along with the environment, determine which types predominate during storage.

Low-heat-processed red meat and poultry products include perishable cured or uncured products that have been subjected to heat treatment (70°C), packaged aerobically or anaerobically, and stored at refrigerated temperature. They include products such as franks, bologna, lunchmeats, and hams. The products, especially those packaged anaerobically and cured, are expected to have a long storage life of 50 days or more.

The microbial sources before heat treatment include the raw meat, ingredients used in formulation, processing equipment, air, and personnel. Heat treatment, especially at an internal temperature of 70^{o}C or higher, kills most microorganisms, except some thermodurics, which include *Micrococcus*, some *Enterococcus*, and maybe some *Lactobacillus* and spores of *Bacillus* and *Clostridium*. The microbial level can be $10^{1–2}$/ g. Following heating, the products, some of which are further processed (such as removing casing or slicing) come in contact with equipment, personnel, air, and water before final packaging. Different types of bacteria, yeasts, and molds, including pathogens, can enter these products, depending on the conditions of the processing plants. Although the initial bacterial level normally does not exceed 10^{2}/g, some of them can be psychrotrophic facultative anaerobic and anaerobic bacteria (*Lactobacillus, Leuconostoc,* some coliforms, *Serratia, Listeria, Clostridium* spp.). During extended storage in vacuum or modified-air packages, even from a low initial level, bacterial population can rise and adversely affect the safety and shelf life of products. This is aggravated by fluctuation in storage temperature and in products having low fat, high pH, and high water activity (A_W).

1.3.2 Raw and Pasteurized Milk

Raw milk can come from cows, buffalo, sheep, and goats, although the largest volume comes from cows. Pasteurized or market milk includes whole, skim, lowfat, and flavored milks, as well as cream, which are pasteurized according to regulatory specifications. Milk is high in proteins and carbohydrates (lactose), which many microorganisms can utilize for growth. Because both raw milk and pasteurized milk contain many types of bacteria as predominant microorganisms, they are refrigerated; yet they have limited shelf life. In raw milk, microorganisms come from inside the udder, animal body surfaces, feed, air, water, and equipment used for milking and storage. The predominant types from inside a healthy udder are *Micrococcus, Streptococcus*, and *Corynebacterium*. Normally, raw milk contains *Flavobacterium*, *Alcaligenes*, and some coliforms and *Bacillus* spp. They can affect the acceptance quality of raw milk by making the flavour and texture undesirable. Some of them can produce heat-stable enzymes (proteinases and lipases), which can also affect the product quality, even after pasteurization of raw milk. Psychrotrophic can multiply in refrigerated raw milk during storage. Microbiological quality of raw and pasteurized milk is monitored in many countries by regulatory agencies. In the U.S., the standard plate counts of raw milk for use as market milk are 1–3 $x10^5$/ml, and for use in product manufacturing are 0.5–1 x 10^6/ml. Grade A pasteurized milk can have standard plate counts of 20,000/ml and ≤10 coliforms/ml. Microorganisms present in pasteurized milk are those that survive pasteurization of raw milk (e.g., the thermodurics) and those that enter after heating and before packaging (e.g., postpasteurization contaminants). Thermodurics surviving pasteurization include *Micrococcus*, some *Enterococcus* (e.g. *E. faecalis*), Streptococcus, some *Lactobacillus* (e.g. *L. viridescens*), and spores of *Bacillus* and *Clostridium*. Postheat contaminants can be coliforms as well as *Pseudomonas, Alcaligenes*, and *Flavobacterium*. Psychrotrophs can grow during refrigerated storage.

1.3.3 Egg Shell and Liquid Egg

Egg shells are contaminated with microorganisms on the outer surface from fecal matter, nesting materials, feeds, air, and equipment. Each shell, depending on the contamination level, can have 10^7 bacteria. Washing helps reduce bacterial level considerably. Eggshells can harbor different types of bacteria, namely *Pseudomonas, Alcaligenes, Proteus, Citrobacter, E. coli, Enterobacter, Enterococcus, Micrococcus,* and *Bacillus*. They can also have *Salmonella* from fecal contamination. Infected ovaries of laying hens can be the source of *Salmonella enteritidis* in the yolk. Liquid egg can be contaminated with bacteria from the shell of washed eggs as well as from the breaking equipment, water, and air. Pasteurization can reduce the numbers to 10^3/ml. Bacteria, especially motile Gram-negative bacteria; can enter through pores of eggshells, particularly if the shells are wet. Several antimicrobial factors present in egg albumin, such as lysozyme, conalbumin (binds iron), avidin (binds biotin), or alkaline pH (8.0 to 9.0), can control bacterial growth. However, if the storage temperature is favorable, they can grow in yolk that is rich in nutrients and has a pH of 7.0. Pasteurization of liquid egg has been designed to destroy pathogens (mainly *Salmonella*) and other Gram-negative rods. Thermoduric bacteria, namely *Micrococcus, Enterococcus* and *Bacillus*, present in the raw liquid egg survive pasteurization.

1.3.4 Fish and Shellfish

This group includes finfish, crustaceans (shrimp, lobster, crabs), and mollusks (oysters, clams, scallops) harvested from aquatic environments. Fish and shellfish are harvested from natural sources and aquacultures and are rich in protein and nonprotein nitrogenous compounds; their fat content varies with type and season. They are very low in carbohydrates except for mollusks that contain about 3% glycogen. The microbial population in these products varies greatly with the

pollution level and temperature of the water. Bacteria from many groups, as well as viruses, parasites, and protozoa, can be present in the raw fish. Muscles of fish and shellfish are sterile, but scales, gills, and intestines harbor microorganisms. Finfish and crustaceans can have 10^{3-8} bacterial cells/g. During feeding, mollusks filter large volumes of water and can thus concentrate bacteria and viruses. Products harvested from marine environments can have halophilic vibrios as well as *Pseudomonas, Alteromonas, Flavobacterium, Enterococcus, Micrococcus,* coliforms, and pathogens such as *V. parahaemolyticus, V. vulnificus*, and *C. botulinum* type E. Freshwater fish generally have *Pseudomonas, Flavobacterium, Enterococcus, Micrococcus, Bacillus,* and coliforms. Fish and shellfish harvested from water polluted with human and animal waste can contain *Salmonella, Shigella, C. perfringens, V. cholerae*, and hepatitis A and Norwalk-like viruses. They can also contain opportunistic pathogens such as *Aeromonas hydrophila* and *Plesiomonas shigelloides*. The harvesting of seafoods, especially shellfish, is controlled by regulatory agencies and water with high coliform populations is closed to harvest. Following harvest, microorganisms can grow rapidly in fish and crustaceans because of high A_w and high pH of the tissue and availability of large amounts of nonprotein nitrogenous compounds. As many of the bacterial species are psychrotrophs, they can grow at refrigerated temperature. Pathogens can remain viable for a long time during storage. Microbial loads are greatly reduced during their subsequent heat processing to produce different products.

1.3.5 Vegetables, Fruits, and Nuts

Vegetables include edible plant components such as leaves, stalks, roots, tubers, bulbs, and flowers. They are relatively high in carbohydrates, with pH values of 5.0 to 7.0. Thus, different types of bacteria, yeasts, and moulds can grow if other conditions are favorable. Fruits are high in carbohydrates, and have a pH of 4.5 or below because of the presence of organic acids, and

some also have antimicrobial essential oils. Nuts can be from the ground (peanuts) or from trees (pecans) and have protective shells and low A_w (0.7). They are converted to nutmeats for further use or to products such as peanut butter. Microorganisms in vegetables vary with the types of vegetables and can come from several sources, such as:

i. soil
ii. water
iii. air
iv. animals
v. insects
vi. birds
vii. harvesting equipment

A leafy vegetable has more microorganisms from the air, whereas a tuber has more from the soil. Microbial levels and types in these products also vary greatly, depending on environmental conditions and conditions of farming and harvesting. Generally, vegetables have $10^{3–5}$ microorganisms/cm^2 or $10^{4–7}$/g. Some of the predominant bacterial types are lactic acid bacteria, *Corynebacterium, Enterobacter, Proteus, Pseudomonas, Micrococcus, Enterococcus,* and spore formers. They also have different types of molds, such as *Alternaria*, *Fusarium*, and *Aspergillus*. Vegetables can have enteric pathogens, especially if animal and human wastes and polluted water are used for fertilization and irrigation. They include *L. monocytogenes, Salmonella, Shigella, Campylobacter, C. botulinum,* and *C. perfringens*. They can also have pathogenic protozoa and parasites. If the vegetables are damaged, then plant pathogens (e.g., *Erwinia*) can also predominate. Many of the microorganisms can cause different types of spoilage of raw products. Pathogens can grow in plant products and cause foodborne diseases (e.g. listeriosis or

botulism). Lactic acid bacteria have important roles in the natural fermentation of vegetables (e.g. sauerkraut). Different methods used to process vegetables and vegetable products greatly reduce the microbial population. Because of their high carbohydrate content and low pH of fruits, it favours the growth of different types of moulds, yeasts, and lactic acid bacteria. In general, microbial populations are $10^{3–6}$/g. Improperly harvested and processed fruits can have pathogens that survive, grow, and cause foodborne disease. Molds, yeasts, and bacteria can cause different types of spoilage. Natural flora, especially yeasts in fruits, can be important in alcohol fermentation. Microorganisms enter nuts from soil (peanuts) and air (tree nuts). During processing, air, equipment, and water can also be the sources contamination. Nuts are protected by shells, but damage on the shell can facilitate microbial contamination. Raw nuts and nutmeats can have $10^{3–4}$ microorganisms/g, with *Bacillus* and *Clostridium* spores, *Leuconostoc*, *Pseudomonas*, and *Micrococcus* predominating. Because of a low A*w*, bacteria do not grow in the products. However, when used as ingredients, they can cause microbiological problems in the products. Moulds can grow in nuts and nutmeats and produce mycotoxins.

1.3.6 Cereal, Starches, and Gums

Cereal includes grains, flour, meals, breakfast cereals, pasta, baked products, dry mixes, and frozen and refrigerated products of cereal grains. Starches include flours of cereals (e.g. corn, rice), tapioca (from plant), potatoes, and other tubers. Gums are used as stabilizers, gelling agents, and film, and are obtained from plants, seaweeds, and microorganisms (e.g. tragacanth, pectin, xanthan, agar, and carrageenan) and as modified compounds (e.g. carboxymethyl cellulose). They are rich in amylose and amylopectin, but can also have simple sugars (e.g. in

grains) and protein (e.g. in lentils). Microbial sources are mainly the soil, air, insects, birds, and equipment. Unprocessed products (grains) may contain high bacterial levels (aerobic plate count of about 10^4/g, coliform of 10^2/g, yeasts and moulds of 10^3/g). They may also contain mycotoxins produced by toxicogenic molds. Processed products may also contain a wide variety of yeasts, moulds, and bacteria. Flours and starches may have higher microbial counts, similar to those of grains, whereas processed products such as breakfast cereals and pasta may contain aerobic plate count of 10^{2-3}/g, coliform of coliforms/100 ml. The indigenous flora are mainly *Flavobacterium*, *Alcaligenes*, and *Micrococcus*. They may also have some *Pseudomonas* as contaminants from outside. They should not have pathogens unless produced under poor sanitation.

1.3.7 Mayonnaise and Salad Dressings

Water-in-oil emulsion products formulated with oil, water, vinegar (about 0.25% acetic acid) or lemon juice, sugar, salt, starch, gum, egg, spices, and vegetable pieces, mayonnaise and salad dressings have a pH between 3.5 and 4.0. Some low-calorie and less sour products containing less acid, less oil, and more water may have a pH of 4.5 or above. Microorganisms are introduced into the products through ingredients, equipment, and air. However, except for aciduric microorganisms, most others die, especially when stored for a long time at room temperature. Among aciduric microorganisms, moulds (*Geotrichum* and *Aspergillus* spp.), yeasts (*Saccharomyces* spp.), and several species of *Lactobacillus* (*L. fructivorans, L. brevis*) and some *Bacillus* spp. (*B. subtilis, B. mesentericus*) have been isolated. Normally, their numbers should not exceed 10/g. If pathogens are introduced (e.g. Salmonella through eggs), they are expected to be killed rapidly; however, they may survive longer in lowcalorie, high-pH products kept at refrigerated temperatures.

1.3.8 Spices and Condiments

Spices are plant products (seed, flower, leaf, bark, roots, or bulb) used whole or ground, singly or mixed. Condiments are spices blended with other components and have a saucelike consistency (catsup, mustard). They are used in relatively small amounts for aroma and colour. Some spices, unless given antimicrobial treatments (irradiation, because ethylene oxide is not permitted anymore), may contain microorganisms as high as 10^{6-7}/g. The most important are spores of moulds, *Bacillus*, and *Clostridium* spp. Also, micrococci, enterococci, yeasts, and several pathogens such as *Salmonella* spp., *S. aureus*, and *B. cereus* have been found. They can also have mycotoxins. Although used in small amounts, they can be the source of spoilage and pathogenic microorganisms in food. Some spices such as cloves, allspice, and garlic have antimicrobial properties.

1.3.9 Canned Foods

Canned foods include those packed in hermetically sealed containers and given high heat treatment. The products with a pH of 4.6 or above are given heat treatments to obtain commercial sterility, but those with a pH below 4.6 are given heat treatments ca. 100^{o}C. Canned foods prepared and processed to obtain commercial sterility can have spores of thermophilic spoilage bacteria, namely *Bacillus stearothermophilus*, *C. thermosaccharolyticum*, and *Desulfotomaculum nigrificans*. Their major sources in the products are soil and blanching water as well as sugar and starches used as ingredients. In canned products stored at 30^{o}C or below, thermophilic spores do not germinate to cause spoilage. However, if the cans are temperature-abused to 40^{o}C or higher, the spores germinate; subsequently, the cells multiply and spoil the products. If the canned products are given lower heat treatment (ca. 100^{o}C), spores of mesophilic bacteria that include both spoilage (*B. coagulans, B. licheniformis, C. sporogenes, C. butyricum*)

and pathogenic types (*B. cereus, C. perfringens, C. botulinum*), along with the spores of thermophiles, survive. In low-pH products, particularly in tomato products, *B. coagulans* spores can germinate and cells can multiply and cause spoilage. Other sporeformers can germinate and grow in high-pH products. *S. aureus* toxins, if present in raw products, are not destroyed by the heat treatment of the canned products and can thus cause food poisoning following consumption of the products.

1.3.10 Sugars and Confectioneries

Refined sugar is obtained from sugar cane and beets. Sugar can have thermophilic spores of *B. stearothermophilus, B. coagulans, C. thermosaccharolyticum,* and *D. nigrificans*, as well as mesophilic bacteria (e.g. *Lactobacillus* and *Leuconostoc*), yeasts, and moulds. When sugars are used as ingredients in food products, the spores can survive and cause spoilage of the products. Pathogens are not present in refined sugar unless contaminated. In liquid sugar, mesophiles can grow. Refined sugar, used in canned products or to make liquid sugar, has strict microbiological standards.

Confectioneries include a large variety of products with a sweet taste. These products have low A_w ($\leq$0.84) and some have low pH. They may contain many types of bacteria, yeasts, and moulds, but their microbiological standards are well regulated. Although they may harbour *Lactobacillus, Leuconostoc*, spores of *Bacillus* and *Clostridium*, and yeasts and moulds, only a few osmotolerant yeasts and moulds can grow. However, when used as additives in other foods, confectioneries can be a source of these microbes. If ready-to-consume products are contaminated with pathogens, either from raw materials, environment, or personnel, they can cause foodborne diseases.

1.3.11 Soft Drinks, Fruit and Vegetable Drinks, Juices, and Bottled Water

Soft drinks are nonalcoholic beverages containing water, sweeteners, acids, flavouring, colouring and emulsifying agents, and preservatives. Some may contain fruit juices and be carbonated or noncarbonated, with a pH of 2.5 to 4.0. Fruit juices (100%) have a pH of 4.0 or below. Vegetable juices (e.g. tomato) can have a pH of 4.5 or above. Bottled water is obtained from either natural springs or drilled wells and handled under conditions that prevent contamination. Soft drinks can have different types of microorganisms, but only aciduric microorganisms, such as moulds, yeasts, lactic acid bacteria, and acetic acid bacteria, can multiply. In carbonated beverages, some yeasts being microaerophilic can grow; in beverages with fruit juices, *Lactobacillus* and *Leuconostoc* species can grow. In noncarbonated beverages, moulds (*Geotrichum*) and *Acetobacter* and *Gluconobacter* spp. can also grow. Most of these come from the processing environment and equipment. In fruit juices, moulds, yeasts, *Lactobacillus* spp. (*L. fermentum, L. plantarum*), *Leuconostoc* spp. (*L. mesenteroides*), and acetic acid bacteria can grow. Spoilage of fruit juices by acid-resistant sporeforming species from the genus *Alicyclobacillus* has currently been recognized. Some pathogens (e.g. acid-tolerant *Salmonella* spp. and *E. coli O157:H7* strains in orange juice and apple cider) can remain viable for a long time (30 days or more) in the acid products. Vegetable juices can have moulds, yeasts, and lactic acid bacteria along with *B. coagulans, C. butyricum,* and *C. pasteurianum*. Bottled water should not contain more than 10 to 100 bacteria and >10 coliforms/100 ml. The indigenous floras are mainly *Flavobacterium, Alcaligenes,* and *Micrococcus*. They may also have some *Pseudomonas* as contaminants from outside. They should not have pathogens unless produced under poor sanitation.

Self- Assessment Exercises 1

1. Write the sources of Microorganism in vegetables
2. List the predominant microorganism in spices and condiments

1.4 Summary

Normal microbial population in a food comes from those that enter from different sources as well as from growth of the contaminants before a food is examined. It is expected that a food that is produced under proper sanitary conditions and preserved properly will have lower microbial load. Information on normal microbial load helps determine microbiological quality of a food and also to set up microbiological standards and specifications. Mere microbial presence does not reduce the quality of food, except in the case of some pathogens. It is necessary for microorganisms to grow or multiply in a food to bring definite changes in quality.

1.5 References/Further Readings

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Silliker, J.H. (1980). Ed., Microbial Ecology of Foods, Vol. II, Academic Press, New York.

Vanderzant, C. and Splittstoesser, D.F. (1992). Eds., Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.

1.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. In Vegetables Microorganisms come from several sources, such as:

i. soil

ii. water

iii. air

iv. animals

v. insects

vi. birds

vii. harvesting equipment

2. The most important are spores of moulds, *Bacillus*, and *Clostridium* spp. Also, *micrococci*, *enterococci*, yeasts, and several pathogens such as *Salmonella* spp., *S. aureus*, and *B. cereus* have been found. They can also have mycotoxins.

UNIT 2 BEHAVIORS OF NATURAL FLORA IN FOOD

CONTENTS

2.1 Introduction

The microbial groups important in foods consist of several species and types of bacteria, yeasts, moulds, and viruses. Although some algae and protozoa as well as some worms (such as nematodes) are important in foods, they are not included among the microbial groups in this unit. Bacteria, yeasts, molds, and viruses are important in food for their ability to cause foodborne

diseases and food spoilage and to produce food and food ingredients. Many bacterial species and some moulds and viruses, but not yeasts, are able to cause foodborne diseases. Most bacteria, moulds, and yeasts, because of their ability to grow in foods can potentially cause food spoilage. Several species of bacteria, moulds, and yeasts are considered safe or food grade, or both, and are used to produce fermented foods and food ingredients. Among the four major groups, bacteria constitute the largest group because of their ubiquitous presence and rapid growth rate, even under conditions where yeasts and moulds cannot grow. Bacteria are considered the most important microorganism in food spoilage and foodborne diseases. Prion or proteinaceous infectious particles have recently been identified to cause transmissible spongiform encephalopathies (TSEs) in humans and animals. However, their ability to cause foodborne diseases is not clearly understood.

2.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the classes of Microorganisms
- Analyse the behavours of natural flora in foods
- Discuss the morphology and structure of microorganisms in foods
- Write the important Microorganisms in foods

2.3 Behaviors of Natural Flora in Food

2.3.1 Classification of Microorganisms

Living cellular organisms, on the basis of phylogenetic and evolutionary relationships, were grouped originally in five kingdoms, in which bacteria belonged to procaryotes and the eucaryotic moulds and yeasts were grouped under fungi. In the 1970s, the procaryotic domain

was changed to Eubacteria (with murine on cell wall) and Archaebacteria (without murine on cell wall). In the 1990s, this was changed to Bacteria and Archaea, respectively.

Archaea include most extremophiles and are not important to food microbiology. Viruses are not considered as living cells and are not included in this classification system. For the classification of yeasts, molds, and bacteria, several ranks are used after the kingdom: divisions, classes, orders, families, genus (plural genera), and species. The basic taxonomic group is the species. Several species with similar characteristics form a genus. Among eucaryotes, species in the same genus can interbreed. This is not considered among procaryotes, although conjugal transfer of genetic materials exists among many bacteria. Several genera make a family, and the same procedure is followed in the hierarchy. In food microbiology, ranks above species, genus, and family are seldom used. Among bacteria, a species is regarded as a collection of strains having many common features. A strain is the descendent of a single colony (single cell). Among the strains in a species, one is assigned as the type strain, and is used as a reference strain while comparing the characteristics of an unknown isolate. Several methods are used to determine relatedness among bacteria, yeasts, and moulds for taxonomic classification.

In yeasts and moulds, morphology, reproduction, biochemical nature of macromolecules, and metabolic patterns are used along with other criteria.

For bacterial morphology, Gram-stain characteristics, protein profiles, amino acid sequences of some specific proteins, base composition (% of G + C), nucleic acid (DNA and RNA) hybridization, nucleotide base sequence, and computer-assisted numerical taxonomy are used. Protein profile, amino acid sequence, base composition, DNA and RNA hybridization, and nucleotide base sequence are directly or indirectly related to genetic makeup of the organisms and thus provide a better chance in comparing two organisms at the genetic level. In mol % G +

C ratio, if two strains differ by 10% or more, they are most likely not related. Similarly, in a hybridization study, two strains are considered the same if their DNAs have 90% or more homology. For the nucleotide base sequence, the sequences in 16S rRNA among strains are compared. A sequence of about 1500 nucleotide bases over a stretch of 16S rRNA is most conserved, so related strains should have high homology.

In numerical taxonomy, many characteristics are compared, such as morphological, physiological, and biochemical. Each characteristic is given the same weightage. Two strains in the same species should score 90% or more. Evolutionary relationships among viruses, if any, are not known. Their classification system is rather arbitrary and based on the types of disease they cause (such as the hepatitis virus, causing inflammation of the liver), nucleic acid content (RNA or DNA, single stranded or double stranded), and morphological structures. In food, two groups of viruses are important: the bacterial viruses (bacteriophages) of starter culture bacteria and some foodborne pathogenic bacteria, and the human pathogenic viruses associated with foodborne diseases.

2.3.2 Nomenclature of Microorganisms

The basic taxonomic group in bacteria, yeasts, and moulds is the species, and each species is given a name. The name has two parts (binomial name): the first part is the genus name and the second part is the specific epithet. Both parts are Latinized; when written, they are italicized (or underlined), with the first letter of the genus written in a capital letter (e.g. *Saccharomyces cerevisiae*, *Penicillium roquefortii*, and *Lactobacillus acidophilus*). A bacterial species can be divided into several subspecies (subsp. or ssp.) if the members show minor but consistent differences in characteristics. Under such conditions, a trinomial epithet (subspecific epithet) is used (e.g. *Lactococcus lactis* ssp. *lactis* or *Lactococcus lactis* ssp. *cremoris*). In some instances,

ranks below subspecies are used to differentiate strains recognized by specific characters (e.g. serovar (antigenic reaction), biovar (producing a specific metabolite) and phagovar (sensitive to a specific phage). Such ranks have no taxonomic importance but can be practically useful (e.g., *Lactococcus lactis ssp*. *lactis* biovar diacetilactis is a *Lactococcus lactis ssp. lactis* strain that produces diacetyl, an important flavor compound in some fermented dairy products. Each strain of a species should be identified with a specific strain number, which can be alphabetic or numeric or a mixture of both (e.g. *Pediococcus acidilactici LB923*). At the family level, bacterial names are used as plural adjectives in feminine gender and agree with the suffix "aceae" (e.g. *Enterobacteriaceae*). The species and strains in a genus can be represented collectively, either using "spp." after genus (e.g. *Lactobacillus* spp.) or plural forms of the genus (e.g. *lactobacilli* for *Lactobacillus*; *lactococci* for *Lactococcus*; *leuconostocs* for *Leuconostoc*, or *salmonellae* for *Salmonella*)

The scientific names of bacteria are given according to the specifications of the International Code of Nomenclature of Bacteria. The International Committee on Systematic Bacteriology of the International Union of Microbiological Association examines the validity of each name and then publishes the approved lists of bacterial names from time to time. A new name (species or genus) must be published in the International Journal of Systematic Bacteriology before it is judged for inclusion in the approved list. Any change in name (genus or species) has to be approved by this committee. When writing the name of the same species more than once in an article, it is customary to use both genus and specific epithet the first time and abbreviate the genus name subsequently. In the Bergey's Manual of Systematic Bacteriology, only the first letter is used (e.g. *Listeria monocytogenes* and then *L. monocytogenes*). The same system is used in most publications in the United States (U.S). However, it creates confusion when one article

has several species with the same first letter in the genus (e.g. *Lactobacillus lactis, Leuconostoc lactis,* and *Lactococcus lactis* as *L. lactis*). The viruses, as indicated previously, have not been given specific taxonomic names as given for bacteria. They are often identified with alphabetic or numeric designation, or a combination of both (e.g., T4 or l bacteriophages), the disease they produce (e.g., hepatitis A, causing liver inflammation), or by other methods (e.g., Norwalk-like viruses, causing a type of foodborne gastroenteritis in humans).

2.3.2 Morphology and Structure of Microorganisms in Foods

2.3.3.1 Yeasts and Moulds

Both yeasts and moulds are eucaryotic, but yeasts are unicellular whereas moulds are multicellular. Eucaryotic cells are generally much larger (20 to 100 µm) than procaryotic cells (1 to 10 µm). Eucaryotic cells have rigid cell walls and thin plasma membranes. The cell wall does not have mucopeptide, is rigid, and is composed of carbohydrates. The plasma membrane contains sterol. The cytoplasm is mobile (streaming) and contains organelles (mitochondria, vacuoles) that are membrane bound. Ribosomes are 80S type and attached to the endoplasmic reticulum. The DNA is linear (chromosomes), contains histones, and is enclosed in a nuclear membrane. Cell division is by mitosis (asexual reproduction); sexual reproduction, when it occurs, is by meiosis. Moulds are nonmotile, filamentous, and branched. The cell wall is composed of cellulose, chitin, or both. A mould (thallus) is composed of large numbers of filaments called hyphae. An aggregate of hyphae is called mycelium. A hypha can be nonseptate, septate-uninucleate, or septate-multinucleate. A hypha can be vegetative or reproductive. The reproductive hypha usually extends in the air and form exospores, either free (conidia) or in a sack (sporangium). Shape, size, and color of spores are used for taxonomic classification. Yeasts are widely distributed in nature. The cells are oval, spherical, or elongated,

about 5–30 x 2–10 µm in size. They are non-motile. The cell wall contains polysaccharides (glycans), proteins, and lipids. The wall can have scars, indicating the sites of budding. The membrane is beneath the wall. The cytoplasm has a finely granular appearance for ribosomes and organelles. The nucleus is welldefined with a nuclear membrane.

2.3.3.2 Bacterial Cells

Bacteria are unicellular, most ca. 0.5–1.0 x 2.0–10 µm in size, and have three morphological forms: spherical (cocci), rod shaped (bacilli), and curved (comma). They can form associations such as clusters, chains (two or more cells), or tetrads. They can be motile or nonmotile. Cytoplasmic materials are enclosed in a rigid wall on the surface and a membrane beneath the wall. Nutrients in molecular and ionic form are transported from the environment through the membrane by several but specific mechanisms. The membrane also contains energygenerating components. It also forms intrusions in the cytoplasm (mesosomes). The cytoplasmic material is immobile and does not contain organelles enclosed in a separate membrane. The ribosomes are 70S type and are dispersed in the cytoplasm. The genetic materials (structural and plasmid DNA) are circular, not enclosed in nuclear membrane, and do not contain basic proteins such as histones. Both gene transfer and genetic recombination occur, but do not involve gamete or zygote formation. Cell division is by binary fission. Procaryotic cells can also have flagella, capsules, surface layer proteins, and pili for specific functions. Some also form endospores (one per cell). On the basis of Gram-stain behavior, bacterial cells are grouped as Gram-negative or Gram-positive. Gram-negative cells have a complex cell wall containing an outer membrane (OM) and a middle membrane (MM). The OM is composed of lipopolysaccharides (LPS), lipoprotein (LP), and phospholipids. Phospholipid molecules are arranged in a bilayer, with the hydrophobic part (fatty acids) inside and hydrophilic part (glycerol and phosphate) outside. LPS

and LP molecules are embedded in the phospholipid layer. The OM has limited transport and barrier functions. The resistance of Gram-negative bacteria to many enzymes (lysozyme, which hydrolyzes mucopeptide), hydrophobic molecules (SDS and bile salts), and antibiotics (penicillin) is due to the barrier property of the OM. LPS molecules also have antigenic properties. Beneath the OM is the MM, composed of a thin layer of peptidoglycan or mucopeptide embedded in the periplasmic materials that contain several types of proteins. Beneath the periplasmic materials is the plasma or inner membrane (IM), composed of a phospholipid bilayer in which many types of proteins are embedded. Gram-positive cells have a thick cell wall composed of several layers of mucopeptide (responsible for thick rigid structure) and two types of teichoic acids. Some species also have a layer over the cell surface, called surface layer protein (SLP). The wall teichoic acid molecules are linked to mucopeptide layers, and the lipoteichoic acid molecules are linked to both mucopeptide and cytoplasmic membrane. Teichoic acids are negatively charged (because of phosphate groups) and may bind to or regulate the movement of cationic molecules in and out of the cell. Teichoic acids have antigenic properties and can be used to identify Gram-positive bacteria serologically

Viruses

Viruses are regarded as noncellular entities. Bacterial viruses (bacteriophages) important in food microbiology are widely distributed in nature. They are composed of nucleic acids (DNA or RNA) and several proteins. The proteins form the head (surrounding the nucleic acid) and tail. A bacteriophage attaches itself to the surface of a host bacterial cell and inoculates its nucleic acid into the host cell. Subsequently, many phages form inside a host cell and are released outside following lysis of the cell. Several pathogenic viruses have been identified as causing foodborne diseases in humans. However, because they are difficult to detect in foods, the involvement of

other pathogenic viruses in foodborne diseases is not properly known. The two most important viruses implicated in foodborne outbreaks are hepatitis A and Norwalk-like viruses. Both are single-stranded RNA viruses. Hepatitis A is a small, naked, polyhedral enteric virus ca. 30 nm in diameter. The RNA strand is enclosed in a capsid.

Self- Assessment Exercises 1

1. List five features of bacteria
2. What type of reproduction occur in yeasts

2.4 Important Microorganisms in Food

2.4.1 Important Mould Genera

Moulds are important in food because they can grow even in conditions in which many bacteria cannot grow, such as low pH, low water activity (A_w), and high osmotic pressure. Many types of moulds are found in foods. They are important spoilage microorganisms. Many strains also produce mycotoxins and have been implicated in foodborne intoxication. Many are used in food bioprocessing. Finally, many are used to produce food additives and enzymes. Some of the most common genera of moulds found in food are listed here.

Aspergillus: It is widely distributed and contains many species important in food. Members have septate hyphae and produce black-colored asexual spores on conidia. Many are xerophilic (able to grow in low A_w) and can grow in grains, causing spoilage. They are also involved in spoilage of foods such as jams, cured ham, nuts, and fruits and vegetables (rot). Some species or strains produce mycotoxins (e.g. *Aspergillus flavus* produces aflatoxin). Many species or strains are also used in food and food additive processing. *A. oryzae* is used to hydrolyze starch by α-amylase in

the production of sake. *A. niger* is used to process citric acid from sucrose and to produce enzymes such as β-galactosidase.

Alternaria: Members are septate and form dark-colored spores on conidia. They cause rot in tomatoes and rancid flavour in dairy products. Some species or strains produce mycotoxins e.g. *Alternaria tenuis.*

Fusarium: Many types are associated with rot in citrus fruits, potatoes, and grains. They form cottony growth and produce septate, sickle-shaped conidia e.g. *Fusarium solani*.

Geotrichum: Members are septate and form rectangular arthrospores. They grow, forming a yeastlike cottony, creamy colony. They establish easily in equipment and often grow on dairy products (dairy mold) e.g. *Geotrichum candidum.*

Mucor: It is widely distributed. Members have nonseptate hyphae and produce sporangiophores. They produce cottony colonies. Some species are used in food fermentation and as a source of enzymes. They cause spoilage of vegetables e.g. *Mucor rouxii*.

Penicillium: It is widely distributed and contains many species. Members have septate hyphae and form conidiophores on blue-green, brush-like conidia head. Some species are used in food production, such as *Penicillium roquefortii* and *P. camembertii* in cheese. Many species cause fungal rot in fruits and vegetables. They also cause spoilage of grains, breads, and meat. Some strains produce mycotoxins e.g. Ochratoxin A.

Rhizopus: Hyphae are aseptate and form sporangiophores in sporangium. They cause spoilage of many fruits and vegetables. *Rhizopus stolonifer* is the common black bread mold.

2.4.2 Important Yeast Genera

Yeasts are important in food because of their ability to cause spoilage. Many are also used in food bioprocessing. Some are used to produce food additives.

Saccharomyces: Cells are round, oval, or elongated. It is the most important genus and contains heterogenous groups. *Saccharomyces cerevisiae* variants are used in baking for leavening bread and in alcoholic fermentation. They also cause spoilage of food, producing alcohol and CO_2.

Pichia: Cells are oval to cylindrical and form pellicles in beer, wine, and brine to cause spoilage. Some are also used in oriental food fermentation e.g. *Pichia membranaefaciens*.

Rhodotorula: They are pigment-forming yeasts and can cause discoloration of foods such as meat, fish, and sauerkraut e.g. *Rhodotorula glutinis*.

Torulopsis: Cells are spherical to oval. They cause spoilage of milk because they can ferment lactose (e.g. *Torulopsis versatilis*). They also spoil fruit juice concentrates and acid foods.

Candida: Many species spoil foods with high acid, salt, and sugar and form pellicles on the surface of liquids. Some can cause rancidity in butter and dairy products e.g. *Candida lipolyticum*.

Zygosaccharomyces: Cause spoilage of high-acid foods, such as sauces, ketchups, pickles, mustards, mayonnaise, salad dressings, especially those with less acid and less salt and sugar e.g. *Zygosaccharomyces bailii*.

2.4.3 Important Viruses

Viruses are important in food for three reasons: Some are able to cause enteric disease, and thus, if present in a food, can cause foodborne diseases. Hepatitis A and Norwalk-like viruses have been implicated in foodborne outbreaks. Several other enteric viruses, such as poliovirus, echo virus, and Coxsackie virus, can cause foodborne diseases. In some countries where the level of sanitation is low, they can contaminate foods and cause disease. Some bacterial viruses are used to identify some pathogens (*Salmonella spp., Staphylococcus aureus* strains) on the basis of the sensitivity of the cells to a series of bacteriophages at appropriate dilutions. Bacteriophages are

used to transfer genetic traits in some bacterial species or strains by a process called transduction (e.g. in *Escherichia coli* or *Lactococcus lactis*). Finally, some bacteriophages can be very important because they can cause fermentation failure. Many lactic acid bacteria, used as starter cultures in food fermentation, are sensitive to different bacteriophages. They can infect and destroy starter-culture bacteria, causing product failure. Among the lactic acid bacteria, bacteriophages have been isolated from many species in the genera *Lactococcus, Streptococcus, Leuconostoc,* and *Lactobacillus*; no bacteriophage of *Pediococcus* is yet known.

2.4.4 Important Bacterial Groups in Foods

Among the microorganisms found in foods, bacteria constitute major important groups. This is many different species can be present in foods, and also have rapid growth rate, ability to utilize food nutrients, and ability to grow under a wide range of temperatures, aerobiosis, pH, and water activity, as well as to better survive adverse situations, such as survival of spores at high temperature. Bacteria of importance in foods have been arbitrarily divided into several groups on the basis of similarities in certain characteristics. This grouping does not have any taxonomic significance.

i. Lactic Acid Bacteria

They are bacteria that produce relatively large quantities of lactic acid from carbohydrates. Species mainly from genera *Lactococcus, Leuconostoc, Pediococcus, Lactobacillus*, and *Streptococcus thermophilus* are included in this group.

ii. Acetic Acid Bacteria

They are bacteria that produce acetic acid, such as *Acetobacter aceti.*

iii. Propionic Acid Bacteria

They are bacteria that produce propionic acid and are used in dairy fermentation e.g. *Propionibacterium freudenreichii*.

iv. Butyric Acid Bacteria

They are bacteria that produce butyric acid in relatively large amounts. Some *Clostridium spp*. such as *Clostridium butyricum* is an example.

v. Proteolytic Bacteria

They are bacteria that can hydrolyze proteins because they produce extracellular proteinases. Examples are the genera *Micrococcus, Staphylococcus, Bacillus, Clostridium, Pseudomonas, Alteromonas, Flavobacterium, Alcaligenes,* some in *Enterobacteriaceae,* and *Brevibacterium*.

vi. Lipolytic Bacteria

They are bacteria that are able to hydrolyze triglycerides because they produce extracellular lipases. Species in *genera Micrococcus, Staphylococcus, Pseudomonas, Alteromonas,* and *Flavobacterium* are examples.

vii. Saccharolytic Bacteria

They are bacteria that are able to hydrolyze complex carbohydrates. Examples include Species in the genera *Bacillus, Clostridium, Aeromonas, Pseudomonas,* and *Enterobacter.*

viii. Thermophilic Bacteria

They are bacteria that are able to grow at 50^{o}C and above. Species from genera *Bacillus, Clostridium, Pediococcus, Streptococcus*, and *Lactobacillus* are examples.

ix. Psychrotrophic Bacteria

They are bacteria that are able to grow at refrigerated temperature ($\leq 5^{o}$C). Some species from *Pseudomonas, Alteromonas, Alcaligenes, Flavobacterium, Serratia, Bacillus, Clostridium,*

Lactobacillus, Leuconostoc, Carnobacterium, Brochothrix, Listeria, Yersinia, and *Aeromonas* are in this group.

x. Thermoduric Bacteria

They are bacteria that are able to survive pasteurization temperature treatment. Examples are some species from *Micrococcus, Enterococcus, Lactobacillus, Pediococcus, Bacillus* (spores), and *Clostridium* (spores).

xi. Halotolerant Bacteria

They are bacteria that are able to survive high salt concentrations (≥10%). They include some species from *Bacillus, Micrococcus, Staphylococcus, Pediococcus, Vibrio*, and *Corynebacterium*.

xii. Aciduric Bacteria

They are bacteria that are able to survive at low pH (<4.0). Some species from *Lactobacillus, Pediococcus, Lactococcus, Enterococcus* and *Streptococuss* are this group.

xiii. Osmophilic Bacteria

They are bacteria that can grow at a relatively higher osmotic environment than that needed for other bacteria. Some species from genera *Staphylococcus, Leuconostoc*, and *Lactobacillus* are in this group. They are much less osmophilic than yeasts and molds.

xiv. Gas-Producing Bacteria

They are bacteria that produce gas (CO_2, H_2, H_2S) during metabolism of nutrients. E.g. species from genera *Leuconostoc, Lactobacillus, Propionibacterium, Escherichia, Enterobacter, Clostridium*, and *Desulfotomaculum* are in this group.

xv. Slime Producers

They are bacteria that produce slime because they synthesise polysaccharides. Some species or strains from *Xanthomonas, Leuconostoc, Alcaligenes, Enterobacter, Lactococcus*, and *Lactobacillus* are examples.

xvi. Spore Formers

They are bacteria having the ability to produce spores. Species from *Bacillus, Clostridium,* and *Desulfotomaculum* are included in this group. They are further divided into aerobic sporeformers, anaerobic sporeformers, flat sour sporeformers, thermophilic sporeformers, and sulfide-producing sporeformers.

xvii. Aerobes

They are bacteria that require oxygen for growth and multiplication. Species from *Pseudomonas, Bacillus,* and *Flavobacterium* are in this group.

xviii. Anaerobes

They are bacteria that cannot grow in the presence of oxygen e.g. species of *Clostridium*.

xix. Facultative Anaerobes

They are bacteria that are able to grow in both the presence and absence of oxygen. *Lactobacillus, Pediococcus, Leuconostoc, enteric pathogens*, and some species of *Bacillus*, *Serratia*, and coliforms are included in this group.

xx. Coliforms

Species from *Escherichia, Enterobacter*, *Citrobacter*, and *Klebsiella* are included in this group. They are used as an index of sanitation.

xxi. Fecal Coliforms

Mainly *Escherichia coli* is included in this group. They are also used as an index of sanitation.

xxii. Enteric Pathogens

Pathogenic *Salmonella, Shigella, Campylobacter, Yersinia, Escherichia, Vibrio, Listeria,* hepatitis A, and others that can cause gastrointestinal infection are included in this group.

Because of the importance of these bacterial groups in food, many laboratory methods are designed to detect a specific group instead of a specific genus or species. Similarly, control methods are sometimes designed to destroy or prevent growth of a specific group.

Self- Assessment Exercises 2

1. List four bacteria that are used as index of sanitation
2. State the pathogens that are identified usingbacteria viruses

2.5 Summary

Two major aspects have been discussed in this chapter: the nomenclature system of bacteria, which is rapidly changing because of the development of new molecular biology techniques, and the morphological and physiological characteristics of the microorganisms important in food. A food microbiologist should be knowledgeable about these facets. The sources of these microorganisms in food are discussed below.

Sources of Microorganisms in Foods

i. **Plants (Fruits and Vegetables):** The inside tissue of foods from plant sources are essentially sterile, except for a few porous vegetables (e.g., radishes and onions) and leafy vegetables (e.g., cabbage and Brussels sprouts). Some plants produce natural antimicrobial metabolites that can limit the presence of microorganisms. Fruits and vegetables harbor microorganisms on the surface; their type and level vary with soil condition, type of fertilizers and water used, and air quality. Molds, yeasts, lactic acid bacteria, and bacteria from the genera of *Pseudomonas,*

Alcaligenes, Micrococcus, Erwinia, Bacillus, Clostridium, and *Enterobacter* can be expected from this source. Pathogens, especially of enteric types, can be present if the soil is contaminated with untreated sewage. Proper methods used during growing (such as use of treated sewage or other types of fertilizers), damage reduction during harvesting, quick washing with goodquality water to remove soil and dirt, and storage at low temperature before and after processing can be used to reduce microbial load in foods of plant origin.

ii. **Animals:** Animals normally carry many types of indigenous microorganisms in the digestive, respiratory, and urinogenital tracts, the teat canal in the udder, as well as in the skin, hooves, hair, and feathers. Their numbers, depending on the specific organ, can be very high (large intestinal contents can have as high as 10^{10} bacteria/g). Many, as carriers, can harbor pathogens such as *Salmonella serovars*, pathogenic *Escherichia coli, Campylobacter jejuni, Yersinia enterocolitica*, and *Listeria monocytogenes* without showing symptoms. Laying birds have been suspected of asymptomatically carrying *Salmonella enteritidis* in the ovaries and contaminating the yolk during ovulation. Disease situations, such as mastitis in cows and intestinal, respiratory, and uterine infections, as well as injury can change the ecology of normal microflora. Similarly, poor husbandry resulting in fecal contamination on the body surface and supplying contaminated water and feed can also change their normal microbial flora. Fish and shellfish also carry normal microflora in the scales, skin, and digestive tracts. Water quality, feeding habits, and diseases can change the normal microbial types and level. Pathogens such as *Vibrio parahaemolyticus, V. vulnificus*, and *V. cholerae* are of major concern from these sources. Many spoilage and pathogenic microorganisms can get into foods of animal origin during production and processing. Milk can be contaminated with fecal materials on the udder surface, egg shells

with fecal material during laying, meat with the intestinal contents during slaughtering, and fish with intestinal contents during processing.

iii. **Air:** Microorganisms are present in dust and moisture droplets in the air. They do not grow in dust, but are transient and variable, depending on the environment. Their level is controlled by the degree of humidity, size and level of dust particles, temperature and air velocity, and resistance of microorganisms to drying. Generally, dry air with low dust content and higher temperature has a low microbial level. Spores of *Bacillus spp., Clostridium spp.*, and *molds*, and cells of some Grampositive bacteria (e.g., *Micrococcus spp*. and *Sarcina spp.*), as well as yeasts, can be predominantly present in air. If the surroundings contains source of pathogens (e.g., animal and poultry farms or a sewage-treatment plant), different types of bacteria, including pathogens and viruses (including bacteriophages), can be transmitted through the air. Microbial contamination of food from the air can be reduced by removing the potential sources, controlling dust particles in the air, using positive air pressure, reducing humidity level, and installing UV light.

iv. **Soil:** Soils, especially the type used to grow agricultural produce and raise animals and birds, contain several types of microorganisms. Because microorganisms can multiply in soil, their numbers can be very high (billions/g). Many types of *molds*, *yeasts*, and bacterial genera (e.g., *Enterobacter, Pseudomonas, Proteus, Micrococcus, Enterococcus, Bacillus,* and *Clostridium*) can enter foods from the soil. Soil contaminated with fecal materials can be the source of enteric pathogenic bacteria and viruses in food. Sediments where fish and marine foods are harvested can also be a source of microorganisms, including pathogens, in those foods. Different types of parasites can also get in food from soil. Washing and avoiding soil contamination can reduce microorganisms in foods from this source.

v. **Sewage:** Sewage, especially when used as fertilizer for crops, can contaminate food with microorganisms. The most significant of which are different enteropathogenic bacteria and viruses. This can be a major concern with organically grown food and many imported fruits and vegetables, in which untreated sewage and manure might be used as fertilizer. Pathogenic parasites can also get in food from sewage. To reduce incidence of microbial contamination of foods from sewage, it is better not to use sewage as fertilizer and if used, it should be efficiently treated to kill the pathogens. Effective washing of foods following harvesting is also important.

vi. **Water:** Water is used to produce, process, and, under certain conditions, store foods. It is used for irrigation of crops, drinking by food animals and birds, raising fishery and marine products, washing foods, processing (pasteurization, canning, and cooling of heated foods) and storage of foods, washing and sanitation of equipment, and processing and transportation facilities. Water is also used as an ingredient in many processed foods. Thus, water quality can greatly influence microbial quality of foods. Contamination of foods with pathogenic bacteria, viruses, and parasites from water has been recorded. Wastewater can be recycled for irrigation. However, chlorine-treated potable water should be used in processing, washing, sanitation, and as an ingredient. Although potable water does not contain coliforms and pathogens (mainly enteric types), it can contain other bacteria capable of causing food spoilage, such as *Pseudomonas*, *Alcaligenes* and *Flavobacterium*. Improperly treated water can contain pathogenic and spoilage microorganisms.

vii. **Humans:** Between food production and consumption, it comes in contact with different people handling the foods. They include not only people working in farms and food processing plants, but also those handling foods at restaurants, catering services, retail stores, and at home. Human carriers have been the source of pathogenic microorganisms in foods that later caused foodborne

diseases, especially with ready-to-eat foods. Lack of aesthetic sense and personal hygiene, dirty hands and clothes and hair can be major sources of microbial contamination in foods. The presence of minor cuts and infection in hands and face and mild generalized diseases like flu, strep throat, or hepatitis A can amplify the situation. In addition to spoilage bacteria, pathogens such as *S. aureus, Salmonella serovars, Shigella spp.,* pathogenic *E. coli*, and hepatitis A can be introduced into foods from human sources.

viii. **Food Ingredients:** Many of the ingredients added to prepared or fabricated foods can be the source of both spoilage and pathogenic microorganisms. Various spices generally have very high population of molds and bacterial spores. Starch, sugar, and flour might have spores of thermophilic bacteria. Pathogens have been isolated from dried coconut, egg, and chocolate. The ingredients should be produced under sanitary conditions and given antimicrobial treatments. Also, setting up acceptable microbial specifications for the ingredients will be important in reducing microorganisms in food from this source.

ix. **Equipment**: A wide variety of equipment is used in harvesting, slaughtering, transporting, processing, and storing foods. Many types of microorganisms from air, raw foods, water, and personnel can get into the equipment and contaminate foods. Depending on the environment and time, microorganisms can multiply and, even from a low initial population, reach a high level and contaminate large volumes of foods. Also, when processing equipment is used continuously for a long period of time, microorganisms initially present can multiply and act as a continuous source of contamination in the product produced subsequently. In some equipment, small parts, inaccessible sections, and certain materials might not be efficiently cleaned and sanitized. These dead spots can serve as sources of both pathogenic and spoilage microorganisms in food. Small equipment, such as cutting boards, knives, spoons, and similar articles, because of improper

cleaning, can be sources of cross contamination. *Salmonella, Listeria, Escherichia, Enterococcus, Micrococcus, Pseudomonas, Lactobacillus, Leuconostoc, Clostridium, Bacillus spp.,* and *yeasts* and *molds* can get in food from equipment. Proper cleaning and sanitation of equipment at prescribed intervals are important to reduce microbial levels in food.

x. **Other Sources**: Foods might be contaminated with microorganisms from several other sources, namely packaging and wrapping materials, containers, flies, vermins, birds, house pets, and rodents. Many types of packaging materials are used in food. Because they are used in products ready for consumption and in some cases without further heating, proper microbiological standards or specifications for packaging materials are necessary. Flies, vermins, birds, and rodents in food processing and food preparation and storage facilities should be viewed with concern as they can carry pathogenic microorganisms. House pets can also harbor pathogens; proper care should be taken not to contaminate food from this source.

2.6 References

Ray, B (2004). Fundamental food microbiology, 3rd edition CRC Press, London New York DC. 625 Pages.

