
UNIT 13 MICROBIAL

Structure

- 13.0 Objectives
- 13.1 Introduction
- 13.2 Microbiological Profile of Harvested Fruits and Vegetables
 - Sources of Microorganisms on Fresh Fruits and Vegetables
 - Factors Affecting Type and Number of Microorganism on Fresh Fruits and Vegetables
 - Human Pathogens Associated with Fresh Fruits and Vegetables
- 13.3 Standards for Water for Human Consumption
 - Sources of Contaminants in Drinking Water
 - Contamination Due to Harmful Microorganisms
 - Drinking Water Standards
- 13.4 Microbiology of Canned Fruits
 - History of Canning
 - Basic Principle of Canning
 - Spoilage of Canned Products
 - Clostridium Botulinum* A Major Threat in Canned Products
- 13.5 Microbiological Standards for Processed Foods
 - Purpose of Microbiological Standards
 - Sampling
 - Microbiological Assessment
 - Categories of Food Based on Microbial Quality
- 13.6 Let Us Sum Up
- 13.7 Key Words
- 13.8 Answers to Check Your Progress Exercises
- 13.9 Some Useful Books

13.0 OBJECTIVES

After studying this unit, you should be able to explain:

- fresh fruits and vegetables;
- canned and processed food products; and
- drinking water.

13.1 INTRODUCTION

In this unit we will make you aware about the types of micro flora present on the surface of fresh fruits and vegetables, the sources of these microorganisms and the characteristics of pathogenic microorganisms. We will also brief you about quality standards for drinking water and the human pathogen present in contaminated water. Canning is an established way of food preservation. You will learn about the history of canning, spoilage of canned products with special reference to *Clostridium botulinum*. After that we will brief you about the microbial limits for processed foods.

13.2 MICROBIOLOGICAL PROFILE OF HARVESTED FRUITS AND VEGETABLES

The consumption of fresh fruits and vegetables is increasing as consumers strive to eat healthy diets. Global trade in fruits and vegetables and changing horticultural practices have enabled this year-round abundance to be possible, as well as adding new varieties of fresh produce to the market. During the last few decades pre-prepared minimally processed fruits and vegetables have become popular among the consumers. These products include pre-washed pre-cut salads items, grated vegetables, prepared fruit salads, or fruit combinations. Most of these products are generally eaten raw without further processing. Some products are packed in modified atmospheres to provide extension of shelf life both in relation to the potential acceptable quality and safety of the product.

Since minimum processing is required for fresh and fresh-cut fruits and vegetables, which omits any effective microbial elimination step, results in food products that naturally would carry microorganisms, some of which may be potentially hazardous to human health.

13.2.1 Sources of Microorganisms on Fresh Fruits and Vegetables

Fruits and vegetables can become contaminated whilst growing in fields, or during harvest, handling, processing, distribution and use. However, there are certain factors, which contribute to the microbiological contamination of these products with pathogens. Table 13.1 lists the sources of pathogenic microorganisms on fresh produce and conditions that influence their survival and growth.

Contamination can arise as a consequence of treating soil with organic fertilizers such as manure and sewage sludge and from irrigation water. Manure, bio-solids and irrigation water should be of a quality that does not introduce pathogens to the treated commodity. The potential of organic farming to contaminate fruits and vegetables with pathogens has to be investigated. Harvesting at the appropriate time and storing the harvested products under controlled conditions will help to retard growth of post-harvest spoilage and pathogenic microorganisms. Humid and warm storage conditions encourage the growth of microbial contaminants. The use of additional post-harvest procedures could reduce the contamination level of fruits and vegetables. Washing with water of potable quality can reduce the microbial load. Although a wide range of different agents are available for disinfecting/sanitizing fresh produce their efficacy is variable and none is able to ensure elimination of pathogens. Fruits and vegetables carry a natural non-pathogenic epiphytic micro flora. During growth, harvest, transportation and further processing and handling the produce can, however, be contaminated with pathogens from human or animal sources. The microbial composition of the different forms of organic fertilizer will vary depending on its origin and further treatment. The quality of the water used for irrigation and as a carrier for plant protection products, fertilizers and frost protection products has to be related to the potential risk it can cause at a later stage. Technologies for irrigation are important for the control of spreading microbiological hazards. The use of drip irrigation instead of flooding or spray irrigation should reduce waterborne contamination and aerosols. However, heavy rains and wind may provide other opportunities for the transfer of microorganisms from soil to plant surfaces.

Table 13.1: Sources of pathogenic microorganisms on fresh produce and conditions that influence their survival and growth

Pre-harvest
<ul style="list-style-type: none"> • Soil • Irrigation water • Green or inadequately composted manure • Air (dust) • Wild and domestic animals • Human handling • Water for other uses (for example, pesticides, foliar treatments, growth hormones)
Post-harvest
<ul style="list-style-type: none"> • Human handling (workers, consumers) • Harvesting equipment • Transport containers (field to packing shed) • Air (dust) • Wash and rinse water • Sorting, packing, cutting and further-processing equipment • Ice • Transport vehicles • Improper storage (temperature, physical environment) • Improper packaging (includes new packaging technologies) • Cross contamination (other foods in storage, preparation and display areas) • Improper handling after wholesale or retail purchase • Cooling water (for example, hydro cooling)