2.7 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1.

i. Bacteria are unicellular
ii. Most ca. 0.5–1.0 x 2.0–10 µm in size
iii. Have three morphological forms: spherical (cocci), rod shaped (bacilli), and curved (comma).
iv. They can form associations such as clusters, chains (two or more cells), or tetrads.
v. They can be motile or nonmotile.
vi. Cytoplasmic materials are enclosed in a rigid wall on the surface and a membrane beneath the wall.
vii. Nutrients in molecular and ionic form are transported from the environment through the membrane by several but specific mechanisms.

viii. The membrane also contains energygenerating components.
ix. It also forms intrusions in the cytoplasm (mesosomes).
x. The cytoplasmic material is immobile and does not contain organelles enclosed in a separate membrane.
xi. The ribosomes are 70S type and are dispersed in the cytoplasm.

Any five points

2. Asexual reproduction

Answers to Self-Assessment Exercises 2

1. *Escherichia, Enterobacter, Citrobacter,* and *Klebsiella*
2. Some bacterial viruses are used to identify some pathogens *Salmonella spp.*, *Staphylococcus aureus* strains

UNIT 3 USES OF NATURAL FLORA IN FOOD INDUSTRY

CONTENTS

3.3 Importance of Microorganisms in Food Industry

3.3.3 Fermentation of Foods

3.3.2 Lactic Starter Cultures

3.3.3 Other Starter Cultures

3.3.4 Yeasts and Moulds

3.4 Summary

3.5 References/Further Reading

3.6 Possible Answers to Self-Assessment Exercises

3.1 Introduction

Beneficial microorganisms are used in foods in several ways. These include actively growing microbial cells, nongrowing microbial cells, and metabolic by-products and cellular components of microorganisms. An example of the use of growing microbial cells is the conversion of milk to yogurt by bacteria. Nongrowing cells of some bacteria are used to increase shelf life of refrigerated raw milk or raw meat. Many by-products, such as lactic acid, acetic acid, some essential amino acids, and bacteriocins produced by different microorganisms, are used in many foods. Finally, microbial cellular components, such as single-cell proteins (SCPs), dextran, cellulose, and many enzymes, are used in food for different purposes. These microorganisms or their by-products or cellular components have to be safe, food grade, and approved by regulatory agencies. When the microbial cells are used in such a way that they are consumed live with the food (as in yogurt), it is very important that they and their metabolites have no detrimental effect on the health of the consumers. When a by-product (such as an amino acid) or a cellular component (such as an enzyme) is used in a food, the microorganisms producing it have to be regulated and approved, and the by-product and cellular component have to be safe. If a food-

grade microorganism is genetically modified, its use in food has to be approved, especially if the genetic material used is obtained from a different source or is synthesized. Thus, the microorganisms used for these purposes have to meet some commercial and regulatory criteria. Characteristics of some microorganisms used in the processing of foods (designated as fermented foods) are discussed in this unit. Many of these microorganisms are used to produce several by-products and cellular components used in foods.

 **3.2 Learning Outcomes**

By the end of this unit, you will be able to:

- Discuss the Microbiology of fermented foods
- Discuss the importance of Bacteria in food industry
- Analyse the benefit of yeast and moulds in fermentation

 **3.3 Importance of Microorganisms in Food Industry**

3.3.1 Fermentation of Foods

Food fermentation involves a process in which raw materials are converted to fermented foods by the growth and metabolic activities of the desirable microorganisms. The microorganisms utilize some components present in the raw materials as substrates to generate energy and cellular components, to increase in population, and to produce many usable by-products (also called end products) that are excreted in the environment. The unused components of the raw materials and the microbial by-products (and sometimes microbial cells) together constitute fermented foods. The raw materials can be milk, meat, fish, vegetables, fruits, cereal grains, seeds, and beans, fermented individually or in combination. Globally, more than 3,500 types of fermented foods are produced. Many ethnic types are produced and used in small localities by

small groups of people. Many of the fermented foods consumed currently have been produced and consumed by humans for thousands of years. The process not only produced new foods but also helped preserve the excess of raw materials both of plant and animal origins. The basic principles developed by the ancient civilizations are used even today to produce many types of fermented foods by a process known as natural fermentation. In this method, either the desirable microbial population naturally present in the raw materials or some products containing the desirable microbes from a previous fermentation (called back slopping), are added to the raw materials. Then the fermentation conditions are set so as to favour growth of the desirable types but prevent or retard growth of undesirable types that could be present in the raw materials. In another type of fermentation, called controlled or pure culture fermentation, the microorganisms associated with fermentation of a food are first purified from the food, identified, and maintained in the laboratory. When required for the fermentation of a specific food, the microbial species associated with this fermentation are grown in large volume in the laboratory and then added to the raw materials in very high numbers. Then the fermentation conditions are set such that these microorganisms grow preferentially to produce a desired product. These microbial species, when used in controlled fermentation, are also referred to as starter cultures. Many of these microbial species are present in raw materials that are naturally fermented, along with other associated microorganisms, some of which may contribute to the desirable characteristics of the products.

3.3.2 Lactic Starter Cultures

Bacterial species from 12 genera are included in a group designated as lactic acid bacteria because of their ability to metabolize relatively large amounts of lactic acids from carbohydrates. The genera include *Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Lactobacillus,*

Enterococcus, Aerococcus, Vagococcus, Tetragenococcus, Carnobacterium, Weissella, and *Oenococcus*. Many of the genera have been created recently from previously existing genera and include one or a few species. For example, *Lactococcus* and *Enterococcus* were previously classified as *Streptococcus* Group N and Group D, respectively. *Vagococcus* is indistinguishable from *Lactococcus*, except that these bacteria are motile. *Weissella* and *Oenococcus* are separated from *Leoconostoc*. *Tetragenococcus* includes a single species that was previously included with *Pediococcus (Pediococcus halophilus*). *Carnobacterium* was created to include a few species that were previously in genus *Lactobacillus* and are obligatory heterofermentative. However, species from the first five genera, i.e., *Lactococcus, Leuconostoc, Pediococcus, Streptococcus,* and *Lactobacillus,* are used as starter cultures in food fermentation. The status of others, except *Tetragenococcus halophilus* and *Oenococcus oeni*, with respect to use in food, is not yet clear.

i. Lactococcus

This genus includes several species, but only one species, *Lactococcus lactic*, has been widely used in dairy fermentation. It has three subspecies (ssp.), ssp. *lactis*, ssp. *cremoris*, and ssp. *hordniae*, but only the first two are used in dairy fermentation. The biovar *L. lactis* ssp. *lactis* biovar diacetylactis is also used in dairy fermentation. The cells are ovoid, ca. 0.5 to 1.0 µm in diameter, present in pairs or short chains, nonmotile, nonsporulating, and facultative anaerobic to microaerophilic. They grow well between 20 and 30^{o}C, but do not grow in 6.5% NaCl or at pH 9.6. In a suitable broth they can produce ca. 1% L (+)-lactic acid and reduce the pH to ca. 4.5. Subsp. *cremoris* can be differentiated from subsp. *lactis* by its inability to grow at 40ºC, in 4% NaCl, ferment ribose, and hydrolyze arginine to produce NH_3. Biovar diacetylactis, as compared with others, produces larger amounts of CO_2 and diacetyl from citrate. They are

generally capable of hydrolyzing lactose and casein and also ferment galactose, sucrose, and maltose. The natural habitats are green vegetation, silage, the diary environment, and raw milk.

***ii. Streptococcus*:**

Only one species, *Streptococcus thermophilus*, has been used in dairy fermentation. A change in designation to *S. salivarius* ssp. *thermophilus* has been suggested but not made. The Gram-positive cells are spherical to ovoid, 0.7 to 0.9 µm in diameter, and exist in pairs to long chains. The cells grow well at 37 to 40ºC, but can also grow even at 52ºC. They are facultative anaerobes.

***iii. Leuconostoc*:**

The Gram-positive cells are spherical, arranged in pairs or in chains, non-motile, non-sporulating, catalase negative and facultative anaerobes. The species grow well between 20 and 30ºC, with a range of 1 to 37ºC. Glucose is fermented to D (–)-lactic acid, CO_2, ethanol, or acetic acid, with the pH reduced to 4.5 to 5.0. The species grow in milk but may not curdle. Also, arginine is not hydrolyzed. Many form dextran while growing on sucrose. Citrate is utilized to produce diacetyl and CO_2. Some species can survive 60ºC for 30 min. *Leuconostoc* species are found in plants, vegetables, silage, milk and some milk products, and raw and processed meats. Five known species are: *Leuconostoc mesenteroides, L. paramesenteroides, L. lactis, L. carnosum,* and *L. gelidum. L. mesenteroides* has three subspecies: subsp. *mesenteroides*, ssp. *dextranicum*, and ssp. *cremoris*. *L. mesenteroides* ssp. *cremoris* and *L. lactis* are used in some dairy and vegetable fermentation. Many of these species, particularly *L. carnosum* and *L. gelidum*, have been associated with spoilage of refrigerated vacuum-packaged meat products. *Leuconostocs* are morphologically heterogenous and may contain genetically diverse groups of

bacteria. Recently, two new genera have been produced from it: *Weisella* and *Oenococcus*. *O. oeni* is found in wine and related habitant and is used for malolactic fermentation in wine.

iv. Pediococcus

The cells are spherical and form tetrads, but can be present in pairs. Single cells or chains are absent. They are Gram-positive, non-motile, non-sporulating, facultative anaerobes. They grow well between 25 and 40^{o}C; some species grow at 50^{o}C. They ferment glucose to L (+)- or DL-lactic acid, some species reducing the pH to 3.6. Some species can ferment sucrose, arabinose, ribose, and xylose. Lactose is not generally fermented, especially in milk, and milk is not curdled. Some strains may have weak lactose-hydrolyzing capability, especially in broth containing lactose as a carbohydrate source. Some species are found in plants, vegetables, silage, beer, milk, and fermented vegetables, meats, and fish. The genus has seven to eight species, of which *P. pentosaceus* and *P. acidilactici* are used in vegetables, meat, cereal, and other types of fermented foods. They have also been implicated in ripening and flavour production of some cheeses as secondary cultures. These two species are difficult to differentiate, but compared with *P. acidilactici, P. pentosaceus* ferments maltose, does not grow at 50ºC, and is killed at 70ºC in 5 min. *P. halophilus,* used in fermentation of high-salt products, is now classified as *T. halophilus*.

v. Lactobacillus

The genus *Lactobacillus* includes a heterogenous group of Gram-positive, rodshaped, usually non-motile, non-sporulating, facultative anaerobic species that vary widely morphologically and in growth and metabolic characteristics. Cells vary from very short to very long rods, slender or moderately thick, often bent, and can be present as single cells or in short to long chains. While growing on glucose, depending on a species, they produce either only lactic acid [L (+), D(–), or DL] or a mixture of lactic acid, ethanol, acetic acid, and CO_2. Some species also produce

diacetyl. Many species utilize lactose, sucrose, fructose, or galactose, and some species can ferment pentoses. Growth temperature can vary from 1 to 50ºC, but most that are used as starter cultures in controlled fermentation of foods grow well between 25 to 40ºC. Several species involved in natural fermentation of some foods at low temperature can grow well from 10 to 25ºC. While growing in a metabolizable carbohydrate, depending on a species, the pH can be reduced between 3.5 and 5.0. They are distributed widely and can be found in plants, vegetables, grains, seeds, raw and processed milk and milk products, raw, processed, and fermented meat products, and fermented vegetables. Some are found in the digestive tract of humans, animals, and birds. Many have been associated with spoilage of foods. Among the large number of species, some have been used in controlled fermentation (dairy, meat, vegetables, and cereal), some are known to be associated with natural fermentation of foods, a few are consumed live for their beneficial effect on intestinal health, and some others have an undesirable effect on foods. On the basis of their metabolic patterns of hexoses and pentoses, the species have been divided into three groups:

i. Group I ferment hexoses (and disaccharides such as lactose and sucrose) to produce mainly lactic acids and do not ferment pentoses (such as ribose, xylose, or arabinose).
ii. Group II, depending on the carbohydrates and the amounts available, either produces mainly lactic acid, or a mixture of lactic, acetic, and formic acids, ethanol, and CO_2.
iii. Group III species ferment carbohydrates to a mixture of lactate, acetate, ethanol, and CO_2.

The three *Lactobacillus delbrueckii* subspecies are used in the fermentation of dairy products, such as some cheeses and yogurt. They grow well at 45ºC and ferment lactose to produce large amounts of D (–) lactic acid. β-galactosidase in these subspecies is constitutive. *L. acidophilus*

and *L. reuteri* are considered beneficial intestinal microbes (probiotic) and present in the small intestine. *L. acidophilus* is used to produce fermented dairy products and also either added to pasteurized milk or made into tablets and capsules for consumption as probiotics. It metabolizes lactose, and produces large amounts of D(–)-lactic acid. However, in *L. acidophilus*, β-galactosidase is generally inducible. *L. helveticus* is used to make some cheeses and ferment lactose to lactic acid (DL). *L. casei* ssp. *casei* is used in some fermented dairy products. It ferments lactose and produces L(+)-lactic acid. Some strains are also used as probiotic bacteria. Strains of *L. casei* ssp. *rhamnosus* (also called *L. rhamnosus*) are now used as a probiotic bacterium. Some strains of *L. johnsonii* are also used in probiotics. *L. plantarum* is used in vegetable and meat fermentation. It produces DL-lactic acid. *L. curvatus* and *L. sake* can grow at low temperatures (2 to 4ºC) and ferment vegetable and meat products. *L. sake* is used to ferment sake wine. *L. kefir* is important in the fermentation of kefir (ethnic fermented sour milk). *L. sanfrancisco* is associated with other microorganisms in the fermentation of San Francisco sourdough bread. *L. viridescens, L. curvatus,* and *L. sake* are associated with spoilage of refrigerated meat products.

vi. Oenococcus

O. oeni, previously designated as *L. oeni*, has the general characteristics of *Leuconostoc* spp. It is found in the winery environment. It is sometimes used to accelerate malolactic fermentation in wine. The cells transport malate in wine and metabolize it to lactic acid and CO_2. This process reduces the acidity of wine.

3.3.3 Other Starter Cultures

i. Bifidobacterium

Bifidobacterium are morphologically similar to some *Lactobacillus* spp. and were previously included in the genus *Lactobacillus*. The cells are Gram-positive, rods of various shapes and sizes, present as single cells or in chain of different sizes. They are non-spore forming, non-motile, and anaerobic, although some can tolerate O_2 in the presence of CO_2. The species grow optimally at 37 to 41ºC, with a growth temperature range of 25 to 45ºC. They usually do not grow at a pH above 8.0 or below 4.5. They ferment glucose to produce lactic and acetic acid in a 2:3 molar ratio without producing CO_2, and also ferment lactose, galactose, and some pentoses

They have been isolated from faeces of humans, animals, and birds and are considered beneficial for the normal health of the digestive tract. They are present in large numbers in the faeces of infants within 2 to 3 days after birth, and usually present in high numbers in breast-fed babies. They are usually found in the large intestine and many species of *Bifidobacterium* have been isolated from the faeces of humans and animals. Some of these include *Bifidovacterium bifidum, B. longum, B. brevis, B. infantis*, and *B. adolescentis*. All have been isolated in humans; however, some species are more prevalent in infants than in adults. Some of these species have been added to dairy products to supply live cells in high numbers to restore and maintain intestinal health in humans.

ii. **Propionibacterium**

The genus includes species in the classical or dairy *Propionibacterium* group and the cutaneous or acne *Propionibacterium* group. The cells are Gram-positive, pleomorphic thick rods 1 to 1.5 µm in length, and occur in single cells, pairs, or short chains with different configurations. They are non-motile, non-sporulating, anaerobic (can also tolerate air), catalase positive, and ferment glucose to produce large amounts of propionic acid and acetic acid. Some species ferment lactose, sucrose, fructose, galactose, and some pentoses. They grow optimally at 30 to 37ºC and

some species form pigments. They have been isolated from raw milk, some types of cheeses, dairy products, and silage. Four species of diary *Propionibacterium* are included in the genus: *Propionibacterium freudenreichii, P. jensenii, P. thoenii,* and *P. acidipropionici*. All the four species are associated with natural fermentation of Swiss-type cheeses, but *P. freudenreichii* has been used as a starter culture in controlled fermentation.

iii. **Brevibacterium**

The genus contains a mixture of coryniform bacterial species, some of which have important applications in cheese production and other industrial fermentations. *Brevibacterium linens* is used in cheese ripening as it has extracellular proteases. The cells are non-motile, Gram-positive, and capable of growing in high salt and wide pH ranges.

iv. **Acetobacter**

A species in this genus, *A. aceti*, is used to produce acetic acid from alcohol. The cells are Gram-negative; aerobic; rods (0.5 to 1.5 μm); occurring as single cells, pairs, or chains; and can be motile or non-motile. They are obligate aerobes, catalase positive, and oxidize ethanol to acetic acid and lactic acid to CO_2 and H_2O. They grow well from 25 to 30ºC. They are found naturally in fruits, sake, palm wine, cider, beer, sugar cane juice, tea fungus, and soil. Some species synthesize large amounts of cellulose.

3.3.4 Yeasts and Moulds

Many yeasts and molds are important in food, but most are involved with the spoilage of food and mycotoxin production. Several are used in food bioprocessing; however, genetic improvements are being made to improve their desirable characteristics.

i. **Yeasts**

Among the many types of yeasts, only a few have been associated with fermentation of foods and alcohol, production of enzymes for use in food, production of SCPs, and as additives to impart desirable flavour in some foods. The most important genus and species used is *Saccharomyces cerevisiae*. It has been used to leaven bread and produce beer, wine, distilled liquors, and industrial alcohol; produce invertase (enzyme); and flavour some foods (soups). Many strains have been developed to suit specific needs. The cells are round, oval, or elongated. They multiply by multipolar budding or by conjugation and formation of ascospores. The strains are generally grouped as bottom yeasts or top yeasts. Top yeasts grow very rapidly at 20ºC, producing alcohol and CO_2. They also form clumps that, because of rapid CO_2 production, float at the surface. In contrast, bottom yeasts grow better at 10 to 15ºC, grow slowly and produce CO_2 slowly, do not clump, and thus settle at the bottom. Top yeasts and bottom yeasts are used according to the need of a particular fermentation process. *Candida utilis* has been used to produce SCPs. It is false yeast (*Fungi imperfecti*) and reproduces by budding (not by conjugation). The cells are oval to elongated and form hyphae with large numbers of budding cells. They are also involved in food spoilage.

Kluyveromyces marxianus and *K. marxianus* var. *lactis* can hydrolyze lactose and have been associated with natural fermentation, along with other yeasts and lactic acid bacteria, of alcoholic dairy products such as kefir. They have also been associated with spoilage of some dairy products. They are used to produce β-galactosidase (lactase) for use commercially to hydrolyze lactose. The enzyme is now used to produce low-lactose milk.

ii. **Moulds**

Although most moulds are associated with food spoilage and many form mycotoxins while growing in foods, other species and strains are used in processing of foods and to produce additives and enzymes for use in foods. Moulds are multicellular, filamentous fungi. The filaments (hyphae) can be septate or nonseptate and have nuclei. They divide by elongation at the tip of a hypha or by forming sexual or asexual spores on a spore-bearing body. Among many genera, several species from the genera *Aspergillus* and *Penicillium*, and a few from *Rhizopus* and *Mucor*, have been used for beneficial purposes in food to identify a non-mycotoxin-producer strain in the case of natural fermentation, but should be an important consideration in the selection of strains for use in controlled fermentation. *Aspergillus oryzae* is used in fermentation of several oriental foods, such as sake, soy sauce, and miso. It is also used as a source of some food enzymes. *A. niger* is used to produce citric acid and gluconic acid from sucrose. It is also used as a source of the enzymes pectinase and amylase. *Penicillium roquefortii* is used for ripening of Roquefort, Gorgonzola, and blue cheeses. Some strains can produce the neurotoxin roquefortin. *P. camembertii* is used in Camembert cheese and *P. caseicolum* is used in Brie cheese. They are also used to produce the enzyme glucose oxidase.

Self- Assessment Exercises 1

1. What is the most important genus and species of yeast used in food processing?
2. State the three groups of Lactobacillus species on the basis of their metabolic patterns of hexoses and pentoses.

3.4 Summary

Food-grade bacteria, yeasts, and moulds are used in different combinations to produc several thousands of fermented foods worldwide by natural or controlled fermentation of milk, meat, fish, egg, fruits, vegetables and others. The species and strains used as starter cultures in controlled fermentation should not only be safe and regulated but also be able to produce desirable characteristics in the fermented foods. These characteristics are the result of metabolic breakdown of carbohydrates, proteins, and lipids present in the food.

3.5 References/Further Readings

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 **3.6 Possible Answers to Self-Assessment Exercises**

Answers to Self-Assessment Exercise

1. The most important genus and species of yeast used in food processing is *Saccharomyces cerevisiae*.
2. On the basis of their metabolic patterns of hexoses and pentoses, *Lactobacillus* species are divided into three groups:

i. Group I ferment hexoses (and disaccharides such as lactose and sucrose) to produce mainly lactic acids and do not ferment pentoses (such as ribose, xylose, or arabinose).

ii. Group II, depending on the carbohydrates and the amounts available, either produces mainly lactic acid, or a mixture of lactic, acetic, and formic acids, ethanol, and CO_2.

iii. Group III species ferment carbohydrates to a mixture of lactate, acetate, ethanol, and CO_2.

UNIT 4 FACTORS INFLUENCING THE GROWTH OF MICROORGANISMS IN FOOD

CONTENTS

4.1 Introduction

The ability of microorganisms to grow or multiply in a food is determined by the food environment as well as the environment in which the food is stored, designated as the intrinsic and extrinsic environment of food, respectively. It is not possible to study the influence of any one factor on growth independently as the factors are interrelated. Instead, the influence of any one factor at different levels on growth is compared keeping other factors unchanged

4.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the Intrinsic Factors or Food Environment
- Analyse the extrinsic factors foods

4.3 Factors That Affect the Growth of Microoranism in Foods

4.31 Intrinsic Factors or Food Environment

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

i. Nutrients and Growth

Microbial growth is accomplished through the synthesis of cellular components and energy. The necessary nutrients for this process are derived from the immediate environment of a microbial cell and, if the cell is growing in a food, it supplies the nutrients. These nutrients include carbohydrates, proteins, lipids, minerals, and vitamins. Water is not considered a nutrient, but it is essential as a medium for the biochemical reactions necessary for the synthesis of cell mass and energy. All foods contain these five major nutrient groups, either naturally or added, and the amount of each nutrient varies greatly with the type of food. Generally, meat is rich in protein, lipids, minerals, and vitamins but poor in carbohydrates. Foods from plant sources are rich in carbohydrates but can be poor sources of proteins, minerals, and some vitamins. Some foods such as milk and many prepared foods have all five nutrient groups in sufficient amounts for microbial growth. Microorganisms normally present in food vary greatly in nutrient requirements, with bacteria requiring the most, followed by yeasts and moulds. Microorganisms also differ greatly in their ability to utilize large and complex carbohydrates (e.g. starch and cellulose), large proteins (e.g. casein in milk), and lipids. Microorganisms capable of using these molecules do so by producing specific extracellular enzymes (or exoenzymes) and hydrolyzing the complex molecules to simpler forms outside before transporting them inside the cell. Moulds

are the most capable of doing this. However, this provides an opportunity for a species to grow in a mixed population even when it is incapable of metabolizing the complex molecules. Microbial cells, following death and lysis, release intracellular enzymes that can also catalyze breakdown of complex food nutrients to simpler forms, which can then be utilized by other microorganisms.

1. **Carbohydrates in Foods**

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

- **Monosaccharides**

  Hexoses: glucose, fructose, mannose, galactose

  Pentoses: xylose, arabinose, ribose, ribulose, xylulose

- **Disaccharides**

  Lactose (galactose + glucose)

  Sucrose (fructose + glucose)

  Maltose (glucose + glucose)

- **Oligosaccharides**

  Raffinose (glucose + fructose + galactose)

  Stachyose (glucose + fructose + galactose + galactose)

- **Polysaccharides**

  Starch (glucose units)

  Glycogen (glucose units)

  Cellulose (glucose units)

  Inulin (fructose units)

Hemicellulose (xylose, galactose, mannose units)

Dextrans (a-1, 6 glucose polymer)

Pectins

Gums and mucilages

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

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

2. Proteins in Foods

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

Microorganisms differ greatly in their ability to metabolize food proteins. Most transport amino acids and small peptides in the cells; small peptides are then hydrolyzed to amino acids inside the cells, such as in some *Lactococcus* spp. Microorganisms also produce extracellular proteinases and peptidases to hydrolyze large proteins and peptides to small peptides and amino acids before they can be transported inside the cells. Soluble proteins are more susceptible to this hydrolytic action than are the insoluble proteins. Hydrolysis of food proteins can be either undesirable (texture loss in meat) or desirable (flavour in cheese). Microorganisms can also metabolize

different NPN compounds found in foods. Amino acids inside microbial cells are metabolized by different pathways to synthesize cellular components, energy, and various by-products. Many of these byproducts can be undesirable (e.g. NH_3 and H_2S production causes spoilage of food, and toxins and biological amines cause health hazards) or desirable (e.g. some sulfur compounds give cheddar cheese flavour). Production of specific metabolic products is used for the laboratory identification of microbial isolates from food. An example of this is the ability of *Escherichia coli* to produce indole from tryptophan, which is used to differentiate this species from non-indole-producing related species (e.g. *Enterobacter* spp.).

3. Lipids in Foods

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

In general, moulds are more capable of producing these enzymes. However, certain bacterial groups such as *Pseudomonas*, *Achromobacter*, and *Alcaligenes* can produce these enzymes. Lysis of dead microbial cells in foods causes release of intracellular lipases and oxidases, which

then can carry out these reactions. The action of these enzymes are associated with spoilage (such as rancidity) in many foods, whereas in other foods the enzymes are credited for desirable flavors (such as in mold-ripened cheeses). Some beneficial intestinal microorganisms, such as *Lactobacillus acidophilus* strains, can metabolize cholesterol and are believed to be capable of reducing serum cholesterol levels in humans.

4. Minerals and Vitamins in Foods

Microorganisms need several elements in small amounts, such as phosphorous, calcium, magnesium, iron, sulfur, manganese, and potassium. Most foods have these elements in sufficient amounts. Many microorganisms can synthesize B vitamins, and foods also contain most B vitamins. Most foods contain different carbohydrates, proteins, lipids, minerals, and vitamins in sufficient amounts to supply necessary nutrients for the growth of moulds, yeasts, and bacteria (especially Gram-negative bacteria normally present in foods). Some foods may have limited amounts of one or a few nutrients for rapid growth of some Gram-positive bacteria, especially some fastidious *Lactobacillus* species. When their growth is desired, some carbohydrates, essential amino acids, and B vitamins may be added to a food. It is not possible to control microbial growth in a food by restricting nutrients.

ii. Growth Factors and Inhibitors in Food

Foods can also have some factors that either stimulate growth or adversely affect growth of microorganisms. The exact nature of growth factors is not known, but they are naturally present in some foods. An example is the growth factors in tomatoes that stimulate growth of some *Lactobacillus* species. These factors can be added to raw materials during food bioprocessing or to media to isolate some fastidious bacteria from foods. Foods also contain many chemicals, either naturally or added, that adversely affect microbial growth. Some of the natural inhibitors

are lysozyme in egg, agglutinin in milk, and eugenol in cloves. The inhibitors, depending on their mode of action, can prevent or reduce growth.

iii. Water Activity and Growth

1. Principle

Water activity (A_w) is a measure of the availability of water for biological functions and relates to water present in a food in free form. In a food system, total water or moisture is present in free and bound forms. Bound water is the fraction used to hydrate hydrophilic molecules and to dissolve solutes, and is not available for biological functions; thus, it does not contribute to A_w. The A_w of a food can be expressed by the ratio of water vapor pressure of the food (P, which is <1) to pure water (which is 1).

2. Aw of Food

The A_w of food ranges from ca. 0.1 to 0.99. The A_w values of some food groups are as follows: cereals, crackers, sugar, salt, dry milk, 0.10 to 0.20; noodles, honey, chocolate, dried egg, jam, jelly, dried fruits, parmesan cheese, nuts, 0.60 to 0.85; fermented sausage, dry cured meat, sweetened condensed milk, maple syrup, 0.85 to 0.93; evaporated milk, tomato paste, bread, fruit juices, salted fish, sausage, processed cheese, 0.93 to 0.98; and fresh meat, fish, fruits, vegetables, milk, eggs, 0.98 to 0.99. The A_w of foods can be reduced by removing water (desorption) and increased by the adsorption of water, and these two parameters can be used to draw a sorption isotherm graph for a food. The desorption process gives relatively lower A_w values than the adsorption process does at the same moisture content of a food. This has important implications in the control of a microorganism by reducing the A_w of a food. The A_w of a food can be reduced by several means, such as adding solutes, ions, hydrophilic colloids, and freezing and drying.

3. Aw and Microbial Growth

The free water in a food is necessary for microbial growth. It is necessary to transport nutrients and remove waste materials, carry out enzymatic reactions, synthesize cellular materials, and take part in other biochemical reactions, such as hydrolysis of a polymer to monomers (proteins to amino acids). Each microbial species or group has an optimum, maximum, and minimum A_w level for growth. The minimum A_w values for growth of microbial groups are as follows: most moulds, 0.8, with xerophilic moulds as low as 0.6; most yeasts, 0.85, with osmophilic yeasts, 0.6 to 0.7; most Gram-positive bacteria, 0.90; and Gram-negative bacteria, 0.93. Some exceptions are growth of *Staphlococcus aureus* at 0.85 and halophilic bacteria at 0.75. The A_w need for spore-forming bacteria to sporulate and the spores to germinate and the toxin-producing microorganisms to produce toxins is generally higher than the minimum A*w* for their growth. Also, the minimum A_w for growth in an ideal condition is lower than that in a non-ideal condition. As an example, if minimum A*w* for growth of a bacterial strain at pH 6.8 is 0.91, then at pH 5.5, it can be 0.95 or more. When the A_w is reduced below the minimum level required for growth of a microorganism, the cells remain viable for a while. But if the A_w is reduced drastically, microbial cells in a population lose viability, generally rapidly at first and then more slowly. This information is used to control spoilage and pathogenic microorganisms in food as well as enhance the growth of desirable types in food bioprocessing (adding salt in processing of cured ham) and in laboratory detection of microorganisms (adding salt to media to enumerate *S. aureus*).

iv. pH and Growth

1. Principle

pH indicates the hydrogen ion concentrations in a system and is expressed as $-\log [H^+]$, the negative logarithm of the hydrogen ion or proton concentration. It ranges from 0 to 14, with 7.0 being neutral pH. The $[H^+]$ concentrations can differ in a system, depending on what acid is present. Some strong acids used in foods, such as HCl and phosphoric acid, dissociate completely. Weak acids, such as acetic or lactic acids, remain in equilibrium with the dissociated and undissociated forms:

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

$CH3\ COOH\ [H^+] + [CH3COO^-]$, pH of 0.1 *N* CH3COOH is 2.9

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

2. pH of Food

Depending on the type, the pH of a food can vary greatly. On the basis of pH, foods can be grouped as high-acid foods (pH below 4.6) and low-acid foods (pH 4.6 and above). Most fruits, fruit juices, fermented foods (from fruits, vegetables, meat, and milk), and salad dressings are high-acid (low-pH) foods, whereas most vegetables, meat, fish, milk, and soups are low-acid (high-pH) foods. Tomato, however, is a high-acid vegetable (pH 4.1 to 4.4). The higher pH limit of most low-acid foods remains below 7.0; only in a few foods, such as clams (pH 7.1) and egg albumen (pH 8.5), does the pH exceed 7.0. Similarly, the low pH limit of most high-acid foods remains above 3.0, except in some citrus fruits (lemon, lime, grapefruit) and cranberry juice, in which the pH can be as low as 2.2. The acid in the foods can either be present naturally (as in fruits), produced during fermentation (as in fermented foods), or added during processing (as in salad dressings). Foods can also have compounds that have a buffering capacity. Foods such as

milk or meat, because of good buffering capacity, does not show pH reduction when compared with a vegetable product in the presence of the same amount of acid.

v. pH and Microbial Growth

The pH of a food has a profound effect on the growth and viability of microbial cells. Each species has an optimum and a range of pH for growth. In general, molds and yeasts are able to grow at lower pH than do bacteria, and Gram-negative bacteria are more sensitive to low pH than are Gram-positive bacteria. The pH range of growth for molds is 1.5 to 9.0; for yeasts, 2.0 to 8.5; for Gram-positive bacteria, 4.0 to 8.5; and for Gram-negative bacteria, 4.5 to 9.0. Individual species differ greatly in lower pH limit for growth; for example, Pediococcus acidilactici can grow at pH 3.8 and Sta. aureus can grow at pH 4.5, but normally Salmonella cannot. The lower pH limit of growth of a species can be a little higher if the pH is adjusted with strong acid instead of a weak acid (due to its undissociated molecules). Acid-resistant or tolerant strains can acquire resistance to lower pH compared with the other strains of a species (e.g., acid-resistant Salmonella). When the pH in a food is reduced below the lower limit for growth of a microbial species, the cells not only stop growing but also lose viability, the rate of which depends on the extent of pH reduction. This is more apparent with weak acids, especially with those that have higher dissociation constant (pK), such as acetic acid vs. lactic acid (with pK values 4.8 and 3.8, respectively). This is because at the same pH, acetic acid has more undissociated molecules than lactic acid does. The undissociated molecules, being lipophilic, enter into the cell and dissociate to generate H+ in the cytoplasm. This causes a reduction in internal pH, which ultimately destroys the proton gradient between the inside and the outside of the cells and dissipates proton motive force as well as the ability of the cells to generate energy. The information on the influence of pH on growth and viability of microbial cells is important to

develop methods to prevent the growth of undesirable microorganisms in food (e.g., in acidified foods; see Chapter 35), used to produce some fermented foods (e.g., sequential growth of lactic acid bacteria in sauerkraut fermentation), and to selectively isolate aciduric microorganisms from food (e.g., yeasts and molds in a medium with pH 3.5).8,9 Acquired acid tolerance by pathogens and spoilage bacteria can impose problems in their control in low-pH foods.

vi. Redox Potential, Oxygen, and Growth

1. Principle

The redox or oxidation–reduction (O–R) potential measures the potential difference in a system generated by a coupled reaction in which one substance is oxidized and a second substance is reduced simultaneously. The process involves the loss of electrons from a reduced substance (thus it is oxidized) and the gain of electrons by an oxidized substance (thus it is reduced). The electron donor, because it reduces an oxidized substance, is also called a reducing agent while the electron recipient is called an oxidizing agent. The redox potential, designated as Eh, is measured in electrical units of millivolts (mV). In the oxidized range, it is expressed in +mV, and in reduced range in –mV. In biological systems, the oxidation and reduction of substances are the primary means of generating energy. If free oxygen is present in the system, then it can act as an electron acceptor. In the absence of free oxygen, oxygen bound to some other compound, such as NO_3 and SO_4 can accept the electron. In a system where no oxygen is present, other compounds can accept the electrons. Thus, presence of oxygen is not a requirement of O–R reactions.

2. Redox Potential in Food

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

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

3. Redox Potential and Microbial Growth

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

+100 to –250 mV or lower. However, this varies greatly with concentrations of reducing components in a food and the presence of oxygen. Moulds, yeasts, and *Bacillus, Pseudomonas, Moraxella*, and *Micrococcus* genera are some examples that have aerobic species. Some examples of facultative anaerobes are the lactic acid bacteria and those in the family *Enterobacteriaceae*. The most important anaerobe in food is *Clostridium*. An example of a microaerophile is *Campylobacter* spp. The Eh range indicates that in each group some species are stricter in their Eh need than others. Although most moulds are strict aerobes, a few can tolerate less aerobic conditions. Similarly, yeasts are basically aerobic, but some can grow under low Eh (below +300 mV). Many *clostridial* species can grow at Eh +100 mV, but some need –150 mV or less. The presence or absence of oxygen and the Eh of food determine the growth capability of a particular microbial group in a food and the specific metabolic pathways used during growth to generate energy and metabolic by-products. This is important in microbial spoilage of a food (such as putrification of meat by *Clostridium* spp. under anaerobic conditions) and to produce desirable characteristics of fermented foods (such as growth of *Penicillium* species in blue cheese under aerobic conditions). This information is also important to isolate microorganisms of interest from foods (such as *Clostridium laramie*, a strict anaerobe from spoiled meat) in the laboratory.

4.3.2 Extrinsic Factors

Extrinsic factors important in microbial growth in a food include the environmental conditions in which it is stored. These are temperature, relative humidity, and gaseous environment. The relative humidity and gaseous condition of storage, respectively, influence the A_w and Eh of the food.

A. Temperature and Growth

1. Principle

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

2. Food and Temperature

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

3. Microbial Growth and Viability

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

(1) Thermophiles (grow at relatively high temperature), with optimum ca. 55^{o}C and range from 45 to 70^{o}C.

(2) Mesophiles (grow at ambient temperature), with optimum at 35^{o}C and range 10 to 45^{o}C.

(3) Psychrophiles (grow at cold temperature), with optimum at 15°C and range –5 to 20°C. However, these divisions are not clear-cut and overlap each other. Two other terms used in food microbiology are very important with respect to microbial growth at refrigerated temperature and survival of microorganisms to low heat treatment or pasteurization, because both methods are widely used in the storage and processing of foods.

Psychrotrophs are microorganisms that grow at refrigerated temperature (0 to 5°C), irrespective of their optimum range of growth temperature. They usually grow rapidly between 10 and 30°C. Moulds; yeasts; many Gramnegative bacteria from genera *Pseudomonas*, *Achromobacter*, *Yersinia*, *Serratia*, and *Aeromonas*; and Gram-positive bacteria from genera *Leuconostoc*, *Lactobacillus, Bacillus, Clostridium*, and *Listeria* are included in this group.

Microorganisms that survive pasteurization temperature are designated as thermodurics. They include species from genera *Micrococcus, Bacillus, Clostridium, Lactobacillus, Pediococcus*, and *Enterococcus*. Bacterial spores are also included in this group. They have different growth temperatures and many can grow at refrigerated temperature as well as thermophilic temperature. When the foods are exposed to temperatures beyond the maximum and minimum temperatures of growth, microbial cells die rapidly at higher temperatures and relatively slowly at lower temperatures. Microbial growth and viability are important considerations in reducing food spoilage and enhancing safety against pathogens, as well as in food bioprocessing. Temperature of growth is also effectively used in the laboratory to enumerate and isolate microorganisms from foods.

Self- Assessment Exercises 1

1. Based on temperature requirement, write the three groups of microorganisms and their temperature ranges.
2. What is a pH?
3. What are the major groups of carbohydrate?

4.4 Summary

The physical and chemical environments control microbial growth within the growth range mainly by influencing their metabolic process associated with synthesis of energy and cellular components. Beyond the growth range, these factors, either individually or in combination, can be used to control microbial growth and even to destroy them. Actual growth is accomplished through the metabolism of various nutrients present in a food.

4.5 Glossary

Aerobes: bacteria that require oxygen for growth and multiplication.

Anaerobes: bacteria that cannot grow in the presence of oxygen.

Condiments: spices blended with other components and have a sauce-like consistency (catsup, mustard).

Culture: the whole complex of traditional behaviour which has been developed by the human race and is successively learned by each generation.

Dissacharides: sugars that yield two Monosaccharides on hydrolysis.

Food: anything eaten by man or animal to satisfy appetite, meet physiological needs for growth, maintain all body processes and supply energy to maintain body temperature and activities.

Fungi: single-celled or multicellular organism without chlorophyll that reproduces by spores and lives by absorbing nutrients from organic matter.

Macronutrients: These are elements required in large quantity for the normal growth and development of an organism.

Monosaccharides: simple sugars and the most important of them are glucose, fructose and galactose.

pH: the hydrogen ion concentrations in a system and is expressed as $-\log [H^+]$.

Polysaccharides: polysaccharides are sugars that yield more than two monosaccharides.

Sanitation: the maintenance of hygienic conditions, through services such as garbage collection and waste disposal.

Spices: plant products (seed, flower, leaf, bark, roots, or bulb) used whole or ground, singly or mixed for aroma and flavour.

Viruses: intracellular obligate parasite which can trigger dangerous infections in humans when they contaminate our food.

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 4.7 **Possible Answers to Self-Assessment Exercises**

Answers to Self-Assessment Exercises 1

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

(1) Thermophiles (grow at relatively high temperature), with optimum ca. 55^{o}C and range from 45 to 70^{o}C.

(2) Mesophiles (grow at ambient temperature), with optimum at 35^{o}C and range 10 to 45^{o}C.

(3) Psychrophiles (grow at cold temperature), with optimum at 15^{o}C and range –5 to 20^{o}C.

2. pH indicates the hydrogen ion concentrations in a system and is expressed as –log [H^{+}], the negative logarithm of the hydrogen ion or proton concentration.
3. Monosaccharides, Disaccharides, Oligosaccharides and Polysaccharides

MODULE 2

UNIT 1 INDICATOR ORGANISM

CONTENTS

1.1 INTRODUCTION

All pathogenic microorganisms implicated in foodborne diseases are considered enteric pathogens, except *Staphylococcus aureus, Bacillus cereus, Clostridium botulinum* (except in the case of infant botulism), *C. perfringens*, and toxicogenic moulds. This means they can survive and multiply or establish in the gastrointestinal (GI) tract of humans, food animals, and birds. A

food contaminated directly or indirectly with fecal materials from these sources may theoretically contain one or more of these pathogens and can thus be potentially hazardous to consumers. To implement regulatory requirements and ensure consumer safety, it is necessary to know that a food is either free of some enteric pathogens, such as *Salmonella serovars* and *Escherichia coli O157:H7*, or contains low levels of some other enteric pathogens, such as *Yersinia enterocolitica* and *Vibrio parahaemolyticus*. The procedures used to isolate and confirm a pathogen from a food involve several steps, take a relatively long time, and are costly. Some of the new tests involving molecular biology techniques require high initial investment and highly skilled technicians. In a modernized, large commercial operation, involving procurement of ingredients from many countries, processing of different products, warehousing, distribution over a large area, and retail marketing, it is not practical or economical to test the required number of product samples from each batch for all the pathogens or even those that are suspected of being present in a particular product. Instead, food samples are examined for the number (or level) of groups or a species of bacteria that are of faecal (enteric) origin, usually present in higher density than pathogens, but usually considered to be non-pathogenic. Their presence is viewed as resulting from direct or indirect contamination of a food with faecal materials and indicates the possible presence of enteric pathogens in the food. These bacterial groups or species are termed indicators of enteric pathogens. Although *S. aureus, C. botulinum, C. perfringens*, and *B. cereus* can be present in the faecal matters of humans and food animals, they, along with toxigenic moulds, are not considered classical enteric pathogens. Their presence in a food is not normally considered to be because of faecal contamination, and the indicators of enteric pathogens are not very effective for the purpose. To determine the presence of these microorganisms and their toxins, specific methods recommended for their detection and

identification should be used. The aerobic plate count (APC) or standard plate count (SPC) is not an indicator of possible presence of pathogens. Instead it is an indicator of the microbiological quality of a food as well as a measure of the level of sanitation used in the handling (processing and storage) of a food.

Criteria for Ideal Indicators

Several criteria were suggested for selecting a bacterial group or species as an indicator of enteric foodborne pathogens. Some are listed with brief explanations:

1. The indicator should preferably contain a single species or a few species with some common and identifiable biochemical and other characteristics in order to be able to identify them from the many different types of microorganisms that might be present in a food.

2. The indicator should be of enteric origin, that is, it should share the same habitat as the enteric pathogens and is present when and where the pathogens are likely to be present.

3. The indicator should be nonpathogenic so that its handling in the laboratory does not require safety precautions as required for pathogens.

4. The indicator should be present in the fecal matter in much higher numbers than the enteric pathogens so they can be easily detected (enumerated or isolated) even when a food is contaminated with small amounts of fecal matter.

5. The indicator should be detected (enumerated or isolated) and identified within a short time, easily, and economically, so that a product, following processing, can be distributed quickly, and several samples from a batch can be tested.

6. The indicator should be detected by using one or more newly developed molecular biology techniques for rapid identification.

7. The indicator should be detected (enumerated or isolated) even in the presence of large numbers of associated microorganisms, which can be achieved by using compounds that inhibit growth of associated microorganisms but not of the indicator.

8. The indicator should have a growth and survival rate in a food as that of the enteric pathogens. It should not grow slower or die off faster than the pathogens in a food. If it dies off more rapidly than the pathogen, then, theoretically, a food can be free of the indicator during storage but can still have pathogens.

9. The indicator should not suffer sublethal injury more (in degree) than the pathogens do when exposed to physical and chemical stresses. If the indicator is more susceptible to sublethal stresses, it will not be detected by the selective methods used in the enumeration, and a food may show no or very low acceptable levels of the indicator even when the pathogens are present at higher levels.

10. The indicator should preferably be present when the pathogens are present in a food; conversely, it should be absent when the enteric pathogens are absent. Unless such correlations exist, the importance of an indicator to indicate the possible presence of a pathogen in a food reduces greatly.

11. There should preferably be a direct relationship between the level of an indicator present and the probability of the presence of an enteric pathogen in a food. This will help set up regulatory standards or specifications for an indicator limit for the acceptance or rejection of a food for consumption. For this criterion, it is very important to recognize whether the high numbers of an indicator in a food have resulted from a high level of initial contamination (and a greater chance for the presence of a pathogen) or from their growth in the food from a very low initial

contamination (in which case a pathogen may not be present even when the indicator is present in high numbers).

It is apparent that no single bacterial group or species will be able to meet all the criteria of an ideal indicator. Several bacterial groups or species satisfy many of these criteria. The characteristics, advantages, and disadvantages of some of the important and accepted indicator bacterial groups and species (of enteric pathogens) are described here.

1.2 Learning Outcomes

By the end of this unit, you should be able to:

- Discuss Indicator Organisms
- Write the criteria for Ideal Indicators
- Analyse the different groups of Indicator Organisms

1.3 Indicator Bacteria Groups and Species

1.3.1 Coliform Group

A. Coliforms

In the coliform group, coliforms, faecal coliforms, and *E. coli* will be discussed.

They are not separate, as both faecal coliforms (mostly *E. coli*) and *E. coli* belong to coliforms. Depending on the situation, food, water, and environmental samples are examined for one or more of the three.

1. **Organisms and Sources**

The term coliform does not have taxonomic value; rather, it represents a group of species from several genera, namely, *Escherichia, Enterobacter, Klebsiella, Citrobacter*, and probably *Aeromonas and Serratia*. The main reason for grouping them together is their many common

characteristics. They are all Gram-negative, nonsporeforming rods; many are motile, are facultative anaerobes resistant to many surface-active agents, and ferment lactose to produce acid and gas within 48 hours at 32 or 35°C. Some species can grow at higher temperature (44.5°C), whereas others can grow at 4 to 5°C. All are able to grow in foods except in those that are at pH $\leq$ 4.0 (a few that are acid resistant can grow or survive) and $A_w \leq 0.92$. All are sensitive to low-heat treatments and are killed by pasteurization. They can be present in faeces of humans and warm-blooded animals and birds. Some can be present in the environment and contaminate food. Thus, some *Klebsiella* spp. and *Enterobacter* spp. are found in soil, where they can multiply and reach high population levels. Some are found in water and plants.

2. Occurrence and Significance in Food

Coliforms are expected to be present in many raw foods and food ingredients of animal and plant origin. In some plant foods, they are present in very high numbers because of contamination from soil. Because they can grow in foods, some even at refrigerated temperature, a low initial number can reach a high level during storage. The occurrence of some coliforms of nonfecal origin and their ability to grow in many foods reduce the specificity of coliforms as an indicator of fecal contamination in raw foods. In contrast, in heat-processed (pasteurized) products, their presence is considered postheat-treatment contamination from improper sanitation. In heat-processed foods, their presence (even in small numbers) is viewed with caution. Thus, in heat-processed foods, their specificity as an indicator is considered favorably (more as an indicator of improper sanitation than fecal contamination). Several selective media have been recommended to determine coliform numbers in food samples. These are selective-differential media and differ greatly in their recovery ability of coliforms. The results are based on the ability of most coliforms to ferment lactose and produce gas and are available in 1 to 2 days. The presence of

sublethally stressed or injured cells can considerably reduce the recovery in selective media. Several other factors, such as high temperature of melted agar media in pour plating, high acidity of a food, and presence of lysozymes (egg-based products) in a food, can further reduce the enumeration of stressed cells. Modified detection methods have been developed to recover injured coliforms. Even with some disadvantages, coliforms are probably the most useful and most extensively used indicators.

B. Faecal Coliforms

1. Organisms and Sources

Fecal coliform bacteria also constitute a group of bacteria and include those coliforms whose specificity as fecal contaminants is much higher than that of coliforms. This group includes mostly *E. coli*, along with some *Klebsiella* and *Enterobacter* spp. Non-faecal coliforms are eliminated by using a high incubation temperature (44.5 ± 0.2 or $45.0 \pm 0.2^{o}C$) for 24 hours in selective broths containing lactose. Lactose fermentation, with the production of gas, is considered a presumptive positive test.

2. Occurrence and Significance in Food

Some faecal coliforms are present in raw foods of animal origin. They can be present in plant foods from contaminated soil and water. High numbers can be due to either gross contamination or growth from a low initial level, probably because of improper storage temperature. Their presence in heat-processed (pasteurized) foods is probably because of improper sanitation after heat treatment. In raw foods that are to be given heat treatment, their presence, even in high numbers (10^3/g or /ml) is not viewed gravely; if the numbers go higher, some importance is given to contamination of fecal matter, improper sanitation, and possible presence of enteric pathogens. A need for corrective measures becomes important. In contrast, in heated products

and ready-to-eat products (even raw), their presence, especially above a certain level, is viewed cautiously for possible faecal contamination and presence of enteric pathogens. A food can be accepted or rejected based on the numbers present. This group is extensively used as an indicator in foods of marine origin (shellfish) and in water and wastewater.

C. *Escherichia coli*

1. Organisms and Sources

In contrast to either coliforms or fecal coliforms, *E. coli* has a taxonomic basis. It includes only the *Escherichia* spp. of the coliform and faecal coliform groups. *E. coli* strains conform to the general characteristics described for coliform groups. Biochemically, they are differentiated from other coliforms by the indole production from tryptone, methyl red reduction due to acid production (red coloration), Voges Proskauer reaction (production of acetyl-methyl carbinol from glucose) and citrate utilization as a C-source (IMViC) reaction patterns. *E. coli* Type I and Type II give IMViC reaction patterns, respectively, of + + – – and – + – –. The – + – – reaction pattern of *E. coli* Type II could also be due to slow or low production of indole from tryptone or peptone. The IMViC tests are conducted with an isolate obtained after testing a food sample for coliform group or fecal coliform group. However, there is a concern now about the adequacy of these reaction patterns to identify *E. coli* types. Initially, *E. coli* types were used as indicators of faecal contamination and possible presence of enteric pathogens (in food), with the considerations that they are non-pathogenic and occur normally in the GI tract of humans, animals, and birds in high numbers. However, it is now known that some variants and strains of *E. coli* are pathogenic (e.g. *E. coli O157:H7*). None of the methods mentioned previously are able to differentiate pathogenic from non-pathogenic *E. coli* strains but can be achieved only through specific tests designed to identify different pathogenic *E. coli* strains.

3. Occurrence and Significance in Food

E. coli is present in the lower intestinal tract of humans and warm-blooded animals and birds. Its presence in raw foods is considered an indication of direct or indirect fecal contamination. Direct fecal contamination occurs during the processing of raw foods of animal origin and because of poor personal hygiene of food handlers. Indirect contamination can occur through sewage and polluted water. In heat-processed (pasteurized) foods, its presence is viewed with great concern. Its value as an indicator of fecal contamination and the possible presence of enteric pathogens is much greater than that of coliform and fecal coliform groups. However, the time to complete the tests (IMViC) is relatively long (5 days). Some direct plating methods have been developed that give an indication of *E. coli* in a shorter time. There are several other inadequacies of *E. coli* as an indicator. *E. coli* strains may die at a faster rate in dried, frozen, and low-pH products than some enteric pathogens, and some enteric pathogens can grow at low temperatures (0 to 2^{o}C), at which *E. coli* strains can die. *E. coli* strains can be injured by sublethal stresses in higher degrees than some enteric pathogens, and may not be effectively detected by the recommended selective media unless a prior resuscitation (repair) step is included.

1.3.2 *Enterobacteriaceae* Group

The methods recommended for detecting coliforms and faecal coliforms and *E. coli* are based on the ability of these bacterial species to ferment lactose to produce gas and acid. In contrast, some enteric pathogens do not ferment lactose, such as most *Salmonella serovars*. Thus, instead of only enumerating coliforms or faecal coliforms in a food, enumeration of all the genera and species in the *Enterobacteriaceae* family is advocated because this family includes not only coliforms but also many genera and species that are enteric pathogens, enumeration of the whole group can be a better indicator of the level of sanitation, possible fecal contamination, and

possible presence of enteric pathogens. In European countries, this concept has been used to a certain degree. The method includes the enumeration of organisms from colony-forming units in a selective-differential agar medium containing glucose instead of lactose.

This concept is criticized because many species in the *Enterobacteriaceae* are not of fecal origin; many are found naturally in the environment, including plants, and those that form typical colonies because of glucose fermentation in the selective medium do not all belong to this family. However, in heat-processed foods (all are sensitive to pasteurization) and ready-to-eat foods, their presence in high numbers should have public health significance.

1.3.3 *Enterococcus* Group

1. Characteristics and Habitat

The genus *Enterococcus* is relatively new and includes many species that were previously grouped as faecal *Streptococci* and other *Streptococci*. They are Grampositive, non-sporeforming, non-motile cocci or coccobacilli, catalase negative, and facultative anaerobes. They can grow between 10 and 45^{o}C, and some species can grow at 50^{o}C. Some require B vitamins and amino acids for growth. Some can survive pasteurization temperature. They are more resistant than most coliforms to refrigeration, freezing, drying, low pH, NaCl, and water. They are found in the intestinal tracts of humans and warm- and cold-blooded animals, birds, and insects. Some can be species specific whereas others can be present in humans, warm-blooded animals, and birds. Among the currently recognized species, several are found in the intestine of humans and food animals and birds, including *Enterococcus faecalis*, *E. faecium*, *E. durans*, *E. gallinarum*, *E. avium*, and *E. hirae*. Many have been found in vegetation, processing equipment, and processing environments. Once established, they can continue to multiply in the equipment and environment and are often difficult to completely remove. They are found in sewage and

water, especially polluted water and mud. They probably do not multiply in water, but can survive longer than many coliforms. They can grow in most foods.

2. **Occurrence and Significance in Food**

Enterococcus can get in different foods through faecal contamination or through water, vegetation, or equipment and processing environments, and may not be of faecal origin. In this respect, its value as an indicator of faecal contamination and possible presence of enteric pathogens in food is questionable. Also, the ability of some strains to survive pasteurization temperature (being thermodurics) reduces their value as an indicator. On the other hand, their better survivability in dried, frozen, refrigerated, and low-pH foods and water can make them favourable as indicators. Currently, their presence in high numbers, especially in heat-processed foods, can be used to indicate their possible presence in high numbers in raw materials and improper sanitation of the processing equipment and environment. They have been used to determine the sanitary quality of water in shellfish beds and are considered to be better as indicators than coliforms for shellfish. Some strains have also been associated with foodborne gastroenteritis, probably as opportunistic pathogens.

Self- Assessment Exercises 1

1. What are coliforms?
2. Write three bacteria in faecal coliform group.
3. Write a strain of E. coli that is pathogenic

1.4 Summary

In food microbiology, the concept of indicator bacteria was introduced to measure the sanitary quality of pasteurized foods (e.g., milk). Its purpose was to indicate the possible presence of enteric pathogens (e.g., Salmonella) in the food. Over time, the suitability of several bacterial groups has been considered and this aspect has been discussed in this chapter. It is apparent that selection of a suitable indicator and its level may change with food types and for raw, processed, and ready-to-eat food.

1.5 References/Further Readings

Ray, B (2004). Fundamental food microbiology, 3rd edition CRC Press, London New York DC. 625 Pages.

1.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. The term coliform does not have taxonomic value; rather, it represents a group of species from several genera, namely, *Escherichia*, *Enterobacter*, *Klebsiella*, *Citrobacter*, and probably *Aeromonas and Serratia*.
2. This group includes mostly *E. coli*, along with some *Klebsiella* and *Enterobacter* spp.
3. *E. coli O157:H*

UNIT 2 PATHOGENIC AND SPOILAGE MICROORGANISMS

CONTENTS

2.1 Introduction

The microbial groups important in foods consist of several species and types of bacteria, yeasts, moulds, and viruses. Although some algae and protozoa as well as some worms (such as nematodes) are important in foods, they are not included among the microbial groups in this unit. Bacteria, yeasts, moulds, and viruses are important in food for their ability to cause foodborne diseases and food spoilage and to produce food and food ingredients. Many bacterial species and some moulds and viruses, but not yeasts, are able to cause foodborne diseases. Most bacteria, moulds, and yeasts, because of their ability to grow in foods (viruses cannot grow in foods), can potentially cause food spoilage. Several species of bacteria, moulds, and yeasts are considered safe or food grade, or both, and are used to produce fermented foods and food ingredients. Among the four major groups, bacteria constitute the largest group. Because of their ubiquitous

presence and rapid growth rate, even under conditions where yeasts and moulds cannot grow, they are considered the most important in food spoilage and foodborne diseases. Prion or proteinaceous infectious particles have recently been identified to cause transmissible spongiform encephalopathies (TSEs) in humans and animals. However, their ability to cause foodborne diseases is not clearly understood.

2.2 Learning Oucomes

By the end of this unit, you will be able to:

- Discuss the spoilage bacteria
- Discuss the spoilage Yeasts and Moulds

2.3 Pathogenic and Spoilage Microorganisms

2.3.1 Bacteria

i. *Acinetobacter*

Acinetobacter is a genus of gram-negative bacteria belonging to the gamma proteobacteria. Acinetobacter species are non-motile and oxidase-negative and occur in pairs as observed under magnification. Young cultures show rod shaped morphology. They are strict aerobes that do not reduce nitrates. They are important soil and water organisms and are also found on many foods especially refrigerated fresh products.

ii. *Bacillus cereus*

B.cereus is a thick long rod shaped gram-positive, catalase positive, aerobic, spore former, and the organism is important in food borne illness. It is a normal inhabitant of soil and is isolated from a variety of foods. It is quite often a cause of diarrheal illness due to the consumption of desserts, meat, dishes, dairy products, rice, pasta etc that are cooked and kept at room

temperature as it is thermoduric. Some of the *B. cereus* strains are psychrotrophic as they grow at refrigeration temperature. *B. cereus* is spread from soil and grass to cows' udders and into the raw milk. It is also capable of establishing in cans, producing proteolytic and amyloltic enzymes, and also phoslipase C (lecithinase). The production of these enzymes by these organisms can lead to the spoilage of foods. The diarrheal illness is caused by an enterotoxin produced during the vegetative growth of *B. cereus* in small intestine. The bacterium has a maximum growth temperature around 48°C to 50°C and pH range 4.9 to 9.3. Like other spores of mesophilic *Bacillus* species, spores of *B. cereus* are also resistant to heat and survive pasteurization temperature.

iii. Bacillus subtilis

Bacillus subtilis known also as the hay *bacillus* or grass *bacillus* is a Gram-positive, catalase-positive bacterium commonly found in soil. A member of the genus *Bacillus*, *B. subtilis* is thin short rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. *B. subtilis* produces the proteolytic enzyme subtilisin. *B. subtilis* spores can survive the extreme heat during cooking and is responsible for causing ropiness a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides in spoiled bread dough. A strain of *B. subtilis* formerly known as

iii. ***Corynebacterium***

Corynebacterium is a genus of gram-positive rod-shaped bacteria. They are widely distributed in nature and are mostly innocuous. Some are useful in industrial settings such as *C. glutamicum*. Others can cause human disease. *C. diphtheriae*, for example, is the pathogen responsible for diphtheria. Some species are known for their pathogenic effects in humans and other animals. Perhaps the most notable one is *C. diphtheriae*, which acquires the capacity to produce

diphtheria toxin only after interacting with a bacteriophage. Diphtheria toxin is a single, 60,000 molecular weight proteins composed of two peptide chains, fragment A and fragment B, held together by a disulfide bond.

v. Clostridium perfringens

C. perfringens is a Gram-positive encapsulated anaerobic non-motile bacterium commonly found on meat and meat products. It has the ability to cause food borne disease. It is a toxin producing organism-produces *C. perfringens* enterotoxin and β -toxin that are active on the human GI tract. It multiplies very rapidly in food (doubling time < 10 minutes). Spores are resistant to radiation, desiccation and heat and thus survive in incompletely or inadequately cooked foods. However, it tolerates moderate exposure to air. Vegetative cells of C. perfringens are also somewhat heat tolerant as they have relatively high growth temperature (43°C -45 °C) and can often grow at 50°C. They are not tolerant to refrigeration and freezing. No growth occurs at 6°C. *C. perfringens* is present in soil and the other natural environment.

vi. Clostridium botulinum

C. botulinum produces the most potent toxin known. It is a gram-positive anaerobic rod shaped bacterium. Oval endospores are formed in stationary phase cultures. There are seven types of *C. botulinum* (A to G) based on the serological specificity of the neurotoxin produced. Botulism is a rare but very serious disease. The ingestion of neurotoxin produced by the organism in foods can lead to death. However, the toxin (a protein) is easily inactivated by heat. The organism can grow at temperature ranging from 10-48°C with optimum growth temperature at 37°C. Spores are highly heat resistant. The outgrowth of spores is inhibited at pH < 4.6, NaCl> 10% or water activity< 0.94. Botulinum spores are probably the most radiation resistant spores of public health concern. Contamination of foods is through soil and sediments where they are commonly

present. The organism grows under obligate anaerobic conditions and produces toxin in under processed (improper canning) low acid foods at ambient temperature.

vii. *Campylobacter*

Campylobacter are gram negative non spore forming rods. *Campyloleacter jejuni* is an important food borne pathogen. It is one of the many species within the genus *Campylobacter*. *Campylobacter* species *C.jejuni and C. coli* cause diarrhea in humans. The organism is heat sensitive (destroyed by milk pasteurization temperature). It is also sensitive to freezing. The organism belongs to the family *Campylobactereaceae*. The organisms are curved, S-shaped, or spiral rods that may form spherical or coccoids forms in old cultures or cultures exposed to air for prolonged periods. Most of the species are microaerophilic. It is oxidase and catalase positive and does not grow in the presence of 3.5% NaCl or at 25 °C or below. The incidences reported for gastroenteritis by this organism are as high as in case of *Salmonella*. The organism is commonly present in raw milk, poultry products, fresh meats, pork sausages and ground beef.

Viii. Enterococcus (E. faecium, E. faecalis)

Enterococcus is a genus of lactic acid bacteria, is a gram positive cocci that often occur in pairs (diplococci) or short chains and are difficult to distinguish from *Streptococci* on physical characters. The two species are commensal organisms in the intestine of humans. The *Enterococci* are facultative anaerobic organisms non-spore forming that grows optimally at 35°C. However, they tolerate wide range of environmental conditions (10-45°C) pH (4.5 to 10.5), quite high NaCl concentration (6.5%) and can survive heating at 60°C for 30 minutes. Catalase-negative, oxidase negative-bacteria of the genes *Enterococcus* are ubiquitous organisms that often occur in large numbers on vegetables, plant materials and foods especially those of animal origin such as meat and dairy products. *Enterococci* also constitute a large preparation of

autochthonous bacteria associated with the mammalian gastro-intestinal tract. The resistance of *Enterococci* to pasteurization temperatures and their adaptability to different substrates and growth conditions in food products manufactured from raw materials and in heat treated food products is of great significance. *Enterococci* may constitute an important part of the microflora of fermented cheese and meats.

ix. Escherichia coli:

E. coli strains are associated with food borne gastroenteritis. These are Gram-negative asprogeneous rods that ferment lactose and produce dark colonies with a metallic sheen on Endo agar. The organism grows well on a large number of media and in many foods. They grow over a wide range of temperature (4 to 46 °C) and pH (4.4 to 9.0). However, they grow very slowly in foods held at refrigerator temperatures (5°C). They belong to the family *Enterobacteriaceae*. The organism is also an indicator of faecal pollution. The organism is also capable of producing acid and gas and off-flavours in foods. *E. coli* strains involved in foodborne illness can be placed into five groups:

a. Enteropathogenic *E. coli* (EPEC)

b. Enterotoxigenic *E. coli* (ETEC)

c. Enteroinvasive *E. coli* (EIEC)

d. Enterohemorrhagic *E. coli* (EHEC)

e. Facultatively enteropathogenic *E. coli* (FEEC).

The organism also grows in the presence of bile salts. The primary habitat of *E.coli* is the intestinal tract of most warm blooded animals. *E.coli 0157: H7* strains are unusually tolerant of acidic environments.

x. Lactococcus

L. lactis subsp. *lactis L. lactis* subsp. *cremoris L. lactis* subsp. *lactis* biovar diaectylactis *Lactococcus* is a genus of lactic acid bacteria that were formerly included in the genus *Streptococcus* Group N (Group N *Streptococci*). They are known as homofermentors meaning that they produce a single product of glucose fermentation. They are Gram-positive, catalase negative, non-motile coccus that is found singly, in pairs or in chains. Some of the strains of *lactococci* are known to grow at or below 7 °C. *Lactococci* are intimately associated with dairy products. These organisms are commonly used in the dairy industry in the manufacture of fermented dairy products like cheeses. They can be used in single strain starter cultures or in mixed strain cultures with other lactic acid bacteria such as *Lactobacillus and Streptococcus.*

Their main purpose in dairy production is the rapid acidification of milk. This causes drop in the pH of fermented product which prevents the growth of spoilage and pathogenic bacteria. These bacteria also play a role in the flavour of the final product. Dairy *lactococci* have also been exploited for several industrial fermentations in the biotechnology industry. They are easily grown at industrial scale up on cheap whey-based media. *Lactococcus lactis* subsp. *lactis* includes species formerly designated as *S. lactis subsp. lactis. L. lactis* subsp. *cremoris* is distinguished from *L. Lactis* subsp. *lactis* by the inability to:

i. Grow at 40 °C
ii. Grow in 4% NaCl
iii. Hydrolyse arginine
iv. Ferment ribose.

Lactobacillus (L. bulgaricus, L. helveticus. L. plantarum, L. acidophilus, L. casei, L. lactis, L. fermentum): The organisms belonging to this important genus are rods usually long and slender and in some of the species form chains. They are aerotolerant/microaerophilic but some

ferment sugars chiefly to lactic acids if they are homofermentative. The hetero fermentative species, besides lactic acid, also produce small amount of acetic acid, carbon dioxide and trace amounts of volatile compounds such acetaldehyde and alcohol. The homofermentative species of *Lactobacillus* include *L. bulgaricus, L. casei, L. helveticus, L. lactis, L. acidophilus* and grow optimally at 37 °C. *L. fermentum, L. brevis* are the typical example of hetero fermentative Lactobacillus and grow well at higher temperatures. Lactobacilli are of considerable importance in foods as they ferment sugar to lactic acid and other desirable flavouring compounds and are thus used in the production of fermented plant dairy and meat products. However, they are also implicated in the spoilage of wine and beer. The organism normally occurs on plant surfaces silage, manure and dairy products. They are quite fastidious in their nutritional requirements as they are unable to synthesize certain vitamins they require and, therefore, media need to be supplemented with these vitamins for their growth.

xi. Leuconostoc

Leuconostoc is a genus of gram-positive bacteria, placed within the family of *Leuconostocaceae*. They are generally ovoid cocci often forming chains. *Leuconostoc* spp. is intrinsically resistant to vancomycin and is catalase-negative (which distinguishes them from *Staphylococci*). All species within this genus are heterofermentative and are able to produce dextran from sucrose. They are generally slime-forming. Blamed for causing the 'stink' when creating a sourdough starter, some species are also capable of causing human infection. *Leuconostoc* spp. along with other lactic acid bacteria such as *Pediococcus* and *Lactobacillus* spp, is responsible for the fermentation of cabbage, to sauerkraut. In this process the sugars in fresh cabbage are transformed to lactic acid which gives it a sour flavour and good keeping qualities.

xii. Listeria monocytogenes

Listeria monocytogenes in foods has attracted worldwide attention due to the serious illness it causes in human. The *Listeria* is Gram positive non-spore forming, non-acidfast rods. The organism is catalase positive and produces lactic acid from glucose and other fermentable sugars. The organism grows well in brain heart infusion (BHI), trypticase soy, and tryptose broths. However, the medium should be fortified with B. vitamins and the amino acids. It is a mesophilic organism with optimal growth temperature 37°C but it can also grow at refrigerator temperature. Strains grow over the temperature range of 1°C to 45°C and pH range 4.1 to 9.6. *Listeria monocytogenes* is widely distributed in nature and can be isolated from decaying vegetation, soil, animal feces, sewage, silage and water. The organism has been found in raw milk, pork, raw poultry, ground beef and vegetables. The most significant virulence factor associated with *L. monocytogenes* is listeriolysin O. The virulent strains produce β-hemolysis on blood agar and acid from rhamnose. *L. monocytogenes* grows well in moderate salt concentrations (6.5%). *L. monocytogenes* is unique among foodborne pathogens while other pathogens excrete toxins or multiply in the blood stream, *L. monocytogenes* enters the host‘s cells and grows inside the cell. In humans it crosses the intestinal barrier after entering by the oral route. Ready to Eat (RTE) foods that are preserved by refrigeration pose a special challenge with regard to *L. monocytogenes* infection.

xiii. ***Micrococcus***

Micrococcus occurs in a wide range of environments, including water, dust, and soil. *Micrococci* are Gram positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and typically appear in tetrads. *Micrococcus* has a substantial cell wall, which may comprise as much as 50% of the cell mass. Some species of *Micrococcus*, such as *M. luteus*, *M. roseus* (red)

produce yellow or pink colonies when grown on mannitol salt agar. *Micrococcus* is generally thought to be a saprophytic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune systems, such as HIV patients.

xiv. ***Proteus***

Since it belongs to the family of *Enterobacteriaceae*, general characters have been applied on this genus: It is oxidase negative, but catalase and nitrate reductase positive. Three species *P. vulgaris, P. mirabilis,* and *P. penneri* are opportunistic human pathogens. *Proteus* includes pathogens responsible for many human urinary tract infections. *P. mirabilis* causes wound and urinary tract infections. Most strains of *P. mirabilis* are sensitive to ampicillin and cephalosporins but *P. vulgaris* is not sensitive to these antibiotics. However, this organism is isolated less often in the laboratory and usually only targets immune suppressed individuals. *P. vulgaris* occurs naturally in the intestines of humans and a wide variety of animals, manure, soil,, and polluted waters. *P. mirabilis,* once attached to urinary tract, infects the kidney more commonly than *E. coli. P. mirabilis* are often found as free-living organisms in soil and water.

xv. Pseudomonas fluorescens

Pseudomonas fluorescens is a common Gram-negative, rod-shaped, motile bacterium. The organism is psychrotrophic in nature and grows at refrigeration temperature (7°C). It has an extremely versatile metabolism and can be found in the soil and in water. It is an obligate aerobe, but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. Optimal temperature for growth of *Pseudomonas fluorescens* is 25-30 °C. It is oxidase positive a*nd* a nonsaccharolytic organism. Heat-stable lipases and proteases are produced by *Pseudomonas fluorescens* and other similar *Pseudomonads*. These enzymes cause

milk to spoil, by causing bitterness, casein breakdown, and ropiness due to the production of slime and coagulation of proteins.

xvi. Pseudomanas aeruginosa

Pseudomanas aeruginosa is a gram-negative, aerobic, rod-shaped bacterium with unipolar motility. It is an opportunistic human and plant pathogen. Gram-stained *Pseudomonas aeruginosa* bacteria (pink-red rods) secretes a variety of pigments, including pyocyanin (blue green), pyoverdine (yellow-green and fluorescent), and pyorubin (red brown). *P. aeruginosa* is often preliminarily identified by its fluorescence and grape-like or tortilla-like odour in vitro. Definitive clinical identification of *P. aeruginosa* often includes identifying the production of pyocyanin and fluorescein, as well as its ability to grow at 42°C. it is capable of growth in diesel and jet fuel, where it is known as a hydrocarbon-using microorganism (or "HUM bug"), causing microbial corrosion. *P. aeruginosa* is considered by many as a facultative anaerobe.

Xvii. ***Salmonella*** (*S. typhimurium, S. typhi, S.enteritidis*)

Salmonella spp. has been reported to be a leading cause of foodborne illnesses in humans. Foodborne salmonellosis scores over all other foodborne bacterial illnesses in humans. Enteric fever is a serious human disease associated with typhoid and paratyphoid strains. *Salmonella* belong to the family *Enterobacteriaceae*. The optimum growth temperature is 37-45 °C. The organism can also grow at about 7°C in foods. It ferments carbohydrates with its production of acid and gas. *Salmonella* are oxidase negative, catalase positive and grow on citrate as a sole carbon source and produce H_2S. Some *Salmonella* strains can grow at higher temperatures (54 °C) while others exhibit psychrotrophic properties. The organism has the ability to grow at pH values ranging from 4.5 to 9.5, with an optimum pH growth at 6.5 to 7.5. it is facultatively anaerobic, Gram-negative, non-spore forming, rod-shaped (2-4 μm) bacteria belonging to the

family. Milk, meat and poultry are principle vehicles of human foodborne salmonellosis. Ingestion of only a few *salmonella* cells can be infectious. Low levels of *Salmonellae* in finished food products may, therefore, be of serious public health consequence.

Xviii. Serratia

Serratia is a genus of gram-negative, facultatively anaerobic, rod-shaped bacteria of the *Enterobacteriaceae* family. The most common species in the genus, *S. marcescens*, is normally the only pathogen and usually causes nosocomial infections. However, rare strains of *S. plymuthica*, *S. liquefaciens*, *S. rubidaea*, and *S. odoriferae* have caused diseases through infection. Members of this genus produce characteristic red pigment, prodigiosin.

xix. Staphylococcus aureus

Staphylococcus aureus is commonly associated with humans. It is a gram-positive catalase-positive coccus. It is the common cause of foodborne gastroenteritis known as staphylococcal food poisoning. Staphylococcal gastroenteritis is caused by the ingestion of food that contains one or more enterotoxins which are produced by some strains of *S. aureus.* Although enterotoxin production is believed generally to be associated with coagulase and thermo nuclease producing *S. aureus* strains, many species of *Staphylococcus* that produce neither coagulase nor DNase are also known to produce enterotoxin.

xx. Shigella

Bacillary dysentery, or shigellosis, is caused by *Shigella* species. *Shigella* is a member of the family *Enterobacteriaceae*. The growth temperature varies from 10 to 48 °C. *Shigella* does not usually survive well in low pH foods. It is sensitive to ionizing radiations. *Shigella* species are non-motile, oxidase negative produce acid only from sugars and do not grow on citrate as sole carbon source. Bacillary dysentery, or shigellosis is caused by ingestion of contaminated foods,

and in some instances, it subsequently leads to rapid dissemination through contaminated faeces from infected individuals. The infective dose may be as low as 100 cells. Contamination of foods usually does not occur at the processing plant but rather through an infected food handler. Humans are the natural reservoir of *Shigella* and the organism is spread through the fecal-oral route.

xxi. *Vibrio*

Vibrio cholerae and *V. parahaemolyticus* are the two important species of the genus *Vibrio*. *Vibrio cholerae* causes cholera, one of the few foodborne illnesses with epidemic and pandemic potential. *Vibrio cholerae* are Gram-negative straight or curved rods and belong to the family *Vibrionaceae*. Important distinctions within the species are made on the basis of productions of cholera enterotoxin (CT) and serogroup. *Vibrio cholerae* is part of the normal free-living bacterial flora in estuarine areas. Amongst the many different enrichment broths described for the isolation of vibrios but alkaline peptone water is the most commonly used. Though *V. parahaemolyticus* can grow in the presence of 1-8% NaCl, the best growth occurs in the salt concentration of 2 to 4%.

xxii. Yersinia

Yersinia enterocolitica and *Yersinia pestis* are the two important human pathogens while *Y.enterocolitica* causes foodborne gastroenteritis, *Y. pestis* is an agent of human plague. *Y. enterocolitica* also known as newly emerging human pathogen is a heterogeneous species that is divisible into a large number of subgroups. *Y. enterocolitica* is unusual because it can grow at temperatures below 4°C. The generations time at the 28- 30 °C (Optimum growth temperature) is about 34 minutes. It also survives in frozen foods but grows better in processed foods such as pasteurized milk, vacuum packed meat, boiled eggs, boiled fish, and cottage cheese. Both the

species can grow over a pH range of 4 to 10 (optimum pH is 7.6) and tolerate alkaline environment well. They are motile at a temperature < 30 °C. However, both these organisms are susceptible to pasteurization, ionizing and ultraviolet (UV) irradiation. The organism can also tolerate upto 5% NaCl. Infections with *Yersinia* species are due to transmittance of the organism from animals to humans. The organism is frequently present in pork, lamb, poultry and dairy products.

2.3.2 Fungi

2.3.2.1 Yeasts

Yeasts have been associated with foods since earliest times, both as beneficial agents and as major causes of spoilage and economic loss. Current losses to the food and dairy industry caused by *yeast* spoilage are estimated at several billion dollars. As new food ingredients and new food manufacturing technologies are introduced, novel food spoilage yeasts are emerging to present additional problems. To date over 70 biological species of yeasts have been described and thousands of different varieties have been shown to exist in all kinds of natural and artificial habitats. Yeasts may be viewed as being unicellular fungi in contrast to the moulds, which are multi-cellular. Yeasts can be differentiated from bacteria by their larger cell size and their oval, elongate, elliptical, or spherical cell shapes. Typical yeast cells range from 5 to 8 µm in diameter, with some being even larger. Older yeast cultures tend to have smaller cells. Most of those of importance in foods divide by budding or fission. Yeasts can grow in presence of various types of organic acids such as lactic, citric and tartaric acid and also over a wide range of acid pH and in up to 18% ethanol. Many grow in the presence of 55-60% sucrose. Many colours are produced by *yeasts*, ranging from creamy to pink to red. The asco and arthospores of some are quite heat resistant.

i. ***Candida***: Members of the *Candida* genus form shining white colonies and cells contain no carotenoid pigments. Candida tropicalis is the most prevalent in foods in general Some members are involved in the fermentation of cocoa beans, as a component of kefir grains, and many other products, including beers, and fruit juices.

ii. ***Debaromyces***: *Debaromyces* is one of the most prevalent *yeast* genera in the dairy products. It can grow in 24% NaCl and at an A_w as low as 0.65.

iii. ***Kluyveromyces***: *Kluyveromyces* spp. produces β-galctosidase and are vigorous fermenters of sugars including lactose. *K. marxianus* is one of the two most prevalent *yeasts* in dairy products, kefir grain and causes cheese spoilage.

iv. ***Rhodotorula*:** The genus *Rhodotorula* contains many psychrotrophic species that are found on fresh poultry, shrimp, fish and beef. Some grow on the surface of butter.

v. ***Saccharomyces*:** *Saccharomyces* are ascosporogenous *yeasts* that multiply by lateral budding and produce spherical spores in asci. They are diploid and do not ferment lactose. All bakers'brewers', wine and champagne *yeasts* are *S. cerevisiae*. They are found in Kefir grains and can be isolated from wide range of foods. *S. cerevisiae* rarely causes spoilage.

vi. ***Torulaspora***: *Torulaspora* multiplies by lateral budding. They are strong fermenters of sugars. *Torula delbrueckii* is the most prevalent species.

2.3.2.2 Moulds

Moulds are filamentous fungi that grow in the form of tangled mass that spreads rapidly and may cover several inches of area in a very short period. It is also referred to as mycelial growth. Mycelium is composed of branches of filaments referred to as hyphae. The molds of great

importance in foods multiply by ascospores or conidia. The ascospores of some of the mold genera are notable for their extreme degrees of heat resistance.

i. *Alternaria*

Alternaria spp. form septate mycelia with conidiophores and large brown conidia are produced. They cause brown to black rots of fruits, apples, and figs. Some species produce mycotoxins.

ii. *Aspergillus*

The *Aspergillus* spp. appears yellow to green to black on a large number of foods. Some species cause spoilage of oils. *A. niger* produces β-galactosidase, glucoamylase, invertase, lipase and pectinase. *A. oryz*ae produces α-amylase. Two species *A. flavus* and *A. parasiticus* produce aflatoxins, and others produce ochratoxin A and sterigmatocystin.

iii. *Geotrichum* The yeast like fungi, Geotrichum are also referred to as dairy mould .

iv. *Mucor* and *Rhizopus*

Mucor species that produce non-septate hyphae are prominent food spoilers. Similarly, Rhizopus spp. also produces non-septate hyphae but give rise to stolons and rhizoids. *R. stolonifer* is by far the most common species in foods and is also referred to as "bread mold". Other important genera of moulds related to spoilage of foods are *Neurospora*, *Thamnidium*, *Trichothecium*, *Penicillium* and *Cladosporium*.

Self- Assessment Exercises 1

1. *How can L. lactis* subsp. *Cremoris* be distinguished from *L. Lactis* subsp. *Lactis?*
2. Write the five groups of E.coli involved in foodborne illness.

2.4 Summary

Growth of microorganisms in food to a high level causes detectable changes in the quality of food, which is generally termed as spoilage. Spoilage characteristics differ with the differences in microbial type and the food component being metabolized. The foodborne diseases also differ according to the microorganism and its products.

2.5 References

Ray, B (2004). Fundamental food microbiology, 3rd edition CRC Press, London New York DC. 625 Pages.

2.6 Possible Answers to Self-Assessment Exercise

1. *L. lactis* subsp. *cremoris* is distinguished from *L. Lactis* subsp. *lactis* by the inability to:

i. Grow at 40 °C

ii. Grow in 4% NaCl

iii. Hydrolyse arginine

iv. Ferment ribose.

2. *E. coli* strains involved in foodborne illness can be placed into five groups:

a. Enteropathogenic *E. coli* (EPEC)

b. Enterotoxigenic *E. coli* (ETEC)

c. Enteroinvasive *E. coli* (EIEC)

d. Enterohemorrhagic *E. coli* (EHEC)

e. Facultatively enteropathogenic *E. coli* (FEEC).

UNIT 3 PRINCIPLE OF MICROBIAL FOOD SPOILAGE

CONTENTS

3.1 INTRODUCTION

Spoilage of food refers to any visible or invisible change which can make food or product derived from food unacceptable for human consumption. Spoilage of food not only causes health hazard to the consumer but also cause large economic losses. Spoilage not only leads to loss of nutrients from food but also causes change in original flavour and texture. It is estimated that about 25% of total food produced is spoilt due to microbial activities only despite range of preservation methods available. Hence, the spoilage of food is not only a health hazard but also carry lot of economic significance too.

In all, the food spoilage is considered a complex phenomenon whereby a combination of microbial and biochemical activities take place leading to the production of various types of metabolites which aid in spoilage.

All foods undergo varying degrees of deterioration after harvest and during storage, leading to losses in nutritional value, safety, and esthetic appeal (colour, texture, flavour). Fruit, vegetables and root crops are very perishable and, if care is not taken in their harvesting, handling and transport, they will soon decay and become unfit for human consumption. Estimates of production losses in developing countries are hard to judge, but some authorities put losses of sweet potatoes, plantain, tomatoes, bananas and citrus fruit as high as 50 percent of what is grown. Reduction in these losses, particularly if they can be avoided economically, would be of great significance to growers and consumers alike.

All fruits, vegetables and root crops are living plant parts containing 65 to 95 percent water and they continue their life processes after harvest. The post-harvest life of produce depends on the rate at which stored food reserves are used up and the rate of water loss. The changes that occur

not only lead to reduced quality but can also make the product more susceptible to contamination with microorganisms.

Food by nature is subject to deterioration either by chemical or microbial means. The shelf-life will be influenced by factors such as:

1. Nature of the product (nutritional composition)
2. Packaging
3. Temperature

In order to optimize the storage quality and extend shelf-life of fresh and value-added products, a clear understanding of the role of the following factors in food spoilage is important:

i. Chemical components in the food
ii. Environmental conditions
iii. Initial microbial load
iv. Nature and types of microorganisms present

Deterioration, or undesirable quality changes, may be the result of biological, microbiological, biochemical/physiological or physical changes in the product. Factors identified as causes of deterioration usually encourage the conditions that lead to quality losses. These factors are usually the result of inadequate training of product handlers, inadequate or non-existent storage structures, unsuitable or inadequate technologies for handling and storing product, ineffective quality control, and adverse/extreme environmental conditions. In addition, time is an important factor in the spoilage of produce.

3.2 Learning Outcomes

By the end of this unit, you will be able to:

- Write the major sources of food deterioration

- Discuss the role of temperature, packaging and composition of food in food spoilage
- Explain the influence of initial microbial load and the nature and types of microorganisms present
- Discuss the extrinsic and intrinsic factors of food that affect deterioration

3.3 Causes of Food Spoilage

Foods are often classified on the basis of their stability as: non- perishable, semi-perishable and perishable.

1. **Non-perishable foods:** Hermetically sealed and heat processed (canned) foods are generally regarded as non-perishable. However, they may become perishable under certain circumstances when an opportunity for recontamination is afforded following processing. Such an opportunity may arise if the cans are faulty, or if there is excessive corrosion resulting in internal gas formation and eventual bursting of the can. Spoilage may also take place when the canned food is stored at unusually high temperatures: thermophilic spore-forming bacteria may multiply, causing undesirable changes such as flat sour spoilage.

2. **Semi-perishable foods:** Low moisture content foods such as dried fruit and vegetables are classified as semi-perishable. Frozen foods, though basically perishable, may be classified as semi-perishable provided that they are properly stored at freezer temperatures.

3. Perishable foods: The majority of foods such as meat and fish, milk, eggs and most fresh fruits and vegetables are classified as perishable unless they have been processed in some way.

3.3.1 Chemical factors

Deterioration may result from chemical reactions (via endogenous enzymes) or through interactions involving one or more of the macronutrients present in food and food products. Enzymes are proteins that occur naturally in plant tissues and catalyze a number of important

biochemical reactions. Some enzyme-catalyzed reactions are beneficial while others result in quality deterioration. Examples of reactions involving endogenous enzymes include:

1. The post-harvest spoilage of fruit and vegetables
2. Oxidation of phenolic substances in plant tissues by phenolase
3. Sugar - starch conversion in plant tissues by amylases
4. Post-harvest demethylation of pectic substances in plant tissues (leading to softening of plant tissues during ripening, and firming of plant tissues during processing.
5. Development of off-flavours through the breakdown of lipid components; and loss of color and undesirable browning.
6. Catalyze fermentation of sugars, breakdown of ascorbic acid, and many other deterioration reactions. Bruising, ripening, cutting, temperature, and presence of co-factors (sFe and Mg) increase the rate of degradative enzyme activity.

3.3.2 Physical Factors

One major undesirable physical change in food powders is the absorption of moisture as a consequence of an inadequate barrier provided by the package; this results in caking. It can occur either as a result of a poor selection of packaging material in the first place, or failure of the package integrity during storage. In general, moisture absorption is associated with increased cohesiveness.

Anti-caking agents are very fine powders of an inert chemical substance that are added to powders with much larger particle size in order to inhibit caking and improve flow-ability. At higher activities, however, ($aw > 0.45$) the observed time to caking is inversely proportional to water activity, and at these levels anti-caking agents are completely ineffective. It appears that

while they reduce inter-particle attraction and interfere with the continuity of liquid bridges; they are unable to cover moisture sorption sites.

3.3.3 Microorganism

Microorganisms can make both desirable and undesirable changes to the quality of foods depending on whether or not they are introduced as an essential part of the food preservation process or arise unintentionally and subsequently grow to produce food spoilage.

Bacteria and fungi (yeasts and moulds) are the two major groups of microorganisms found in foods. Bacteria are generally the fastest growing and outgrow fungi in conditions favourable to both. Spoilage microorganisms including bacteria, fungi, and viruses are major causes of food deterioration. These organisms may cause softening, off-colour, and off-flavour in foods. Disease causing microorganisms (pathogens) will result in illness of those consuming the food if present in sufficient quantity. Fruits and vegetables offer considerable resistance to microbial activity, however, the softening that usually accompanies aging of products and mechanical injuries increase the susceptibility of produce to microorganisms.

The species of microorganisms which cause the spoilage of particular foods are influenced by two factors:

1. The nature of the foods
2. Their surroundings/environment.

These factors are referred to as intrinsic and extrinsic parameters. The intrinsic parameters are an inherent part of the food and include pH, water activity, nutrient content, antimicrobial constituents and biological structures. The extrinsic parameters of foods are those properties of the storage environment that affect both the foods and their microorganisms. The growth rate of

the microorganisms responsible for spoilage primarily depends on these extrinsic parameters: temperature, relative humidity and gas compositions of the surrounding atmosphere.

3.3.4 Effect of Deterioration on Food Quality

Chemical, physical and biological changes which occur during handling, processing and storage of foods lead to deterioration in sensory and nutritional quality of foods.

3.3.4.1 Sensory Quality

i. Lipid Oxidation

Lipid oxidation rate is influenced by light, local oxygen concentration, high temperature, the presence of catalysts (transition metals such as iron and copper) and water activity. Control of these factors can significantly reduce the extent of lipid oxidation in foods.

ii. Non-enzymatic browning

Non-enzymatic browning is one of the major causes of deterioration which occurs during storage of dried and concentrated foods. The non-enzymatic browning or Maillard reaction can be divided into three stages:

1. Early Maillard reactions which are chemically well-defined steps without browning.
2. Advanced Maillard reactions which lead to the formation of volatile or soluble substances
3. Final Maillard reactions leading to insoluble brown polymers.

iii. Colour changes

Chlorophylls: Almost any type of food processing or storage causes some deterioration of the chlorophyll pigments. Phenophytinisation is the major change and it is acid catalyzed reaction accelerated by heat. Dehydrated products such as green peas and beans packed in clear glass containers can undergo photo-oxidation and loss of desirable colour.

Anthocyanins: These are a group of more than 150 reddish water- soluble pigments that are very widespread in the plant kingdom. The rate of anthocyanin destruction is pH dependent, being greater at higher pH values. Of interest from a packaging point of view is the ability of some anthocyanins to form complexes with metals such as Al, Fe, Cu and Sn. These complexes result in an undesirable change in the colour of the pigment (red sour cherries react with tin to form a purple complex). Since metal packaging materials such as cans could be sources of these metals, they are usually coated with special organic linings to avoid these undesirable reactions.

Carotenoids: The carotenoids are a group of mainly lipid soluble compounds responsible for most of the yellow and red colours of plant and animal products. The main cause of carotenoid degradation in foods is oxidation. The pigments may auto-oxidize by reaction with atmospheric oxygen at rates dependent on light, heat and the presence of antioxidants.

iv. Flavour changes

Enzymatically generated compounds derived from long-chain fatty acids play an extremely important role in the formation of characteristic flavours. In addition, these types of reactions can lead to significant off-flavours. Enzyme-induced oxidative breakdown of unsaturated fatty acids occurs extensively in plant tissues and these yield characteristic aromas associated with some ripening fruits and disrupted tissues. The permeability of packaging materials is of importance in retaining desirable volatile components within packages, or in permitting undesirable components to permeate through the package from the ambient atmosphere.

3.3.4.2 Nutritional Quality

The four major factors that affect nutrient degradation to varying extents are light, oxygen concentration, temperature, and water activity. However, due to the diverse nature of the various nutrients, chemical heterogeneity within each class of compounds and complex interactions of

the above variables, generalizations about nutrient degradation in foods will inevitably be broad ones.

Ascorbic acid (Vitamin C) is the most sensitive vitamin in foods and its stability vary markedly as a function of environmental conditions such as pH and the concentration of trace metal ions and oxygen. The nature of the packaging material can significantly affect the stability of ascorbic acid in foods. The effectiveness of the material as a barrier to moisture and oxygen as well as the chemical nature of the surface exposed to the food are important factors. For example, problems of ascorbic acid instability in aseptically packaged fruit juices have been encountered because of oxygen permeability of the package and the oxygen dependence of the ascorbic acid degradation reaction. Also, because of the preferential oxidation of metallic tin, citrus juices packaged in cans with a tin contact surface exhibit greater stability of ascorbic acid than those in enamelled cans or glass containers. The aerobic and anaerobic degradation reactions of ascorbic acid in reduced moisture foods have been shown to be highly sensitive to water activity, the reaction rate increasing in an exponential fashion over the water activity range of 0.1-0.8.

Self- Assessment Exercises 1

1. What are the four major factors that affect nutrient degradation?
2. What are the two factors that influence the species of microorganisms which cause the spoilage of particular food?

3.4 Contamination and spoilage of foods

3.4.1 Spoilage of cereals and cereal products

Cereals are important foods which provide bulk of our dietary requirements. They are also source of carbohydrates which are metabolized by body for energy generation. Besides, cereals

also provide minerals, proteins and vitamins. Nigeria produces a large variety of cereals such wheat, maize, barley, millets, etc. Various types of products are prepared from cereals.

Cereal products can be broadly classified into the following groups:

- Whole cereals where only the husk of the grain is removed such as rice, wheat, gram, lentils, etc.
- Milled grain products are made by removing the bran and usually the germ of the seed and then crushing the kernel into various sized pieces to produce wheat flour, maida, semolina, etc.
- Processed cereals like weaning food, breakfast cereals, etc.
- Ready mixes like cake mix, idli mix, vada mix etc.

India is self-sufficient in grain production and is the second largest rice producer in the world with a 20% share. But due to constantly increasing population there is still a shortfall in cereals. A large amount of these cereals is spoilt every year due to various factors.

Spoilage Factors

The grains are low moisture commodities and are less susceptible to spoilage, hence, have greater shelf life. The spoilage mainly occurs due to moisture absorption during storage leading to fungal growth at high temperature and humidity. Before bulk packaging and storage, the whole grains are usually fumigated to reduce microbial load and increase storage period. The three factors influencing the quality of cereals are:

Physical factors

Physical losses are caused by spillages occurring due to use of faulty packaging materials.

Physiological

Physiological losses occur as a result of respiration and heating in grains, temperature, humidity and oxygen.

Biological factors

Biological losses occur due to microorganisms, insects, rodents, and fungi.

The sources of contamination in cereals are:

- Soil
- Air
- Insects
- Natural microflora of harvested grains

3.4.1.1 Cereal Grains and Flours

At initial stages, the grains are contaminated by *Pseudomonas*, *Micrococci*, *Lactobacillus* and *Bacillus*. The initial bacterial and mold population varies from one grain to another. Due to low moisture content, grains and flours usually have long shelf life if properly harvested or stored under proper conditions. Spoilage of stored grains by molds is attributed to the following factors:

1. Type and number of microorganisms
2. Moisture content of more than 12-13%
3. Storage temperature
4. Physical damage

Most common species of molds are *Aspergillus, Rhizopus, Mucor and Fusarium*. A significant aspect of spoilage of molds is production of mycotoxins which may pose danger to health.

The process of flour making such as washing and milling reduces the microbial load of product. Moisture content of less than 15% does not allow growth of molds. Most molds and bacteria in

flours can grow only above 17% moisture, thus moistening of flours is essential for spoilage by microorganisms.

Spoilage of Bread

Bread is prepared from flours which undergo fermentation for which desirable microorganisms must grow. If this fermentation exceeds the required limits, it causes souring. Excessive growth of proteolytic bacteria reduces the gas holding capacity which is otherwise required for dough rising. Spoilage of bread is usually of two types namely: moldiness and ropiness. During bread making, it is baked at very high temperature thereby leaving less chances of survival of microorganisms. Thus, the contamination usually occurs when cooling is done as well as during packing, handling and from the environment. The molds which are prevalent are *Rhizopus stolonifer* (referred as bread mold), *Penicillium expansum, Aspergillus niger*. Mucor and Geotrichum also develop.

The ropiness in bread is usually due to bacterial growth and is considered more prevalent in homemade breads. The main causative organism is *Bacillus subtilis* or *B. licheniformis*. These are spore forming bacteria with their spores surviving baking temperatures. These spores can germinate into vegetative cells, once they get suitable conditions as heat treatment activates them. In ropiness, the hydrolysis of bread flour protein takes place by proteinases. Starch is hydrolysis by amylases encourages ropiness. The manifestation of ropiness is development of yellow to brown color and soft and sticky surface accompanied by odor.

Another type of spoilage of bread is chalky bread which is caused by growth of yeast like *Endomycosis fibuligera* and *Trichosporon* variable and characterized by development of white chalk like spots.

An unusual spoilage of bread is Red or Bloody bread, which is due to the growth of the bacteria *Serratia marcescens*. This organism produces brilliant red color on starchy foods giving blood like appearance. *Neurospora* and *Geotrichum* may also be involved in imparting pigmentation during spoilage of bread.

3.4.2 Contamination and Spoilage of Vegetables

Vegetables form an integral part of diet due to their role in providing various types of vital nutrients such as carbohydrates, minerals, vitamins, roughages etc. Vegetables being a part of fresh produce, contain high moisture which makes them highly perishable foods and hence more prone to spoilage. Microorganisms gain entry into vegetables from various sources including:

1. Soil
2. Water
3. Diseased plant
4. Harvesting and processing equipment
5. Handlers
6. Packaging and packing material
7. Contact with spoiled vegetables

The conditions in which vegetables are stored and transported after harvesting also contribute to rate of spoilage.

Types of Spoilage in Vegetables

The microbial spoilage of vegetables is predominately of following types

i. Spoilage due to pathogens

The plant pathogens which infect stems, leaves, roots, flowers, fruits, and other parts of the plant causing different plant diseases.

ii. Spoilage due to saprophytes

Vegetables have general microflora inhabiting them which grows under certain conditions and spoil them. There are certain secondary invaders which may enter the healthy food or grow after growth of pathogens. It is well known that plant diseases are mostly caused by fungi. Thus, most of the spoilage causing pathogens in vegetables is fungi. Fungi have specific characteristics when spoiling food as it leads to mushy areas which may be water soaked. The fungi produce characteristic spores which may be pigmented. The pigmentation helps in identification of the type of spoilage by fungi. The bacterial diseases too cause spoilage of vegetables but to a lesser extent.

Spoilage in vegetables is largely affected by composition of vegetable. The non-acidic foods are spoiled by bacterial rot while acidic foods with dry surfaces are more prone to mold spoilage. The product on which organism grows and types of organisms growing largely determine the character of spoilage.

Bacterial Soft Rot

i. Caused by *Erwinia carotovora* and *Pseudomonas* such as *P. marginalis*. *Bacillus* and *Clostridium species* are also implicated.

ii. Breaks down pectin, giving rise to a soft, mushy consistency, sometimes a bad odour and water-soaked appearance.

iii. Vegetables affected include onions, garlic, beans, carrot, beets, lettuce, spinach, potatoes, cabbage, cauliflower, radishes, tomatoes, cucumbers and watermelons.

Fungal spoilage of vegetables

Penicillium, Cladosporium, Rhizopus and *Aspergillus species* are responsible for various defects in vegetables. Gray mold rot caused by *Botrytis cinera* in vegetables is favoured by high humidity and warm temperature

3.4.3 Contamination and Spoilage of Fruits

Fruits are natural sources of minerals, vitamins besides carbohydrates and other essential substances. Naturally fresh fruits and juices made out of them contain high amount of water thereby making them highly prone to attack by microorganisms. Most of the fruits are naturally provided with coatings and coverings in the form of skins but these are fragile enough to be easily disturbed by various biological and mechanical factors. Like vegetables, fruits being foods of plant origin get contaminated through different sources by a variety of microorganisms which may play significant role in their spoilage. These are soil, water, diseased plant, harvesting and processing equipment, handlers, packaging and packing material and contact with spoiled fruits.

Microorganisms associated with spoilage in fruits and juices

The microorganisms associated with fruits depend on the structure of fruit. The fruits contain different organic acids in varying amounts and the predominately found of acids are citric acid, malic acid and tartaric acid. The low pH of fruits restricts the proliferation of various types of organisms.

Due to the low pH, a large number of microorganisms are restricted to grow on fruits. Fungi are most dominating organisms to grow on fruits because of the ability of yeasts and molds to grow under acidic conditions. A small number of bacteria which are aciduric grow on fruits. The dry conditions prevailing on the skin and surface do not allow the growth of certain microorganisms and these plants also produce certain antimicrobial components too. Despite the high water

activity of most fruits, the low pH leads to their spoilage being dominated by fungi, both yeasts and molds.

a. Yeasts

Yeasts are unicellular fungi which normally reproduce by budding. Of the 215 species important in foods, about 32 genera are associated with fruits and fruit products. Only a few species of yeasts are pathogenic for man and other animals but none of the pathogenic species are common contaminants of fruits and fruit products. Fruit that has been damaged by birds, insects, or pathogenic fungi usually contain very high yeast populations. The yeasts are introduced into the exposed tissue, often by insects, and are able to use the sugars and other nutrients to support their growth. Types of yeasts growing in fruits depend upon the nature of the fruit and the strain of yeast. Growth of a strongly fermentative type such as certain strains of *Saccharomyces cerevisiae* may produce sufficient CO_2 to burst the container. Growth of some species in a clear fruit juice may produce only slight haze and sediment. Carbon dioxide and ethanol are the predominant metabolic products of yeasts but other products such as glycerol, acetaldehyde, pyruvic acid, and a - ketoglutaric acid are also formed. Oxidative yeasts such as species of *Brettanomyces* produce acetic acid in wines and other fruit products. Although yeasts produce hydrolytic enzymes which degrade pectins, starch, and certain proteins, enzymatic activity is usually much less than that exhibited by other aciduric microorganisms (molds in particular).

b. Moulds

Moulds are filamentous fungi which are important group of microflora of fruits and fruit products. Some of the members are xerophilic, thereby having potential to spoil foods of low water activity such as dried fruits and fruit juice concentrates. Some of the species have heat resistant spores such as ascospores which can survive the commercial pasteurization treatments

that are given to most fruit products. Growth of moulds on processing equipment such as wooden tanks can result in the generation of off-flavors in wines, juices, and other fruit products. Mould-infected raw fruit may become soft after processing because pectinases will not inactivated by the thermal treatment. The metabolic products of many molds are toxic to humans, e.g. mycotoxins.

Moulds are aerobic microorganisms, but many of them are very efficient scavengers of oxygen. Due to this property of molds, processed fruits, including those hermetically sealed in cans or glass, are susceptible to spoilage. In case of limited vegetative growth, evidence of spoilage may be the changes produced by fungal enzymes such as the breakdown of starch or pectins while in case of heavy growth, colonies develop in the headspace or as strands throughout a beverage or similar product.

Some types of spoilage by fungi

Rhizopus stolonifer cause soft and mushy food, cottony growth of mold.

Anthracnose

Colletotrichum lindemuthianum, cause spotting of leaves and fruits and seed pods

Downy mildew:

Initially, the lesions tend to be small and confined to the upper surface of wrapper leaves. As the areas enlarge, they turn from light green or yellowish to brown and become soft. It is caused by *Phytophthora*.

Watery soft rot

This rot occurs on the lower part of water soaked and light or pinkish brown called leads. A white cottony mold spreads over the decayed tissue and the lead eventually becomes a watery mall.

c. Bacteria:

Various groups of bacteria have ability to grow on fruits and its juices. These bacteria by virtue of their diversity in metabolism grow on fruits and produce different types of compounds. The major groups of bacteria of bacteria that grow on the fruits:

- Lactic acid bacteria
- Acetic acid bacteria
- Spore forming bacteria

Lactic acid bacteria

The lactic acid bacteria are Gram-positive, catalase negative, rod-shaped (lactobacilli), or cocci organisms which can grow under anaerobic conditions. The homo fermentative species produce mainly lactic acid from hexose sugars; the hetero fermenters produce one molecule of lactic acid, one molecule of carbon dioxide, and a two-carbon compound, which is usually acetic acid or ethanol or a combination of the two.

Growth of lactic acid bacteria in juices and other fruit products cause the formation of haze, gas, acid, and a number of other changes. Certain hetero-fermentative *lactobacilli* lead to slime in cider. The *lactobacilli* and *leuconostocs* that are present in citrus juices generate acetylmethylcarbinol and diacetyl, compounds that give the juices an undesirable, butter-milk-like flavor. Lactic acid bacteria have the ability to decarboxylate malic acid to lactic acid. This malo-lactic fermentation is often desirable in high-acid wines because the acidity is reduced and desirable flavors are produced. *Oenococcus oenos* is the most acid and alcohol-tolerant species often isolated from wines that are undergoing a malo-lactic fermentation.

Acetic acid bacteria

These are Gram negative, aerobic rods having two genera, namely: *Acetobacter* and *Gluconobacter*. These species oxidize ethanol to acetic acid under acidic condition, *Acetobacter* species can oxidize acetic acid to carbon dioxide thus, the genus is called over oxidizer. The bacteria are obligate aerobes, so, juices, wines, and cider are most susceptible to spoilage while held in tanks prior to bottling. Some strains of *Acetobacter pasteurianus* and *Gluconobacter oxydans* produce microfibrils composed of cellulose, which leads to formation of flocs in different fruit juice beverages.

Spore forming bacteria

Spores are heat resistant, so, the role of organisms producing spores is important in heat treated of juices and beverages. Various spore formers such as *Bacillus coagulans, B. subtilis, B. macerans, B. pumilis, B. sphaericus* , and *B. pantothenticus* have been found to grow in different types of wines. Some of these organisms have also been involved in canned fruits. Sporeforming *bacilli* that actually prefer a low pH have been responsible for spoilage of apple juice and a blend of fruit juices.

3.4.4 Contamination and Spoilage of Meat and Meat Products

The microbiological profile of meat products presented to the consumers is the sum total of the slaughtered animal health, conditions under which it was reared, quality of slaughtering, processing, packaging and conditions under which the meat was stored. Meat pathogens can cause self-limiting human enteric diseases or systemic and fatal infections of the immunocompromised, the elderly and the young. Meat can act as an ideal substrate for microbial proliferation. Major meat associated pathogenic bacteria include *Clostridium perfringens,*

Staphylococcus aureus, Salmonella spp, pathogenic strains of Escherichia coli, Campylobacter spp, Yersinia enterocolitica, Listeria monocytogenes and Aeromonas hydrophila.

Microorganisms Associated With Meat during Processing

Meat spoilages indicate:

i. Color changes

ii. Textural changes

iii. Development of off-flavour or off-odor or slime as a result of microbial growth.

Salmonella is the primary microbial challenge of poultry *and Escherichia coli* O157: H7 is the primary microbial to the beef industry. *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 two 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.

Gram-negative bacteria (Aerobes): *Neisseriaceae: Psychrobacter immobilis, P. phenylpyruvica, Acinetobacter spp., A. twoffii, A. Johnsonii, Pseudomonadaceae: Pseudomonas fluorescens, P. lundensis, P. fragi,* and *P. putida*

Gram-positive bacteria: *Brochothrix thermosphacta, Kurthia zophii, Staphylococcus spp., Clostridium estertheticum, Clostridium frigidicarnis, Clostridium casigenes,* and *Clostridium* sp.

Meat Spoilage

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 its shelf life. The surface of beef carcasses may contain anywhere from 101 to 107 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 for microbial growth. 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 is 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 species* are associated with spoilage of vacuum-packaged meats. "Blown pack" meat spoilage is characterized by excessive gas formation with off-odours 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; hence, they are a minor component of spoilage flora.

Spoilage of Processed Meat

Addition of sodium chloride, nitrites and/or nitrates, along with various other seasonings, emulsifiers, and preservatives to ground or whole muscle meats changes the environment significantly and also the spoilage flora of processed meats. Dried and dry-fermented meats generally do not support microbial growth although process deviations may allow growth of some organisms. Spoilage organisms can grow on fresh and cooked cured meats, so they are best stored chilled, under a vacuum or modified atmosphere. *Pseudomonas spp*. are not usually important causes of spoilage in processed meats because of their sensitivity to curing salts and heat pasteurization and their inability to grow well in meats packed with a vacuum or high carbon dioxide atmosphere. But these bacteria may spoil refrigerated processed meats when

packages have been opened and there has been insufficient curing. Some cold and salt tolerant Enterobacteriaceae have been found to cause spoilage in some specific processed meats, such as ham or bacon.

Lactic acid bacteria (LAB) are the group of bacteria primarily associated with spoilage of processed meats. They produce sour off-flavors, gas, slime, and greening, and this spoilage may be more severe than in fresh meat because of the presence of added carbohydrates. Competitive ability of different LAB strains is related to pH and water activity of the meat, cooking and storage temperatures and oxygen and carbon dioxide levels. Spore formers (*Clostridium* and *Bacillus spp.*) are usually not a spoilage problem in processed meats because of the presence of nitrite and other curing salts. But faulty cooking/cooling procedures, including long cooling periods and temperature abuse, has allowed the growth of these organisms in some cases. Spores of these organisms may be introduced with spices or other ingredients. Yeasts cause some spoilage in processed meats but are generally only important when sulfite is used as a preservative or when meats have been irradiated or stored aerobically in the cold. In some sausages, slime may be produced along with vinegary or malty off-odours.

3.4.5 Contamination and Spoilage of Milk and Milk Products

Milk secreted from an uninfected animal's udder is sterile. It becomes contaminated during milking, cooling and/or storage. It is an excellent medium for the growth of bacteria, yeasts and moulds that are the common contaminants of any food material. Their rapid growth, particularly at high ambient temperatures can spoil the milk for liquid consumption and for manufacturing dairy products.

Spoilage of milk

As a result of microbial growth or biochemical activities, cause undesirable changes in milk and, are responsible for spoilage. Milk producer should be aware of the sources of microorganisms causing rapid changes, conditions favoring their growth, and methods of preventing their activity. The manufacturer of milk products must contend with problems similar to those of producer of milk and additional ones also, as milk products (butter, cheeses, etc.) are frequently stored for longer periods, during which there may be further decrease quality. The problem of spoilage is especially important with the cheeses. They require ripening, since conditions must be favorable for growth of certain desirable microorganisms and may also allow the growth and development of undesirable ones.

The initial microbial quality of raw milk is quite crucial for the production of good quality dairy foods. Spoilage is a term used to describe the deterioration of a foods texture, color, odor or flavor to the point, where it becomes unsuitable for human consumption. Microbial spoilage of food often involves the degradation of protein, carbohydrates, and fats by the microorganisms or their enzymes. The microorganisms that are mainly involved in spoilage of milk are psychrotrophs and are destroyed by pasteurization; however, some like *Pseudomonas fluorescens and Pseudomonas fragican* produce proteolytic and lipolytic extracellular enzymes that are heat stable and capable of causing spoilage. Some species and strains of *Bacillus, Clostridium, Corynebacterium, Arthrobacter, Lactobacillus, Microbacterium, Micrococcus,* and *Streptococcus* can survive pasteurization and grow at refrigeration temperatures that can cause spoilage problems in milk and milk products.

Sources of Microbial Contamination of Milk

Microbial contamination of milk can be from the internal and/ or external sources as described below.

Interior of udder

Varying numbers of bacteria are found in aseptically drawn milk with the reported counts of <100-10,000 CFU/ml from normal udder, but an anticipated average is 500-1000 CFU/ml in advanced countries. Microorganisms enter the udder through the duct at the teat tip that varies in length (from 5-14 mm) and its surface is heavily keratinized. This keratin layer retains the milk residues and exhibit antimicrobial activity.

During progress of a milking, bacteria are present in the largest numbers at the beginning and then gradually decrease. This is mainly due to the mechanical dislodging of bacteria, particularly in teat canal, where the numbers are probably highest. Because of this, discarding of first few streams of milk helps in lowering the counts of microbes in milk.

Micrococci are slow growing, mostly non-pathogenic but if allowed to grow, they cause acid formation and proteolysis. Streptococcus agalactiae may be present even in non-clinical mastitis and thus it appears to be a natural inhabitant of udder. *Corynebacterium bovis*, a Gram positive rod has been found in large numbers. It is non-pathogenic, but if grown causes rancidity. If an animal is infected from mastitis, microbial contamination from within the udder of animal contributes notably to the total numbers of microbes in the bulk milk, when compared with the milk originated from a healthy animal. The influence of mastitis on the total bacterial count of milk depends on the type of the infecting microbe. Most common microbial agents of mastitis in milch animals are *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli*, and *Corynebacterium pyogenes.*

Exterior of udder

In addition, to the udder infections, unclean udder and teats of animal also contribute significantly to the total bacterial counts of milk. The microbes that are naturally associated with the skin of the animals as well as those derived from the environment, where the cow is housed and milked are predominant in the milk. The environmental conditions such as soil, manure, mud, feed or bedding; determines what kind of microbes will dominate in milk. Udder and teat become soiled with dung, mud, bedding material such as saw dust and straw. With heavily soiled udder teats the counts may be up to100,000 cfu/ml. The bedding material in winter has high number of bacteria, mainly psychrotrophs, coliforms and *Bacillus spp*. Udder microflora is not affected much by simple washing but economy washing with sodium hypochlorite accompanied by drying, helps in reducing the number of microbes. Categories of microbes that occurs in the exterior of udder are:

1. Predominantly *Micrococci* and coagulase negative *Staphylococci* exist.
2. Next, on the teat surface are faecal *Streptococci*, but gram-negative bacteria including coliforms are less. Coliforms do not survive well on teat surface.
3. Aerobic thermoduric organisms are entirely *Bacillus spp*. The more frequent are *B. licheniformis, B. subtilis, B. pumilis* and less frequent ones are *B. cereus, B. circulans* and *B. firmus*.
4. Teat surface may also contain *Clostridia* spores that are usually found in cows' fodder, bedding and feces.

Psychrotrophic and thermoduric bacteria predominate on the teat surfaces. The psychrotrophs that can grow at 7^{o}C and below are mostly Gram negative rods, and the major ones are *Pseudomonas fluorescens, Alcaligenes* and *Flavobacterium*. On the other hand, thermodurics on

teat surfaces are often bacterial spores (a dormant and nonreproductive structure; highly resistant to radiations, desiccation, lysozymes, high temperature, starvation and disinfectants) that are typically found in the soil. When these spores enter the bulk milk, they may survive during pasteurization and cause a number of post-pasteurization problems.

Coat of cow

The coat serves as a vehicle to contribute bacteria directly to milk. The hairs around udder, flanks and tail contribute to the higher bacterial count in milk. The coat may indirectly contribute microbes into air, especially *Bacillus spp*. The coat may carry bacteria from the stagnant water pools, especially ropiness causing milk microbes.

Animal shed and surroundings

Milk produced on farms with poor hygiene practices may undergo significant spoilage and have a shorter shelf-life compared to milk produced under hygienic conditions.

Microbes associated with the bedding materials include:

i. Coliforms

ii. Spore-formers

iii. *Staphylococci*

iv. *Streptococci*

v. Other Gram negative bacteria

Milking staff

Soiled clothes and hands increase the risk of contamination of milk and milking equipment many folds. Milker with infected wounds on hands contributes pathogenic *Streptococcus spp*. and *micrococci*. If wet hand milking is practiced, the microorganisms present in lubricants like fore-milk, water or saliva of the milker and bacteria from hands and teats will enter the milk.

The common microbial pathogens from humans causing diseases such as typhoid, paratyphoid and dysentery may contaminate the milk. Microbial pathogens causing scarlet fever, septic sore throat, diptheria, cholera etc. also contaminate the milk.

Milking equipment (storage containers and transportation systems)

Improperly cleaned milking and cooling equipment are one of the main sources of milk contamination. Milk residues left on the equipment contact surfaces supports the growth of a variety of microbes. The tanker and collecting pipes are also the potential sources of contamination, if not adequately cleaned. In addition, biofilms can easily build up on the enclosed, hard to clean surfaces.

Unclean or improperly cleaned milk cans and lids if they are still moist, results in multiplication of thermophilic bacteria like *Bacillus cereus*. Improperly sterilized milking machines contain thermoduric *Micrococci*, *Bacillus spp*. and *Microbacterium spp*. compared to coliforms and *Streptococci*. Rubber hoses predominantly contribute to *Pseudomonads* rather than thermodurics.

Water supplies

At dairy farms, the water can be a predominant source of microbial contamination. Water used in production should be of good bacteriological quality. Inadequately or uncleaned, storage tanks, untreated water supplies from natural sources like bore wells, tanks and rivers, may also be contaminated with the fecal microbes (*Coliforms*, *Streptococci* and *Clostridia*). In addition, a wide variety of saprophytic bacteria (*Pseudomonas*, *Coliforms*, other Gram negative rods, *Bacillus* spores, *Coryneform* bacteria and lactic acid bacteria) may also be present in water and may contaminate the milk potentially. The warm water used for udder washing is potent source of *Pseudomonas* and *Coliforms* which may even cause mastitis.

Airborne contamination

Air contains dust, moisture and bacteria; hence its entry should be minimized in milk. *Micrococci, Coryneforms, Bacillus* spores, *Streptococci*, and Gram negative rods are the major genera present in air. The more air is incorporated into milk the faster the bacterial growth.

Colour changes in milk

i. Affected by amount and yellowness of butter fat, thinness of milk, content of blood and pus and feed of animal.

ii. Blue milk - *Pseudomonas syncyanea, Streptococcus lactis, Actinomycetes* or *Geotrichum* mold.

iii. Yellow milk - *Pseudomonas synxantha, Flavobacterium.*

iv. Red milk - *Serratia marcescens, Brevibacterium erythrogenes, Micrococcus roseus.*

v. Brown milk - *Pseudomonas putrefaciens, P.fluorescens*.

Spoilage of Butter

The cream quality and the sanitary conditions used in the butter processing are the major determinants of microbiological quality of butter.

All three major groups of organisms - bacteria (e.g., *Pseudomonas spp*.), yeasts (e.g., *Candida* spp.), and molds (e.g., *Geotrichum*) have been implicated in spoilage of butter on the surface causing flavour defects such as putridity, rancidity and/or fishy flavour as well as surface discoloration.

The flavour defects in unsalted butter have been attributed to growth of coliforms, *Enterococcus* and *Pseudomonas* in water-phase of butter.

Microorganisms of concern in the spoilage of butter are mainly psychrotrophs, which are predominantly Gram-negative, rod shaped microorganisms. The main characteristic of

psychrotrophs which makes them important in the spoilage of butter, is their ability to survive at low temperatures (3–7ºC) and the production of enzymes (lipase and protease) which catalyze the hydrolysis of lipids and proteins in the butter, respectively.

The rich nutrient content of the butter also makes it susceptible to spoilage microorganisms other than *Pseudomonas* such as *Serratia, Acinetobacter* and *Moraxella*. Two main types of butter spoilage are color change at the surface (surface taint) and rancidity.

The major culprit for both is believed to be *Pseudomonas spp. Shewanella putrefaciens* (formerly *Alteromonas putrefaciens*) or *Flavobacterium spp*. which also play role in development of surface taints in butter. Some species such as *P. putrefaciens* are able to grow on butter surface and produce a putrid odour within a relatively short period of time (7–10 days) at refrigeration temperature. The odour is suggested to be the result of releasing organic acids such as isovaleric acid. Black discoloration and shunk-like odour are also developed in butter by *Pseudomonas nigrificans* and *Pseudomonas mephitica* respectively.

Pseudomonas fragi and, in rare cases, *Pseudomonas fluorescens* as well as non-microbial lipases degrade milk fat into free fatty acids leading to hydrolytic rancidity in butter.

Hydrolytic rancidity in butter can also be the result of activity of *Micrococcus spp*. and molds such as *Rhizopus, Geotrichum, Penicillium* and *Cladosporium.*

3.4.6 Contamination and Spoilage of Poultry

Poultry is 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 such as *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).

The primary causes of poultry products spoilage are:

i. Prolonged distribution or storage time.

ii. Inappropriate storage temperature

iii. High initial bacterial counts

iv. High post-rigor meat pH

Spoilage factors

Companies are able to prevent prolonged storage times by properly rotating their stock. Product that is to be sold in locations far from the processing plant should be transported at temperatures that are below freezing point (26^{o}F), but not sufficient to freeze the muscle tissue.

Inappropriate storage temperatures or fluctuations in storage temperature are the most avoidable causes of spoilage. Temperature abuse can occur during distribution, storage, retail display or handling of the product by the consumer. Processors can determine whether product has been temperature abused by monitoring temperature or evaluating bacterial populations throughout the distribution system.

Initial bacterial counts on broiler carcasses may have a direct effect on the shelf-life of fresh product as well. The initial number of bacteria on poultry is generally a function of grow-out procedures, production practices, and plant and processing sanitation. Higher numbers of spoilage bacteria on the chicken immediately after processing, translates to more rapid spoilage. High post-rigor meat pH is often caused by stress on the birds during grow-out or transportation. This reduces the shelf-life of the meat by up to six days and is due to the fact that spoilage

bacteria multiply much more rapidly on meat that is at a pH of 6.2 than on meat that is at a normal post-rigor pH of 5.4-5.6.

Bacteria responsible for spoilage

Research demonstrates that the populations of bacteria high in number on the carcass immediately after processing are not the ones that grow under refrigeration and spoil carcasses. The bacteria found after carcasses spoil are very difficult to find on carcasses at the time of processing. After processing, the spoilage bacteria are present in very low numbers, but they can multiply rapidly to cause spoilage odours and slime.

These spoilage bacteria are called psychrotrophic bacteria because they are able to multiply under cold conditions. Fresh poultry products held long enough at refrigerator temperatures will spoil as a result of the growth of psychrotrophic bacteria. In contrast, the bacteria that exist in higher numbers at the time of processing on the skin of chickens and in their intestinal tracts are primarily mesophiles. These bacteria do not multiply to an appreciable degree at refrigerator temperatures. *Salmonella, E. coli* and other bacteria found on chickens are mesophiles. When a company conducts an "Aerobic Plate Count" or "Total Plate Count" on a chicken carcass, it is measuring the mesophiles.

Origin of spoilage bacteria

Spoilage bacteria on the carcass immediately after processing come from the feathers and feet of the live bird, the water supply in the processing plant, the chill tanks and processing equipment. These spoilage bacteria are not usually found in the intestines of the live bird. High populations of *Acinetobacter* (108cfu/g) have been found on the feathers of the bird and may originate from the deep litter. Other spoilage bacteria, such as *Cytophaga* and *Flavobacterium,* are often found in chill tanks but are rarely found on carcasses.

The psychrotrophic spoilage bacteria on chicken carcasses immediately after slaughter are generally *Acinetobacter* and pigmented *pseudomonads*. Although strains of nonpigmented *Pseudomonas* produce off-odours and off-flavors on spoiled poultry, initially, they are difficult to find on carcasses *and P. putrefaciens* (*Shewanella putrefaciens*) is rarely found.

Spoilage species

The bacterial genera most isolated in high numbers on spoiled poultry were *Pseudomonas fluorescens, putida,* or *fragi* or *Shewanella putrefaciens*. Identification of the genus and species most responsible for spoiling poultry is important because, once identified, it is easier to understand the mechanisms by which they produce spoilage. High numbers (105 cfu/cm^2) of psychrotrophic spoilage bacteria are required on poultry surfaces before off-flavors, off-odors and appearance defects are able to be detected organoleptically. Researchers have reported that higher numbers of bacteria ($3.2x10^7$ to $1x10^9$ cfu/cm^2) were required to produce slime than were needed for odor to become noticeable.

Causes of spoilage defects

Spoilage is caused by the accumulation of metabolic by-products or the action of extracellular enzymes produced by psychrotrophic spoilage bacteria as they multiply on poultry surfaces at refrigeration temperatures. Some of these by-products become detectable as off-odours and slime, as bacteria utilize nutrients on the surface of meats.

Off-odours do not result from breakdown of the protein in skin and muscle, as previously thought, but from the direct microbial utilization of low molecular weight nitrogenous compounds such as amino acids, which are present in skin and muscle. Concentrations of free amino acids increase as proteolysis occurs throughout the storage period. It has been demonstrated that measurement of these free amino acids, due to the production of

aminopeptidases and subsequent breakdown of protein, may be used to rapidly determine the bacteriological quality of beef.

Development of off-odors and slime

Microorganisms appear first in damp pockets on the carcass, such as folds between the foreleg and breast of a carcass, and their dispersion is promoted by condensation which occurs when a cold carcass is exposed to warm, damp air.

An ester-like odor, which was described as a "dirty dishrag" odour, can develop on cut-up chickens. Off-odour precedes slime formation and is considered the initial sign of spoilage in most cases. Immediately after off-odours are detected, many small, translucent, moist colonies may appear on the cut surfaces and skin of the carcass. Eventually, meat surfaces become coated with tiny drop-like colonies, which increase in size and coalesce to form a slimy coating.

In the final stages of spoilage, the meat may begin to exhibit a pungent ammoniacal odour in addition to the dirty dishrag odour, which may be attributed to the breakdown of protein and the formation of ammonia or ammonia-like compounds.

Effects of cold storage

Under refrigeration (< 5^{o}C), psychrotrophic bacterial populations are able to multiply on broiler carcasses and produce spoilage defects; however, the mesophilic bacteria that predominate on the carcass initially remain the same or decrease in number. This phenomenon may be explained by examining the metabolic changes that occur in these groups of bacteria as they are exposed to refrigerator temperatures.

Cellular lipids: Naturally, mesophilic bacteria cease to proliferate below a certain environmental temperature because as temperature decreases so does their cellular absorption of nutrients.

Psychrotrophic bacteria species typically exhibit no such temperature-induced difference; hence, they are able to grow rapidly at refrigerator temperatures.

Lipase production: Research has demonstrated that the amount of lipase produced by psychrotrophic bacteria increases as a result of exposure of the bacteria to cold temperatures. This means that *Pseudomonas* is able to breakdown fat equally well when on chicken in the refrigerator or at room temperature, hence, its capability to spoil chicken.

Proteolytic activity: Research has shown that production of proteolytic enzymes by *Pseudomonas fluorescens* was higher when this bacterium was cultured at lower temperatures. This means that, as with fat, *Pseudomonas* is able to breakdown protein more effectively at refrigeration temperature than at room temperature and this makes the bacterium ideally suited to spoil chicken.

Shelf life: From time to time premature spoilage will occur. In order for companies to assess this problem, they often conduct aerobic plate counts on products. This microbiological method is inappropriate for this purpose because measuring mesophilic bacteria on chicken does not indicate what is happening with spoilage bacteria. Aerobic plate counts (APC) may miss up to 99.9% (3 logs) of spoilage bacteria on the surface of the product.

To measure spoilage bacteria, samples should be plated and incubated at 7^{o}C for 10 days. In this way, the bacteria that grow and produce colonies on the plate will be the ones responsible for spoiling the product.

3.4.6.1 Contamination and Spoilage of Eggs and Egg Products

Most freshly laid eggs are sterile, at least inside, but the shells soon become contaminated by fecal matter from the hen, by the cage or nest, by wash water if the eggs are washed, by handling, and perhaps by the material in which the eggs are packed. The total number of

microorganisms per shell of a hen's egg has been reported to range from 10^2 to 10^7 with a mean of about 10^5. The types of microorganisms recovered from the shell are diverse. *Salmonella* species may be on the shell or in the egg as laid, build up during processing, and appear in significant numbers in frozen or dried eggs.

Factors influencing the contamination of eggshells

The spoilage of eggs is related to eggshell contamination and the ability of some bacteria to penetrate the egg. The type and level of egg contamination of the eggshell surface are related to the hygienic conditions in which the hens are reared, the breeding environment, the breeding practices, the housing system, the geographical area, and the season.

Contamination may also occur during egg transport and/or packaging in farms or in the conditioning centre, either through the environment or from one egg to another.

Even though the microflora of the eggshell surface varies, the spoilage flora of the egg content tends to be less diversified, highlighting the fact that the intrinsic egg barriers have a strong influence on the invasiveness of spoilage bacteria.

The cuticle, shell and shell membranes are barriers preventing the penetration of microorganisms from the surface into the egg content. Nevertheless, the cuticle which is resistant to water and microorganism penetration may crack rapidly over time or during egg manipulation.

The effectiveness of this protective coating is therefore limited. The shell, a calcified proteinic layer, represents a physical barrier but is rather ineffective because of the possible transfer of microorganisms through the pores, particularly if condensed water is present on the eggshell.

The presence of eggshell cracks or micro-cracks increases the risk of contamination. The manipulation of eggs, especially in the conditioning centres, increases the risk of egg cracking.

The external and internal shell membranes represent effective filters composed of antibacterial glycoprotein fibres, which may play a role in protection against penetration.

In addition to these physical barriers, egg white, similar to an intracellular fluid, is an important line of defence against invading bacteria because it represents an unfavourable environment for microbial development (poor nutrient, exhibiting an alkaline pH, and high viscosity and heterogeneity), and because it contains several molecules expressing antimicrobial activities, such as lysozyme, ova transferrin, proteinase inhibitors (cystatin, ova mucoid and ovoinhibitor), and vitamin binding proteins (riboflavin binding protein, avidin- and thiamine-binding proteins).

The integrity of egg barriers is essential to prevent microbial penetration and proliferation.

Spoilage of Eggs

Some of the defects of eggs are obvious from their general appearance, others are shown by candling with transmitted light and some show up only in a broken egg.

Defects in the fresh egg

Fresh eggs may exhibit cracks, leaks, loss of bloom or gloss, or stained or dirty spots on the exterior as well as "meat spots", general bloodiness, or translucent spots in the yolk when candled.

Changes during egg storage: The changes that take place in eggs while they are being held or stored may be divided into those due to non-microbial causes and the changes caused by microorganisms.

a. Changes Not Caused by Microorganisms

1. Untreated eggs lose moisture during storage and hence lose weight. The amount of shrinkage is shown to the candler by the size of the air space or air cell at the blunt end of the egg, a large cell indicating much shrinkage.

2. The change in the physical state of the contents of the egg, as shown by candling or by breaking out the egg.

3. The albumin (egg white) becomes thinner and more watery as the egg ages, and the yolk membrane becomes weaker.

4. When an old egg is broken onto a flat dish, the thinness of the white is more evident, and the weakness of the yolk membrane permits the yolk to flatten out or even break. By contrast, a broken fresh egg shows a thick white and a yolk that stands up strongly in the form of a hemisphere.

5. During storage, the alkalinity of the white of the egg increases from a normal pH of about 7.6 to about 9.5. Any marked growth of the chick embryos in fertilized eggs also serves to condemn the eggs.

6. The poorer the egg, the more the movement is there of yolk and the nearer it approaches the shell when it is twirled during candling.

b. Changes Caused by Microorganisms

To cause spoilage of an undamaged shell egg, the causal organisms must do the following:

(1) Contaminate the shell.

(2) Penetrate the pores of the shell to the shell membranes (usually the shell must be moist for this to occur).

(3) Grow through the shell membranes to reach the white (or to reach the yolk if it touches the membrane).

(4) Grow in the egg white, despite the previously mentioned unfavourable conditions there, to reach the yolk, where they can grow readily and complete spoilage of the egg.

In general, more spoilage of eggs is caused by bacteria than by molds and the types of bacterial spoilage, or "rots," of eggs go by different name. The primary ones are:

i. **Green rots:** Caused primarily by *Pseudomonas fluorescens*, a bacterium that grows at 0^{o}C. The rot is named because of the bright-green colour of the white during early stages of development. This stage is noted with difficulty in candling but shows up clearly when the egg is broken. Odour is lacking or is fruity or "sweetish." The contents of eggs so rotted fluoresce strongly under ultraviolet light.

ii. **Colourless rots:** This may be caused by *Pseudomonas, Acinetobacter, Alcaligenes*, certain coliform bacteria, or other types of bacteria. These rots are detected readily by candling, for the yolk usually is involved, except in very early stages, and disintegrates or at least shows a white incrustation. The odour varies from a scarcely detectable one to fruity to "highly offensive."

iii. **Black rots:** Where the eggs are almost opaque to the candling lamp because the yolks become blackened and then break down to give the whole egg contents a muddy-brown colour. The odour is putrid, with hydrogen sulphide evident, and gas pressure may develop in the egg. Species of *Proteus* most commonly cause these rots, although some species of *Pseudomonas* and *Aeromonas* can also cause black rots. *Proteus melanovogenes* causes an especially black coloration in the yolk and a dark colour in the white. The development of black rot and of red rot usually means that the egg has at some time been held at temperatures higher than those ordinarily used for storage.

iv. **Pink rots:** Pink rots occur less often, and red rots are still more infrequent. Pink rots are caused by strains of *Pseudomonas*. They resemble the colourless rots, except for a pinkish precipitate on the yolk and a pink colour in the white.

v. **Red rots:** Red rots caused by species of *Serratia*, are mild in odour and are not offensive.

Spoilage of Eggs by Fungi: The spoilage of eggs by fungi goes through stages of mold growth that give the defects their names.

Pin spot molding: Very early mold growth is termed pin-spot molding because of the small, compact colonies of molds appearing on the shell and usually just inside it. The colour of these pin spots varies with the kind of mold. *Penicillium* species cause yellow or blue or green spots inside the shell, *Cladosporium* species give dark-green or black spots, and species of *Sporotrichum* produce pink spots.

Superficial fungal spoilage: In storage atmospheres of high humidity, a variety of molds may cause spoilage, first in the form of fuzz or "whiskers" covering the shell and later as more luxuriant growth. When the eggs are stored at near-freezing temperatures, the temperatures are high enough for slow mycelial growth of some molds but too low for sporulation, while other molds may produce asexual spores. Molds causing spoilage of eggs include species of *Penicillium, Cladosporium, Sporotrichum, Mucor, Thamniidium, Botrytis, Alternaria*, and other genera.

Fungal rotting: The final stage of spoilage by molds is fungal rotting, after the mycelium of the mold has grown through the pores or cracks in the egg. Jellying of the white may result, and coloured rots may be produced, e.g., fungal red rot by Sporotrichum and a black colour by Cladosporium, the cause of black spot of eggs as well as of other foods. The hyphae of the mold

may weaken the yolk membrane enough to cause its rupture, after which the growth of the mold is stimulated greatly by the food released from the yolk.

Off-flavours sometimes are developed in eggs: Mustiness may be caused by a number of bacteria, such as *Achromobacter perolens, Pseudomonas graveolens,* and *P. mucidolens*. The growth of Streptomyces on straw or elsewhere near the egg may produce musty or earthy flavours that are absorbed by the egg. Molds growing on the shell also give musty odours and tastes. A hay odour is caused by *Enterobacter cloacae*. Fishy flavours are produced by certain strains of *Escherichia coli*. The "cabbage-water" flavour may appear before rotting is obvious. Off-flavors, such as the "coldstorage taste", may be absorbed from packing materials.

Self- Assessment Exercises 2

1. List the sources of contamination in cereals
2. Describe the two types of microbial spoilage of vegetables

3.5 Summary

Food spoilage can occur as a result of the activities of enzymes, bacteria or fungi. The mixture of the food composition and packaging materials also influence food deterioration. Also, environmental, temperature and storage conditions play major roles in food deterioration.

Major causes of food deterioration include the following: growth and activities of microorganisms, principally bacteria, yeasts and moulds, activities of natural food enzymes, insects, parasites and rodents, temperature, both heat and cold, moisture and dryness, air and in particular oxygen, light, and time.

Extrinsic factors controlling the rate of food deterioration reactions are mainly: Effect of temperature; Effect of water activity; Effect of gas atmosphere; and Effect of light.

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3.7 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. The four major factors that affect nutrient degradation to varying extents are light, oxygen concentration, temperature, and water activity.
2. The species of microorganisms which cause the spoilage of particular foods are influenced by two factors:

   i. The nature of the foods

   ii. Their surroundings/environment.

Answers to Self-Assessment Exercises 2

1. The sources of contamination in cereals are:

- Soil
- Air
- Insects
- Natural microflora of harvested grains

2. The microbial spoilage of vegetables is predominately of following types

i. **Spoilage due to pathogens:** The plant pathogens which infect stems, leaves, roots, flowers, fruits, and other parts of the plant causing different plant diseases.

ii. **Spoilage due to saprophytes:** Vegetables have general microflora inhabiting them which grows under certain conditions and spoil them. There are certain secondary invaders which may enter the healthy food or grow after growth of pathogens. It is well known that plant diseases are mostly caused by fungi.

UNIT 4 FOOD SPOILAGE BY INSECTS AND RODENTS AND THEIR MICROBIAL RELATIONSHIP

CONTENTS

4.1 INTRODUCTION

Spoilage of food refers to any visible or invisible change which can makes food or product derived from food unacceptable for human consumption. Spoilage of food not only causes health hazard to the consumer but also cause large economic losses. Spoilage not only leads to loss of

nutrients from food but also causes change in original flavour and texture. Hence, the spoilage of food is not only a health hazard but also carry lot of economic significance too.

Most of the foods linked to food infections or food intoxications are from animal sources. Pathogens have been found to enter the food supply through animal carriers, animal hosts or improper handling procedures. The major groups of pests affecting the food products include rodents (especially murine rodents), birds (mainly crows, pigeons, seagulls, starlings and sparrows), reptiles (such as different species of lizards), insects (e.g. cockroaches, mosquitoes, flies, beetles, ants, wasps, bees, bugs, lice), and stored product insects (such as beetles, weevils, moths, ticks and mites), as well as domestic animals. Rodents which represent more than 40% of species diversity within the class Mammalia have a worldwide distribution and can be found in every habitat. The rats and squirrels are small rodents which live in close association with humans. These rodents are well known as a source of foodborne diseases. They are attracted by food supplies but do not venture far from their nesting sites or shelters. Three basic requirements that a rodent need are harborage, food, and water. Decrease in rodent populations distributed in an area usually is expected if one or more of these items are missing. Because of their ecology, these rodents nest close to food sources, eat poultry feed, contaminate food with their droppings, urine and filth and ruin crops, food containers and packaging much more than they eat. Furthermore, they have good reproduction capabilities and pose a particular threat as they live close to humans and livestock. Pathogenic agents can also be introduced onto soils, water supplies, vegetables and fruits by rodents. Moreover, they are considered as important pests around farms and homes due to physical damage to the building and equipment (such as gnawing on electrical wiring and damage to machinery, power failures or operational shutdowns due to

electrical or mechanical malfunctions, burrowing under walls and concrete walkways causing shifting and cracks).

All fruits, vegetables and root crops are living plant parts containing 65 to 95 percent water and they continue their life processes after harvest. The post-harvest life of produce depends on the rate at which stored food reserves are used up and the rate of water loss.

Food shelf-life will be influenced by factors such as:

1. Nature of the product (nutritional composition)
2. Packaging
3. Temperature

Time is an important factor in the spoilage of produce.

4.2 Learning Outcomes

By the end of this unit, you will be able to:

- Write the major sources of food deterioration
- Discuss the role of temperature, packaging and composition of food in food spoilage
- Discuss the extrinsic and intrinsic factors of food that affect deterioration
- Analyse other forms of deterioration arising from insect infestation and rodent attacks.

4.3 Causes of Deterioration and Spoilage

4.3.1 Chemical factors

Deterioration may result from chemical reactions (via endogenous enzymes) or through interactions involving one or more of the macronutrients present in food and food products. Enzymes are proteins that occur naturally in plant tissues and catalyze a number of important

biochemical reactions. Some enzyme-catalyzed reactions are beneficial while others result in quality deterioration. Examples of reactions involving endogenous enzymes include:

1. the post-harvest spoilage of fruit and vegetables
2. oxidation of phenolic substances in plant tissues by phenolase
3. sugar - starch conversion in plant tissues by amylases
4. post-harvest demethylation of pectic substances in plant tissues (leading to softening of plant tissues during ripening, and firming of plant tissues during processing).
5. Development of off-flavours through the breakdown of lipid components; and loss of color and undesirable browning.
6. Catalyze fermentation of sugars, breakdown of ascorbic acid, and many other deterioration reactions. Bruising, ripening, cutting, temperature, and presence of co-factors (Fe and Mg) increase the rate of degradative enzyme activity.

4.3.2 Physical Factors

One major undesirable physical change in food powders is the absorption of moisture as a consequence of an inadequate barrier provided by the package; this results in caking. It can occur either as a result of a poor selection of packaging material in the first place, or failure of the package integrity during storage. In general, moisture absorption is associated with increased cohesiveness.

Anti-caking agents are very fine powders of an inert chemical substance that are added to powders with much larger particle size in order to inhibit caking and improve flow-ability. At higher activities, however, ($a_w > 0.45$) the observed time to caking is inversely proportional to water activity, and at these levels anti-caking agents are completely ineffective. It appears that

while they reduce inter-particle attraction and interfere with the continuity of liquid bridges; they are unable to cover moisture sorption sites.

4.3.3 Biological Factors

The presence of pests and/or their droppings is a cause for alarm. Pests can spread disease-causing organisms to foods. Pests such as insects, rodents and birds are often identified as causes of biological deterioration of produce. They also cause damage to the surface of fruits and vegetables leading to greater susceptibility to invasion by microorganisms that can cause food spoilage and/or diseases to consumers. Proper sanitation in all produce handling and storage areas is the most effective weapon against these pests. Microorganisms can make both desirable and undesirable changes to the quality of foods depending on whether or not they are introduced as an essential part of the food preservation process or arise unintentionally and subsequently grow to produce food spoilage.

4.3.3.1 Insect Pests

Many species of insect are found in stored foods but few causes damage and losses. Some of them are beneficial because they attack insects and pests. Among several insects that attack stored products, weevils are very important.

Sitophilus oryzae L. (Rice weevil): They attack cereals like rice and cereal products such as paste, flour and biscuits

Sitophilus zeamais (Maize weevil): They attack maize, sorghum and other cereals Insects are the major causes of post harvest grain losses. They penetrate the kernels and feeding on the surfaces and the endosperm. They remove selectively the nutritious part of the food and encourage the development of bacteria and increase the moisture content of the food. Insect infestation will eventually lead to other storage problems. They give off moisture which can cause grain

moisture contents to increase enough to create a mould problem. Mould activity will in turn raise temperatures and result in an increased rate of insect reproduction. Greater numbers of insects create more moisture, and the cycle is repeated at an ever increasing rate. Insects also cause quality deterioration through their excreta as they consume. Insects are generally not a problem in grain stored for less than 10 months or a year if the grain is at its safe moisture and low temperature of storage.

Warm humid environments promote insect growth, although most insects will not breed if the temperature exceeds about 35 C° or falls below 10 C°. Also many insects cannot reproduce satisfactorily unless the moisture content of their food is greater 11%. The main categories of foods subject to pest attack are cereal grains and cereal-based products, other seeds used as food, dairy products such as cheese and milk powders, dried fruits, dried and smoked meats and nuts.

The presence of insects and insect excrete in packaged foods may render products unsaleable, causing considerable economic loss, as well as reduction in nutritional quality, production of off-flavours and acceleration of decay processes due to creation of higher temperatures and moisture levels. Early stages of infestation are often difficult to detect; however, infestation can generally be spotted not only by the presence of the insects themselves but also by the products of their activities such as webbing, clumping together of food particles and holes in packaging materials.

Unless plastic films are laminated with foil or paper, insects are able to penetrate most of them quite easily, the rate of penetration usually being directly related to film thickness. Thicker films are more resistant than thinner films, and oriented films tend to be more effective than cast films. The looseness of the film has also been reported to be an important factor, loose films being more easily penetrated than tightly fitted films. Generally, the penetration varies depending on

the basic resin from which the film is made, on the combination of materials, on the package structure, and of the species and stage of insects involved. The relative resistance to insect penetration of some flexible packaging materials is as follows:

i. **Excellent resistance:** polycarbonate and poly-ethylene-terephthalate

ii. **Good resistance:** cellulose acetate, polyamide, polyethylene (0.254 mm), polypropylene (biaxially oriented) and poly-vinyl- chloride (unplasticised).

iii. **Fair resistance:** acrylonitrile; poly-tetra-fluoro-ethylene and polyethylene (0.123 mm).

iv. **Poor resistance:** regenerated cellulose, corrugated paper board, kraft paper, polyethylene (0.0254 - 0.100 mm), paper/foil/polyethylene laminate pouch and poly-vinylchloride (plasticized).

Prevention and control of insect infestation

• Spray the inside of the storage with protective insecticides 2 to 3 weeks before new grain is added.

• Treat the grain with an approved insecticide as the storage is filled.

• Top-dress the grain with an approved insecticide after the storage has been filled and the grain surface has been leveled.

Controlling insects with insecticides, including fumigants, rather than using preventative methods incurs great cost. In addition, infestation generally results in dissatisfied customers and related marketing problems that develop from a poor reputation in marketing channels. The most unfortunate consequence of not managing grain properly is the loss of money, time, and effort to produce the grain (seed, fertilizer, field pest management, harvesting, threshing costs). Store preparation, drying and cooling are the main ways to protect against grain storage pests.

Changing temperatures and increasing moisture contents at the surface of grain bulks may allow residual infestations to develop. Occasional control failures due to poor management may require remedy. Most storage insects carry over from previously stored grain, so it is important to detect any residual infestations. Chemical grain treatment may be justified if persistent infestations cannot be controlled by drying and/or cooling. Protectants include:

• Liquid pesticides which may be applied to store fabric or grain itself to kill insects and mites.

• Fumigants which eliminate infestations within a few days.

• Smoke generators (not fumigants), primarily used to control flying insects.

• Diatomaceous earth which is not regarded as a pesticide.

4.3.3.2 Rodents

Three species of rodents are major pest of stored products:

• Rattus rattus (Black rat)

• Rattus norvegieus (Brown rat)

• Mus musculus (House mouse) Rodents consume cereal crops and damage sacks and building structures. They contaminate much great portion of the grain with their urine and droppings than they consume. Rodents can daily consume about 10% of their body weight. Poisoning and preventing their access to stored commodities can control them. Biological control also applied to stop rat damages. Generally, rats transmit diseases (typhus, rabies, trichomaisis) and destroy and damage building structures. Regardless of storage period, grain pest can invade the stored grain and affect the quantity and its quality.

Rodents carry pathogens on their feet and/or in their intestinal tracts and are known to harbour *Salmonella* of serotypes frequently associated with human food-borne infections. In addition to

the public health consequences of rodent populations in close proximity to humans, they also compete intensively with humans for food.

Rodents gnaw to reach sources of food and drink and to keep their teeth short. Their incisor teeth are so strong that rodents have been known to gnaw through lead pipes and unhardened concrete, as well as sacks, wood and flexible packaging materials. Proper sanitation in food processing and storage areas is the most effective weapon in the fight against rodents, since all packaging materials apart from metal and glass containers can be attacked by rodents.

Signs of Rodent's Infestation

Signs of pest infestation are:

- Presence of rodent faeces or small droppings
- Animal footprints
- Greasy smear marks along walls or tail streaks
- Nest holes or burrows
- Evidence of gnawed holes
- Physical damage to food or Food packaging material and property
- Damaged crops and plants
- Visual sighting of live rodents
- Take of bait at baiting points or from pest traps
- Musty smell where heavy infestations exist.

Rodent's Infections of Significant Public Health Importance.

Rodents have the capability to spread many human pathogens. They carry different pathogens on their skins and in their digestive system such as *Borrelia* spp., *Campylobacter* spp., *Clostridium* spp., *Cryptosporidium parvum, Escherichia coli, Leptospira* spp., *Listeria* spp.,

Mycoplasma spp., *Salmonella* spp., *Staphylococcus aureus*, *Streptobacillus moniliformis*, *Toxoplasma* spp., *Trichinella* spp., *Francisella tularensis*, *Yersinia pestis*, and Hantaviruses. They also cause infectious diseases such as campylobacteriosis, ascaridiasis, Bubonic plague, capilariasis, coli.bacillosis, hemorrhagic enteritis, hymenolepiasis, leptospirosis, listeriosis, lyme disease, mycoplasmosis, pasteurellosis, rat bite fever or Haverhill fever, salmonellosis, toxoplasmosis, trichinosis, tularemia and Hantavirus pulmonary syndrome.

i. *Clostridium perfringens* causes one of the most frequent forms of food intoxication. This gram-positive bacterium is widespread in nature and is found in air, soil, water, sewage, and on many food products.

ii. *E.coli* lives in the colons of humans, rodents and other mammals, and help break down waste products. However, whenever they enter the stomach and small intestines, they can cause illness. *E.coli* can be passed out of the body in faeces.

iii. Listeriosis is another foodborne infection caused by the bacteria species *Listeria monocytogenes*. This rod-shaped, gram-positive bacterium is found in soil, water and many species of animals and caused flu-like symptoms. This infection in pregnant women can result in miscarriage or stillbirth. Nearly 25% of serious cases result in death.

iv. *Salmonella* is a genus of rod-shaped, gramnegative bacteria which cause an illness called salmonellosis. *S. enteriditis* and *S. typhymurium* are most frequently isolated form from mice. These pathogenic bacteria can survive on people's hands for hours before being transmitted to foods and are often spread from one food to another by food handlers. Animal and human excreta, insects, soil, dust and raw meat are as the sources of this pathogen.

v. ***Trichinella spiralis*** as a pathogenic roundworm is probably the best known endoparasite that causes foodborne illness. An infection caused by this microscopic worm is known as trichinosis.

It occurs in wild animals, such as bears, boars, rodents and rabbits, as well as humans. This parasite can enter a host as larvae or adult worms through infected food.

vi. Hantavirus pulmonary syndrome (HPS) is a deadly viral disease caused by Sin Nombre, or the "No Name Virus". A wide variety of wild rodents such as cotton rat (Sigmodon hispidus), rice rat (Oryzomys palustris), deer mouse (Peromyscus maniculatus) and (Peromyscus leucopus) are reservours for the virus.

4.3.4 Effect of Deterioration on Food Quality

Chemical, physical and biological changes which occur during handling, processing and storage of foods lead to deterioration in sensory and nutritional quality of foods.

4.3.4.1 Sensory Quality

i. Lipid Oxidation

Lipid oxidation rate is influenced by light, local oxygen concentration, high temperature, the presence of catalysts (transition metals such as iron and copper) and water activity. Control of these factors can significantly reduce the extent of lipid oxidation in foods.

ii. Non-enzymatic browning

Non-enzymatic browning is one of the major causes of deterioration which occurs during storage of dried and concentrated foods. The non-enzymatic browning or Maillard reaction can be divided into three stages:

1. Early Maillard reactions which are chemically well-defined steps without browning.
2. Advanced Maillard reactions which lead to the formation of volatile or soluble substances
3. Final Maillard reactions leading to insoluble brown polymers.

iii. Colour changes

Almost any type of food processing or storage causes some deterioration of the chlorophyll pigments. Phenophytinisation is the major change and it is acid catalyzed reaction accelerated by heat. Dehydrated products such as green peas and beans packed in clear glass containers can undergo photo-oxidation and loss of desirable colour.

iv. Flavour changes

Enzymatically generated compounds derived from long-chain fatty acids play an extremely important role in the formation of characteristic flavours. In addition, these types of reactions can lead to significant off-flavours. Enzyme-induced oxidative breakdown of unsaturated fatty acids occurs extensively in plant tissues and these yield characteristic aromas associated with some ripening fruits and disrupted tissues. The permeability of packaging materials is of importance in retaining desirable volatile components within packages, or in permitting undesirable components to permeate through the package from the ambient atmosphere.

4.3.4.2 Nutritional Quality

The four major factors that affect nutrient degradation to varying extents are light, oxygen concentration, temperature, and water activity. However, due to the diverse nature of the various nutrients, chemical heterogeneity within each class of compounds and complex interactions of the above variables, generalizations about nutrient degradation in foods will inevitably be broad ones.

Ascorbic acid (Vitamin C) is the most sensitive vitamin in foods and its stability vary markedly as a function of environmental conditions such as pH and the concentration of trace metal ions and oxygen. The nature of the packaging material can significantly affect the stability of ascorbic acid in foods. The effectiveness of the material as a barrier to moisture and oxygen as well as the chemical nature of the surface exposed to the food are important factors. For example, problems

of ascorbic acid instability in aseptically packaged fruit juices have been encountered because of oxygen permeability of the package and the oxygen dependence of the ascorbic acid degradation reaction. Also, because of the preferential oxidation of metallic tin, citrus juices packaged in cans with a tin contact surface exhibit greater stability of ascorbic acid than those in enamelled cans or glass containers. The aerobic and anaerobic degradation reactions of ascorbic acid in reduced moisture foods have been shown to be highly sensitive to water activity, the reaction rate increasing in an exponential fashion over the water activity range of 0.1-0.8.

Self- Assessment Exercises

1. What are the factors that determine the relative resistance to insect penetration of some flexible packaging materials?
2. How can you control rodent's spoilage of foods?
3. List at least five disease caused by rodent spoilage of foods

4.4 Summary

Pest rodents infesting or entering food products or properties are a potential source of physical and microbiological hazards. Use of rodenticides, careless storage, and poorly executed rodent control programs may also result in chemical hazards. Hence, good hygiene as well as the five basic points for safe and sanitary food service is strictly advised as follows: protecting food from dirty equipment, vermin, animals, wastes, handling, coughs and sneezes during preparation, storage and service; keeping temperature cold to slow or stop the growth of Microbes for cold foods, also hot foods should be kept hot because heat kills microbes; handling, washing and carefully storing utensils in the correct way to keep them sanitary; cleaning and washing hands with soap and water before work, after using toilet and every time they become contaminated;

checking the health of food service personnel to prevent colds and other diseases from being passed to others.

4.5 Glossary

Food: anything eaten by man or animal to satisfy appetite, meet physiological needs for growth, maintain all body processes and supply energy to maintain body temperature and activities.

Fungi: single-celled or multicellular organism without chlorophyll that reproduces by spores and lives by absorbing nutrients from organic matter.

Sanitation: the maintenance of hygienic conditions, through services such as garbage collection and waste disposal.

Spoilage: any visible or invisible change which can make food or product derived from food unacceptable for human consumption.

Viruses: intracellular obligate parasite which can trigger dangerous infections in humans when they contaminate our food.

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4.7 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. The relative resistance to insect penetration of some flexible packaging materials is as follows:

i. **Excellent resistance:** polycarbonate and poly-ethylene-terephthalate

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iv. **Poor resistance:** regenerated cellulose, corrugated paper board, kraft paper, polyethylene (0.0254 - 0.100 mm), paper/foil/polyethylene laminate pouch and poly-vinylchloride (plasticized).

2. Proper sanitation in food processing and storage areas is the most effective weapon in the fight against rodents, since all packaging materials apart from metal and glass containers can be attacked by rodents.

3. They cause infectious diseases such as campylobacteriosis, ascaridiasis, Bubonic plague, capilariasis, coli.bacillosis, hemorrhagic enteritis, hymenolepiasis, leptospirosis, listeriosis, lyme disease, mycoplasmosis, pasteurellosis, rat bite fever or Haverhill fever, salmonellosis, toxoplasmosis, trichinosis, tularemia and Hantavirus pulmonary syndrome.

MODULE 3

UNIT 1 MICROBIOLOGY OF WATER SUPPLIES

CONTENTS

1.1 Introduction

Water is an essential resource for human survival. Safe drinking-water is essential to health. Water contamination is a common problem to all over the world. There are many contaminating elements of water which makes water unsafe for drinking. Microbial contaminants are one of the commonest of these elements. The microbial contaminants include pathogens like bacteria, viruses, and parasites such as microscopic protozoa and worms. These living organisms can be

spread by human and animal wastes knowingly or unknowingly. Some contaminants can be easily identified by assessing color, odour, turbidity and the taste of the water. However, most cannot be easily detected and require testing to reveal whether water is contaminated or not. As a result of this, the contaminants may result in unappealing taste or odour and staining as well as health effects.

1.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the microbiology of water
- Write the microorganisms that contaminate water
- Discuss water borne infection

1.3 Microbiology of water supplies

1.3.1 Biological contamination of water

Biological contamination of water is caused by the presence of living organisms, such as algae, bacteria, protozoan or viruses. Each of these can cause distinctive problems in water. Algae are in general single celled and microscopic. These are quite abundant and depend on nutrients in water. The nutrients are generally from domestic run-off or industrial pollution. The excess algae growth is not only imparted taste and odor problems in water; it clogs filters, and produces unwanted slime growths on the carriers. There are numerous pathogenic bacteria and can be contaminated with water. They can result in typhoid, dysentery, cholera and gastroenteritis. Although not harmful, some nonpathogenic bacteria may cause taste and odour problems. Protozoans likewise are also single-celled and microscopic organisms such as Giardia and Cryptosporidium commonly found in rivers, lakes, and streams contaminated with animal feces

or which receive wastewater from sewage treatment plants. These may cause diarrhea, stomach cramps, nausea, fatigue, dehydration and headaches. Viruses are the smallest living organisms capable of producing infection and causing diseases. Hepatitis and polio viruses are commonly reported in the contaminated water.

The greatest risk to public health from microbes in water is associated with consumption of drinking-water that is contaminated with human and animal excreta, although other sources and routes of exposure may also be significant. Infectious diseases caused by pathogenic bacteria, viruses and parasites are the most common and widespread health risk associated with drinking-water. The public health burden is determined by the severity and incidence of the illnesses associated with pathogens, their infectivity and the population exposed.

Waterborne pathogens have several properties that distinguish them from other drinking-water contaminants. These include;

• Organisms can cause acute and also chronic health effects.

• Some pathogens can grow in the environment.

• Pathogens are discrete.

• Pathogens are often clustered and adhere to suspended solids in water, and pathogen concentrations vary in time, so that the likelihood of acquiring an infective dose cannot be predicted from their average concentration in water.

• Exposure to a pathogen resulting in disease depends upon the dose, invasiveness and virulence of the pathogen, as well as the immune status of the individual.

• If infection is established, pathogens multiply in their host.

• Certain waterborne pathogens are also able to multiply in food, beverages or warm water systems, perpetuating or even increasing the likelihood of infection.

• Unlike many chemical agents, pathogens do not exhibit a cumulative effect.

1.3.2 Waterborne infections

The pathogens that may be transmitted through contaminated drinking-water all differ in properties, behaviour and resistance. Faecal–oral transmitted pathogens in drinking-water are only through one vehicle of transmission. Contamination of food, hands, utensils and clothing can also play a role, particularly when domestic sanitation and hygiene are poor. Improvements in the quality and availability of water, excreta disposal and general hygiene are all important in reducing faecal–oral disease transmission. Microbial drinking-water safety is not related only to faecal contamination. Although consumption of contaminated drinking-water represents the greatest risk, other routes of transmission can also lead to disease, with some pathogens transmitted by multiple routes. Certain serious illnesses result from inhalation of water droplets in which the causative organisms have multiplied because of warm waters and the presence of nutrients. These include legionellosis, caused by *Legionella* spp., and illnesses caused by the amoebae *Naegleria fowleri* and *Acanthamoeba* spp. Schistosomiasis is a major parasitic disease of tropical and subtropical regions that is transmitted when the larval stage (cercariae), which is released by infected aquatic snails, penetrates the skin. It is primarily spread by contact with water. Ready availability of safe drinking-water contributes to disease prevention by reducing the need for contact with contaminated water resources.

It is conceivable that unsafe drinking-water contaminated with soil or faeces could serve as a carrier of other infectious parasites, such as *Balantidium coli* and certain helminths such as *Fasciola*, *Fasciolopsis*, *Echinococcus*, *Ascaris*, *Trichuris*, *Necator*, and *Taenia solium*. Most of these have their normal mode of transmission as ingestion of the eggs in food contaminated with faeces or faecally contaminated soil. If water used by immunocompromised persons such as the

elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy or those with acquired immunodeficiency syndrome (AIDS) for drinking or bathing contains sufficient numbers of these organisms, they can produce various infections of the skin and the mucous membranes of the eye, ear, nose and throat. Examples of such agents are *Pseudomonas aeruginosa* and species of *Flavobacterium*, *Acinetobacter*, *Klebsiella*, *Serratia*, *Aeromonas* and certain "slow growing" (non-tuberculous) mycobacteria.

Outbreaks of waterborne disease may affect large numbers of persons, and the first priority in developing and applying controls on drinking-water quality should be the control of such outbreaks. Available evidence also suggests that drinking-water can contribute to background rates of disease in non-outbreak situations, and control of drinking-water quality should therefore also address waterborne disease in the general community. Experience has shown that systems for the detection of waterborne disease outbreaks are typically inefficient in countries at all levels of socioeconomic development, and failure to detect outbreaks is not a guarantee that they do not occur; nor does it suggest that drinking-water should necessarily be considered safe. Some of the pathogens that are known to be transmitted through contaminated drinking-water lead to severe and sometimes life-threatening disease. Examples include typhoid, cholera, infectious hepatitis (caused by hepatitis A virus or hepatitis E virus) and disease caused by *Shigella* spp. and *E. coli* O157. Others are typically associated with less severe outcomes, such as self-limiting diarrhoeal disease (e.g., noroviruses, *Cryptosporidium*).

The effects of exposure to pathogens are not the same for all individuals or, as a consequence, for all populations. Repeated exposure to a pathogen may be associated with a lower probability or severity of illness because of the effects of acquired immunity. For some pathogens (e.g., hepatitis A virus), immunity is lifelong, whereas for others (e.g., *Campylobacter*), the protective

effects may be restricted to a few months to years. In contrast, vulnerable subpopulations (e.g., the young, the elderly, pregnant women, the immunocompromised) may have a greater probability of illness or the illness may be more severe, including mortality. Not all pathogens have greater effects in all vulnerable subpopulations. Not all infected individuals will develop symptomatic disease. The proportion of the infected population that is asymptomatic (including carriers) differs between pathogens and also depends on population characteristics, such as prevalence of immunity. Those with asymptomatic infections as well as patients during and after illness may all contribute to secondary spread of pathogens.

Self- Assessment Exercises 1

1. What is water-borne infection?
2. State the properties of water-borne pathogens

1.4 Summary

There are many contaminating elements of water which makes water unsafe for drinking but microbial contaminants are one of the commonest of these elements. The microbial contaminants include pathogens like bacteria, viruses, and parasites such as microscopic protozoa and worms. These living organisms can be spread by human and animal wastes knowingly or unknowingly. Some contaminants can be easily identified by assessing colour, odour, turbidity and the taste of the water. However, most cannot be easily detected and require testing to reveal whether water is contaminated or not. As a result of this, the contaminants may result in unappealing taste or odour and staining as well as health effects.

1.5 References/Further Readings

Eugene W. Nester, Denise G. Anderson., C. Evans Roberts, Jr., and Martha T. Nester (2012). Microbiology: A Human Perspective. 7th edition. McGraw-Hill, a business unit of The McGraw-Hill Companies, Inc., 1221 Avenue of the Americas, New York, NY 10020.

1.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. Water-borne infection is an infection caused by use of contaminated water for drinking, cooking, washing etc.
2. The properties of waterborne pathogens include;

• Organisms can cause acute and also chronic health effects.

• Some pathogens can grow in the environment.

• Pathogens are discrete.

• Pathogens are often clustered and adhere to suspended solids in water, and pathogen concentrations vary in time, so that the likelihood of acquiring an infective dose cannot be predicted from their average concentration in water.

• Exposure to a pathogen resulting in disease depends upon the dose, invasiveness and virulence of the pathogen, as well as the immune status of the individual.

• If infection is established, pathogens multiply in their host.

• Certain waterborne pathogens are also able to multiply in food, beverages or warm water systems, perpetuating or even increasing the likelihood of infection.

• Unlike many chemical agents, pathogens do not exhibit a cumulative effect.

UNIT 2 **WATER CONTAMINATION FROM SEWAGE**

CONTENTS

2.1 Introduction

Sewage or wastewater includes all the material that flows from household plumbing systems, including washing and bathing water and toilet wastes as well as others from business and industrial wastes. In many cities, storm water runoff that flows into street drains enters the system as well. The lack of adequate sanitation and the indiscriminate flow of sewage or waste water for humans is a staggering problem.

2.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss how sewage contaminate water
- Write the microorganisms associated with the sewage contamination of water
- Discuss sewage treatment Processes

2.3 Water contamination from sewage

Contamination of surface waters by faecal viruses from sewage systems is only one sign and dimension of the pollution problem. Accompanying faeces and faecal viruses in sewage discharge are a cocktail of pollutants, including, heavy metal chemicals, pharmaceuticals, and pathogens. Like faecal viruses, these chemicals are good indicators of sewage contamination. It is widely recognized that sewage contamination of surface waters is a global threat to public health and they also affect wildlife and natural habitats. For this reason, wastewater must be treated before discharge since pathogenic microbes can be transmitted through it. If untreated sewage is released into a river or lake that is then used as a source of drinking water, disease can easily spread. If marine waters become contaminated in a similar manner, eating the local shellfish can result in disease. The plants and wildlife living in or around contaminated water often accumulate toxins and pathogens found in sewage. Heavy metal occurrence in predatory fish is associated with an increase in sewage pollution in those waters. Pathogens found in sewage pollution have been shown to cause widespread disease in humans. Because sewage contamination is a global threat that occurs in most areas where humans live and nature is often nearby, it is possible that sewage also globally threatens natural habitats and biodiversity.

Health effect of sewage

People are exposed to sewage by hand-to-mouth contact during eating, drinking and smoking, or by wiping the face with contaminated hands or gloves. Exposure can also occur by skin contact, through cuts, scratches, or penetrating wounds, and from discarded hypodermic needles. Certain organisms can enter the body through the surfaces of the eyes, nose and mouth and by breathing them in as dust, aerosol or mist.

Sewage contains bacteria, fungi, parasites, and viruses that can cause intestinal, lung, and other infections. Bacteria may cause diarrhea, fever, cramps, and sometimes vomiting, headache, weakness, or loss of appetite. Some bacteria and diseases carried by sewage are *E. coli*, shigellosis, typhoid fever, *salmonella*, and cholera. Fungi such as *Aspergillus* and other fungi often grow in compost. These can lead to allergic symptoms (such as runny nose) and sometimes can lead to lung infection or make asthma worse. Parasites including *Cryptosporidium* and *Giardia lamblia* may cause diarrhea and stomach cramps, and even nausea or a slight fever. Most people have no symptoms to roundworm (Ascariasis). Roundworms cause coughing, trouble breathing and/or pain in your belly and blocked intestines. Viruses such as Hepatitis A cause liver disease. Symptoms of Hepatitis A include feeling tired, having pain in your belly, being nauseous, having jaundice (yellow skin), having diarrhea, or not being hungry.

2.3.2 Sewage Treatment

Typical public sewage contains oxygen-demanding materials, sediments, grease, oil, scum, pathogenic bacteria, viruses, salts, algal nutrients, pesticides, refractory organic compounds, heavy metals, and an astonishing variety of refuse ranging from children's socks to sponges. Several characteristics used to describe sewage include turbidity, suspended solids, total dissolved solids, acidity, and dissolved oxygen (in ppm O_2). Biochemical oxygen demand is used as a measure of oxygen-demanding substances. Current processes for the treatment of wastewater can be divided into three main categories: primary treatment, secondary treatment, and tertiary treatment.

Primary Waste Treatment

Primary treatment of wastewater consists of the removal of insoluble matter such as grit, grease, and scum from water. The first step in primary treatment normally is screening to remove or

reduce the size of trash and large solids that get into the sewage system. These solids are collected on screens and scraped off for subsequent disposal. Comminuting devices shred and grind solids in the sewage. Particle size can be reduced to the extent that the particles can be returned to the sewage flow. Grit in wastewater consists of such materials as sand and coffee grounds that do not biodegrade well and generally have a high settling velocity. Grit removal is practiced to prevent its accumulation in other parts of the treatment system, to reduce clogging of pipes and other parts, and to protect moving parts from abrasion and wear. Grit normally is allowed to settle in a tank under conditions of low flow velocity, and it is then scraped mechanically from the bottom of the tank.

Primary sedimentation removes both settleable and floatable solids. During primary sedimentation there is a tendency for flocculent particles to aggregate for better settling, a process that may be aided by the addition of chemicals. The material that floats in the primary settling basin is known collectively as grease. In addition to fatty substances, the grease consists of oils, waxes, free fatty acids, and insoluble soaps containing calcium and magnesium. Normally, some of the grease settles with the sludge and some floats to the surface, where it can be removed by a skimming device.

Secondary Waste Treatment by Biological Processes

Secondary wastewater treatment is designed to remove BOD (biological oxygen demand), usually by taking advantage of the same kind of biological processes that would otherwise consume oxygen in water receiving the wastewater. Secondary treatment by biological processes takes many forms but consists basically of the action of microorganisms provided with added oxygen degrading organic material in solution or in suspension until the BOD of the waste has been reduced to acceptable levels. The waste is oxidized biologically under conditions controlled

for optimum bacterial growth, and at a site where this growth does not influence the environment.

Tertiary Waste Treatment

Tertiary waste treatment or advanced waste treatment is a term used to describe a variety of processes performed on the effluent from secondary waste treatment. The contaminants removed by tertiary waste treatment fall into the general categories:

i. Suspended solids

ii. Dissolved organic compounds

iii. Dissolved inorganic materials, including the important class of algal nutrients.

Each of these categories presents its own problems with regard to water quality. Suspended solids are primarily responsible for residual biological oxygen demand in secondary sewage effluent waters. The dissolved organics are the most hazardous from the standpoint of potential toxicity. The major problem with dissolved inorganic materials is that presented by algal nutrients, primarily nitrates and phosphates, and potentially hazardous toxic metals may be found among the dissolved inorganics.

Self- Assessment Exercises

1. What are the pollutants of sewage?
2. State the effect of sewage contamination on plants and wide life

2.4 Summary

Sewage, especially when used as fertilizer in crops, can contaminate food with microorganisms, the most significant of which are different enteropathogenic bacteria and viruses. This can be a major concern with organically grown food and many imported fruits and vegetables, in which

untreated sewage and manure might be used as fertilizer. Pathogenic parasites can also get in food from sewage. To reduce the incidence of microbial contamination of foods from sewage, it is better not to use sewage as fertilizer or If used, it should be efficiently treated to kill the pathogens. Also, effective washing of foods following harvesting is important.

2.5 References/Further Readings

Eugene W. Nester, Denise G. Anderson., C. Evans Roberts, Jr., and Martha T. Nester (2012). Microbiology: A Human Perspective. 7th edition. McGraw-Hill, a business unit of The McGraw-Hill Companies, Inc., 1221 Avenue of the Americas, New York, NY 10020.

Ray, B (2005). Fundamental Food Microbiology 3rd edition. Boca Raton London New York Washington, D.C. 625 pages.

Manahan, S. E (2001). Water Treatment, Fundamentals of Environmental Chemistry Boca Raton: CRC Press LLC, 42 pages

2.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. Viruses, heavy metal chemicals, pharmaceuticals, and pathogens.
2. The plants and wildlife living in or around contaminated water often accumulate toxins and pathogens found in sewage.

UNIT 3 WATER CONTAMINATION FROM HANDLING AND DUST

CONTENTS

3.1 Introduction

Water is used to produce, process, and, under certain conditions, store foods. It is used for irrigation of crops, drinking by food animals and birds, raising fishery and marine products, washing foods, processing and storage of foods, washing and sanitation of equipment, and processing and transportation facilities. Water is also used as an ingredient in many processed foods. Therefore, the quality of water can greatly influence microbial quality of foods. Contamination of foods with pathogenic bacteria, viruses, and parasites from water has been recorded. Wastewater can be recycled for irrigation; however, chlorine-treated potable water, should be used in processing, washing, sanitation, and as an ingredient. Although potable water does not contain coliforms and pathogens, it can contain other bacteria capable of causing food spoilage, such as *Pseudomonas*, *Alcaligenes*, and *Flavobacterium*. Improperly treated water can contain pathogenic and spoilage microorganisms and to overcome this problem, many food processors use water, especially as an ingredient, that has a higher microbial quality than that of potable water

 **3.2** Learning Outcomes

By the end of this unit, you will be able to:

- Discuss water contamination from handling
- Discuss water contamination from dust

 **3.3 Water contamination from handling and dust**

Human activities, industrialization and agricultural practices contribute significantly to the degradation and contamination of environment which contrarily affects the water bodies. Industries are the main cause of water pollution. Various toxic chemicals, organic and inorganic substances, toxic solvents and volatile organic chemicals may be released in industrial production into aquatic ecosystems. Without adequate treatment, they cause water pollution. With continued urbanization, wastewater from industrial production has gradually increased impacting the human health negatively. Pesticides, nitrogen fertilizers and organic farm wastes from agriculture are significant causes of water pollution. Dusts from Agricultural activities contaminate the water with nitrates, phosphorus, pesticides, soil sediments, salts and pathogens making them unsafe for use. Water contamination dust most of the times take the form of inorganic “water pollution”. This involves inorganic water poisons brought about by industrial releases from power plants and chemical waste as industrial byproducts. Heavy metals from engine vehicles and corrosive mine. Untreated or partially treated water contaminated with processing dusts poses risks to the environment and health. These dusts have an adverse impact on health through drinking water.

Self- Assessment Exercises 1

1. How do dusts contaminate water?
2. State the bacteria that can be easily found in potable water.

3.4 Summary

Pollution in water resources is any change in the properties of water; physical, chemical and biological. This can be due to the throwing of pollutants; solid, liquid and gaseous substances into the sources of water that causes damage to the economy & health and safety of the population.

3.5 References/Further Reading

Eugene W. Nester, Denise G. Anderson., C. Evans Roberts, Jr., and Martha T. Nester (2012). Microbiology: A Human Perspective. 7th edition. McGraw-Hill, a business unit of The McGraw-Hill Companies, Inc., 1221 Avenue of the Americas, New York, NY 10020.

3.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercise

1. Dusts contaminate water with nitrates, phosphorus, pesticides, soil sediments, salts and pathogens making them unsafe for use.
2. Potable water contains bacteria such as *Pseudomonas, Alcaligenes,* and *Flavobacterium*.

UNIT 4 WATER TREATMENT PROCESS

CONTENTS

4.1 Introduction

Treatment of drinking water is designed to eliminate pathogenic microbes as well as harmful chemicals. Water is made to flow into a reservoir and is allowed to stand long enough for the particulate matter to settle out. Subsequently the water is transferred to a tank where it is mixed with a chemical that causes suspended materials to coagulate using a coagulant such as aluminum sulfate. Sedimentation of the mixture follows in a tank, where the coagulated materials are allowed to slowly sink to the bottom. Some microbes and other substances are trapped and thereby removed as well as they form colloids. Following the removal of the coagulated materials, the water is filtered using a thick bed of sand and gravel, to remove various microbes including bacteria and protozoan cysts and oocysts. Additional filtration can be done to remove organic chemicals that may be harmful or give undesirable tastes and odours. This process is done using an activated charcoal filter, which adsorbs dissolved chemicals. Filtration is important since microorganisms growing in biofilms on the filter materials use carbon from the water as it passes. This lowers the organic carbon content that might remain.

4.2 Learning Outcomes

By the end of this unit, you should be able to:

- Discuss water treatment processes

4.3 Water Treatment Processes

Coagulation

Coagulation is the process by means of which the colloidal particles in water are destabilized so that they form flocs through the process of flocculation that can be readily separated from the water. Destabilization is achieved through the addition of chemicals (coagulants) to the water. Different chemicals can be used as coagulants. The most common coagulants are:

- Aluminium sulphate, also known as alum $Al_2(SO_4)_3.16\ H_2O$
- Ferric chloride, $FeCl_3$ is also commonly used as coagulant
- Hydrated lime
- Polymeric coagulants including Dadmacs and polyamines which form white or brown flocs when added to water. š
- Polyelectrolytes are mostly used to assist in the flocculation process and are often called flocculation aids.
- Aluminium polymers such as poly-aluminium chloride
- Activated silica
- Bentonite and/or kaolin

Flocculation

Flocculation involves the stirring of water to which a coagulant has been added at a slow rate, causing the individual particles to “collide”. A simple mechanical stirrer can be used for

flocculation or a specially designed channel with baffles to create the desired flow conditions can also be used to flocculate the particles in water. The basis of the design of a flocculation channel is that the flow velocity of the water has to be reduced from a high initial value to a much lower value to enable large, strong aggregates to form. If the flow velocity is too high the aggregates may break up again, causing settling of the broken flocs to be incomplete. Flocculation is controlled through the introduction of energy into the water (through paddles or by means of baffles in the flocculation channel) to produce the right conditions (required velocity gradient) for flocs to grow to the optimum size and strength. If too high, shear forces become large and this may result in break-up of aggregates. Aggregates and flocs are removed from water by means of separation processes such as sedimentation and sand filtration; or flotation and sand filtration.

Sedimentation and Flotation

Sedimentation or settling is the simple process in which particles that are heavier than water settle to the bottom of a container in which the suspension is held, or through which the suspension flows. On the basis of the concentration of particles and the tendency of particles to interact, four types of settling behaviour can be distinguished: discrete particle settling, flocculent settling, hindered/zone settling, and compression settling.

Effective sedimentation and removal of particles from water depends mainly on the effectiveness of the coagulation-flocculation process and on the proper design of the sedimentation tank. If coagulation is not effective, the forces that keep particles apart will persist. If flocculation is not effective, the opportunity for collision between particles and floc growth will not be optimal and large floc aggregates that settle readily will not be formed. If coagulationflocculation is effective but the design of the sedimentation tank is such that flocs deteriorate due to poor inlet design, or

flocs cannot settle due to too high up-flow velocity or short-circuiting, the effluent water will still have a high turbidity.

Flotation

Flotation involves the formation of small air bubbles in water that has been coagulated. The bubbles attach to the flocs causing them to rise to the surface where they are collected as a froth that is removed from the top of the flotation unit. Air is dissolved under pressure in a small amount of water in a device called a saturator. This water that is saturated with dissolved air is added to the main stream of water that is to be treated. When the pressure is released after the saturated water is mixed with the water to be treated, the dissolved air comes out of solution in the form of very fine bubbles.

Both sedimentation and flotation remove the bulk of the flocs from the water. However, most of the time a small amount of flocs or non-flocculated colloidal material remains in the water. This material has to be removed to ensure a low enough turbidity in the water by means of sand filtration. A sufficiently low turbidity level is required for effective disinfection of the water and to remove all traces of cloudiness from the water.

Filteration

Some form of filtration will always be found at a water treatment plant. While coagulation, flocculation, sedimentation and flotation play important roles and will remove the bulk of the particles from the raw water, they alone will not meet the strict quality standards that are required of drinking water. Filtration is the only process that is capable of removing very small particles down to the level required. Filtration had been viewed as a simple aesthetic polishing step to complete a chain of processes, starting with chemical dosing and ending with filtration. In the past decade or more, there has been a renewed interest in filtration, as it turned out that the

process is the only one that can be completely relied on to remove a number of newly discovered health hazards, namely the protozoan cysts and oocysts common to most raw water sources in the world. Higher standards for filtration performance have thus been globally adopted and water supply utilities are taking a new, critical look at their filtration facilities.

Disinfection

Disinfection is the most important stage of water treatment and is a process specifically designed for the reduction of the number of pathogenic organisms present. As disinfection is the final safeguard against water-borne microbial disease, the application of disinfectants is of utmost importance as it is the last point at which the water quality can be affected. It is essential that disinfectants and their dosage rates are selected such that the chemical demand of the water is satisfied and the desired residual after initial contact is achieved and maintained throughout the distribution system up to the consumer. Regular monitoring of disinfectant residuals at the purification plant and throughout the distribution system, in parallel with microbiological examination, is essential to evaluate and control the disinfection process. In addition to the destruction of pathogens, disinfection improves the general microbiological quality of the water. This helps to maintain the water quality in long distribution lines and reticulation systems. The introduction of two water treatment processes, namely filtration and chlorination, had a significant impact on reducing the number and severity of incidents of water borne diseases. In the preparation of drinking water the term disinfection is used to describe the process of destroying or inactivating pathogenic organisms. The sole purpose of disinfection of drinking water is to specifically destroy pathogenic organisms, thereby eliminating the possibility of water-borne diseases.

Types of Disinfectants and Modes of Disinfection

Mircoorganisms can be removed, inhibited or killed by various physical processes, physical agents or chemical agents. š

Physical processes: These include gravity separation and filtration. Gravity separation (sedimentation and flotation) and filtration play a very important role in the removal of bacteria, viruses and protozoan cysts.

Physical agents: These include heating and irradiation. Heating water by boiling or by solar energy in small transparent containers is only possible on a very small scale and is more suited to situations where no other form of disinfection is available. Concentrated UV light as a mode of disinfection for drinking water is gaining popularity and has been proven to be very effective in the inactivation of microorganisms.

Chemical agents: These are by far the most popular means of disinfection in the drinking water industry and many alternatives, each with its own particular application, are available. The most commonly used chemicals are chlorine gas (Cl_2), calcium hypochlorite [$Ca(OCl)_2$], sodium hypochlorite [NaOCl], chlorine dioxide [ClO_2], monochloramne [NH_2Cl], ozone [O_3], hydrogen peroxide [H_2O_2], potassium permanganate [$KMnO_4$], iodine [I_2],and bromine [Br_2]. While the chlorine based disinfectants have historically been the most popular products to use, the unique properties of other compounds such as ozone have caused a rapid increase in their use.

Self- Assessment Exercise

1. Outline the most common coagulants used in water treatment.
2. List the chemical used in the treatment of water.

4.4 Summary

Aggregates and flocs are removed from water by means of separation processes such as sedimentation and sand filtration; or flotation and sand filtration. Subsequent to the removal of the coagulated materials, the water is filtered to remove various microbes including bacteria and protozoan cysts and oocysts. Filtration is important since microorganisms growing in biofilms on the filter materials use carbon from the water as it passes. This lowers the organic carbon content that might remain. The application of disinfectants is of utmost importance as it is the last point at which the water quality can be affected.

4.5 Glossary

Sanitation: the maintenance of hygienic conditions, through services such as garbage collection and waste disposal.

Drying: removal of water from food and is one of the oldest and simplest methods of preserving food.

Refrigeration: process of lowering the temperature in a given space and maintaining it for the purpose of chilling foods, preserving certain substances, or providing an atmosphere conducive to bodily comfort.

Sewage: all the material that flows from household plumbing systems, including washing and bathing water and toilet wastes as well as others from business and industrial wastes.

Spoilage: any visible or invisible change which can makes food or product derived from food unacceptable for human consumption.

4.6 References

Eugene W. Nester, Denise G. Anderson., C. Evans Roberts, Jr., and Martha T. Nester (2012). Microbiology: A Human Perspective. 7th edition. McGraw-Hill, a business unit of The McGraw-Hill Companies, Inc., 1221 Avenue of the Americas, New York, NY 10020.

Frik Schutte (2006). Handbook for the Operation of Water Treatment Works. The Water Research Commission The Water Institute of Southern Africa, TT 265/06. 242 pages

4.7 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercise

1. The most common coagulants are:

- Aluminium sulphate, also known as alum $Al_2(SO_4)_3.16\ H_2O$
- Ferric chloride, $FeCl_3$ is also commonly used as coagulant
- Hydrated lime
- Polymeric coagulants including Dadmacs and polyamines which form white or brown flocs when added to water. š
- Polyelectrolytes are mostly used to assist in the flocculation process and are often called flocculation aids.
- Aluminium polymers such as poly-aluminium chloride
- Activated silica
- Bentonite and/or kaolin

2. The most commonly used chemicals are chlorine gas (Cl_2), calcium hypochlorite [$Ca\ (OCl)_2$], sodium hypochlorite [NaOCl], chlorine dioxide [ClO_2], monochloramne [NH_2Cl], ozone [O_3], hydrogen peroxide [H_2O_2], potassium permanganate [$KMnO_4$], iodine [I_2],and bromine [Br_2].

Module 4

Unit 1 Food and water borne diseases

Unit 2 Food infections and toxicants

Unit 3 Identification of food poisoning microorganisms

Unit 4 Control of food poisoning microorganisms

UNIT 1 FOOD AND WATER BORNE DISEASES

CONTENTS

1.1 Introduction

Foodborne illness, more commonly referred to as food poisoning, is the result of eating contaminated, spoiled, or toxic food. Human illnesses from the consumption of foods contaminated with biological factors are what is known as foodborne diseases. Every day, humans and animals consume food materials contaminated with microbes which adversely affect their health condition. The Centers for Disease Control and Prevention (CDCP), reports that one in every six people in the United States gets sick each year from eating contaminated food. This is number is even more developing countries where there is poor hygiene level and more people handle food materials poorly. With this, it means that more people are affected as part of the more than 1,000 outbreaks that happen each year. Foodborne illness is caused by unintentional contamination of food materials with microorganisms. Although biological hazards cause the majority of illnesses, some may be due to chemical contamination. We will be focusing more in the biological hazards on the course of our study.

A number of factors contribute to the outbreak of foodborne disease. While contamination can occur at any level of the food processing, it all affect the health of humans. Before consumption, foods are exposed to many different environments and conditions. These dictate whether a pathogen present initially will survive or be killed, whether recontamination can occur, or whether a pathogen can multiply to reach a high population to cause disease. Factors such as the presence of a pathogen in the raw materials with a potential of contaminating the finished

products, the ability of a pathogen to grow optimally in a particular type of food, quality control failure during processing, and a higher possibility of contaminating the finished products by food handlers are all factors that causes food poisoning.

Generally, foodborne illness is favoured by the following conditions;

- The microorganism or its toxin must be present in food.
- The food must be suitable for the microorganism to grow.
- The temperature must be suitable for the microorganism to grow.
- Enough time must be given for the microorganism to grow and to produce a toxin.
- The food must be eaten.

It is worthy of note that foods contaminated with pathogenic microorganisms usually do not look, taste or smell bad. Owing to this, it is impossible to determine whether a food is contaminated with pathogenic microorganisms without microbiological testing. To avoid potential problems in foods, it is very important to control or eliminate these microorganisms in food products.

Bacteria and viruses are the most common cause of food poisoning. The symptoms and severity of food poisoning vary, depending on which bacteria or virus has contaminated the food. Some Parasites are also implicated in some incidence of food poisoning. the most common foodborne parasites are protozoa, roundworms, and tapeworms.

Molds, Toxins, and Contaminants: Most food poisoning is caused by bacteria, viruses, and parasites rather than toxic substances in the food. But some cases of food poisoning can be linked to either natural toxins or added chemical toxins. Allergens: Food allergy is an abnormal response to a food triggered by your body's immune system. Some foods, such as nuts, milk,

eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat or soybeans, can cause allergic reactions in people with food allergies.

Sympyoms of food poisoning

Symptoms can vary and the length of time for the symptoms to appear depends on the source of the infection, but it can range from as little as 1 hour to as long as 28 days.

Common cases of food poisoning will typically include at least three of the following symptoms:

i. abdominal cramps
ii. loss of appetite
iii. diarrhoea
iv. vomiting
v. headaches
vi. mild fever
vii. weakness
viii. nausea

Symptoms of potentially life-threatening food poisoning include:

i. diarrhoea persisting for more than three days
ii. a fever higher than 101.5°F
iii. difficulty seeing or speaking
iv. symptoms of severe dehydration, which may include dry mouth, passing little to no urine, and difficulty keeping fluids down
v. bloody urine

1.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss food poisoning and cross contamination
- Write the types of botulism
- Evaluate the main sources of mineral poisoning
- Write the symptoms of bacteria and mineral poisoning

1.3 Causes and Management of Food Poisoning

Direct or indirect contamination of food can cause infections in man. The transmission of these infections by food depends on the following conditions:

i. The presence of a food that supports the growth of the microorganism.

ii. The inoculation of foods with sufficient number of microorganisms from a patient with clinical disease, or a carrier, or contaminated environment.

iii. Contamination at suitable temperatures for a period long enough to permit the growth of the organism or the elaboration of the toxin.

iv. The absence of suitable treatment or processing of the food to inactivate the organism or the toxin.

v. Ingestion of food by the host.

This series of events usually occurs in a setting where:

a. There is a reservoir of organism in man, animals, or the environment

b. Knowledge and practice of food hygiene and personal hygiene is inadequate to prevent transmission of the organism

c. Sanitation facilities are insufficient to prevent contamination of the environment with human excreta and its transfer to food.

Contamination of food leading to food poisoning can occur as a result of the way in which the food is handled and prepared. The major causes of food poisoning are:

1.3.1 Inadequate Cooking

Inadequate cooking of contaminated raw food and inadequate reheating of pre-cooked food will not produce heat sufficient enough to kill microorganisms on the food.

1.3.2 Food Storage Conditions

Cooked food should not be kept at temperatures that favour the growth of bacteria. *Bacillus cereus* forms spores that are relatively resistant to heat and these spores are commonly found on cereal grains. If cooked rice is kept warm, the spores germinate and the organisms grow and produce its toxin.

1.3.3 Cross - Contamination between Raw and Cooked Food

Cross-contamination is a problem in domestic food preparation, but may also occur, sometimes with dramatic effects, in industrial food processing.

1.3.4 Poor Personal Hygiene in Food Handlers

Personal hygiene begins at home, with the essential elements for good hygiene being a clean body, clean hair and clean clothing. Hair in food can be a source of both microbiological and physical contamination. Hairnets and beard covers should be worn to assure food product integrity. Long-sleeved smocks should be worn to cover arm hair. Clean uniforms, aprons and other outer garments that are put on after the employee gets to work can help minimize contamination. While working, clothing should be kept reasonably clean and in good repair. Removal of smocks, laboratory coats or aprons should take place when leaving the work area to

go to the employee break room, restroom or exiting the building. Personal items such as meals and snacks should be stored in a locker or break room area that is located away from processing areas or areas where equipment and utensils are washed.

The only jewelry allowed in a food plant is a plain wedding band and/or one small post earring in each ear. No other jewelry is to be worn because it may fall into the product, it can present a safety hazard and it cannot be adequately sanitized against bacterial transmission. It should be removed prior to entering the processing facility. Employees must wear different colored smocks when going from a raw processing part of the establishment to the cooked processing side. They should also step into a sanitizer footbath between the two processing areas to eliminate the bacteria on their shoes.

No employee who is affected with, has been exposed to, or is a carrier of a communicable disease, the flu or a respiratory problem, or any other potential source of microbiological contamination shall work in any area where there is a reasonable possibility that food or food ingredients can be contaminated. The number one symptom of a foodborne illness is diarrhoea. Other symptoms include fever, dizziness, vomiting, and sore throat with fever or jaundice. Any employee with these symptoms should not be allowed to work around food. If an employee has been diagnosed with a foodborne illness, exclude them from the establishment, and contact the local health department. The health department must be notified if the employee has been diagnosed with one of the following foodborne illnesses: *Salmonella typhi*, *Shigella species*, *shiga* toxin producing *E. coli*, or hepatitis A virus.

1.3.5 Ingestion of Toxins

The ingestion of naturally occurring poisons present in mushrooms, toadstools, fish and shellfish and other contaminants causes food poisining. Food poisoning from mushrooms such as *Amanita*

phalloides or muscaria, results in sweating, cramps, diarrhoea, confusion and sometimes convulsions. Patients usually recover within 24 hours if the infecting mushroom is *Amanita phalloides*, however, liver damage is common, leading to jaundice. Remissions may occur, but the mortality rate is about 60 percent or higher. Fish poisoning can result from Pacific types such as sea bass, Caribbean types such as Cavallas, Scrombroid types such as Mackerel, and Tetraodon types such as Puffers. Symptoms include numbness of the limbs, joint aches, chills and fever. Muscle weakness and paralysis can also occur, and death may result within 24 hours.

1.3.6 Ingestion of Heavy Metals

Ingestion of heavy metals like lead and mercury can cause acute nausea, vomiting and diarrhoea and may cause respiratory or nervous system damage over a long term. The severity of the symptoms depends on the metal and the dose, as well the patient. Treatment includes bed rest, fluids and blood or plasma expanders in severe cases where shock is anticipated.

Self- Assessment Exercises 1

1. What are the Symptoms of potentially life-threatening food poisoning?
2. Outline the major causes of food poisoning

1.4 Types of Food Poisoning Organisms

Food poisoning organisms can be classified into four groups, depending on the mechanism involved in causing disease:

a. Organisms that produce toxin in the food
b. Organisms that multiply in the intestinal tract and produce toxins that causes the symptoms

c. Organisms that invade the body but generally remain in the region of the intestinal tract or cause widespread systemic infection

d. Other microbial infection

1.4.1 Organisms that Produce Toxin in the Food

The main examples of organisms that produce toxin in the food are *Clostridium botulinum*, *Staphylococcus aureus* and some strains of *Bacillus cereus*. The problem here is more of intoxication than infection and if the food contains a significant amount of the toxin, subsequent cooking will not reduce the risk of food poisoning.

a. *Clostridium botulinum* Food Borne Poisoning

Botulism is food poisoning caused by eating food containing a poisonous bacterium called *Clostridium botulinum*. There are three main types of botulism viz: food-borne botulism caused by eating foods that contain the botulism toxin; wound botulism caused by toxin produced from a wound infected with *Clostridium botulinum* and infant botulism caused by consuming the spores of the botulinum bacteria which then grow in the intestines and release toxin. The three forms of botulism are fatal and cause medical emergencies.

Food-borne botulism is particularly dangerous because eating a batch of *Clostridium botulinum* contaminated food can poison a large number of people. The *Clostridium botulinum* is found in the soil but grows in many meats and vegetables *Clostridium botulinum* spores are killed by boiling while the toxins may be destroyed by moist heat at 80° C for 30 minutes. The spores grow best in the absence of oxygen and this makes improperly processed foods in sealed containers a perfect environment for their growth.

If food contaminated by the bacterium *Clostridium botulinum* is not properly canned or bottled, the bacteria are able to produce a toxin called ‘botulin’, which produces the disease botulism.

Within 8 to 36 hours of ingestion of the contaminated food, the botulin toxin paralyses nerves regulating muscle function, resulting in respiratory failure, as the muscles that control breathing weaken. The toxin also affects the central nervous system and interrupts nerve impulses, but the mind continues to function normally. The symptoms of botulism usually appear 18 to 36 hours after ingestion of the contaminated food. Disability progresses from difficulty in walking and swallowing, with impaired vision and speech, to occasional convulsions, and ultimately to paralysis of the respiratory muscles, suffocation, and death, all within a few hours or days, depending on the amount of toxin ingested.

The most direct way to confirm diagnosis is to demonstrate the presence botulin in the patient's serum or stool by injecting serum or stool into mice and looking for signs of botulism. Botulism antitoxin may be effective if administered early. Surgical opening of the trachea and use of a respirator may be lifesaving. Physicians may try to remove contaminated food still in the gut by inducing vomiting or by using enemas. The respiratory failure and paralysis that occur with severe botulism may require a patient to be on a ventilator for weeks. Research into the use of botulism in biological warfare has produced a toxoid, an inactivated poison for use in a vaccine, to induce immunity.

b. Staphylococcus Food Borne Poisoning

The most common species of Staphylococcus is *Staphylococcus aureus*, which is found on the skin, mouth, external ear and in the nostrils of many healthy individuals. Another species of staphylococcus called *Staphylococcus epidermidis* is very widespread but is not normally pathogenic. These bacteria can not cause serious infections under the right conditions. They may infect wounds or give rise to endocarditis (inflammation of the heart membrane) if the host's immune system is weak. They may also cause pneumonia and internal abscesses. They do not

form spores but can survive for several weeks in dry conditions. Some strains can withstand high temperatures; they do not often grow outside the body, but may do so in meat, milk or dirty water.

The various species of Staphylococcus multiply rapidly at room temperature and may directly infect the gastrointestinal tract. Due to careless food handling, workers may sneeze or cough on food or may have infected pimples or wounds on the hands or face and transmit the bacteria to the food. *Staphylococcus aureus* infections are characterised by the presence of pus and formation of abscesses.

Staphylococcus is responsible for skin pustules (pimple containing pus), boils and carbuncles (severe skin abscess), impetigo (contagious skin infection forming pimples and sores), infections of wounds and burns, breast abscesses, whitlow, osteomyelitis, bronchopneumonia, septicaemia, acute endocarditis, food poisoning and scalded skin syndrome. The Symptoms of Staphylococal infection includes nausea, vomiting and diarrhoea which develop within 1 to 8 hours after exposure to the bacteria. Treatment is usually by combination of fluid and electrolyte replacement but deaths rarely occur.

1.4.2 Organisms that Multiply in the Intestinal Tract and Produce Toxins that Causes the Symptoms

Organisms may multiply in the intestinal cavity (for example, *Bacillus Cereus* and *Clostridium perfringens*) and produce relatively rapid symptoms after eating the contaminated food and the infection lasts for only a day or so. Other organisms, including the various pathogenic strains of *Escherichia coli*, *Aeromonas species* and *Vibrio cholerae* invade and multiply inside the cells of the intestinal wall and secrete toxins. The onset of symptoms from such organisms is typically one to two days and the symptoms may last for several days.

a. *Escherichia coli* Food Borne Poisoning

E. coli infection is a potentially fatal form of food poisoning caused by certain strains of the bacterium *Escherichia coli*. About 5 million *E. coli* normally inhabit the human and animal intestinal tract, and are vital to processing vitamins in the diet. However, a number of strains are pathogenic and causes gastroenteritis. Strains known as entero-pathogenic strains are associated with undercooked meat, and are a common cause of diarrhoea in infants, but rarely produce gastroenteritis in adults. Other "entero-toxicogenic" strains are the main cause of "travellers' diarrhoea". A relatively large number of *E. coli* (100 million or more) are normally required to cause infections, which are generally associated with food and water contaminated by faeces.

Entero-invasive strains *E. coli* invade cells of the intestines, causing dysentery, with bloody diarrhoea. These are highly virulent strains, and ingestion of just a few organisms may cause infection. Outbreaks of such infection have been associated with undercooked hamburgers and unpasteurised milk. The entero-haemorrhagic strains are also highly virulent, causing both bloody diarrhoea and possibly fatal systemic infection. In particular, the strain *E. coli* 0157:H7, which also exists in animals and humans, is thought to be a virally infected, highly toxic strain of the *E. coli*. Ingestion of as few as 10 organisms may cause intestinal haemorrhaging and possible kidney failure. The fatality rate from the infection is 50 per cent in children and the elderly. The main source of infection is undercooked contaminated beef. Once infected, people in confined areas can transmit the pathogen.

Certain rare strains of the bacteria *Escherichia coli* cause food poisoning in young children, the elderly, and people with impaired immune systems. *E. coli* 0157:H7 normally found in the intestines and faecal matter of humans and animals can survive in meat if the meat is not cooked beyond 155°F. Outbreaks are due mainly to contaminated cooked meats bought from local retail

butchers. These incidences emphasize the need for improved food regulations, preparation and hygiene as bacteria from meat surfaces are incorporated during grinding and cutting, and subsequent insufficient cooking.

Symptoms of *E. coli* infection appear after four to nine days and include bloody diarrhoea, cramping, pain, and fever. Complications of *E. coli* infection include septicaemia, kidney failure and brain damage. Currently there is no cure for *E. coli* infection. Patients recover once the infection has run its course, although digestive and renal problems may persist. Prevention of *E. coli* infection is by maintaining high standards of food hygiene. The standards food hygiene includes always washing the hands before handling food, scalding the utensils used to prepare meat and keeping raw meat separate from other foods and thoroughly cooking of food to 70° C.

b. *Vibrio cholerae* Infection

Vibrio cholerae cause cholera - a severe infectious disease endemic to tropical countries and occasionally spreading to temperate climates. The major means of infection is through the use of contaminated water in the preparation of foods such as fruits and vegetables. Ready-to-eat foods may be contaminated by storage in contaminated containers or by sprinkling with contaminated water. The symptoms of cholera are diarrhoea and the loss of water and electrolytes in the stool. In severe cholera, the patient develops violent diarrhoea, vomiting, thirst, muscle cramps and sometimes, circulatory collapse. Death can occur as quickly as a few hours after the onset of symptoms. The mortality rate is greater than 50% in untreated cases, but falls to less than 1% with effective treatment. Prevention of the disease is a matter of sanitation and treatment consists mainly of intravenous or oral replacement of fluids and salts containing the correct mixture of sodium, potassium, chloride, bicarbonate and glucose. A vaccine made from dead

bacteria is commercially available and offers partial protection for a period of three to six months after immunization.

1.4.3 Organisms that Invade the Body but generally remain in the Region of the Intestinal Tract and/or Cause Widespread Systemic Infection

Microorganisms like *Campylobacter*, *Salmonella*, *Shigella* and *Yersinia* remain in the intestinal tract. The onset of symptoms is relatively slow and the infection may persist for weeks. Organisms that invade and cause systemic infections in the body include *Listeria monocytogenes*, *Salmonella typhi* and *Salmonella paratyphi*. The onset of symptoms may occur many days after consuming the contaminated food and symptoms may persist for many weeks.

a. *Salmonella* Food Borne Infection

Salmonella is transmitted through contaminated poultry, eggs and other foods. Three species are recognised: *Salmonella typhi, S. cholerasius* and *S. enteritidis* which have more than 1,400 antigenically distinct serotypes. *S. typhi* cause typhoid fever. *S. typhimurium* - a serotype of *S. enteritidis* causes salmonella gastroenteritis, a type of food poisoning characterised by abdominal pain, fever, nausea and vomiting, and diarrhoea. The incubation period is 8 to 48 hours, and an attack may last from three to seven days. Mild cases usually are treated with anti-diarrhoeal remedies while more severe cases require antibiotics. *S. enteritidis* occurs in most flocks of hens, thus undercooked chicken or eggs are the usual source of infection. Careful cleaning and thorough cooking of food prevent salmonella infections.

b. Typhoid Fever

Typhoid fever is an acute infectious disease caused by the bacillus *Salmonella typhi* transmitted through milk, water, or solid food contaminated by faeces of typhoid victims or of carriers. The incubation period of *Salmonella typhi* lasts one to three weeks. The bacteria collect in the small

intestine, from where they enter the bloodstream and induce the first symptoms - chills followed by high fever. Victims may also experience headache, cough, vomiting and diarrhoea. The disease spontaneously subsides after several weeks in most instances, but in about 20 percent of untreated cases the disease progresses to pneumonia, intestinal haemorrhage and even death.

Control of typhoid includes pasteurization of milk, purification of water supplies, the recognition of carriers, improvement of sewerage facilities and inoculation of people exposed to the disease, such as hospital employees and travellers to areas with poor sanitary facilities.

1.4.4 Other Microbial Infection

Clostridium perfringens is found mainly in poultry products and it causes mild form of food poisoning. Symptoms last only a day and starts about 8 – 22 hours after ingestion and include abdominal pain, nausea, diarrhoea and vomiting.

Shigella is found in chicken spread, fruit and fish salad. It is characterized by sudden appearance of abdominal pains, cramps, diarrhoea, fever and vomiting with the presence of blood, pus and mucous in stools of about 35% of infected patients.

Self- Assessment Exercises 2

1. Write the four groups Food poisoning organisms can be classified into, depending on the mechanism involved in causing disease.
2. What is typhoid fever and how is it transmitted.

3.6 Summary

Food poisoning may occur as a result of non-control of environmental temperature requirement of foods leading to growth of microbial spores including *Clostridium* and *Staphylococcus species*. It can also occur from heavy metals contaminations and cross-contaminations.

Food poisoning can result from poor environmental and personal hygiene, storage conditions and contaminations by microorganisms and heavy metals. The most deadly and pathogenic microorganisms include 3 types of *Clostridium botulinum* and *Staphylococcus aureus.* Mineral poisoning occurs from heavy metals (lead and mercury) contaminations. Other microorganisms that cause food poisoning include:

1. *Escherichia coli* especially in children and the elderly.

2. *Vibrio cholerae* which causes cholera.

3. Salmonella transmitted through contaminated poultry, eggs and certain foods.

4. *Salmonella typhi* which causes typhoid fever is transmitted by milk, water or solid food contaminated by faeces of carriers.

The recommended control pressures against food poisoning include:

1. Good hygiene in the handling, processing and storage of foods.

2. Application of low Temperature: Refrigeration at less than $4^{o}C$ and deep-freezers at less than $17^{o}C$.

3. Application of proper Health Education by applying methods from contacts and information media.

3.7 References/Further Readings

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3.8 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. Symptoms of potentially life-threatening food poisoning include:

i. diarrhoea persisting for more than three days

ii. a fever higher than 101.5°F

iii. difficulty seeing or speaking

iv. symptoms of severe dehydration, which may include dry mouth, passing little to no urine, and difficulty keeping fluids down

v. bloody urine

2. Inadequate Cooking, Food Storage Conditions, Cross - Contamination between Raw and Cooked Food, Poor Personal Hygiene in Food Handlers, Ingestion of Toxins and Ingestion of Heavy Metals.

Answers to Self-Assessment Exercises 2

1.

a. Organisms that produce toxin in the food
b. Organisms that multiply in the intestinal tract and produce toxins that causes the symptoms
c. Organisms that invade the body but generally remain in the region of the intestinal tract or cause widespread systemic infection
d. Other microbial infection

2. Typhoid fever is an acute infectious disease caused by the bacillus *Salmonella typhi* transmitted through milk, water, or solid food contaminated by faeces of typhoid victims or of carriers.

UNIT 2 FOOD INFECTIONS AND TOXICANTS

CONTENTS

2.1 Introduction

Foodborne diseases in humans result from the consumption of either food or water contaminated with viable pathogenic bacterial cells and/or spores or food containing toxins produced by toxigenic bacteria and molds.

Many foodborne infections occur at people's homes, due to poor hygiene and poor food handling. It's as easy as preparing food without proper hand washing after visiting the toilet. Cross-contamination is also a risk, for instance if raw meat and lettuce are both chopped on the same cutting board. Even using the same knife to chop both could cause contamination by foodborne pathogens. Eating meat or fish that is not properly cooked or eating raw shellfish, increases the risk of food-borne infections. The common pathogens implicated in many foodborne outbreaks are *Salmonella, E. coli, Listeria, and norovirus*. Different *Salmonella serovars* were involved in the outbreaks because many animals harbour *Salmonella* as carriers in the digestive tract and thus can contaminate meat, eggs, and dairy products and the environment.

Processed fish (smoked) contain large numbers of *L.monocytogenes*. In recent years, fish consumption has increased, and foodborne disease outbreaks from fishery products have also increased. Similarly, fruits and vegetables can be contaminated with *E. coli, L. Monocytogenes, and Salmonella* from untreated manure/fertilizer of animal origin, irrigation water, and the soil. In contrast, salads, which are handled extensively, can be contaminated with several pathogens of human origin.

2.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss food poisoning and foodborne infection
- Write various food toxicants
- Write the symptoms of foodborne infection

2.3.1 Foodborne infection

Foodborne infection is an illness that occurs from the consumption of food and water contaminated with enteropathogenic bacteria or viruses. Simply put as the ingestion of food containing live bacteria which grow and establish themselves in the human intestinal tract. This infection causes inflammation of the stomach and bowels. Often times, the inflammation graduates into diarrhoea, nausea, vomiting, abdominal pain, abdominal cramps and sometimes fever which can last between one and three days.

*Salmonellosis (*Typhoid*)* caused by serovars of *Salmonella* is a major food borne infection implicated in water, egg, meat and milk and their products.

Characteristics of foodborne infections

Organisms implicated in foodborne infection must be;

1. Live cells of the enteric pathogens have to be consumed through food.

2. The surviving cells from gastric environment must penetrate through the mucus membrane and establish in the epithelial cells of the intestines, multiply, and produce toxins and other virulence factors.

3. Dose levels that cause infection vary greatly.

4. Symptoms generally occur between 18-24 hours of ingestion, which, depending on the pathogen, can be both enteric and non-enteric in nature.

5. Enteric symptoms are local and result from enteric infection and the effect of toxins.

6. Non-enteric symptoms result when the pathogens or their toxins pass through the intestine and invade or affect other internal organs and tissues. Symptoms depend on the types of organs and tissues affected but are accompanied by fever.

Examples of pathogens include *Listeria monocytogenes, enterohemorrhagic E. coli (EHEC), V. vulnificus,* and Hepatitis A virus, *Salmonella enterica*.

2.3.2 Foodborne intoxication

The ingestion of food containing toxins formed by bacteria is termed Foodborne intoxication. This results from the growth of bacteria in the food item. The live microorganism does not have to be consumed. In foodborne intoxication, a toxin must be present in the contaminated food in an active form. Once the microorganisms have grown and produced a toxin in a food, there is no need of viable cells during the consumption of the food for illness to occur. Foodborne intoxication or food poisoning of microbial origin occurs from the ingestion of a food containing a preformed toxin from bacteria, such as Staphylococcus aureus, Bacillus cereus, and Clostridium botulinum, and mycotoxin from molds.

Characteristics of foodborne intoxication

Some general characteristics of food intoxication include the following;

1. The toxin is produced by a pathogen while growing in a food.

2. A toxin can be heat labile or heat stable.

3. Ingestion of a food containing an active toxin, not viable microbial cells, is necessary for poisoning (except for infant botulism or hidden botulism, in which viable spores need to be ingested).

4. Symptoms generally occur quickly, as early as 30 minutes after ingestion.

5. Symptoms differ with the type of toxin ingested; enterotoxins produce gastrointestinal symptoms, and neurotoxins produce neurological symptoms.

6. The febrile symptom is not present.

Toxicants are toxic substances that are found in foods that can produce harmful effects on ingestion by humans and animals. These harmful substances can be both biological and chemical toxicants. *Staphylococcal* food poisoning also known as staphylococcal gastroenteritis or staph food poisoning, caused by toxins of *Staphylococcus aureus*, and *Clostridium botulinum* are considered the most frequently occurring foodborne diseases worldwide.

Mycotoxins are toxic compounds that are naturally produced by certain types of moulds (fungi) which grow on numerous foodstuffs such as cereals, dried fruits, nuts and spices. Their growth can occur either before harvest or after harvest, during storage, on/in the food itself often under warm, damp and humid conditions. Exposure to mycotoxins can happen either directly by eating infected food or indirectly from animals that are fed contaminated feed, in particular from milk.

Food-borne mycotoxins have acute symptoms appearing quickly after consumption of food products contaminated with mycotoxins. Other mycotoxins occurring in food have been linked to long-term effects on health, including the induction of cancers and immune deficiency. Of the

several hundred mycotoxins identified so far, about a dozen have gained the most attention due to their severe effects on human health and their occurrences in food.

Aflatoxins produced by certain moulds (Aspergillus flavus and Aspergillus parasiticus) which grow in soil, decaying vegetation, hay, and grains are amongst the most poisonous mycotoxins. The toxins can also be found in the milk of animals that are fed contaminated feed, in the form of aflatoxin M1. Large doses of aflatoxins can lead to acute poisoning (aflatoxicosis) and can be life threatening, usually through damage to the liver.

Foodborne toxico-infection

A third class of this classification is the toxico-infection. This is an illness that occurs from the ingestion of a large number of some viable pathogenic bacterial cells through contaminated food and water. In essence, the bacterial cells sporulate, colonize, or die and release their toxins for before they produce the symptoms. The pathogenic microorganisms associated with these foodborne illnesses, are mostly bacterial species and strains, usually considered nonpathogenic, but are capable of causing gastroenteritis in immunocompromised or susceptible individuals. For this reason, they are often referred to as opportunistic pathogens and are normally required to be alive and with high infective dose when consumed.

2.3.3 Symptoms of Food Poisoning

Most food poisoning doesn't go undetected. They show glaring symptoms after a period of time. These symptoms can vary depending on the source of the infection as well the immune level of persons. The length of time it takes for symptoms to appear can range from as little as 1 hour to as long as 28 days. The commonest cases of food poisoning typically present at least three of the following symptoms;

- Abdominal cramps

- Diarrhoea
- Nausea or vomiting
- Loss of appetite
- Mild fever
- Weakness
- Nausea
- Headaches

The symptoms of food poisoning can be life-threatening when diarrhoea persists for more than three days, fever higher than 101.5°F or there is difficulty seeing or speaking. This is because they can lead to severe dehydration.

Some Microbial Foodborne Diseases and their Causative Pathogens

Types of Disease	**Causative Microorganism**	**Microbial Group**	**Major Symptom Type**
Infection			
Salmonellosis	Over 2000 Salmonella enterica serovars.	Bacteria	Diarrhea
Shigellosis	Four Shigella species	Bacteria,	Bloody mucoid diarrhea
Brucellosis	Brucella abortus	Bacteria	Gastric and nongastric
Listeriosis	Listeria monocytogenes	Bacteria	Fever, meningitis, abortion, diarrhea
Enterohemorrhagic *Escherichia coli* (EHEC)	*E. coli O157:H7, E. coli O26:H11*	Bacteria,	Hemorrhagic diarrhea, Hemolytic uremic syndrome (HUS)

Enteropathogenic *E. coli* (EPEC)	*E. coli O111:H12*	Bacteria	Hemorrhagic diarrhea
Vibrio parahaemolyticus gastroenteritis	Pathogenic strains of *V. parahemolyticus*	Bacteria	Diarrhea, hepatitis
Intoxication			
Staph poisoning	*Staphylococcus aureus*	Bacteria,	Vomiting, diarrhea
Mycotoxin poisoning	Mycotoxin producing strains (e.g., *Aspergillus flavus*)	Molds	Carcinogenic, Hepatotoxic
Botulism Clostridium	Botulinum Bacteria,	Bacteria,	Neurologic
Toxico-infection			
Cholera Vibrio	Cholerae	Bacteria,	Diarrhea
Clostridium perfringens gastroenteritis	*C. perfringens*	Bacteria	Diarrhea, vomiting
Escherichia coli gastroenteritis	Enterotoxigenic *E. coli* (ETEC) serotype O15:H11	Bacteria	Travellers' diarrhea

Self- Assessment Exercise 1

1. List the symptoms of food poisoning
2. Write eight (8) example of foodborne illnesses

2.4 Summary

Pathogenic microorganisms can be transmitted to humans by a number of routes. Diseases which result from pathogenic microorganisms are of three types: Food borne infection, intoxication and toxico-infection.

2.5 References/Further Readings

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2.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. The commonest cases of food poisoning typically present at least three of the following symptoms;

- Abdominal cramps
- Diarrhoea
- Nausea or vomiting
- Loss of appetite
- Mild fever
- Weakness
- Nausea

- Headaches

2. Salmonellosis, Shigellosis, Brucellosis, Listeriosis, Enterohemorrhagic *Escherichia coli* (EHEC), Enteropathogenic *E. coli* (EPEC). *Vibrio* parahaemolyticus gastroenteritis, *Botulism Clostridium*, Cholera *Vibrio*, Staph poisoning, *Clostridium perfringens* gastroenteritis, Mycotoxin poisoning, etc.

UNIT 3 IDENTIFICATION OF FOOD POISONING MICROORGANISMS

Content

3.1 Introduction

Microbial food spoilage occurs as a consequence of either microbial growth in a food or release of microbial extracellular and intracellular enzymes in the food environment. Some of the detectable parameters associated with spoilage of different types of foods are changes in colour, odour, and texture; formation of slime; accumulation of gas (or foam); and accumulation of liquid. Spoilage by microbial growth occurs much faster than spoilage by microbial extra- or intracellular enzymes in the absence of viable microbial cells. Between initial production and final consumption, different methods are used to preserve the acceptance qualities of foods,

which include the reduction of microbial numbers and growth. Yet microorganisms grow and cause food spoilage, which for some foods could be relatively high.

 3.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the types of food poisoning
- Discuss the food poisoning organisms

 **3.3 Food Poisoning Organisms**

Food poisoning organisms can be classified into four groups, depending on the mechanism involved in causing disease:

e. Organisms that produce toxin in the food
f. Organisms that multiply in the intestinal tract and produce toxins that causes the symptoms
g. Organisms that invade the body but generally remain in the region of the intestinal tract or cause widespread systemic infection
h. Other microbial infection

3.3.1 Organisms that Produce Toxin in the Food

The main examples of organisms that produce toxin in the food are *Clostridium botulinum*, *Staphylococcus aureus* and some strains of *Bacillus cereus*. The problem here is more of intoxication than infection and if the food contains a significant amount of the toxin, subsequent cooking will not reduce the risk of food poisoning.

c. *Clostridium botulinum* Food Borne Poisoning

Botulism is food poisoning caused by eating food containing a poisonous bacterium called *Clostridium botulinum*. There are three main types of botulism viz: food-borne botulism caused by eating foods that contain the botulism toxin; wound botulism caused by toxin produced from a wound infected with *Clostridium botulinum* and infant botulism caused by consuming the spores of the botulinum bacteria which then grow in the intestines and release toxin. The three forms of botulism are fatal and cause medical emergencies.

Food-borne botulism is particularly dangerous because eating a batch of *Clostridium botulinum* contaminated food can poison a large number of people. The *Clostridium botulinum* is found in the soil but grows in many meats and vegetables *Clostridium botulinum* spores are killed by boiling while the toxins may be destroyed by moist heat at 80° C for 30 minutes. The spores grow best in the absence of oxygen and this makes improperly processed foods in sealed containers a perfect environment for their growth.

If food contaminated by the bacterium *Clostridium botulinum* is not properly canned or bottled, the bacteria are able to produce a toxin called 'botulin', which produces the disease botulism. Within 8 to 36 hours of ingestion of the contaminated food, the botulin toxin paralyses nerves regulating muscle function, resulting in respiratory failure, as the muscles that control breathing weaken. The toxin also affects the central nervous system and interrupts nerve impulses, but the mind continues to function normally. The symptoms of botulism usually appear 18 to 36 hours after ingestion of the contaminated food. Disability progresses from difficulty in walking and swallowing, with impaired vision and speech, to occasional convulsions, and ultimately to paralysis of the respiratory muscles, suffocation, and death, all within a few hours or days, depending on the amount of toxin ingested.

The most direct way to confirm diagnosis is to demonstrate the presence botulin in the patient's serum or stool by injecting serum or stool into mice and looking for signs of botulism. Botulism antitoxin may be effective if administered early. Surgical opening of the trachea and use of a respirator may be lifesaving. Physicians may try to remove contaminated food still in the gut by inducing vomiting or by using enemas. The respiratory failure and paralysis that occur with severe botulism may require a patient to be on a ventilator for weeks. Research into the use of botulism in biological warfare has produced a toxoid, an inactivated poison for use in a vaccine, to induce immunity.

b. Staphylococcus Food Borne Poisoning

The most common species of Staphylococcus is *Staphylococcus aureus*, which is found on the skin, mouth, external ear and in the nostrils of many healthy individuals. Another species of staphylococcus called *Staphylococcus epidermidis* is very widespread but is not normally pathogenic. These bacteria can not cause serious infections under the right conditions. They may infect wounds or give rise to endocarditis (inflammation of the heart membrane) if the host's immune system is weak. They may also cause pneumonia and internal abscesses. They do not form spores but can survive for several weeks in dry conditions. Some strains can withstand high temperatures; they do not often grow outside the body, but may do so in meat, milk or dirty water.

The various species of Staphylococcus multiply rapidly at room temperature and may directly infect the gastrointestinal tract. Due to careless food handling, workers may sneeze or cough on food or may have infected pimples or wounds on the hands or face and transmit the bacteria to the food. *Staphylococcus aureus* infections are characterised by the presence of pus and formation of abscesses.

Staphylococcus is responsible for skin pustules (pimple containing pus), boils and carbuncles (severe skin abscess), impetigo (contagious skin infection forming pimples and sores), infections of wounds and burns, breast abscesses, whitlow, osteomyelitis, bronchopneumonia, septicaemia, acute endocarditis, food poisoning and scalded skin syndrome. The Symptoms of Staphylococal infection includes nausea, vomiting and diarrhoea which develop within 1 to 8 hours after exposure to the bacteria. Treatment is usually by combination of fluid and electrolyte replacement but deaths rarely occur.

3.3.2 Organisms that Multiply in the Intestinal Tract and Produce Toxins that Causes the Symptoms

Organisms may multiply in the intestinal cavity (for example, *Bacillus Cereus* and *Clostridium perfringens*) and produce relatively rapid symptoms after eating the contaminated food and the infection lasts for only a day or so. Other organisms, including the various pathogenic strains of *Escherichia coli*, *Aeromonas species* and *Vibrio cholerae* invade and multiply inside the cells of the intestinal wall and secrete toxins. The onset of symptoms from such organisms is typically one to two days and the symptoms may last for several days.

a. *Escherichia coli* Food Borne Poisoning

Escherichia coli is a harmless, Gram negative, motile, nonsporulating, rod-shaped, facultative anaerobic bacterium. It is a normal inhabitant of the intestinal tract of humans and warm-blooded animals, and birds. Because it is normally present in millions per gram of the content of the large intestine, for a long time it has been used as an index organism of possible fecal contamination and the presence of enteric pathogens in food and water. *E. coli* strains cause diarrhea, particularly in infants, and they are designated as Enteropathogenic *Escherichia coli* (EPEC). Current evidence indicates that pathogenic strains of *E. coli* are more than one type. They are

subdivided into six groups based on their ability to produce toxins and to adhere to and to invade epithelial cells. They are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), and diffuse-adhering *E. coli* (DAEC). *E. coli* O157:H7 is the principal serotype associated with enterohemorrhagic colitis. The organism is destroyed by pasteurization temperatures and time and killed at 64.3°C in 9.6 seconds. The cells survive well in food at –20°C.

E. coli infection is a potentially fatal form of food poisoning caused by certain strains of the bacterium *Escherichia coli*. About 5 million *E. coli* normally inhabit the human and animal intestinal tract, and are vital to processing vitamins in the diet. However, a number of strains are pathogenic and causes gastroenteritis. Strains known as entero-pathogenic strains are associated with undercooked meat, and are a common cause of diarrhoea in infants, but rarely produce gastroenteritis in adults. Other "entero-toxicogenic" strains are the main cause of "travellers' diarrhoea". A relatively large number of *E. coli* (100 million or more) are normally required to cause infections, which are generally associated with food and water contaminated by faeces.

Entero-invasive strains *E. coli* invade cells of the intestines, causing dysentery, with bloody diarrhoea. These are highly virulent strains, and ingestion of just a few organisms may cause infection. Outbreaks of such infection have been associated with undercooked hamburgers and unpasteurised milk. The entero-haemorrhagic strains are also highly virulent, causing both bloody diarrhoea and possibly fatal systemic infection. In particular, the strain *E. coli* 0157:H7, which also exists in animals and humans, is thought to be a virally infected, highly toxic strain of the *E. coli*. Ingestion of as few as 10 organisms may cause intestinal haemorrhaging and possible kidney failure. The fatality rate from the infection is 50 per cent in children and the elderly. The

main source of infection is undercooked contaminated beef. Once infected, people in confined areas can transmit the pathogen.

Certain rare strains of the bacteria *Escherichia coli* cause food poisoning in young children, the elderly, and people with impaired immune systems. *E. coli* 0157:H7 normally found in the intestines and faecal matter of humans and animals can survive in meat if the meat is not cooked beyond 155°F. Outbreaks are due mainly to contaminated cooked meats bought from local retail butchers. These incidences emphasize the need for improved food regulations, preparation and hygiene as bacteria from meat surfaces are incorporated during grinding and cutting, and subsequent insufficient cooking.

Symptoms of *E. coli* infection appear after four to nine days and include bloody diarrhoea, cramping, pain, and fever. Complications of *E. coli* infection include septicaemia, kidney failure and brain damage. Currently there is no cure for *E. coli* infection. Patients recover once the infection has run its course, although digestive and renal problems may persist. Prevention of *E. coli* infection is by maintaining high standards of food hygiene. The standards food hygiene includes always washing the hands before handling food, scalding the utensils used to prepare meat and keeping raw meat separate from other foods and thoroughly cooking of food to 70° C.

d. *Vibrio cholerae* Infection

Vibrio cholerae cause cholera - a severe infectious disease endemic to tropical countries and occasionally spreading to temperate climates. The major means of infection is through the use of contaminated water in the preparation of foods such as fruits and vegetables. Ready-to-eat foods may be contaminated by storage in contaminated containers or by sprinkling with contaminated water. The symptoms of cholera are diarrhoea and the loss of water and electrolytes in the stool. In severe cholera, the patient develops violent diarrhoea, vomiting, thirst, muscle cramps and

sometimes, circulatory collapse. Death can occur as quickly as a few hours after the onset of symptoms. The mortality rate is greater than 50% in untreated cases, but falls to less than 1% with effective treatment. Prevention of the disease is a matter of sanitation and treatment consists mainly of intravenous or oral replacement of fluids and salts containing the correct mixture of sodium, potassium, chloride, bicarbonate and glucose. A vaccine made from dead bacteria is commercially available and offers partial protection for a period of three to six months after immunization.

3.3.3 Organisms that Invade the Body but generally remain in the Region of the Intestinal Tract and/or Cause Widespread Systemic Infection

Microorganisms like *Campylobacter*, *Salmonella*, *Shigella* and *Yersinia* remain in the intestinal tract. The onset of symptoms is relatively slow and the infection may persist for weeks. Organisms that invade and cause systemic infections in the body include *Listeria monocytogenes*, *Salmonella typhi* and *Salmonella paratyphi*. The onset of symptoms may occur many days after consuming the contaminated food and symptoms may persist for many weeks.

c. Salmonella Food Borne Infection

Salmonella is transmitted through contaminated poultry, eggs and other foods. Three species are recognised: *Salmonella typhi, S. cholerasius* and *S. enteritidis* which have more than 1,400 antigenically distinct serotypes. *S. typhi* cause typhoid fever. *S. typhimurium* - a serotype of *S. enteritidis* causes salmonella gastroenteritis, a type of food poisoning characterised by abdominal pain, fever, nausea and vomiting, and diarrhoea. The incubation period is 8 to 48 hours, and an attack may last from three to seven days. Mild cases usually are treated with anti-diarrhoeal remedies while more severe cases require antibiotics. *S. enteritidis* occurs in most flocks of

hens, thus undercooked chicken or eggs are the usual source of infection. Careful cleaning and thorough cooking of food prevent salmonella infections.

d. Typhoid Fever

Typhoid fever is an acute infectious disease caused by the bacillus *Salmonella typhi* transmitted through milk, water, or solid food contaminated by faeces of typhoid victims or of carriers. The incubation period of *Salmonella typhi* lasts one to three weeks. The bacteria collect in the small intestine, from where they enter the bloodstream and induce the first symptoms - chills followed by high fever. Victims may also experience headache, cough, vomiting and diarrhoea. The disease spontaneously subsides after several weeks in most instances, but in about 20 percent of untreated cases the disease progresses to pneumonia, intestinal haemorrhage and even death.

Control of typhoid includes pasteurization of milk, purification of water supplies, the recognition of carriers, improvement of sewerage facilities and inoculation of people exposed to the disease, such as hospital employees and travellers to areas with poor sanitary facilities.

3.3.4 Other Microbial Infection

Clostridium perfringens is found mainly in poultry products and it causes mild form of food poisoning. Symptoms last only a day and starts about 8 – 22 hours after ingestion and include abdominal pain, nausea, diarrhoea and vomiting.

Shigella is found in chicken spread, fruit and fish salad. It is characterized by sudden appearance of abdominal pains, cramps, diarrhoea, fever and vomiting with the presence of blood, pus and mucous in stools of about 35% of infected patients.

***a. Staphylococcal* food intoxication**

Staphylococcal food poisoning also known as *staphylococcal* gastroenteritis, or *staph* food poisoning, is caused by toxins of *Staphylococcus aureus*. It is considered to be one of the most

frequently occurring foodborne diseases worldwide. *S. aureus* are gram-positive cocci which occurs singly or in grape-like clusters and are nonmotile, noncapsular, and nonsporulating. *S. aureus* can be isolated from foods by culturing.

An enumeration technique is used in one or more selective differential agar media to determine the load of viable cells of *S. aureus* followed by several biochemical tests, such as hemolysis, coagulase, thermonuclease reactions, or ability of a pure culture to produce enterotoxin, are performed to link the potential causes of the food poisoning outbreaks. In serological methods, the enterotoxins are purified and examined by one of the several recommended immunological methods. Not only are these tests very sensitive, but they also allow the identification of the types of enterotoxins involved in a food poisoning case.

b. **Botulism by *Clostridium Botulinum***

Botulism is caused by the consumption of food containing the potent botulinum toxin of *Clostridium botulinum*. Botulism occurs from the ingestion by the infant of *C. botulinum* spores that germinate, grow, and produce toxins in the Gastrointestinal tract of humans and cause specific symptoms. *C. botulinum* is a Gram-positive rod which occurs as single cells or in small chains. Many of them are motile, obligate anaerobes and form single terminal spores. Cells are sensitive to low pH. *C. botulinum* can be determined by enumeration techniques using selective agar media and anaerobic incubation, followed by biochemical and toxicological testing. The presence of toxins in the food is more often tested. This involves injection of a food extract intraperitoneally to mice. Development of characteristic neurological symptoms, followed by death in 92 hours suggests the presence of toxins.

c. ***Salmonella Enterica***

Salmonella enterica serovar Typhi and *Paratyphi* were considered the major causes of worldwide foodborne and waterborne diseases in humans caused by *Salmonella*. The *Salmonella* cells are Gram-negative, nonsporulating, facultative anaerobic motile rods. They form gas while growing in media containing glucose. Generally, they ferment dulcitol, but not lactose; utilize citrate as carbon source; produce hydrogen sulfide, decarboxylate lysine, and ornithine; do not produce indole; and are negative for urease. They are mesophilic with an optimum growth temperature between 35°C and 37°C but generally have a growth range of 5°C–46°C. They are killed by pasteurization temperatures and sensitive to low pH (4.5 or below) and do not multiply at $\mathbf{A_W}$ 0.94, especially in combination with a pH at 5.5 and below. The cells survive under frozen and dried states for a long time. They are capable of multiplying in many foods without affecting the acceptance qualities. In recent years, Salmonella outbreaks associated with low-moisture foods, including peanuts, almonds, peanut butter, spices, and wheat flours indicate that they can survive at AW of as low as 0.2 for an extended period of time (over 1000 days).

The methods involve pre-enrichment of a sample of food in a nutrient broth, followed by selective enrichment, streaking on a selective-differential agar medium, and biochemical and serological confirmation. Several rapid methods, based on specific immunological characteristics and nucleotide base sequence in the nucleic acids, have been developed.

d. Campylobacter Species

Campylobacter species cause human gastroenteritis. *C. jejuni* and *C. coli*. *C. jejuni* is a gram-negative, motile, nonsporulating, rod-shaped bacterium. The cells are small, fragile, and spirally curved. The strains are microaerophilic and catalase and oxidase positive. The strains require a microaerophilic environment of approximately 5% oxygen, 8% CO_2, and 87% N_2 for growth. Growth temperature ranges between 32°C and 45°C with optimum approximately 42°C. They

grow better in amino acids than in carbohydrates. They generally grow slowly and are not good competitors while growing with other bacteria. They generally do not grow well in many foods. They are sensitive to many environmental parameters, including oxygen (in air), NaCl (above 2.5%), low pH (below pH 5.0), temperature (below 30°C), heat (pasteurization), and drying.

Isolation of *Campylobacter* spp. from a suspected sample requires specific methods. After developing the method and incorporating it to isolate suspected foodborne pathogens, *C. jejuni* has been confirmed as a causative agent in many foodborne illnesses. The foods implicated most often in *campylobacteriosis* were raw milk and improperly cooked chicken. Although several *Campylobacter* spp. have been associated with foodborne campylobacteriosis, *C. jejuni* has been isolated in most incidents.

Self- Assessment Exercises 1

1. Write the four groups Food poisoning organisms can be classified into, depending on the mechanism involved in causing disease.
2. What is typhoid fever and how is it transmitted.

3.4 Summary

Food poisoning may occur as a result of non-control of environmental temperature requirement of foods leading to growth of microbial spores including *Clostridium* and *Staphylococcus species*. It can also occur from heavy metals contaminations and cross-contaminations.

Food poisoning can result from poor environmental and personal hygiene, storage conditions and contaminations by microorganisms and heavy metals. The most deadly and pathogenic microorganisms include 3 types of *Clostridium botulinum* and *Staphylococcus aureus.* Mineral

poisoning occurs from heavy metals (lead and mercury) contaminations. Other microorganisms that cause food poisoning include:

1. *Escherichia coli* especially in children and the elderly.

2. *Vibrio cholerae* which causes cholera.

3. Salmonella transmitted through contaminated poultry, eggs and certain foods.

4. *Salmonella typhi* which causes typhoid fever is transmitted by milk, water or solid food contaminated by faeces of carriers.

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 **3.6 Possible Answers to Self-Assessment Exercises**

Answers to Self-Assessment Exercises 1

1.

e. Organisms that produce toxin in the food

f. Organisms that multiply in the intestinal tract and produce toxins that causes the symptoms

g. Organisms that invade the body but generally remain in the region of the intestinal tract or cause widespread systemic infection

h. Other microbial infection

2. Typhoid fever is an acute infectious disease caused by the bacillus *Salmonella typhi* transmitted through milk, water, or solid food contaminated by faeces of typhoid victims or of carriers.

UNIT 4 CONTROL OF FOOD POISONING MICROORGANISMS

Content

4.1 Introduction

The impact of microorganisms in food products directly affects man. Although some microorganisms are desirable for the production of bioprocessed food, many are unwanted as they cause food spoilage and foodborne diseases. The activities of pathogenic microorganisms, poses health threat thereby their activities must be regulated and controlled to avoid poisoning. Controlling the growth and metabolic activities of microorganisms helps to stop food spoilage and foodborne diseases as well as preserve the quality of food by controlling access of the microorganisms in foods, physically removing the microorganisms present in foods**,** preventing or reducing the growth of microorganisms and germination of spores present in foods and killing microbial cells and spores present in foods.

There are several methods of preserving food and controlling food poisoning such as drying and salting, high temperature treatment, low temperature storge addition of antimicrobials. Some of

the methods can be used individually or in combination to achieve the goals of stopping food spoilage. Irrespective of the methods used, it is important to recognize that a control method is more effective when a food has fewer microbial cells and when the cells are in the exponential growth phase and are injured.

4.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss Temperature-Based Methods of controlling food poisoning Microbes
- Discuss Non-Temperature-Based Methods of controlling food poisoning Microbes

4.3 Control of Food Poisoning Microorganisms

4.3.1 Temperature-Based Methods

a. Pasteurization: This preservation method is a controlled condition which involves exposing the food to high temperature for a short period of time, enough to destroy the organisms and reduce the number of spoilage organisms. Although this temperature is high enough to destroy the organisms, it isn't enough to change the taste and flavour of the food.

b. Cooking: Cooking is the exposure of food material to heat enough to destroy non spore forming organisms. It is like pasteurization only that heat distribution may be uneven and this helps the survival of some organisms.

c. Canning: This is a **v**ery effective means of controlling food spoilage and poisoning. This method of food preservation process destroys all spoilage and pathogenic organism which grows at normal temperature. Steam under pressure (autoclaving) is used to destroy the endospores of *Clostridium botulinum*. It is deployed mostly to preserve low acid foods which do not require high temperature.

d. Refrigeration: the use of refrigerator is an important preservation of food to stop spoilage. This method involves the slowing down of the growth rate of microbes. This makes the pathogens unable to multiply at low temperatures.

e. Freezing: like refrigeration, this involves the use of very low temperature to stop the microbial growth because ice affects biological reactions. The presence of ice damages microbial cells and kills them since they can't grow in the ice crystals.

f. Lowering the pH: Lowering the pH of foods either by adding acids or aiding fermentation helps to stop food spoilage and poisoning as some pathogenic organisms do not survive in low pH.

g. Drying: Drying foods inhibits microbial growth by decreasing the available moisture.

h. Addition of antimicrobial chemicals: The addition of antimicrobial chemicals such as organic acids helps to inhibit fungal growth.

i. Irradiation: Irradiation with Gamma rays destroys pathogenic microorganisms without significantly changing the flavour of the food.

4.3.2 Non-Temperature-Based Methods

Food Hygiene at Home and the Catering Industry

Food hygiene is the major means of controlling food poisoning and attitude to the importance of food hygiene will depend upon their awareness, education and the standard of living they can afford. Food hygiene regulations have been brought into force in many countries of the world to protect the public and reduce the number of outbreaks of food poisoning. These regulations must be followed by anyone responsible for handling food in the food business.

Food hygiene is divided into three main sections: personal hygiene, environmental hygiene and food hygiene practice.

a. Personal Hygiene

Prevention of food poisoning starts with personal hygiene as the food poisoning bacteria can be found on human skin, hair, and clothes, and in ears, noses, mouths and faeces. If people touch affected parts of their bodies during the preparation of food, they can transfer the bacteria to the food; hence, hands must always be washed before working with food, especially after visiting the toilet. The bacterium *Staphylococcus aureus* is found in the human nose, infected wounds and boils, so cuts and grazes must be covered to avoid food contamination. Clean and protective clothing such as aprons or overalls should also be worn during food preparation.

Food handlers should not work with food if they are suffering from or are carriers of food-poisoning infections as they can accidentally contaminate foods. Food handlers should not smoke, chew tobacco, cover their hairs and beards hats and nets or spit around food preparation areas. Always wash your hands in hot soapy water before preparing each dish at home, after changing a diaper or wiping a nose of a child, or handling any animal. Wash fruits and vegetables in lukewarm water to get rid of insects and pesticide residue. Skinning, peeling and boiling are the best ways to cleanse foodstuff.

b. Environmental Hygiene

Environments where food is stored and prepared must be kept clean and free from pests and pets. Dirt, soil and food residues can harbour bacteria and pests. Hot water with detergents solution be used to wipe down and clean surfaces, equipment, floors and walls. All utensils - cutting boards and countertops should be washed with hot soapy water after preparing each. Food waste should be regularly removed from the food preparation area. The danger zone is anywhere between 5°C and 63°C and bacteria grows very well at 37^{o}C. Temperature control is important; cold food must be stored correctly then cooked at a temperature high enough to kill bacteria. Although the

refrigerator can inhibit the growth of dangerous bacteria, the temperature of refrigerator and freezer should not be greater than 4°C and –17°C respectively.

c. Food Hygiene Practice

The most serious cross contamination occurs between raw foods and cooked foods, so they should not be stored together or prepared using the same equipment. Keep raw meat, poultry or seafood separate from other food at all times. Never put cooked food unto a dish that formerly held raw meat, fish or poultry, unless that dish has been washed thoroughly with hot soapy water.

cook all food items thoroughly because if the internal heat of food exceeds 70°C, even briefly, almost all bacteria, viruses and parasites will be killed. Poultry should be cooked even more than that, up to 80°C. Reheated foods should be brought to a temperature of 75°C or it should be hot and steaming. Avoid eating poultry that is still pink inside, eggs with runny yolks or whites, or fish that is not yet opaque and that you cannot readily flake apart with a fork.

When dining out, make sure the restaurant you visit satisfies the health standards required by law. Take-away meals should be eaten within 2 hours from the time of purchase and If time elapses reheat the food to a temperature not less than 75°C.

d. Health Education

Health education programmes are concerned with turning knowledge into the following action:

i. Changing food habits to incorporate: boiling of drinking water, cooking all food and avoiding raw meat and fish.

ii. Taking specific precautions including: adequate cooking of food and the avoidance of foods and food preparation methods that have caused outbreaks in the past.

iii. Avoiding long delays in consuming prepared food and following approved food sanitation methods and procedures.

iv. Giving positive support to community activities such as improvement of water supply and the construction and use of latrines.

v. Accepting expert advice on food hygiene and control of enteric diseases.

Health education methods include both person-to-person contacts and the use of mass information media. The methods must be carefully chosen to match the educational level of the target group and effective use should be made of community leaders in the educational effort. The educational programme should be designed specifically for the community. The Health education programme is difficult since a number of anti-health factors exist. These anti-health factors include ignorance, superstition, lethargy, poverty and opposition from vested interests.

Self- Assessment Exercises 3

1. What are the factors that makes health education programme difficult
2. List thepossible ways of controlling food poisoning

4.4 Summary

The recommended control measures against food poisoning include: good hygiene in the handling, processing and storage of foods. Application of low Temperature such as Refrigeration at less than $4^{o}C$ and deep-freezers at less than $17^{o}C$ and proper Health Education by applying methods from contacts and information media can also be used.

4.5 Glossary

Food: anything eaten by man or animal to satisfy appetite, meet physiological needs for growth, maintain all body processes and supply energy to maintain body temperature and activities.

Fungi: single-celled or multicellular organism without chlorophyll that reproduces by spores and lives by absorbing nutrients from organic matter.

Moulds: filamentous fungi which are important group of microflora of fruits and fruit products.

Sanitation: the maintenance of hygienic conditions, through services such as garbage collection and waste disposal.

Drying: removal of water from food and is one of the oldest and simplest methods of preserving food.

Refrigeration: process of lowering the temperature in a given space and maintaining it for the purpose of chilling foods, preserving certain substances, or providing an atmosphere conducive to bodily comfort.

Sewage: all the material that flows from household plumbing systems, including washing and bathing water and toilet wastes as well as others from business and industrial wastes.

Spoilage: any visible or invisible change which can makes food or product derived from food unacceptable for human consumption.

Viruses: intracellular obligate parasite which can trigger dangerous infections in humans when they contaminate our food.

Yeasts: unicellular fungi which normally reproduce by budding.

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4.7 Possible Answers to Self-Assessment Exercise

1. Ignorance, superstition, lethargy, poverty and opposition from vested interests.

2. Personal hygiene, environmental hygiene and food hygiene practice.

MODULE 5

Unit 1 Laboratory methods of assessing microbiological status of beverages

Unit 2 Laboratory methods of assessing microbiological status of cereals

Unit 3 Laboratory methods of assessing microbiological status of roots and tubers

Unit 4 Laboratory methods of assessing microbiological status of fruits and vegetables

Unit 5 Laboratory methods of assessing microbiological status of meat, fish and dairy products

UNIT 1 LABORATORY METHODS OF ASSESSING MICROBIOLOGICAL STATUS OF BEVERAGES

Content

1.1 Introduction

Microbiological and aseptic testing plays a significant role in such quality assurance. It is not a guarantee of product safety; however, it is one component of an overall food safety system. Before microbiological testing is initiated, prerequisite programs must be in place. These should include programs that are appropriate to the specific operation, such as: Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), Sanitation Practices, Hazard Analysis Critical Control Point (HACCP), Traceability and Recall Management. It helps to ensure the biological stability of the products. This is imperative because only a few microbes are all it takes to spoil large quantities of foods.

Quality control must be adapted in all food processes to ensure food is free from any trace of contamination. The microbiological assessment of food helps in the classification of microbiological quality into one of the following three classes;

- **Satisfactory:** Test results indicating good microbiological quality.
- **Borderline**: Test results that are not unsatisfactory but are also not satisfactory, are on the upper limit of acceptability and which indicate the potential for development of public health problems and of unacceptable risk.
- **Unsatisfactory**: Test results which indicate investigating reasons for high count may be considered. For hygiene indicator organisms, test results that require remedial action. For

pathogens, test results at levels which indicate a product that is potentially injurious to health and/or unfit for human consumption and require immediate remedial action.

Foodborne diseases are an important cause of morbidity and mortality because millions of people fall ill and many die after ingesting food unfit for consumption. They are caused by several microorganisms including *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Salmonella* spp and *pathogenic Escherichia coli,* which cause various consumer disorders.

1.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the Laboratory Methods of Assessing Microbiological Status of Beverages

1.3 Laboratory Methods of Assessing Microbiological Status of Beverages

For quality, safety of consumers and the longer shelf life of foods and beverages, they must meet certain microbiological criteria deemed safe. Because safety is very important, quality assurance can’t be limited to inspection of the final product alone, instead, continuous inspection of incoming raw materials and in-process quality control tests must be performed throughout production. Although there is clearly a place for the direct examination of a beverages for microorganisms, a full microbiological examination usually requires that individual viable cells are encouraged to multiply in broth or culture media. Contaminating indicator organisms grow in the media. By ensuring optimal growth in the elective medium for one organism, it is desirable that conditions are sub-optimal, or even inhibitory, to others. The methods of assessments include:

1.3.1 Enumeration Methods

Enumeration methods of microbiological assessment involves different direct cell counts using direct or cell counter. These techniques are particularly useful for determining the total numbers of microbes, including those that cannot be grown in culture. Unfortunately, they generally do not distinguish between living and dead cells in the specimen.

1.3.2 Microscopic Count

The microscopic count is a direct enumeration method. It is one of the most rapid methods of determining the cell concentration in a suspension. Here, a liquid specimen is added to special glass slide designed specifically for counting cells. The slide has a thin chamber that holds a known volume of liquid atop a microscopic grid. The contents of the chamber can be viewed under the light microscope, so the number of cells in a given volume can be counted precisely. At least 10 million bacteria (10^7) per milliliter are usually required for enough cells to be seen in the microscope field.

1.3.3 The use of cell-counting instruments

A **Coulter counter** is an electronic instrument that counts cells in a suspension as they pass single file through a narrow channel. The suspending liquid must be an electrically conducting fluid, because the machine counts the brief changes in resistance that occur when non-conducting particles such as bacteria pass. A **flow cytometer** is another cell counting instrument that is very useful. It is similar in principle to a Coulter counter except that it measures light scattered by cells as they pass a laser. The instrument can be used to count either total cells or a specific subset that has been stained with a fluorescent dye or tag.

1.3.4 Viable Cell Counts

Viable cell counts determine the number of cells capable of multiplying. They are particularly valuable when working with samples such as food and water that contain too few microbes for a direct microscopic count. In addition, by using appropriate selective and differential media, these methods can be used to count the cells of a particular microbial species.

1.3.5 Plate Counts

Plate counts measure the number of viable cells in a sample by taking advantage of the fact that an isolated microbial cell on a nutrient agar plate will give rise to one colony. A simple count of the colonies determines how many cells were in the initial sample. Plate counts are generally only done if a sample contains more than 100 organisms/ml. Otherwise, few if any cells will be transferred to the plates. In these situations, alternative methods give more reliable results. When counting colonies, the ideal number on a plate is between 30 and 300. Numbers outside of that range are more likely to be inaccurate. Samples usually contain many more cells than that, so they generally must be diluted by a stepwise process called serial dilution. This is done using a sterile liquid called the diluent, often physiological saline (0.85% NaCl in water). Dilutions are normally done in 10-fold increments, making the resulting math relatively simple. Two techniques can be used to plate samples—spread-plate and pour-plate. In the **spread -plate method,** 0.1 to 0.2 ml of the diluted sample is transferred onto a plate of a solidified agar medium. It is then spread over the surface of the agar with a sterilized bent glass rod that resembles a miniature hockey stick. In the **pour -plate method,** 0.1 to 1.0 ml of the diluted sample is transferred to a sterile Petri dish and then overlaid with a melted agar medium cooled to 50°C. At this temperature, agar is still liquid. The dish is then gently swirled to mix the microbial cells with the liquid agar. When the agar hardens, the individual cells become fixed in

place; they form colonies when incubated. Colonies that form on the surface will be larger than those embedded in the medium.

In both methods, the plates are incubated and then the number of colonies is counted. From that number, the concentration of **colony-forming units** (**CFUs)** in the sample can be determined. This measure of viable cells accounts for the fact that microbial cells often attach to one another and then grow to form a single colony. When calculating CFUs, three things must be considered: The number of colonies, the amount the sample was diluted before being plated, and the volume plated.

1.3.6 Membrane Filtration

The Membrane filtration technique is used for liquid samples that contain relatively few cells, as might occur in dilute environments such as natural waters. This method concentrates the microbes by filtration before they are plated. A known volume of liquid is passed through a sterile membrane filter that has a pore size small enough to prevent microorganisms from passing through. The filter is then placed on an appropriate agar medium and incubated. The number of colonies that form on the filter indicates the number of cells in the volume filtered.

1.3.7 Most Probable Number (MPN)

The most probable number (MPN) is a method for estimating the concentration of cells in a specimen. The procedure uses a series of dilutions to determine the point at which subsequent dilutions receive no cells. To determine the MPN, three sets of three or five tubes containing a growth medium are prepared. Each set receives a measured amount of a sample such as water, soil, or food. The amount added is determined, in part, by the expected microbial concentration in that sample. What is important is that the second set receives 10-fold less than the first, and the third set 100-fold less. In other words, each set is inoculated with an amount 10-fold less than

the previous set. After incubation, the presence or absence of turbidity or other indication of growth is noted; the results are then compared against an MPN table, which gives a statistical estimate of the cell concentration.

1.3.8 Measuring Biomass

In this method of microbiological assessment instead of measuring the number of cells, the cell mass can be determined.

1.3.8.1 Turbidity

The cloudiness or turbidity of a microbial suspension is proportional to the concentration of cells, and is measured with a spectrophotometer. This instrument shines light through a specimen and measures the percentage that reaches a light detector. That percentage is inversely proportional to the optical density. To use turbidity to estimate cell numbers, a one-time test must be done to determine the correlation between optical density and cell concentration for the specific organism and conditions under study. Once this correlation has been determined-generally using a direct microscopic count or plate count to determine cell concentration-the turbidity measurement becomes a rapid and relatively accurate assay. One limitation of using turbidity to measure biomass is that the medium must contain a relatively high concentration of cells to be cloudy. A solution containing 1 million bacteria (106) per ml is still perfectly clear, and if it contains 10 times that amount, it is barely turbid. It is important to remember that although a turbid culture indicates that microbes are present, a clear solution does not guarantee their absence.

1.3.8.2 Total Weight

The total weight of a culture can be used to measure growth, but the method is tedious and time-consuming. Because of this, total weight is usually used to study only filamentous organisms that

do not readily separate into the individual cells necessary for a valid plate count. To measure the wet weight, cells in liquid culture are centrifuged and the liquid supernate removed. The weight of the resulting packed cell mass is proportional to the number of cells in the culture. The dry weight can be determined by heating the centrifuged cells in an oven before weighing them.

Self- Assessment Exercises 1

1. What is the disadvantage of using Enumeration Methods
2. Explain membrane filteration techniques

1.4 Summary

Like every other microbiological method of ensuring safety, the laboratory methods of assessing microbiological status of foods are both to ensure quality, and increase the shelf life. Though microbiological criteria or the investigation of an outbreak of foodborne illness may often require the monitoring of certain products for specific pathogens, the difficulties associated with detecting low numbers of pathogens make it impracticable as a routine procedure to be applied without good cause. An alternative to monitoring for specific pathogens is to look for an associated organism present in much larger numbers – an indicator organism.

1.5 References/Further Readings

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Compendium of Microbiological Criteria for Food (2018): Food Standards Australia New Zealand

1.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. Enumeration Methods do not distinguish between living and dead cells in the specimen.
2. The Membrane filtration technique is used for liquid samples that contain relatively few cells, as might occur in dilute environments such as natural waters. This method concentrates the microbes by filtration before they are plated. A known volume of liquid is passed through a sterile membrane filter that has a pore size small enough to prevent microorganisms from passing through. The filter is then placed on an appropriate agar medium and incubated. The number of colonies that form on the filter indicates the number of cells in the volume filtered.

UNIT 2 LABORATORY METHODS OF ASSESSING MICROBIOLOGICAL STATUS OF CEREALS

CONTENT

2.1 Introduction

Several processing technologies and techniques have been widely applied in enhancing the nutritional properties of fermentable cereals products. This includes cooking, sprouting, milling and fermentation. Microorganisms play both essential and deleterious roles in food products. In the fermentation industry, the attributes of the food products produced is largely due to the type, age, composition of the microorganism employed. Typically, the microbial load gradually increases from the first day (0 hours) and attain optimum at 24 – 48 hours of fermentation, before beginning to decline from 72 to 96 hours. The density of the microbes for lactic acid bacteria culture using MRS agar is second to aerobic culture using plate count agar or nutrient agar. Like in the assessment of beverages, samples are collected and cultured to identify contamination of indicator microorganisms. The total plate scounts, as well as most probable number of identified organisms are done to establish baseline of safety.

2.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the Laboratory Methods of Assessing Microbiological Status of Cereals

2.3 Laboratory Methods of Assessing Microbiological Status of Cereals

For quality, safety of consumers and the longer shelf life of foods and beverages, they must meet certain microbiological criteria deemed safe. Because safety is very important, quality assurance can't be limited to inspection of the final product alone, instead, continuous inspection of incoming raw materials and in-process quality control tests must be performed throughout production. Although there is clearly a place for the direct examination of a beverages for microorganisms, a full microbiological examination usually requires that individual viable cells are encouraged to multiply in broth or culture media. Contaminating indicator organisms grow in the media. By ensuring optimal growth in the elective medium for one organism, it is desirable that conditions are sub-optimal, or even inhibitory, to others. The methods of assessments include:

2.3.1 Enumeration Methods

Enumeration methods of microbiological assessment involves different direct cell counts using direct or cell counter. These techniques are particularly useful for determining the total numbers of microbes, including those that cannot be grown in culture. Unfortunately, they generally do not distinguish between living and dead cells in the specimen.

2.3.2 Microscopic Count

The microscopic count is a direct enumeration method. It is one of the most rapid methods of determining the cell concentration in a suspension. Here, a liquid specimen is added to special glass slide designed specifically for counting cells. The slide has a thin chamber that holds a known volume of liquid atop a microscopic grid. The contents of the chamber can be viewed under the light microscope, so the number of cells in a given volume can be counted precisely.

At least 10 million bacteria (10^7) per milliliter are usually required for enough cells to be seen in the microscope field.

2.3.3 Plate Counts

Plate counts measure the number of viable cells in a sample by taking advantage of the fact that an isolated microbial cell on a nutrient agar plate will give rise to one colony. A simple count of the colonies determines how many cells were in the initial sample. Plate counts are generally only done if a sample contains more than 100 organisms/ml. Otherwise, few if any cells will be transferred to the plates. In these situations, alternative methods give more reliable results. When counting colonies, the ideal number on a plate is between 30 and 300. Numbers outside of that range are more likely to be inaccurate. Samples usually contain many more cells than that, so they generally must be diluted by a stepwise process called serial dilution. This is done using a sterile liquid called the diluent, often physiological saline (0.85% NaCl in water). Dilutions are normally done in 10-fold increments, making the resulting math relatively simple. Two techniques can be used to plate samples—spread-plate and pour-plate. In the **spread -plate method,** 0.1 to 0.2 ml of the diluted sample is transferred onto a plate of a solidified agar medium. It is then spread over the surface of the agar with a sterilized bent glass rod that resembles a miniature hockey stick. In the **pour -plate method,** 0.1 to 1.0 ml of the diluted sample is transferred to a sterile Petri dish and then overlaid with a melted agar medium cooled to 50°C. At this temperature, agar is still liquid. The dish is then gently swirled to mix the microbial cells with the liquid agar. When the agar hardens, the individual cells become fixed in place; they form colonies when incubated. Colonies that form on the surface will be larger than those embedded in the medium.

In both methods, the plates are incubated and then the number of colonies is counted. From that number, the concentration of **colony-forming units** (**CFUs)** in the sample can be determined. This measure of viable cells accounts for the fact that microbial cells often attach to one another and then grow to form a single colony. When calculating CFUs, three things must be considered: The number of colonies, the amount the sample was diluted before being plated, and the volume plated.

2.3.4 Most Probable Number (MPN)

The most probable number (MPN) is a method for estimating the concentration of cells in a specimen. The procedure uses a series of dilutions to determine the point at which subsequent dilutions receive no cells. To determine the MPN, three sets of three or five tubes containing a growth medium are prepared. Each set receives a measured amount of a sample such as water, soil, or food. The amount added is determined, in part, by the expected microbial concentration in that sample. What is important is that the second set receives 10-fold less than the first, and the third set 100-fold less. In other words, each set is inoculated with an amount 10-fold less than the previous set. After incubation, the presence or absence of turbidity or other indication of growth is noted; the results are then compared against an MPN table, which gives a statistical estimate of the cell concentration.

Self- Assessment Exercises 1

1. What is the disadvantage of using Enumeration Methods
2. Explain microscopic count techniques

2.4 Summary

Like every other microbiological method of ensuring safety, the laboratory methods of assessing microbiological status of foods are both to ensure quality, and increase the shelf life. Though microbiological criteria or the investigation of an outbreak of foodborne illness may often require the monitoring of certain products for specific pathogens, the difficulties associated with detecting low numbers of pathogens make it impracticable as a routine procedure to be applied without good cause. An alternative to monitoring for specific pathogens is to look for an associated organism present in much larger numbers – an indicator organism.

2.5 References/Further Readings

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2.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. Enumeration Methods do not distinguish between living and dead cells in the specimen.
2. The microscopic count is a direct enumeration method. It is one of the most rapid methods of determining the cell concentration in a suspension. Here, a liquid specimen is added to special glass slide designed specifically for counting cells. The slide has a thin chamber that holds a known volume of liquid atop a microscopic grid. The contents of the chamber can be viewed under the light microscope, so the number of cells in a given volume can be counted precisely. At least 10 million bacteria (10^7) per milliliter are usually required for enough cells to be seen in the microscope field.

UNIT 3 LABORATORY METHODS OF ASSESSING MICROBIOLOGICAL STATUS OF ROOTS AND TUBERS

CONTENT

3.6 Possible answers to Self-Assessment Exercises

3.1 Introduction

Roots and tubers are highly perishable and lot of postharvest losses occur to this commodity during storage due to high physiological activities and activities of microorganisms that enter bruises received during harvesting as well as the inherent high moisture content of fresh roots, which promote both microbial deterioration and unfavourable biochemical changes in the commodity. Microbiological foodborne diseases are typically caused by bacteria or their metabolites, parasites, fungi, virus or toxins. The Microbiological Assessment of root and tubers involves the enumeration, total plate count, fungi count, and coliform count as described below.

3.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the Laboratory Methods of Assessing Microbiological Status of roots and tubers

3.3 Methods of Assessing Microbiological Status of roots and tubers

3.3.1 Enumeration of microorganisms

Samples are cleaned externally with 70% ethanol to disinfect it. An appropriate serial dilution of all the samples is carried out and 0.1 ml of the selected dilution spread on duplicate plates using sterile glass spreader. This technique is used for the enumeration of total aerobic viable count, coliform, fungal and staphylococcal counts on Nutrient Agar and Eosin Methylene Blue (EMB) Agar. Potatoe Dextrose Agar and Baird Parker Agar supplemented with tellurite and egg yolk emulsion, respectively. All cultures are incubated at 37°C for 24 h except for coliform bacteria which is incubated at 37°C and 44°C for 24 h. Media used were prepared according to the

manufacturer's instructions. Samples collected is serially diluted tenfold in which ten grams of each sample is diluted in 90mL peptone water followed by homogenization by horizontal and vertical agitations for a few minutes to obtain 10−1 dilution. Further tenfold serial dilutions are made up to 10^{-5} for colony count. 1mL of volume of each dilution is spread plated on de Man Rogosa Sharpe Agar (MRS) and incubated anaerobically at 35°C for 48 h for the enumeration of lactic acid bacteria, and Plate Count Agar incubated at 32°C for 48h is used for the enumeration of aerobic bacteria. 0.1mL of each of the samples is also plated on Potato Dextrose Agar supplemented with 60 μgmL−1 chloramphenicol for fungal isolation. This is incubated at 28°C for 5 days. The colonies are counted and recorded followed by isolation, purification, and storage on Nutrient Agar slants.

3.3.2 Total Plate Count

For microbial load determination, serial dilutions of each homogenized mixture are prepared upto 10-6 and dilutions 10-2 and 10-4 used for inoculation on nutrient agar media. The inoculated plates are incubated at 37° C for 24 hours. Duplicate plates with 25 to 250 colonies were selected for total count. The number of colonies is multiplied to dilution factor (reciprocal of dilution) in order to find the microbial load.

3.3.3 Most Probable Numbers (MPN)

In this method, dilutions of food samples are prepared and three serial aliquots or dilutions are then planted into 9 or 15 tubes of appropriate medium for the three- or five-tube method, respectively. Numbers of organisms in the original sample are determined by use of standard MPN tables. The method is statistical in nature, and MPN results are generally higher than Standard plate counts (SPC) results. This method was introduced by McCrady in 1915. It is not a precise method of analysis; the 95% confidence intervals for a three-tube test range from 21 to

395. When the three-tube test is used, 20 of the 62 possible test combinations account for 99% of all results, whereas with the five-tube test, 49 of the possible 214 combinations account for 99% of all results.

Advantages MPN

1. It is relatively simple.

2. Results from one laboratory are more likely than SPC results to agree with those from another laboratory.

3. Specific groups of organisms can be determined by use of appropriate selective and differential media.

4. It is the method of choice for determining fecal coliform densities.

Among the drawbacks to its use are the large volume of glassware required (especially for the five-tube method), the lack of opportunity to observe the colonial morphology of the organisms, and its lack of precision.

Self- Assessment Exercises 1

1. What are the advantages of MPN
2. Name the most commonly used indicator in assessing the health risk associated with food consumption

3.4 **Summary**

The examination of foods for the presence, types, and numbers of microorganisms and/or their products is basic to food microbiology. In spite of the importance of this, none of the methods in common use permits the determination of exact numbers of microorganisms in a food product. Although some methods of analysis are better than others, every method has certain inherent limitations associated with its use.

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3.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. **Advantages MPN**

   1. It is relatively simple.

   2. Results from one laboratory are more likely than SPC results to agree with those from another laboratory.

3. Specific groups of organisms can be determined by use of appropriate selective and differential media.

4. It is the method of choice for determining fecal coliform densities.

2. The most commonly used indicator organisms are the coliforms.

UNIT 4 LABORATORY METHODS OF ASSESSING MICROBIOLOGICAL STATUS OF FRUITS AND VEGETABLES

CONTENT

4.1 Introduction

Vegetables and fresh fruits are vital part of a nutritious and healthy diet because they promote a healthy body and mind. Due to their dietary values, fruits and vegetables also harbour a variety of elevated microbial contaminants. Toxins produced by variety of microorganisms play major role in contaminating the food products. Disease causing microorganisms like *E. coli*, *Rhizopus*, *Staphylococcus aureus, Salmonella*, *Clostridium botulinum, Bacillus cereus, Pseudomonas aeruginosa*, *Mucor* species, *Aspergillus* and *Candida* species can contaminate fresh produce.

4.2 Leaning Outcomes

By the end of this unit, you will be able to:

- Discuss the Methods of Assessing Microbiological Status of Fruits And Vegetables

4.3 Isolation of Microorganisms

Eosin Methylene Blue agar, SS (*Salmonella-Shigella*) agar and MacConkey agar are used for the isolation of microbes. The agars are prepared and autoclaved for sterilization at 121° C for 15 minutes. All agars are plated by pour plate method and kept for solidification at room temperature. After solidification, media is inoculated with 1 ml of sample from dilution 10-2 and 10-6, followed by incubation for 24 hours at 37º C. The isolated and distinct colonies are selected and further streaked on separate plates for formation of pure isolated colonies for identification.

4.3.1 Total Plate Count

For microbial load determination, serial dilutions of each homogenized mixture are prepared upto 10-6 and dilutions 10-2 and 10-4 used for inoculation on nutrient agar media. The inoculated plates are incubated at 37° C for 24 hours. Duplicate plates with 25 to 250 colonies were selected for total count. The number of colonies is multiplied to dilution factor (reciprocal of dilution) in order to find the microbial load.

4.3.2 Counts for lactic acid bacteria

Lactic acid bacteria such as *L. mesenteroides* are the primary organisms responsible for spoilage of some fresh-cut produce. In packaged mixed vegetables, the development of Lactic acid bacteria is related to temperature increases, and Lactic acid bacteria counts seem to be high in shredded carrots. Their densities can be estimated on DeMan, Rogosa, Sharpe (MRS) agar

adjusted to pH 5.6 and incubated under anaerobic conditions at 30°C for 48 h. Although an anaerobic system provides an oxygen-free work chamber, a simple and easy way to achieve anaerobic conditions is to use GasPakTM gas generating systems. These are multi-use systems that produce atmospheres suitable for isolating and cultivating anaerobic and microaerophilic bacteria by using gas generating sachets inside the system. Anaerobic conditions for incubation may also be obtained by using sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained.

4.3.3 Enumeration of yeasts and moulds

A great population of yeasts and moulds can be found on raw or fresh-cut fruits and vegetables prior to harvest, during its storage and on the shelf. They form the predominant microorganisms associated with the spoilage of fresh-cut fruit products. Some yeasts and moulds can cause spoilage and some are a public health concern due to their production of mycotoxins. Selective media and lower incubation temperatures are often used to slow or inhibit bacterial growth and thereby select for the outgrowth of yeasts and moulds. Selective bacterial inhibition can be achieved using antibiotics such as chloramphenicol at 100 μg/mL, or through acidification of media such as potato dextrose agar (PDA) with tartaric acid to pH 3.5. Incubation is conducted at 22–25°C for up to 5 days. Chose Sabouraud Dextrose Agar incubated at 28°C for 2 days for yeasts and Malt Extract Agar (MEA) incubated at 25°C for 4 days for moulds. Dichloran rose bengal chloramphenicol agar (DRBC) incubated at 25°C for 5 days is used to recover and enumerate yeasts/moulds.

4.3.4 Quantification of coliform bacteria

In conducting the microbiologic analysis of food in order to assess the health risk associated with its consumption, indicator organisms are often utilized. These usually are present in higher

numbers than true pathogens and are easier to detect. The growth and survival characteristics of these organisms are similar to those of pathogens. The most commonly used indicator organisms are the coliforms. Coliform bacteria are a group of Gram negative, non-spore forming, aerobic or facultative anaerobic rods that ferment lactose forming acid and gas within 48 h at 35°C. *E. coli* is a member of the coliform group. Most coliforms are members of the *Enterobacteriaceae* family. The presence of high numbers of coliforms in foods is often assumed to be indicative of the co-presence of intestinal pathogens, which are usually more difficult to detect and quantify. In addition, their presence has been used to indicate the absence of proper sanitation. When enumerating coliforms, *E. coli* may be present among the mixed population measured. Selective or differential media can be used for estimating *E. coli* levels under these circumstances. Among these is the most probable number (MPN) method.

Self- Assessment Exercises 1

1. Discuss the total plate count techniques
2. Name the most commonly used indicator in assessing the health risk associated with food consumption

4.4 Summary

The laboratory examination of fruits and vegetables for the presence, types, and numbers of microorganisms and/or their products is basic to food microbiology. Due to their dietary values, fruits and vegetables also harbour a variety of elevated microbial contaminants. Toxins produced by variety of microorganisms play major role in contaminating the food products. Disease causing microorganisms like *E. coli, Rhizopus, Staphylococcus aureus, Salmonella, Clostridium botulinum, Bacillus cereus, Pseudomonas aeruginosa, Mucor* species, *Aspergillus* and *Candida* species can contaminate fresh produce.

4.5 References/Further Readings

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4.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. For microbial load determination, serial dilutions of each homogenized mixture are prepared upto 10-6 and dilutions 10-2 and 10-4 used for inoculation on nutrient agar media. The inoculated plates are incubated at 37° C for 24 hours. Duplicate plates with 25 to 250 colonies were selected for total count. The number of colonies is multiplied to dilution factor (reciprocal of dilution) in order to find the microbial load.
2. The most commonly used indicator organisms are the coliforms.

UNIT 5 LABORATORY METHODS OF ASSESSING MICROBIOLOGICAL STATUS OF MEAT, FISH & DAIRY PRODUCTS

CONTENT

5.1 Introdction

Meat, fish & dairy products provide excellent growth media for bacteria when suitable temperatures exist. This is in direct contrast to natural waters, which lack the essential nutrients for pathogens. The introduction of a few pathogens into meat, fish & dairy products become a much more serious problem because of the ability of these substances to support tremendous increases in bacterial numbers. Many milk-borne epidemics of human diseases have been spread by contamination of milk by soiled hands of dairy workers, unsanitary utensils, flies, and polluted water supplies. The same thing can be said for improper handling of foods in the home,

restaurants, hospitals, and other institutions. Bacteriological testing of meat, fish & dairy products may also be performed in this same manner, using similar media and procedures to detect the presence of coliforms. However, most testing by public health authorities is quantitative. Although the presence of small numbers of bacteria in these substances does not necessarily mean that pathogens are lacking, low counts do reflect better care in handling of food and milk than is true when high counts are present.

 **5.2 Learning Outcomes**

By the end of this unit, you will be able to:

- Discuss the Methods of Assessing Microbiological Status of meat, fish & dairy products

 **5.3 Laboratory methods of assessing microbiological status of meat, fish & dairy products**

5.3.1 Standard Plate Count of Milk

The bacterial count in meat, fish & dairy products is the most reliable indication we have of its sanitary quality. Although human pathogens may not be present in a high count, it may indicate a diseased udder, unsanitary handling of meat, fish & dairy products, or unfavorable storage temperatures. In general, therefore, a high count means that there is a greater likelihood of disease transmission. On the other hand, it is necessary to avoid the wrong interpretation of low plate counts, since it is possible to have pathogens such as the brucellosis and tuberculosis organisms when counts are within acceptable numbers.

5.3.2 Direct Microscopic Count of Organisms in Milk

When it is necessary to determine milk quality in a much shorter time than is possible with a standard plate count, one can make a **direct microscopic count** on a slide. This is accomplished by staining a measured amount of meat, fish & dairy samples that has been spread over an area one square centimeter on a slide. The slide is examined under oil and all of the organisms in an entire microscopic field are counted. To increase accuracy, several fields are counted to get average field counts. Before the field counts can be translated into organisms per milliliter, however, it is necessary to calculate the field area. High-quality milk will have very few organisms per field, necessitating the examination of many fields. A slide made of poor-quality milk, on the other hand, will reveal large numbers of bacteria per field, thus requiring the examination of fewer fields. It is widely used for testing raw milk in creamery receiving stations and for diagnosing the types of contamination and growth in pasteurized milk products.

5.3.3 Reductase Test

Meat, fish & dairy products that contains large numbers of actively growing bacteria will have a lowered oxidation-reduction potential due to the exhaustion of dissolved oxygen by microorganisms. The fact that methylene blue loses its color (becomes reduced) in such an environment is the basis for the **reductase test.** In this test, 1 ml of methylene blue (1:25,000) is added to 10 ml of milk. The tube is sealed with a rubber stopper and slowly inverted three times to mix. It is placed in a water bath at 35° C and examined at intervals up to 6 hours. The time it takes for the methylene blue to become colorless is the **methylene blue reduction time** (MBRT). The shorter the MBRT, the lower the quality of the milk. An MBRT of 6 hours is very good. Milk with an MBRT of 30 minutes is of very poor quality. The validity of this test is based on the assumption that all bacteria in milk lower the oxidation-reduction potential at 35°C. Large

numbers of psychrophiles, thermophiles, and thermodurics, which do not grow at this temperature, would not produce a positive test. Raw milk, however, will contain primarily *Streptococcus lactis* and *Escherichia coli,* which are strong reducers; thus, this test is suitable for screening raw milk at receiving stations. Its principal value is that less technical training of personnel is required for its performance.

5.3.4 Bacterial Counts of Meat, fish & dairy products

The standard plate count, as well as the multiple tube test, can be used on Meat, and fish products much in the same manner that they are used on milk and water to determine total counts and the presence of coliforms.

Self- Assessment Exercises 1

1. What is methylene blue reduction time?
2. Explain the Bacterial Counts of Meat, fish & dairy products

5.4 Summary

Bacteriological testing of meat, fish & dairy products may also be performed using similar media and procedures to detect the presence of coliforms. However, most testing by public health authorities is quantitative. Although the presence of small numbers of bacteria in these substances does not necessarily mean that pathogens are lacking, low counts do reflect better care in handling of food and milk than is true when high counts are present.

5.5 Glossary

Sanitation: the maintenance of hygienic conditions, through services such as garbage collection and waste disposal.

Sewage: all the material that flows from household plumbing systems, including washing and bathing water and toilet wastes as well as others from business and industrial wastes.

5.5 References/Further Readings

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5.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. The time it takes for the methylene blue to become colorless is the **methylene blue reduction time** (MBRT).
2. The standard plate count, as well as the multiple tube tests, can be used on Meat, and fish products much in the same manner that they are used on milk and water to determine total counts and the presence of coliforms.

Module 6

Unit 1	Microbiological standards and criteria.
Unit 2	Indices of food sanitary quality.
Unit 3	Microbiology of cheese and beverage fermentation (tea, coffee, vinegar)
Unit 4	Microbiology of fermented milk products (acidophilus milk, yoghurt) and probiotics
Unit 5	Application of microbial enzymes in food industries
Unit 6	Genetically modified foods (foods and genetic engineering)

UNIT 1 MICROBIOLOGICAL STANDARDS AND CRITERIA.

Content

1.1 Introduction

The establishment and use of microbiological criteria, is to serve as a standard and guidelines or specifications for ensuring food safety. Microbiological standards and criteria are set as specifications to determine the usefulness of a food or food ingredient for a particular purpose. As standard, there is zero tolerance set for *Salmonella* in all RTE foods. The criteria are most effectively applied as part of quality assurance programs in which HACCP and other prerequisite programs are in place. On this premise, microbiological criteria for foods continue to evolve as

new information becomes available. The establishment of microbiological criteria for foods in international trade is to serve as guidelines for national standards and policies which will culminate in food safety for all.

1.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the Microbiological standards and criteria.
- Analyse the seven HACCP steps
- Discuss the food Safety Objective

1.3 Microbiological standards and criteria.

Microbiological criteria vary considerably for different countries. There are no general criteria suitable for all foods however; the standard is to ensure that limit of the indictor organisms is not exceeded. Microbiological criteria fall into two main categories: mandatory and advisory. A mandatory criterion is a microbiological standard that normally should contain limits only for pathogens of public health significance, but limits for nonpathogens may be set. An advisory criterion is either a microbiological end product specification intended to increase assurance that hygienic significance has been met or a microbiological guideline that is applied in a food establishment at a point during or after processing to monitor hygiene (it, too, may include nonpathogens). Before recommending a criterion, the product must be in international trade, there must have been a good epidemiological evidence that it has been implicated in foodborne disease, and have associated with it good evidence that a criterion will reduce the potential hazard(s) in Principle.

The Codex definition of a microbiological criterion consists of five components:

- A statement of the organisms of concern and/or their toxins
- The analytical methods for their detection and quantitation
- A sampling plan, including when and where samples are to be taken
- Microbiological limits considered appropriate to the food
- The number of sample units that should conform to these limits.

These five components are embodied in a sampling plan.

Sampling Plans: A sampling plan is a statement of the criteria of acceptance applied to a lot based on appropriate examinations of a required number of sample units by specified methods. It consists of a sampling procedure and decision criteria and may be a two-class or a three-class plan. A two-class plan consists of the following specifications: n, c, m; while a three-class plan requires n, c, m, and M.

n = the number of sample units (packages, beef patties, and so forth) from a lot that must be examined to satisfy a given sampling plan.

c = the maximum acceptable number, or the maximum allowable number of sample units that may exceed the microbiological criterion m. When this number is exceeded, the lot is rejected.

m = the maximum number or level of relevant bacteria per gram; values above this level are either marginally acceptable or unacceptable. It is used to separate acceptable from unacceptable foods in a two-class plan, or, in a three-class plan, to separate good quality from marginally acceptable quality foods. The level of the organism in question that is acceptable and attainable in the food product is m. In the presence/absence situations for two-class plans, it is common to assign m = 0. For three-class plans, m is usually some nonzero value.

M = a quantity that is used to separate marginally acceptable quality from unacceptable quality foods. It is used only in three-class plans. Values at or above M in any sample are unacceptable relative to health hazard, sanitary indicators, or spoilage potential

Microbiological criteria for *Listeria monocytogenes* in RTE foods

COUNTRY	Limit (per g)			
	N	C	M	M
United states	5	0	0 (negative/25 g)	
United Kingdom		5	100	
Canada				
Category 1 ,2	5	0	0 (0/25 g)	
Category 2	5	0	100	
Germany			10,000	

Source: International Commission on Microbiological Specifications for Foods, Microorganisms in Foods: Microbiological Testing in Food Safety Management.

International microbiological standards have been developed. These standards recommend that a series of steps must be taken to manage microbiological hazards for foods intended for international trade. These steps include conducting a risk assessment and an assessment of risk management options, establishing a food safety objective (FSO), and confirming that the FSO is achievable by application of Good Microbiological Practices and Hazard Analysis and Criticl Control Points (HACCPs) for all foods meant for public consumption. A food safety objective (FSO), is a statement showing the frequency or maximum concentration of a microbiological hazard contained in a food that is considered acceptable for consumer protection. For example, in

cheese the *staphylococcal* enterotoxin levels must not exceed 1 mg/100 g. The concentration of aflatoxin in peanuts should not exceed 15 mg/kg.

It is the responsibility of the food industry to apply GMPs and establish appropriate control measures, including critical control points, in all their processes and HACCP plans to ensure quality and safety.

1.3.1 Hazard Analysis Critical Control Point (HACCP)

Hazard is a biological, chemical, or physical agent that is reasonably likely to cause illness or injury in the absence of its control. Hazard Analysis is the process of collecting and evaluating information on hazards associated with the food under consideration to decide which are significant and must be addressed in the HACCP plan. Hazard Analysis Critical Control Points (HACCP) is a system which provides the framework for monitoring the total food system, from harvesting to consumption, to reduce the risk of foodborne illness. The system is designed to identify and control potential problems before they occur. The application of HACCP is based on technical and scientific principles that assure safe food.

HACCP consists of seven steps used to monitor food as it flows through the establishment, whether it is a food processing plant or foodservice operation. The seven steps of the HACCP system address the analysis and control of biological, chemical and physical hazards.

HACCP terminology

Critical Control Point (CCP): A procedure/practice (control) in food handling/preparation that will reduce, eliminate or prevent hazards. It is a “kill” step that kills microorganisms or a control step that prevents or slows their growth.

Hazard: Unacceptable contamination, microbial growth, persistence of toxins or survival of microorganisms that are of a concern to food safety

Monitoring: Checking to determine if the criteria established by the critical control point(s) (CCP) have been achieved

Risk: Probability that a condition(s) will lead to a hazard

Severity: Seriousness of the consequences of the results of a hazard

Formal HACCP seven steps:

1. **Conduct a hazardous analysis.**

The purpose of a hazardous analysis is to develop a list of hazards which are likely to cause injury or illness if they are not controlled. Points to be considered in this analysis can include: skill level of employees; transport of food; serving elderly, sick, very young children, immune-compromised; volume cooling; thawing of potentially hazardous foods; high degree of food handling and contact; adequacy of preparation and holding equipment available; storage, and method of preparation. The next step is to determine if the factors may influence the likely occurrence and severity of the hazard being controlled. Finally, the hazards associated with each step in the flow of food should be listed along with the measures necessary to control the hazard.

2. **Determine Critical Control Points (CCP's)**

A critical control point is any step in which hazards can be prevented, eliminated or reduced to acceptable levels. CCP's are usually practices/procedures which, when not done correctly, are the leading causes of foodborne illness outbreaks. Examples of critical control points include cooking, cooling, re-heating, holding.

3. **Establish Critical Limits**

A critical limit ensures that a biological, chemical or physical hazard is controlled by a CCP. Each CCP should have at least one critical limit. Critical limits must be something that can be monitored by measurement or observation. They must be scientifically and/or regulatory based. Examples include temperature, time, pH, water activity or available chlorine**.**

4. **Establish Monitoring Procedures**

The monitoring system should be easy to use and meet the needs of the food establishment, as well as the regulatory authority. It is important that the job of monitoring be assigned to a specific individual and they be trained on the monitoring technique.

5. **Establish Corrective Actions**

Corrective actions may range, for example, from "continue cooking until the established temperature is reached" to "throw out the product," depending on the severity of the situation. HACCP plans should be established in advance to include the following: who is responsible for implementing the corrective action and what corrective action was taken.

6. **Establish verification procedures**

Verification can be accomplished by expert advice and scientific studies and observations of the flow of food, measurements and evaluations. Another means of verification is an onsite review of the established critical limits. Each CCP will have one independent authority. This verification step provides an opportunity to make modifications to the plan if necessary.

7. **Establish record-keeping and documentation procedures**

Record-keeping and documentation procedures should be simple to complete and include information that illustrates that the established standards are being met. Employees need to be trained on the record-keeping procedures and why it is a critical part of their job. Examples of

records include time/temperature logs, checklists, forms, flowcharts, employee training records, etc.

1.3.2 Food Safety Objective (FSO)

An FSO is a statement of the frequency or maximum concentration of a microbiological hazard in a food considered acceptable for consumer protection. Some of the examples of specific FSOs include:

- Staphylococcal enterotoxin in cheese must not exceed 1 µg/100 g
- Aflatoxin in peanuts should not exceed 15 µg/kg
- *Listeria monocytogenes* in ready-to-eat foods should not exceed 100/g at the time of consumption
- Salmonellae on raw poultry meat should be 106 to 109 cfu/g.

1.3.3 Microbiological Criteria for Various Products

The application of criteria to products in the absence of an HACCP program is much less likely to be successful than when the two are combined. Thus, microbiological criteria are best applied as part of a comprehensive program.

Prior to the development of the HACCP and sampling plan concepts, microbiological criteria (generally referred to as standards at the time) were applied to a variety of products. Below are foods and food ingredients that are covered under microbiological standards of various in the United States.

1. Standards for Starch and Sugar (National Canners Association)

    A. Total thermophilic spore count: of the five samples from a lot of sugar or starch none shall contain more than 150 spores per 10 g, and the average for all samples shall not exceed 125 spores per 10 g.

B. Flat-sour spores: of the five samples, none shall contain more than 75 spores/10 g, and the average for all samples shall not exceed 50 spores per 10 g.

C. Thermophilic anaerobe spores: not more than three (60%) of the five samples shall contain these spores, and in any one sample, not more than four (65%) of the six tubes shall be positive.

D. Sulfide spoilage spores: not more than two (40%) of the five samples shall contain these spores, and in any one sample, there shall be no more than five colonies per 10 g.

2. Standard for "Bottlers" Granulated Sugar, Effective July 1, 1953 (American Bottlers of Carbonated Beverages)

A. Mesophilic bacteria: Not more than 200 per 10 g.

B. Yeasts: Not more than 10 per 10 g.

C. Molds: Not more than 10 per 10 g.

3. Standard for "Bottlers" Liquid Sugar, Effective in 1959 (American Bottlers of Carbonated Beverages). All figures are based on dry-sugar equivalent (D.S.E.)

A. Mesophilic bacteria (a) Last 20 samples average 100 organisms or less per 10 g of D.S.E.; (b) 95% of last 20 counts show 200 or less per 10 g; (c) 1 of 20 samples may run over 200; other counts as in (a) or (b).

B. Yeasts: (a) Last 20 samples average 10 organisms or less per 10 g of D.S.E.; (b) 95% of last 20 counts show 18 or less per 10 g; (c) 1 of 20 samples may run over 18; other counts as in (a) and (b).

C. Molds: Standards like those for yeasts.

4. Standards for Dairy Products

A. From 1965 recommendations of the U.S. Public Health Service.

a. Grade A raw milk for pasteurization: Not to exceed 100,000 bacteria per milliliter prior to commingling with other producer milk; and not exceeding 300,000 per milliliter as commingled milk prior to pasteurization.

b. Grade A pasteurized milk and milk products (except cultured products): Not over 20,000 bacteria per milliliter, and not over 10 coliforms per milliliter.

c. Grade A pasteurized cultured products: Not over 10 coliforms per milliliter.Note: Enforcement procedures for (a), (b), and (c) require a three-out-of-five compliance by samples. Whenever two of four successive samples do not meet the standard, a fifth sample is tested; and if this exceeds any standard, the permit from the health authority may be suspended. It may be reinstated after compliance by four successive samples has been demonstrated.

B. Certified milk (American Association of Medical Milk Commissions, Inc.)

a. Certified milk (raw): Bacterial plate count not exceeding 10,000 colonies per milliliter; coliform colony count not exceeding 10 per milliliter.

b. Certified milk (pasteurized): Bacterial plate count not exceeding 10,000 colonies per milliliter before pasteurization and 500 per milliliter in route samples. Milk not exceeding 10 coliforms per milliliter before pasteurization and 1 coliform per milliliter in route samples.

C. Milk for manufacturing and processing (USDA, 1955)

a. Class 1: Direct microscopic clump count (DMC) not over 200,000 per milliliter.

b. Class 2: DMC not over 3 million per milliliter.

c. Milk for Grade A dry milk products: must comply with requirements for Grade A raw milk for pasteurization above.

D. Dry milk

a. Grade A dry milk products: at no time a standard plate count over 30,000 per gram, or coliform count over 90 per gram.

b. Standards of Agricultural Marketing Service (USDA):

- Instant nonfat: U.S. Extra Grade, a standard plate count not over 35,000 per gram, and coliform count not over 90 per gram.
- Nonfat (roller or spray): U.S. Extra Grade, a standard plate count not over 50,000 per gram; U.S. Standard Grade, not over 100,000 per gram
- Nonfat (roller or spray): Direct microscopic clump count not over 200 million per gram; and must meet the requirements of U.S. Standard Guide. U.S. Extra Grade, such as used for school lunches, has an upper limit of 75 million per gram.

Dried milk (International Dairy Federation proposed microbiological specifications, 1982).

- Mesophilic count: n = 5, c = 2, m = 5×10^4, M = 2×10^5
- Coliforms: n = 5, c = 1, m = 10, M = 100
- Salmonella: n = 15, c = 0, m = 0.

E. Frozen desserts States and cities that have bacterial standards usually specify a maximal count of 50,000 to 100,000 per milliliter or gram. The U.S. Public Health Ordinance and Code sets the limit at 50,000 and recommends bacteriological standards for cream and milk used as ingredients.

5. Standard for Tomato Juice and Tomato Products—Mold-count Tolerances (Food and Drug Administration). The percentage of positive fields tolerated is 2% for tomato juice and 40% for other comminuted tomato products, such as catsup, pur´ee, paste, and so forth. A microscopic field is considered positive when an aggregate length of not more than three mold filaments

present exceeds onesixth of the diameter of the field (Howard mold count method). This method has also been applied to raw and frozen fruits of various kinds, especially berries.

Self- Assessment Exercises 1

1. What is Critical Control Point?
2. What are the five components of Codex definition of a microbiological criterion

 1.4 **Summary**

The criteria are most effectively applied as part of quality assurance programs in which HACCP and other prerequisite programs are in place. On this premise, microbiological criteria for foods continue to evolve as new information becomes available. The establishment of microbiological criteria for foods in international trade is to serve as guidelines for national standards and policies which will culminate in food safety for all.

 **1.5 References/Further Readings**

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Compendium of Microbiological Criteria for Food (2018): Food Standards Australia New Zealand

1.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercises 1

1. **Critical Control Point (CCP):** A procedure/practice (control) in food handling/preparation that will reduce, eliminate or prevent hazards. It is a "kill" step that kills microorganisms or a control step that prevents or slows their growth.
2. The Codex definition of a microbiological criterion consists of five components:
    - A statement of the organisms of concern and/or their toxins
    - The analytical methods for their detection and quantitation
    - A sampling plan, including when and where samples are to be taken
    - Microbiological limits considered appropriate to the food
    - The number of sample units that should conform to these limits.

UNIT 2 INDICES OF FOOD SANITARY QUALITY.

Content

2.1 Introduction

Examination of a product for indicator organisms can provide simple, reliable, and rapid information about process failure, postprocess contamination, contamination from the environment, and the general level of hygiene under which the food was processed and stored.

To serve an indices of food sanitary quality, ideal indicators of product quality or shelf life should meet the following criteria.

- Indicator organisms should be present and detectable in all foods whose quality is to be assessed.
- Their growth and numbers should have a direct negative correlation with product quality.

- They should be easily detected and enumerated and be clearly distinguishable from other organisms.
- They should be enumerable in a short period, ideally within a workday.

2.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the Microbiological standards and criteria.
- Analyse the seven HACCP steps
- Discuss the food Safety Objective

Indices of food sanitary quality

2.3.1 Indicator Microorganisms

Microorganisms used to establish microbiological criteria are called Indicator organisms. The presence of indicator microbes suggests a potential microbial hazard. The presence of generic *Escherichia coli* in a food sample indicates possible fecal contamination. These criteria might be used to address existing product quality or to predict the shelf life of the food. The aerobic plate count (APC) is common technique used to determine the total number of indicator microorganisms in a food product. It may be a component of microbiological criteria assessing product quality when those criteria are used to monitor foods for compliance with standards or guidelines set by various regulatory agencies, monitor foods for compliance with purchase specifications, and monitor adherence to GMPs.

The Howard mold count, yeast and mold count, heat resistant-mold count, and thermophilic-spore count are other methods commonly used to indicate the quality of different food products. intended for use in low-acid, heat-processed canned foods.

2.3.2 Personal Hygiene

Personal hygiene begins at home, with the essential elements for good hygiene being a clean body, clean hair and clean clothing. Hair in food can be a source of both microbiological and physical contamination. Hairnets and beard covers should be worn to assure food product integrity. Long-sleeved smocks should be worn to cover arm hair. Clean uniforms, aprons and other outer garments that are put on after the employee gets to work can help minimize contamination. While working, clothing should be kept reasonably clean and in good repair. Removal of smocks, laboratory coats or aprons should take place when leaving the work area to go to the employee break room, restroom or exiting the building. Personal items such as meals and snacks should be stored in a locker or break room area that is located away from processing areas or areas where equipment and utensils are washed.

The only jewelry allowed in a food plant is a plain wedding band and/or one small post earring in each ear. No other jewelry is to be worn because it may fall into the product, it can present a safety hazard and it cannot be adequately sanitized against bacterial transmission. It should be removed prior to entering the processing facility. Employees must wear different colored smocks when going from a raw processing part of the establishment to the cooked processing side. They should also step into a sanitizer footbath between the two processing areas to eliminate the bacteria on their shoes.

No employee who is affected with, has been exposed to, or is a carrier of a communicable disease, the flu or a respiratory problem, or any other potential source of microbiological contamination shall work in any area where there is a reasonable possibility that food or food ingredients can be contaminated. The number one symptom of a foodborne illness is diarrhoea.

Other symptoms include fever, dizziness, vomiting, and sore throat with fever or jaundice. Any employee with these symptoms should not be allowed to work around food. If an employee has been diagnosed with a foodborne illness, exclude them from the establishment, and contact the local health department. The health department must be notified if the employee has been diagnosed with one of the following foodborne illnesses: *Salmonella typhi, Shigella species,* shiga toxin producing *E. coli,* or hepatitis A virus.

2.3.3 Factors to Consider during Sanitation in foods

To minimize the access of microorganisms in foods, the microbiological quality of the environment to which a food is exposed (food contact surfaces) and the ingredients added to a food should be of good microbiological quality. To achieve these goals, several factors need to be considered, which are briefly discussed here.

i. Plant Design

At the initial designing stage of a food-processing plant, an efficient sanitary program has to be integrated in order to provide maximum protection against microbial contamination of foods. This includes both the outside and the inside of the plant. Some elements to consider are specific floor plan, approved materials used in construction, adequate light, air ventilation, direction of air flow, separation of processing areas for raw and finished products, sufficient space for operation and movement, approved plumbing, water supply, sewage disposal system, waste treatment facilities, drainage, soil conditions, and the surrounding environment. Regulatory agencies have specifications for many of these requirements and can be consulted at the initial stage of planning to avoid costly modifications.

ii. Quality of Water, Ice, Brine, and Curing Solution

Water is used as an ingredient in many foods and is also used in some products after heat treatment. The microbiological quality of this water, especially if the foods are ready-to-eat types, should not only be free from pathogens but also be low (if not free) in spoilage bacteria such as *Pseudomonas spp*. This is particularly important for foods kept at low temperature for extended shelf life. The ice used for chilling unpackaged foods also should not contaminate a food with pathogenic and spoilage bacteria. Water used for chilling products, such as chicken at the final stage of processing, can be a source of cross-contamination of a large number of birds from a single bird contaminated with an enteric pathogen. Similarly, the warm water used to de-feather chicken can be a source of thermoduric bacteria. Brine and curing solutions used in products such as ham, bacon, turkey-ham, and cured beef brisket can be a source of microbial contamination. To reduce this, brine and curing solutions should be made fresh and used daily. Storing brine for extended periods before use may reduce the concentration of nitrite through formation and dissipation of nitrous oxide and may reduce shelf life of the products.

1. **Quality of Air**

Some food-processing operations, such as spray drying of nonfat dry milk, require large volumes of air that come into direct contact with the food. Although the air is heated, it does not kill all the microorganisms present in the dust of the air and thus can be a source of microbial contamination of foods. The installation of air inlets to obtain dry air with the least amount of dust and filtration of the air is important to reduce microbial contamination from this source.

2. **Training of Personnel**

A processing plant should have an active program to teach the plant personnel the importance of sanitation and personal hygiene to ensure product safety and stability. The program should not only teach how to achieve good sanitation and personal hygiene but also monitor the implementation of the program. People with an illness and infection should be kept away from handling the products.

3. Equipment

The most important microbiological criterion to be considered during the design of food processing equipment is that it should protect a food from microbial contamination. This can be achieved if the equipment does not contain dead spots where microorganisms harbor and grow or that cannot be easily and readily cleaned in place or by disassembling. Some of the equipment, such as meat grinders, choppers, or slicers and several types of conveyor systems, may not be cleaned and sanitized very effectively and therefore serve as a source of contamination to a large volume of product. This is particularly important for products that come in contact with equipment surfaces after heat treatment and before packaging.

4. Cleaning of Processing Facilities

Cleaning is used to remove visible and invisible soil and dirt from the food-processing surroundings and equipment. The nature of soil varies greatly with the type of food processed, but chemically it consists of lipids, proteins, carbohydrates, and some minerals. Although water is used for some cleaning, to increase efficiency of cleaning, chemical agents or detergents are used with water. In addition, some form of energy with the liquids, such as spraying, scrubbing, or turbulent flow, is used for better cleaning. Many types of detergents are available, and they are selected based on the need. The effectiveness of a cleaning agent to remove soil from surfaces depends on several characteristics, such as efficiency of emulsifying lipids, dissolving proteins,

and solubilizing or suspending carbohydrates and minerals. Also, a detergent should be noncorrosive, safe, rinsed easily, and compatible, when required, with other chemical agents. The detergents frequently used in food processing facilities are synthetic, which can be anionic, cationic, or nonionic. Among these, anionic detergents are used with higher frequency. Examples of anionic detergents include sodium lauryl sulfate and different alkyl benzene sulfonates and alkyl sulfonates. Each molecule has a hydrophobic or lipophilic (nonpolar) segment and a hydrophilic or lipophobic (polar) segment. The ability of a detergent to remove dirt from a surface is attributed to the hydrophobic segment of a molecule. They dissolve the lipid materials of the soil on the surface by forming micelles with the polar segments protruding outside in the water. The concentration of a detergent at which micelle formation starts is called the critical micelle concentration (CMC), which varies with the detergent. The concentration of a detergent is used above its CMC level.

The frequency of cleaning depends on the products being processed and the commitment of the management to good sanitation. From a microbiological standpoint, prior microbiological evaluation of a product can give an indication about the frequency of cleaning necessary in a particular facility. Cleaning of the equipment is done either after disassembling the equipment or by the CIP system. Because of its efficiency and lower cost, CIP cleaning has become popular. The system uses detergent solutions at a high pressure. Because microorganisms can grow in some detergent solutions, they preferably should be prepared fresh (not exceeding 48 hours).

2.3.4 Sanitation of Food Processing Equipment

Efficient cleaning can remove some microorganisms along with the soil from the food contact surfaces, but cannot ensure complete removal of pathogens. To achieve this goal, food contact surfaces are subjected to sanitation after cleaning. The methods should effectively destroy

pathogenic microorganisms as well as reduce total microbial load. Several physical and chemical methods are used for sanitation of food processing equipment. Physical agents used for sanitation of food processing equipment include hot water, steam, hot air, and UV irradiation. UV irradiation is used to disinfect surfaces. Hot water and steam, although less costly and efficient for destroying vegetative cells, viruses, and spores (especially steam) can be used only in a limited way. Chemical sanitizers are used more frequently than physical sanitizers. Several groups of sanitizers are approved for use in food processing plants. They vary greatly in their antimicrobial efficiency. Some of the desirable characteristics used in selecting a chemical sanitizer are effectiveness for a specific need, nontoxicity, non-corrosiveness, no effect on food quality, easy to use and rinse, stability, and cost effectiveness. Important factors for antimicrobial efficiency are exposure time, temperature, concentrations used, pH, microbial load and type, microbial attachment to surface, and water hardness. Some sanitizers, designated as detergent sanitizers, can both clean and sanitize. They can be used in a single operation instead of first using detergent to remove the soil and then using sanitizers to control microorganisms. The mechanisms of antimicrobial action and the advantages and disadvantages of some of the sanitizers used in food-processing plants are briefly below.

2.3.5 Sanitizing agents

a. Chlorine-Based Sanitizers

Some of the chlorine compounds used as sanitizers include: liquid chlorine, hypochlorites, inorganic or organic chloramines, and chlorine dioxide. Chlorine compounds are effective against vegetative cells of bacteria, yeasts and molds, spores, and viruses. Clostridial spores are more sensitive to chlorine compounds than are bacilli spores. The antimicrobial action of

chlorine compounds is due to the oxidizing effect of chlorine on the –SH group in many enzymes and structural proteins.

The damage to membrane, disruption of protein synthesis, reactions with nucleic acids, and interference with metabolisms has been suggested. The germicidal action of liquid chlorine and hypochlorites is produced by hypochlorous acid (HOCl). It probably enters the cell and reacts with the –SH group of proteins. HOCl is stable at acid pH and is thus more effective; at alkaline pH, it dissociates to H+ and OCl– (hypochlorite ions), which reduces its germicidal effectiveness. They are also less effective in the presence of organic matter. Chloramines (inorganic or organic), such as Chloramine T, release chlorine slowly, but they are less active against bacterial spores and viruses. They are effective, to some extent, against vegetative cells at alkaline pH. Chlorine dioxide is more effective at alkaline pH and in the presence of organic matter. Chlorine compounds are fast acting against all types of microorganisms, less costly, and easy to use. However, they are unstable at higher temperatures and with organic matter, corrosive to metals, can oxidize food, and are less active in hard water.

b. **Iodophores**

Iodophores are prepared by combining iodine with surface-active compounds, such as alkylphenoxypolyglycol. Because of the surface-active compounds, they are relatively soluble in water. Iodophores are effective against Gram-positive and Gram-negative bacteria, bacterial spores, viruses, and fungi. Their germicidal property is attributed to elemental iodine (I_2) and hypoiodous acid, which oxidize the –SH group of proteins, including key enzymes. They are more effective at acidic pH and higher temperatures and in the presence of organic matters, they do not lose germicidal property as rapidly as chlorine does. However, their effectiveness is reduced in hard water. They are fast acting, noncorrosive, easy to use, nonirritating, and stable.

Iodophores are expensive, less effective than hypochlorites against spores and viruses, can cause flavor problems in products, and react with starch.

c. Quaternary Ammonium Compounds

Quaternary ammonium compounds (QACs) can be used as detergent sanitizers because they have cleaning properties along with germicidal abilities. However, they are principally used as sanitizers. They are synthesized by reacting tertiary amines with alkyl halides or benzyl chloride.

The cationic group is hydrophobic and the anionic group is hydrophilic. QACs can act as bactericides in high concentrations and when used in solution. However, they form a film on the equipment surface, in which state are bacteriostatic. They are more effective against Gram-positive bacteria than many Gram-negative bacteria, bacterial spores, fungi, and viruses. The antimicrobial action is produced by the denaturation of microbial proteins and destabilization of membrane functions. They are more effective against microorganisms at acidic pH and higher temperature. Their effectiveness is not greatly reduced in the presence of organic matters. However, they are less effective in hard water. QACs are advantageous as sanitizers because they are highly stable, non-corrosive, non-irritating, non-toxic, show residual bacteriostatic effect, and show detergent effect. The disadvantages are high cost; low activity against many Gram-negative bacteria, spores, and viruses; incompatibility with anionic synthetic detergents; and rinsing requirement before use because of film formation on equipment surfaces. Some Gram-negative bacteria, such as *Pseudomonas spp.*, can grow in diluted QAC solutions.

d. Hydrogen Peroxides (H_2O_2)

H_2O_2 is a very effective germicide and kills vegetative cells, spores, and viruses. It is used for sanitation of equipment and containers used in the aseptic packaging of foods and beverages. Equipment and container surfaces can be sterilized in 15 min with a 30 to 50% solution; the treatment time can be reduced if the temperature of the solution is raised to 65.6 to 71.7°C (150 to 160ºF). Use of H_2O_2 in vapor phase can also be effective in killing microorganisms on food contact surfaces. Organic materials greatly reduce the germicidal effect of H_2O_2.

2.3.6 Microbiological Standards, Specifications, and Guideline

Microbiological standards, specifications, and guidelines are useful in keeping the microbial load of foods at acceptable levels by various methods, one of which is by controlling their access to foods. Microbiological standards of food are set and enforced by regulatory agencies to increase consumer safety and product stability. A standard dictates the maximum microbial level that can be accepted in a food. With proper sanitation and quality control, this level is generally attainable. Some examples are maximum acceptable levels of standard plate counts (SPCs) of Grade A raw milk, 100,000/ml; pasteurized Grade A milk, SPC 20,000/ml and coliforms <10/ml. However, very few foods have microbiological standards while many foods and food ingredients have microbiological specifications.

A specification indicates maximum permissible microbial load for the acceptance of a food or food ingredient. It should be attainable and agreed on by the buyers and sellers of the products. It is not set up or enforced by regulatory agencies. In the U.S., the military has microbiological specifications of foods purchased outside for army rations. For example, dried whole egg has the following specifications: aerobic plate count (APCs), 25,000/g; coliforms, 10/g; and Salmonella,

negative in 25/g. The specifications discourage mixing of a microbiologically poor-quality product with a good quality product.

Microbiological guidelines are generally set either by regulatory agencies or food processors to help generate products of acceptable microbiological qualities. A guideline is set at a level that can be achieved if a food-processing facility uses good cleaning, sanitation, and handling procedures. It also helps detect if a failure has occurred during processing and handling, and thus alerts the processor to take corrective measures.

Self- Assessment Exercises 1

1. What do you understand by hazard analysis critical control point (HACCP)?
2. What are the physical agents used for sanitation of food processing equipment

2.4 Summary

Spoilage and pathogenic microorganisms enter in food from different sources. One of the major objectives to produce a safe food with desirable shelf life is to minimize the access of microorganisms in food from various sources. This can be achieved by proper plant design, training personnel, designing equipment that can be sanitized effectively, and establishing an efficient cleaning and sanitation procedure. Many cleaning and sanitizing chemicals are available commercially. The aim will be to select agents that are suitable for a specific purpose. Adaptation of an efficient and approved procedure (by regulatory agencies) helps meet the required microbiological standards and specifications.

Sanitization in foods can be achieved by proper plant design, training personnel, designing equipment that can be sanitized effectively, and establishing an efficient cleaning and sanitation procedure. Many commercially available cleaning and sanitizing agents are Iodophores,

Chlorine-based compounds; Quaternary Ammonium Compounds, and Hydrogen Peroxides. The aim will be to select agents that are suitable for a specific purpose.

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 **2.6 Possible Answers to Self-Assessment Exercises**

Answers to Self-Assessment Exercises 1

1. Hazard Analysis Critical Control Points (HACCP) is a system which provides the framework for monitoring the total food system, from harvesting to consumption, to reduce the risk of foodborne illness. The system is designed to identify and control potential problems before they occur.
2. Physical agents used for sanitation of food processing equipment include hot water, steam, hot air, and UV irradiation.

UNIT 3 MICROBIOLOGY OF CHEESE AND BEVERAGE FERMENTATION (TEA, COFFEE, VINEGAR)

Content

3.1 Introduction

The word **Fermentation** has many meanings. According to Louis Pasteur it describes life in the absence of oxygen. Fermentation is the process that bacteria use to make energy from carbohydrates in the absence of oxygen. Fermentation involves exposing the raw or starting food materials to conditions that favor growth and metabolism of specific and desirable microorganisms. As the desirable microorganisms grow, they utilize some nutrients and produce some end products. These end products, along with the unmetabolized components of the starting materials, constitute the fermented foods having desirable acceptance qualities, many of which are attributed to the metabolic end products.

With a few exceptions, *Fermentation* describes any biological process that make vinegar, antibiotics, monosodium glutamate, amino acids, citric acid, etc whether oxygen is present or not. Food fermentations are bioprocesses that change food properties while the bacteria generate energy in the absence of oxygen. These changes go far beyond acid production. Fermentations add value to foods by producing flavour compounds and carbonation, altering texture, and increasing nutrient bioavailability.

The production of a fermented product has two related yet separate aspects, one involving the importance of metabolic activities of microorganisms during fermentation and storage of the product and the other involving the parameters used during processing and storage of the product.

3.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the microorganisms involved in fermentation processes
- Discuss the fermentation process
- Discuss the microbiology of cottage cheese

3.3 Microbiology of Cheese and Beverage Fermentation

3.3.1 Microorganisms involved in fermentation processes

The process of breaking carbon sources into desirable end products that benefit man involves the activities of a number of microorganisms. Many desirable species and strains of bacteria, yeasts, and molds are associated with fermentation of foods. Depending on a product, fermentation may be achieved by a single predominating species and strain. However, in most fermentations, a mixed population of several bacterial species and strains, or even bacteria and yeasts or bacteria

and molds, is involved. When a fermentation process involves a mixed population, the members act together to aid the process in a synergistic manner. Maximum growth of a desirable microorganism and optimum fermentation rates are dependent on environmental parameters, such as nutrients, temperature of incubation, oxidation-reduction potential, and pH. In the fermentation process, if the different species in a mixed population need different environmental conditions (e.g., temperature of growth), a compromise is made to facilitate growth of all the species at a moderate rate. Depending on the raw or starting material and a specific need, carbohydrates (dextrose in meat fermentation), salts, citrate, and other nutrients are supplemented.

In some natural fermentation, several species may be involved for the final desirable characteristics of the product. However, instead of growing at the same time, they appear in sequence with the consequence that a particular species predominates at a certain stage during fermentation.

3.3.2 Fermentation process

Foods can be fermented in three different ways, based on the sources of the desirable microorganisms: **Natural fermentation, Back slopping, and Controlled fermentation.**

Natural Fermentation

Many raw materials used in fermentation contain both desirable and associated microorganisms. The conditions of incubation are set to favour rapid growth of the desirable types and no or slow growth of the associated (many are undesirable) types. A product produced by natural fermentation can have some desirable aroma resulting from the metabolism of the associated flora. However, because the natural microbial flora in the raw materials may not always be the same, it is difficult to produce a product with consistent characteristics over a long period of

time. Also, chances of product failure because of growth of undesirable flora and foodborne diseases by the pathogens are high.

Back Slopping

In this method, some products from a successful fermentation are added to the starting materials, and conditions are set to facilitate the growth of the microorganisms coming from the previous product. This is still practiced in the production of many ethnic products in small volumes. Retention of product characteristics over a long period may be difficult because of changes in microbial types. Chances of product failure and foodborne diseases are also high.

Controlled Fermentation

In controlled fermentation, the starting materials may be heat treated and inoculated with a high population (10^6 cells/mL or more) of a pure culture of single or mixed strains or species of the starter culture. Incubation conditions are set for the optimum growth of the starter cultures. Large volumes of products can be produced with consistent and predictable characteristics each day. Generally, there is less chance of product failure and foodborne diseases. However, there may be no growth of desirable secondary flora. As a result, a product may not have some delicate flavor characteristics.

The microbiology of fermented products involves the metabolic activities of microorganisms which gives rise to new products. Fermentation processes involves exposing the raw or starting food materials to conditions that favours growth and metabolism of specific and desirable microorganisms. Microorganisms often known as starter culture grow, and utilize some nutrients to produce some end products. These end products, along with the unmetabolized components of the starting materials, constitute the fermented foods having desirable qualities.

3.3.3 Microbiology of Cottage Cheese

Cheese is a product of milk fermentation made by coagulating the casein in milk with lactic acid. It is produced by lactic acid bacteria, without or with the enzyme rennin. The process is followed by collecting the casein for further processing, which may include ripening. Cottage cheese is made from low-fat or skim milk and has a soft texture with approximately 80% moisture. It is unripened and has a buttery aroma resulting from diacetyl (along with lactic acid and little acetaldehyde). Made from Pasteurized milk, the milk is inoculated with a starter culture, usually containing *Lactococcus cremoris* and *L. lactis*, and then incubated until fermentation products cause the proteins in milk to coagulate. The coagulated proteins, or **curd,** are heated and cut into small pieces to make it easier to drain the liquid waste portion. Unlike most cheeses that undergo further microbial processes called ripening or curing, cottage cheese is unripened. The initial steps of ripened cheese production are the same as those of cottage cheese, except the enzyme rennin is added to the fermenting milk to speed protein coagulation. After the proteins coagulate, the whey (the liquid waste portion) is removed. The curds are then salted, pressed, and shaped into the traditional forms, usually bricks or wheels. The cheese is then ripened, resulting in characteristic textural and flavor changes due to the metabolic activities of naturally occurring or starter lactic acid bacteria. Depending on the type of cheese, ripening can take from several weeks to years. Longer ripening creates more acidic, sharper cheeses.

Some cheeses are inoculated with other bacteria or fungi that give characteristics particular to the kind of cheese. The bacterium *Propionibacterium shermanii* ripens Swiss cheese and gives it the characteristic holes and a nutty flavour. This bacterium ferments organic compounds to produce propionic acid and CO_2. The CO_2 gas causes the holes in the cheese, while the propionic acid gives the typical flavour. Propionic acid also inhibits spoilage organisms.

Processing of cheese from Skim Milk

To get cottage cheese, the skimmed milk is;

- Pasteurized, cooled to 70°F (22.2°C), starter added, and incubated for 12 h at pH 4.7.
- Firm curd set, cut in cubes, and cooked at 125°F (51.7°C) for 50 min or more.
- Whey drained off, stirred to remove more to get dry curd.
- Salted, creamed, and preservative added.
- Packaged and refrigerated.

3.3.4 Coffee

Coffee is made from several species of *Coffea*, including *Coffea arabica*, *C. robusta*, and *C. canephora*, which are grown in South America, Central America, Hawaii, Ethiopia, and India. Coffee cherries, which are much smaller than cocoa pods, contain coffee beans surrounded by fleshy mucilage. The mucilage must be removed to liberate the beans. This can be done mechanically, by enzymes, by dehydration, or by fermentation.

Fermentation plays a much less critical role in coffee production as it contributes very little to the bean flavor or quality than in cocoa production. It is basically important to liberate the beans. When the beans are fermented, they are first mechanically depulped. The beans, covered with residual mucilage, are submerged in tanks of water. Native yeast, molds, LAB, and gram-negative bacteria ferment the mucilage to water-soluble products that can then be washed away. Since the main component of mucilage is pectin, the fermentation is dominated by a pectinolytic population. During the fermentation, which takes 12 to 60 h, the pH of the beans drops to 3.7 from the pH of 5.4 to 6.4 characteristic of the native bean. The beans are then subjected to 10 to 25 days of sun drying characterized by an ill-defined microbial succession. The beans can then be roasted and ground.

3.3.5 Fermentation of Vinegar

Vinegar is made by oxidizing ethanol to acetic acid. The process is of Vinegar fermentation is as ancient as that of wine. It follows the discovery of wine, since vinegar is made from wine or other alcoholic substrates. It was accidentally discovered when *Gluconobacter* or *Acetobacter* spp. contaminated wine and turned it to vinegar. The process occurs in a two-step bioprocess. First, alcoholic fermentation by yeast to produce alcohol. Ethanol from wine or hard cider is mostly used, but vinegar can be made by the fermentation of almost any fruit or starchy material. After the production of alcohol, the ethanol content of the first bioprocess is oxidized to acetic acid. *Gluconobacter oxydans* used in making vinegar is a strict aerobe, therefore the ethanol is oxidized to acetic acid, and oxygen transfer is the rate-limiting step in the process. Earlier processors of vinegar simply left wine in wooden barrels exposed to the air, inoculated it with *Gluconobacter*, and waited. The wine became vinegar after several weeks, or months. With technological dvncemeent,The trickling fermentor is a large wooden box with slatted sides to allow oxygenation. The box is filled with wood shavings, on which the *Gluconobacter* grows as biofilms. A rotating sprayer at the top of the chamber sprays the alcoholic liquid on the top of the chips, and it trickles down over the immobilized *Gluconobacter*, being converted to acetic acid in the process. The large surface area of the bacteria on the wood shavings exposed to oxygen makes this method very efficient. Its demise occurred not due to technical limitations but because the craftsmen who could build trickling fermentors retired or died. The trickling fermentor is probably the first bioreactor using immobilized-cell technology. The most modern method of vinegar production is the submerged culture reactor (Fig. 3.1). By pumping oxygen through these very large fermentors, at rates equal to the volume of the fermentor, large quantities of vinegar can be made in a very short time.

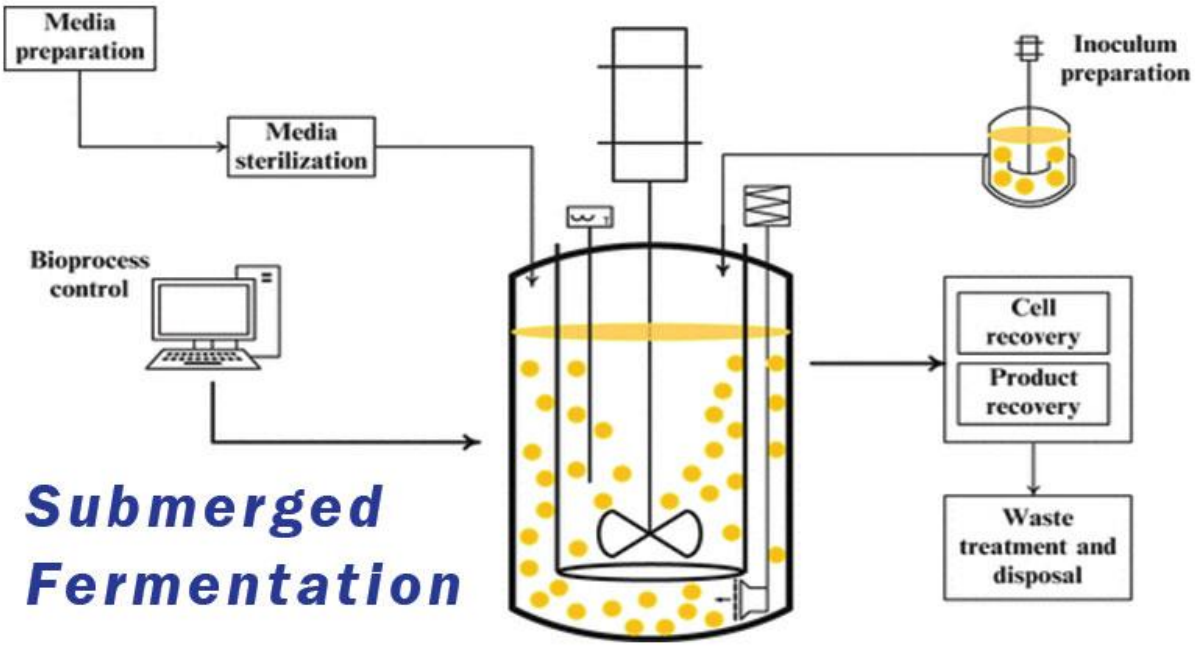

Figure 3.1: Submerged Culture Reactor

Self- Assessment Exercise

1. What are the functions of CO_2 and Propionic acid in cheese?
2. State the three ways of fermenting food

3.4 Summary

. Food fermentations are bioprocesses that change food properties while the bacteria generate energy in the absence of oxygen. These changes go far beyond acid production. Fermentations add value to foods by producing flavour compounds and carbonation, altering texture, and increasing nutrient bioavailability.

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3.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercise

3. The CO_2 gas causes the holes in the cheese, while the propionic acid gives the typical flavour. Propionic acid also inhibits spoilage organisms.
4. Foods can be fermented in the following three different ways:

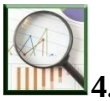
    i. Natural fermentation

    ii. Back slopping

    iii. Controlled fermentation

UNIT 4 MICROBIOLOGY OF FERMENTED MILK PRODUCTS (ACIDOPHILUS MILK, YOGHURT) AND PROBIOTICS

Content



4.1 Introduction

Fermented dairy products can be broadly divided into two groups: fermented milk products and cheeses. In fermented milk products, all the constituents of the milk are retained in the final products with the exception of those partially metabolized by the bacteria. There are many types of fermented milk products produced in different parts of the world. A few are produced by controlled fermentation, and the microbial types and their respective contributions are known. In many others, fermented either naturally or by back slopping, the microbial profiles and their contributions are not exactly known. Many types of lactic acid bacteria and some yeasts are found to predominate microbial flora in these products.

4.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss the microorganisms involved in fermentation processes
- Discuss the fermentation process
- Discuss the microbiology of cottage cheese

4.3 Milk Composition and Quality

The growth of desirable microorganisms and the quality of a fermented dairy product are influenced by the composition and quality of the milk used in a fermentation process. Cow's milk contains approximately 3.2% protein, 4.8% lactose, 3.9% lipids, 0.9% minerals, traces of vitamins, and approximately 87.2% water. Among the proteins, casein in colloidal suspension as calcium caseinate is present in higher amounts than the other two soluble proteins, albumin and globulin. Lactose is the main carbohydrate and is present in solution, and lipids are dispersed as globules of different sizes in emulsion (fat in water). Minerals are present in solution and as colloid with casein. Water-soluble vitamins are present in the aqueous phase, whereas fat-soluble vitamins are present with the lipids. The solid components (ca. 12.8%) are designated as total solids (TS), and TS without lipids are designated as solid-not-fat (SNF; ca. 8.9%). The whey contains principally the water-soluble components, some fat, and water.

The growth of desirable microorganisms can be adversely affected by several components that are either naturally present or have entered in the milk as contaminants. The natural antimicrobials are agglutinins and the lactoperoxidase-isothiocynate system. The agglutinins can induce clumping of starter-culture cells and slow their growth and metabolism. The lactoperoxidase-isothiocynate system can inhibit starter cultures. Antimicrobials can cause

problems only when raw milk is used because both are destroyed by heating milk. Milk can also contain antibiotics, either used in the feed or used to treat animals for some infections, such as mastitis. Their presence can also affect the growth of starter cultures. Some milk can contain heat-stable proteases and lipases produced by some psychrotropic bacteria, such as *Pseudomonas* species, during refrigerated storage of raw milk before pasteurization.

Acidophilus Milk

Traditional acidophilus milk is the product of fermentation by *Lactobacillus acidophilus.* The more readily available sweet acidophilus milk retains the flavor of fresh milk because it is not fermented. Instead, a culture of *L. acidophilus* is added immediately before packaging. The bacteria are only included for their possible health benefits. Some evidence suggests they may aid in the digestion of lactose as well as prevent and reduce the severity of some diarrheal illnesses, but the role they play in the complex interactions of the human intestinal tract is not clear. Unlike most lactic acid bacteria used as starter cultures, *L. acidophilus* can potentially colonize the intestinal tract.

4.3.1 Microbiology of Yogurt Fermentation

Yogurt has traditionally been made using *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *Lactobacillus acidophilus* and *Bifidobacterium* spp. They are often added due to their popularity as probiotics. The first step in yogurt manufacture is to concentrate the milk by 25% using a vacuum dehydrator. Milk solids (5%, wt/wt) are then added and the mixture is heated to 90°C for 30 to 90 min. After the mixture is cooled to 45°C, the starter culture is added at 2% (vol/vol) and the mixture is incubated for 3 to 5 h. The final product has a titratable acidity of 0.8 to 0.9% and about 109 organisms/g. These may die off during cold storage to a population of 106 organisms/g, the minimum required to make a “live and active culture” claim for the yogurt.

Yogurt has a semisolid mass resulting from the coagulation of milk (skim, low, or full fat) by starter-culture bacteria. It has a sharp acidic taste with a flavor similar to walnuts and a smooth mouthfeel. The flavor is a result of the combined effects of acetaldehyde, lactate, diacetyl, and acetate, but 90% of the flavor is a result of the acetaldehyde.

It is generally fermented in batches, but a continuous method has also been developed. The batch process for a low-fat (2%) plain yogurt is as follows:

- Homogenized milk (12% TS) + stabilizer (1%). The stabilizer is added to give desired gel structure.
- Heated to 185°F (85°C) for 30 min, and cooled to 110°F (43.3°C). Heating helps destroy vegetative microbes and slightly destabilize casein for good gel formation.
- Starter added, incubated at 110°F (43.3°C) to pH 4.8 for ca. 6 h, acidity ca. 0.9%. Starter used as either direct vat set (frozen) or bulk culture (2%–3%).
- Quickly cooled to 85°F (29.4°C) in ca. 30 min to slow down further starter growth and acid production, especially by *Lactobacillus* species, agitated, and pumped to filler machine.
- Packaged in containers and cooled by forced air to 40°F (4.4°C). Final cooling by forced air results in a rapid drop in temperature to stop the growth of starters.
- Held for 24 h; pH drops to 4.3.

L. delbrueckii ssp. *Bulgaricus* and *Str. thermophilus* are used in yoghurt processing. Some processors also combine these two with other species, such as *L. acidophilus* and *Bifidobacterium* spp., *Lab. rhamnosus*, or *Lab. casei*. However, in general, they do not compete well in growth with the two yogurt starters. Therefore, they are added in high numbers after fermentation and before packaging. They may not survive well when present in yogurt with the

regular yogurt starter cultures. For a good product, the two starter species should be added at a *Streptococcus*:*Lactobacillus* cell ratio of 1:1; in the final product, the ratio should not exceed 3:2. However, *Lactobacillus* cells are more susceptible to freezing and freeze-drying.

The fermentation is conducted at approximately 110°F (43.3°C). At this temperature, both acid and flavor compounds are produced at the desired level. If the temperature is raised above 110°F, the *Lactobacillus* sp. predominates, causing more acid and less flavor production; at temperatures below 110°F, growth of *Streptococcus* spp. is favoured, forming a product containing less acid and more flavour. The two species show symbiotic growth while growing together in milk. Initially, *Streptococcus* sp. grows rapidly in the presence of dissolved oxygen and produces formic acid and CO_2. The anaerobic condition, formic acid, and CO_2 stimulate growth of *Lactobacillus* sp., which has good exoproteinase and peptidase systems and produces peptides and amino acids from milk proteins (outside the cells) in the milk. Some of the amino acids, such as glycine, valine, histidine, leucine, and methionine, are necessary for good growth of the *Streptococcus* sp., which lacks proteinase enzymes. *Streptococcus* sp. gets these from the milk and grows rapidly until the pH drops to approximately 5.5, at which time the growth of *Streptococcus* sp. slows down. However, growth of *Lactobacillus* sp. continues fairly rapidly until the temperature is reduced to 85°F (29.4 29.4°C), following a drop in pH to 4.8. At 85°F, both grow slowly, but *Streptococcus* sp. has the edge. At 40°F (4.4°C) and a pH of approximately 4.3, both species stop growing.

The two species also have a synergistic effect on growth rate, rate of acid production, and amounts of acetaldehyde formation when growing together as compared with when growing individually. The species growing separately in milk produce approximately 8–10 ppm

acetaldehyde; when grown together, acetaldehyde production increases to a desirable level of 25 ppm or higher.

A low concentration gives a chalky and sour flavor, and too much acetaldehyde can give a green flavor. Similarly, too much diacetyl gives a buttery aroma. Too much acid production during storage causes a sour taste. Proteolysis and accumulation of bitter peptides during storage are associated with bitter flavor. Production of exopolysaccharides by the starter can give a viscous and ropy texture (which can be desirable in some situations). Growth of yeasts during storage can also produce a fruity flavour, especially in yogurt containing fruits and nuts. In coloured, flavoured, and blended yogurt, many of these problems are masked. During long storage, molds can grow on the surface. These are the microbial problems associated with yoghurt production.

4.3.2 Probiotics

Enormous interest has been generated in understanding the role of the intestinal microbial community in brain development, psychological well-being, infectious diseases, inflammatory bowel diseases, and chronic metabolic disorders, such as obesity and diabetes in recent years. A diverse colonization of the microbial community in the gut early in life promotes a balanced immune response and prevents allergic diseases, obesity, and diabetes. Gut microbiota are responsible for insulin resistance, increased adsorption of triglycerides, and chronic low-grade inflammation and show a propensity for development of diabetes.

The belief in the health benefits of fermented foods continued throughout civilization and, even today, remains of interest among many consumers and researchers. Since the discovery of food fermentation, the process has yielded products that had not only better shelf life and desirable qualities but also some health benefits, especially to combat some intestinal ailments. Probiotics is one of those products.

The word “probiotic” is derived from the Greek word meaning “for life.” Probiotics are defined as live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host. Studies have been conducted to determine specific health benefits from the consumption of live cells of beneficial bacteria. Live cells have been consumed from three principal sources:

- From fermented milk products, such as yogurt, which contains live cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* and is supplemented with *Lab. acidophilus* and others, and pasteurized milk, which contains *Lactobacillus acidophilus*;
- As supplementation of foods and drinks with live cells of one, two, or more types of probiotics, such as *Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Lactobacillus casei*, and *Bifidobacterium* species; and
- As pharmaceutical products of live cells of a monoculture or a mixture in the form of tablets, capsules, granules, and freeze-dried sachets. VSL #3 is a commercial product which four species of *Lactobacillus* (*casei*, *plantarum*, *acidophilus*, and *delbrueckii* subsp. *bulgaricus*), three species of *Bifidobacterium* (*longum*, *breve*, and *infantis*), and one strain of *Streptococcus salivarius* subsp. *thermophilus*.

Probiotic preparations consisting of multiple species or strains are functionally superior because of their synergistic effects, and if one culture fails, others can compensate, which is not possible for probiotics of a monoculture. The beneficial effects from consuming these live cells were attributed to their ability to provide protection against enteric pathogens, supply enzymes to help metabolize some food nutrients (such as lactase to hydrolyze lactose), detoxify some harmful food components and metabolites in the intestine, stimulate intestinal immune systems, improve

intestinal peristaltic activity, reduce tumerogenesis (colorectal cancer), and ameliorate chronic disorders, such as ulcerative colitis (UC) and inflammatory bowel disease (IBD).

Several microorganisms have been studied for their beneficial attributes, and the list continues to grow. There have been a few reports indicating the involvement of certain strains of probiotics, such as *Bacillus subtilis*, *Lactobacillus rhamonosus*, and *Saccharomyces cerevisiae* (Boulardii) in causing infections (bacteremia, endocarditis, and septic shock) in humans with underlying conditions. Lactic acid bacteria, especially those used in food fermentation and as probiotics, are considered food grade and have been given the GRAS (generally regarded as safe) status internationally. Although there re issues of safety, which raises concerns of infection, these can be eliminated, in our food chain if the identity of the organisms and their safety is known. There are three possible reasons for the infections that result:

- The true identity of a strain is not known. Many strains currently used, especially as probiotics, have not been correctly identified to the species level, have not been tested for their beneficial properties to humans, or their sources have not been identified. The products may be contaminated with pathogens because they have been produced under unsanitary conditions.
- Being overly anxious for the benefit, an individual may consume a product in large volume. If the individual is immunocompromised and has underlying conditions, the large dose can cause opportunistic infection.
- Many isolates from infections have not been identified correctly to the genus and species levels by modern genetic techniques. By biochemical fermentation pattern only, it is difficult to identify the genus and species of an isolate in many situations.

The incidence of health hazard from beneficial bacteria, even with all the abuses, is very low. If we consider other beneficial attributes, such as the use of antibiotics in the treatment of diseases, the incidence of health risk is much higher. Considering this, it is justifiable to assume that true food-grade lactic acid bacteria are safe. Bioengineered probiotics carrying foreign genes also raise several concerns about their safety, most importantly, the consequences of prolonged consumption of probiotics that are carrying adhesion and invasion genes of pathogens. It is also not known whether individuals with suppressed immunity will respond differently than healthy persons.

Self- Assessment Exercises 1

1. List the organisms used for the traditional production of yogurght.
2. State the beneficial effects of Probiotics

4.4 Summary

The growth of desirable microorganisms can be adversely affected by several components that are either naturally present or have entered in the milk as contaminants. The natural antimicrobials are agglutinins and the lactoperoxidase-isothiocynate system. The agglutinins can induce clumping of starter-culture cells and slow their growth and metabolism. Antimicrobials can cause problems only when raw milk is used because both are destroyed by heating milk. Milk can also contain antibiotics, either used in the feed or used to treat animals for some infections, such as mastitis. Some milk can contain heat-stable proteases and lipases produced by some psychrotropic bacteria, such as *Pseudomonas* species, during refrigerated storage of raw milk before pasteurization. Probiotics (live microorganisms) when administered in adequate amounts confer a health benefit on the host.

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2.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercise

1. Yogurt has traditionally been made using *Streptococcus thermophiles, Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium* spp.
2. The beneficial effects of probiotics are attributed to their ability to provide protection against enteric pathogens, supply enzymes to help metabolize some food nutrients

(such as lactase to hydrolyze lactose), detoxify some harmful food components and metabolites in the intestine, stimulate intestinal immune systems, improve intestinal peristaltic activity, reduce tumerogenesis (colorectal cancer), and ameliorate chronic disorders, such as ulcerative colitis (UC) and inflammatory bowel disease (IBD).

UNIT 5 APPLICATION OF MICROBIAL ENZYMES IN FOOD INDUSTRIES

Content

5.1 Introduction

Many enzymes are used in the processing of food as food additives. The Use of specific enzymes instead of microorganisms has several advantages. A specific substrate can be converted into a specific product by an enzyme through a single-step reaction. Thus, production of different metabolites by live cells from the same substrate can be avoided. In addition, a reaction step can be controlled and enhanced more easily by using purified enzymes. Finally, by using recombinant DNA technology, the efficiency of enzymes can be improved and, by immobilizing, they can be recycled. The main disadvantage of using enzymes is that if a substrate is converted to a product through many steps (such as glucose to lactic acid), microbial cells must be used for their efficient and economical production.

Among the five classes of enzymes, three are predominantly used in food processing: Hydrolases

Isomerases, and Oxidoreductases.

α-Amylase, Glucoamylase, and Glucose Isomerase

Together, these three enzymes are used to produce high-fructose corn syrup from starch. α-Amylase hydrolyzes starch at α-1 position randomly and produces oligosaccharides (containing three hexose units or more, for example, dextrins). Glucoamylase hydrolyzes dextrins to glucose units, which are then converted to fructose by glucose isomerase. α-Amylase is also used in bread making to slow down staling (starch crystallization resulting from loss of water). Partial hydrolysis of starch by α-amylase can help reduce the water loss and extend the shelf life of bread.

 **5.2 Learning Outcomes**

By the end of this unit, you will be able to:

- Discuss the functions of microbial enzymes in food
- Discuss the Flavour Compounds and Flavour Enhancers
- Analyse Single cell proteins

 **5.3.1 Microbial enzymes in food**

Catalase

Raw milk and liquid eggs can be preserved with H_2O_2 before pasteurization. However, the H_2O_2 needs to be hydrolyzed by adding catalase before heat processing of the products.

Cellulase, Hemicellulase, and Pectinase

Because of their ability to hydrolyze respective substrates, the use of these enzymes in citrus juice extraction has increased juice yield. Normally, these insoluble polysaccharides trap juice during pressing. Also, they get into the juice and increase viscosity, causing problems during

juice concentration. They also cloud the juice. By using these hydrolyzing enzymes, such problems can be reduced.

Invertase

Invertase can be used to hydrolyze sucrose to invert sugars (mixture of glucose and fructose) and increase sweetness. It is used in chocolate processing.

Lactase

Whey contains high amounts of lactose. Lactose can be concentrated from whey and treated with lactase to produce glucose and galactose. It can then be used to produce alcohol.

Lipases

Lipases are produced by filamentous fungi, and commercially important lipase-producing fungi include *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp., *Mucor* sp., and *Rhizomucor* sp. Lipases may be used to accelerate cheese flavor along with some proteases.

Proteases

Different proteases are used in the processing of many foods. They are used to tenderize meat, extract fish proteins, separate and hydrolyze casein in cheese making (rennet), concentrate cheese flavor (ripening), and reduce bitter peptides in cheese (specific peptidases.

Immobilized Enzymes

Enzymes are biocatalysts and can be recycled. An enzyme is used only once when added to a substrate in liquid or solid food. In contrast, if the molecules of an enzyme are attached to a solid surface (immobilized), the enzyme can be exposed repeatedly to a specific substrate. The major advantage is the economical use of an enzyme, especially if the enzyme is very costly. Enzymes can be immobilized by several physical, chemical, or mechanical means. The techniques can be divided into four major categories. Some of the cell components, metabolic end products, and

enzymes produced by food-grade and regulatory agency–approved microorganisms that are used in foods as additives to improve the nutritional and acceptance qualities of foods. Recent advances in genetic engineering and metabolic engineering of these bacteria have helped develop strains that can produce many unique products.

These enzymes like other microbial metabolites can be used as food additives to improve nutritional value, flavour, colouur, and texture. Some of these include proteins, essential amino acids, vitamins, aroma compounds, flavor enhancers, salty peptides, peptide sweeteners, colors, stabilizers, and organic acids. Because they are used as ingredients, they need not come only from microorganisms used to produce fermented foods but can be produced by many other types of microorganisms with regulatory approval for safety before use in foods. Many enzymes from bacteria, yeasts, molds, as well as from plant and mammalian sources, are currently used for the processing of foods and food ingredients. Some examples are production of high-fructose corn syrups, extraction of juice from fruits and vegetables, and enhancement of flavor in cheese.

Recombinant DNA technology (or biotechnology) has opened up the possibilities of identifying and isolating genes or synthesizing genes encoding a desirable trait from plant and animal sources or, from microorganisms that are difficult to grow normally, clone it in a suitable vector (DNA carrier) and incorporate the recombinant DNA in a suitable microbial host that will express the trait and produce the specific additive or enzyme economically. In addition, metabolic engineering, by which a desirable metabolite can be produced in large amounts by a bacterial strain, is being used to produce food additives from new sources. The metabolites can then be purified and used as food additives and in food processing, provided they are a safe-to use, generally regarded as safe.

5.3.2 Amino Acids

Proteins of most cereal grains are deficient in one or more of the essential amino acids, particularly methionine, lysine, and tryptophan. To improve the biological values, cereals are supplemented with essential amino acids. Supplementing vegetable proteins with essential amino acids has been suggested to improve the protein quality for people who either do not consume animal proteins (people on vegetarian diets) or do not have enough animal proteins (such as in some developing countries, especially important for children). To meet this demand, as well as for use as nutrient supplements, large amounts of several essential acids are being produced. At present, because of economic reasons, they are mostly produced from the hydrolysis of animal proteins followed by purification.

In recent years, bacterial strains have been isolated, some of which are lactic acid bacteria that produce and excrete large amounts of lysine in the environment. Isolating high-producing strains of other amino acids and developing strains by genetic and metabolic engineering that will produce these amino acids in large amounts can be important for economical production of essential amino acids.

5.3.3 Single-Cell Proteins (SCPs)

Molds, yeasts, bacteria, and algae are rich in proteins, and the digestibility of these proteins ranges from 65% to 96%. Proteins from yeasts, in general, have high digestibility as well as biological value. In commercial production, yeasts are preferred. Some of the species used are from genera *Candida*, *Saccharomyces*, and *Torulopsis*. Some bacterial species have been used, especially from genus *Methylophilus*.

The use of microbial proteins as food has several advantages over animal proteins. There may not be enough animal proteins to feed the growing human population in the future, especially in

many developing countries. Also, microbial proteins can be produced under laboratory settings. Thus, land shortage and environmental calamities (such as drought or flood) can be overcome. They can be produced on many agricultural and industrial wastes. This will help alleviate waste disposal problems and also reduce the cost of production. Microbial proteins can be a good source of B vitamins, carotene, and carbohydrates.

There are some disadvantages of using microbial proteins as human food. They are poor in some essential amino acids, such as methionine. However, this can be corrected by supplementing microbial proteins with the needed essential amino acids. The other problem is that proteins from microbial sources can have high-nucleic acid content (RNA and DNA; 6%–8%), which, in the human body, is metabolized to uric acid. A high serum-acid level can lead to kidney stone formation and gout. However, through genetic manipulations, the nucleic acid content in microbial proteins has been reduced.

Even though, at present, the use of microbial proteins as a protein source in human food is limited, they are being used as a protein source in animal feed. An increase of microbial proteins will automatically reduce the use of grains (such as corn and wheat) as animal feed, which then can be used as human food.

5.3.4 Flavour Compounds and Flavour Enhancers

Flavour compounds and enhancers include those that are associated directly with the desirable aroma and taste of foods and indirectly with the strengthening of some flavors. Many microorganisms produce different types of flavor compounds, such as diacetyl (butter flavor by *Leuconostoc*), acetaldehyde (yogurt flavor by *Lactobacillus acidophilus*), some nitrogenous and sulfur-containing compounds (sharp cheese flavor by *Lactococcus lactis*), propionic acid (nutty flavor by dairy *Propionibacterium*), pyrazines (roasted nutty flavors by strains of *Bacillus*

subtilis and *Lac. lactis*), and terpenes (fruity or flowery flavors by some yeasts and molds). Some natural flavors from plant sources are very costly because only limited amounts are available and the extraction process is very elaborate. By employing biotechnology, they can be produced economically by suitable microorganisms. Natural vanilla flavor (now obtained from plants), if produced by microorganisms, may cut the cost to only a 10th or less. Natural fruit flavors are extracted from fruits. Not only is it costly, but also large amounts of fruits are wasted. The possible production of many of these flavors by microorganisms through recombinant DNA technology is being studied.

Several flavour enhancers are now used to strengthen the basic flavours of foods. Monosodium glutamate (MSG, enhances meat flavor) is produced by several bacterial species, such as *Corynebacterium glutamicum* and *Micrococcus glutamicus*. Also, 5′ nucleotides, such as inosine monophosphate and guanosine monophosphate, give an illusion of greater viscosity and mouthfeel in foods such as soups. They can be produced from *Bac. subtilis*.

Several small peptides, such as lysylglycine, have strong salty tastes. They can be produced by recombinant DNA technology by microorganisms and used to replace NaCl. Sweet peptides, such as monellin and thaumatin from plant sources, can also be produced by microorganisms through gene cloning. At present, the dipeptide sweetener aspartame is produced synthetically, but a method to produce it by microorganisms has been developed. By metabolic engineering, strains of lactic acid bacteria have been developed that can produce large quantities of diacetyl (for the aroma of butter), acetaldehyde (for the aroma of yogurt), α-ketoglutarate (to produce cheese flavor), and other compounds.

Colours

Many bacteria, yeasts, and molds produce different colour pigments. The possibility of using some of them, especially from those that are currently consumed by humans, is being studied. Some of the common fermentative food-grade pigments include yellow, red, and orange pigments from *Monascus* sp. (fungus), astaxanthin (pink-red) from *Xanthophyllomyces dendrorhous* (yeast), arpink red from *Penicillium oxalicum* (fungus), riboflavin from *Ashbya gossypii* (fungus), β-carotene from *Blakeslea trispora* (fungus). The pink-red color pigment astaxanthin gives the red color to salmon, trout, lobster, and crabs. Another red pigment, produced by *Monascus* sp., has been used for a long time in the Orient to make red rice wine. Because pigment production may involve multistep reactions, recombinant DNA techniques to produce some fruit colors by microorganisms may not be economical. However, they can be produced by the plant cell culture technique.

5.3.5 Nutraceuticals and Vitamins

Many vitamins are added to foods and also used regularly by many as supplements. Thus, there is a large market for vitamins, especially some B vitamins and vitamins C, D, and E. Some of these are obtained from plant sources, several are synthesized, and a few are produced by microorganisms. Vitamin C is now produced by yeast by using cheese whey. Microorganisms have also been a source of vitamin D. Many are capable of producing B vitamins.

The possibility of using gene-cloning techniques to improve production of vitamins by microorganisms may not now be very practical or economical. Vitamins are produced through multienzyme systems, and it may not be possible to clone the necessary genes. In recent years, through metabolic engineering, strains of lactic acid bacteria have been developed that when used in dairy fermentation produce high amounts of folate and some cyanocobalamin (B12) in

the fermented products, thereby increasing the nutritional value of the products. In addition, strains of lactic acid bacteria have been developed that produce low-calorie sweeteners, such as mannitol, sorbitol, and tagatose.

Self- Assessment Exercise

1. List the three predominant classes of enzyme used in food processing
2. State the uses of microbial ezymes

5.4 Summary

Microorganisms produce enzymes and many enzymes are used in the processing of food as food additives with several advantages. A specific substrate can be converted into a specific product by an enzyme through a single-step reaction. The production of different metabolites by live cells from the same substrate can be avoided. Also, a reaction step can be controlled and enhanced more easily by using purified enzymes. Microorganisms have also been a source of vitamin D and many are capable of producing B vitamins.

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5.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercise

1. Among the five classes of enzymes, three are predominantly used in food processing: Hydrolases, Isomerases, and Oxidoreductases.
2. These enzymes like other microbial metabolites can be used as food additives to improve nutritional value, flavour, colouur, and texture. Some of these include proteins, essential amino acids, vitamins, aroma compounds, flavor enhancers, salty peptides, peptide sweeteners, colors, stabilizers, and organic acids.

UNIT 6 GENETICALLY MODIFIED FOODS (FOODS AND GENETIC ENGINEERING)

Content

6.1 Introduction

Genetically modified (GM) foods are foods whose genetic makeup has been altered “in a way that does not occur spontaneously. The process of genome manipulation involves the translocation of genes from multiple genetic sources, in a process widely known as recombinant deoxyribonucleic acid (rDNA) technology. Three basic rDNA techniques include transformation, phage introduction, and nonbacterial transformation. Transformation involves enzymatically excising a desired fragment of DNA, inserting it into a vector vehicle, and implanting the vector into a host cell. There is also nonbacterial transformation, where the DNA vector is inserted directly into the nucleus of a cell, instead of a bacterial host cell. Phage induction, incorporates a bacteriophage (that is, *virus*) in place of a bacterial cell, with the same principles as transformation. Using these techniques, rDNA can be used to directly incorporate extraneous genetic material into the food matrix. Furthermore, insertion of rDNA into plant cells for industrial genetic modification primarily includes 2 prominent methods, which are the gene gun method and *Agrobacterium* method. The gene gun method involves bombarding target plant

cells using gene-coated particles of gold or tungsten. Desired rDNA strands are coated on the entire surface of either gold or tungsten micromolecules, which are then propelled towards a plant cell using a vacuum chamber for random insertion into cells. However, the more common of the 2 methods is the use of *Agrobacterium tumefaciens*, a bacterium that parasitizes plants by inserting its DNA plasmid into cells to initiate host colonization. This process removes the DNA sequence that controls metabolism and replaces it with the bacterial rDNA strand. Using these two methods, scientists are able to implement rDNA technology for a myriad of industrial applications.

6.2 Learning Outcomes

By the end of this unit, you will be able to:

- Discuss genetically modified foods
- Discuss the importance of GMF

6.3 Genetically Modified foods

Genetically modified (GM) foods are foods whose genetic makeup has been altered “in a way that does not occur spontaneously. The process of genome manipulation involves the translocation of genes from multiple genetic sources, in a process widely known as recombinant deoxyribonucleic acid (rDNA) technology. Three basic rDNA techniques include transformation, phage introduction, and nonbacterial transformation. Transformation involves enzymatically excising a desired fragment of DNA, inserting it into a vector vehicle, and implanting the vector into a host cell. There is also nonbacterial transformation, where the DNA vector is inserted directly into the nucleus of a cell, instead of a bacterial host cell. Phage induction, incorporates a bacteriophage (that is, *virus*) in place of a bacterial cell, with the same principles as

transformation. Using these techniques, rDNA can be used to directly incorporate extraneous genetic material into the food matrix. Furthermore, insertion of rDNA into plant cells for industrial genetic modification primarily includes 2 prominent methods, which are the gene gun method and *Agrobacterium* method. The gene gun method involves bombarding target plant cells using gene-coated particles of gold or tungsten. Desired rDNA strands are coated on the entire surface of either gold or tungsten micromolecules, which are then propelled towards a plant cell using a vacuum chamber for random insertion into cells. However, the more common of the 2 methods is the use of *Agrobacterium tumefaciens*, a bacterium that parasitizes plants by inserting its DNA plasmid into cells to initiate host colonization. This process removes the DNA sequence that controls metabolism and replaces it with the bacterial rDNA strand. Using these two methods, scientists are able to implement rDNA technology for a myriad of industrial applications.

Important applications of the genetic modification are the delayed or quickened ripening of fruits, herbicide tolerance and insect resistance micronutrient enrichment, and pathogen resistance to bacteria, fungi and viruses. The advantages of genetically modified foods are very widespread, encompassing a variety of aspects of increased food production and health benefits, and are becoming increasingly more prevalent.

With the world's population increasing at an alarming rate, especially in developing countries, there is a major threat posed to food security. Therefore, the magnitude of the introduction of GM crops may have a huge positive impact as it pertains to the ethical guiding principle of justice where a fair, equitable food supply is maintained. Climate change is also another environmental factor threatening food security, which may lead to malnutrition and other health problems due to the lack of food. Both the increasing population and changing climate poses the

ethical dilemma of maintaining stewardship and utilizing available natural resources in a conscientious manner to ensure that they are available for future generations.

While GM foods offer numerous health and agricultural benefits the public outlook on the consequences of genetic pollution and the ethical notions of genetic modification have given well-known infamy to GM foods.

With the increase in GM foods, the notion of ethical eating has also surfaced. These ethical eating issues relies on the “moral consequences of food choices” and food product development. Issues of the morality of genetic modification and its industrial uses have echoed through both public and expert spaces raising many questions. Some of these questions are on the;

- Concerns surrounding the safety of GM food consumption,
- The interference of the natural evolution of organisms, and more recently,
- The potential benefits of GM foods increasing food insecurity

These rising issues is attempting to weigh the disadvantages of GM foods against the benefits, especially since GM foods have the potential to help developing nations in need of economic stimulation and food security.

Some of the advantages of GM foods

i. Better quality food
ii. Higher nutritional yields
iii. Inexpensive and nutritious food, like carrots with more antioxidants.
iv. Foods with a greater shelf life, like tomatoes that taste better and last longer.
v. Food with medicinal benefits, such as edible vaccines - for example, bananas with bacterial or rotavirus antigens.

vi. Crops and produce that require less chemical application, such as herbicide resistant canola.

Some of the disadvantages of GM foods

i. Toxicity (using similar methods to those used for conventional foods).

ii. Tendency to provoke any allergic reaction.

iii. Stability of the inserted gene.

iv. Whether there is any nutritional deficit or change in the GM food.

Health Risks Associated with GM Food Consumption

Research has indicated that animals fed by GM crops have been harmed or even died. Rats exposed to transgenic potatoes or soya had abnormal young sperm; cows, goats, buffalo, pigs and other livestock grazing on Bt-maize, GM cottonseed and certain biotech corn showed complications including early deliveries, abortions, infertility and also many died. Although Agri-biotech companies do not accept the direct link between the GMFs consumption and human health problems, there are some examples given by the opponents. For example: The foodborne diseases such as soya allergies have increased over the past 10 years in USA and UK and an epidemic of Morgellons disease in the US. There are also reports on hundreds of villagers and cotton handlers who developed skin allergy in India. Recent studies have revealed that *Bacillus thuringiensis* corn expresses an allergenic protein which alters overall immunological reactions in the body. The reports by independent GM researchers have lead to a concern about the risks of GMFs and the inherent risks associated with the genetic technology. It is therefore essential that the safety and long-term effects of GM crops should be examined before their release into the food chain by all organizations responsible to produce GMFs. In order to give the public the option of making informed decision about the consumption of GMF, enough information on the

safety tests of such product is required. Unfortunately, such data are scarce due to a number of factors. For example it is hard to compare the nutritional contents of GM crops with their conventional counterparts because the composition of crops grown in different areas might vary depending on the growth and agronomic conditions. Current testing methods being used in biotech companies appear to be inadequate. For instance, only chemical analysis of some nutrients are reported and generally consider the GM crops equal to its conventional crops when no major differences are detected between the compound compositions in both products. Such approach is argued to guarantee that the GM crop is safe enough to be patented and commercially produced. It is strongly believed that animal trials should be used to evaluate the probable toxic effects of genetically modified foods. Herbicide and glyphosphate resistant soybeans as well as GM cotton resistant to insects are claimed to be substantially equal to conventional soybeans or cotton. Another example are from the results of toxicological studies conducted on a variety of animals fed with glyphosate-resistant soybean (GTS) which were shown to be similar for GTS fed and control group. Also, there are some false claims on the improvement of the protein content of GM crops expressing the desired protein from an inserted gene. For example, studies on GM potato and containing soybean glycine gene did not show considerable increase in the protein content or even amino acid profile and as for GM rice the rise in protein content was due to the decline in moisture rather than the increase in protein content. Also, there are some difficulties with assessing the allergenicity of GM crops. When the gene causing allergenicity is known, such as the gene for the alpha-amylase trypsin inhibitors, or cod proteins, it is easier to recognize whether the GMF is allergenic by using in vitro tests. Of course to test the stability of GMF products in the digestive systems, human/animal trials are required and data bank studies are effective. Since insertion of a non-allergenic gene might cause

over expression of already existing minor allergen, it is difficult to specifically identify whether a new GM crop with a gene transferred from a source with unknown allergenicity is allergenic before its introduction to the food chain.

Self- Assessment Exercise

1. State the disadvantages of GMF
2. What is a GMF?

6.4 Summary

With the world's population increasing at an alarming rate, especially in developing countries, there is a major threat posed to food security. Therefore, the magnitude of the introduction of GM crops may have a huge positive impact as it pertains to the ethical guiding principle of justice where a fair, equitable food supply is maintained.

6.5 Glossary

Hazard: Unacceptable contamination, microbial growth, persistence of toxins or survival of microorganisms that are of a concern to food safety

Monitoring: Checking to determine if the criteria established by the critical control point(s) (CCP) have been achieved

Moulds: filamentous fungi which are important group of microflora of fruits and fruit products.

Processing: changing plants or animals into what we recognize and buy as food.

Risk: Probability that a condition(s) will lead to a hazard

Sanitation: the maintenance of hygienic conditions, through services such as garbage collection and waste disposal.

Severity: Seriousness of the consequences of the results of a hazard

Sewage: all the material that flows from household plumbing systems, including washing and bathing water and toilet wastes as well as others from business and industrial wastes.

Spoilage: any visible or invisible change which can makes food or product derived from food unacceptable for human consumption.

Viruses: intracellular obligate parasite which can trigger dangerous infections in humans when they contaminate our food.

Yeasts: unicellular fungi which normally reproduce by budding.

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6.6 Possible Answers to Self-Assessment Exercises

Answers to Self-Assessment Exercise

1a. Toxicity (using similar methods to those used for conventional foods)

b. Tendency to provoke any allergic reaction.

c. Stability of the inserted gene.

d. Whether there is any nutritional deficit or change in the GM food.

2. A genetically modified food (GMF) is a food whose genetic makeup has been altered in a way that does not occur spontaneously. The process of genome manipulation involves the translocation of genes from multiple genetic sources, in a process widely known as recombinant deoxyribonucleic acid (rDNA) technology