13.2.2 Factors affecting Type and Number of Microorganism on Fresh Fruits and Vegetables

Fruits and vegetables normally carry a non-pathogenic epiphytic micro flora. The majority of bacteria found on the surface of plants are usually Gram-negative and belong either to the *Pseudomonas* group or to the *Enterobacteriaceae*. Many of these organisms are normally non-pathogenic for humans. The numbers of bacteria present will vary depending on seasonal and climatic variation and may range from 10^4 to 10^8 per gram. The inner tissues of fruits and vegetables are usually regarded as sterile. However, bacteria can be present in low numbers as a result of the uptake of water through certain irrigation or washing procedures. If these waters are contaminated with human pathogens these may also be introduced. The survival or growth of contaminating microorganisms is affected by intrinsic, extrinsic and processing factors. Factors of importance are nutrient composition, pH, presence of scales and fibers, redox potential, temperature and gaseous atmosphere. Mechanical shredding, cutting and slicing of the produce open the plant surfaces to microbial attack. About two thirds of the spoilage of fruits and vegetables is caused by molds. Members of the genera *Penicillium*, *Aspergillus*, *Sclerotinia*, *Botrytis* and *Rhizopus* are commonly

involved in this process. The spoilage is usually associated with cellulolytic or pectinolytic activity, which causes softening of tissues, and weakening of plant structures. These structures are important barriers to prevent growth in the products by contaminating microbes.

13.2.3 Human Pathogens Associated with Fresh Fruits and Vegetables

However, risk profile surveys on the microbiological contamination of fruits and vegetables eaten raw demonstrates, potential for a wide range of these products to become contaminated with microorganisms, including human pathogens. The range of microorganisms associated with outbreaks linked to fresh produce encompasses bacteria, viruses and parasites. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the *Enterobacteriaceae*. Of these, *Salmonella* and *Escherichia coli* O157 are of particular concern. Outbreaks of illness caused by bacteria, viruses and parasites have been linked epidemiologically to the consumption of a wide range of vegetables and, to a lesser extent fruits. Surveillance of vegetables has indicated that these foods can be contaminated with various bacterial pathogens, including *Salmonella*, *Shigella*, *E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter*. Table 13.2 shows the characteristics of some microbial pathogens that have been linked to outbreaks of fresh fruits and vegetable associated illness.

Table 13.2: Characteristics of some microbial pathogens that have been linked to outbreaks of produce associated illness

Microorganism	Typical Incubation Period	Symptoms	Infectious Dose (Number of cells)	Source
BACTERIA				
<i>Clostridium botulinum</i>	12 to 36 h	Nausea, vomiting, fatigue, dizziness, dryness of mouth and throat, muscle paralysis, difficulty in swallowing, double or blurred vision, drooping eyelids, and breathing difficulties	Intoxication growth and toxin production in food	Soil, lakes, streams, decaying vegetation, reptiles
<i>Escherichia coli</i> O157:H7	2 to 5 d	Bloody diarrhoea, abdominal pain. Can lead to hemolytic uremic syndrome and kidney failure especially in children and the elderly	10 to 1000	Animal feces, especially cattle, deer and human; cross contamination from raw meat
<i>Salmonella</i> spp.	18 to 72 h	Abdominal pain, diarrhoea, chills, fever, nausea, vomiting	10 to 100,000	Animal and human feces; cross contamination from raw

**Safe Chemicals and
Microbial Limits for
Different Foods**

				meat, poultry, or eggs
<i>Shigella</i> spp.	1 to 3 d	Abdominal pain, diarrhoea, fever, vomiting	~10	Human feces
<i>Listeria monocytogenes</i>	1 d to 5 or more wk	Febrile gastroenteritis in healthy adults; may lead to spontaneous abortion or stillbirth in pregnant women; severe septicemia and meningitis in neonates and immunocompromised adults; mortality may be 20 to 40%	Unknown dependent upon health of individual	Soil, food processing environments
PARASITES				
<i>Cryptosporidium</i> spp.	1 to 12 d	Profuse watery diarrhoea, abdominal pain, anorexia, vomiting	~30	Animal and human feces
<i>Cyclospora</i> spp.	1 to 11 d	Watery diarrhoea, nausea, anorexia, abdominal cramps (duration 7 to 40 d)	Unknown, probably low	Others? specific environmental sources unknown at this time
VIRUSES				
Hepatitis A	25 to 30 d	Fever, malaise, anorexia, nausea, abdominal pain, jaundice, dark urine	10 to 50	Human feces and urine
Norwalk/ Norwalk-like virus	12 to 48 h	Vomiting diarrhoea, malaise, fever, nausea, abdominal cramps	Unknown, probably low	Human feces, vomitus

However, multiplication of the pathogen is also essential for causing any damage. Some microorganisms cause illness only when ingested in high numbers (for example, *Clostridium perfringens*), while in other cases, the infectious dose is thought to be dependent upon the susceptibility of the individual (most infectious agents). Illness due to *Staphylococcus aureus*, *Bacillus cereus*, or *Clostridium botulinum* is a result of the production of toxins in the food, and it is the toxins that are responsible (sometimes in the absence of viable cells) for symptoms of the disease. These toxins are only produced by multiplying cells. This requires favourable growth conditions.

Check Your Progress Exercise 1



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

1. What are the sources of microbial contamination on fresh fruits and vegetables?

.....

.....

.....

.....

.....

2. What are the factors that affect the survival and growth of microorganisms on fruits?

.....

.....

.....

.....

.....

3. Name few fungi responsible for spoilage of fresh fruits and vegetables?

.....

.....

.....

.....

.....

4. Name two bacteria associated with outbreaks linked to fresh fruits and vegetables?

.....

.....

.....

.....

.....

13.3 STANDARDS OF WATER FOR HUMAN CONSUMPTION

Clean water is one of the most important needs of our bodies. It is a sad fact that something as essential to life as clean drinking water can no longer be granted to us. According to research articles and news, most tap and well water now are not safe for drinking due to heavy industrial and environmental

pollution. We have reached to a point that, all sources of our drinking water, including municipal water systems, wells, lakes, rivers, and even glaciers, contain some level of contamination.

13.3.1 Sources of Contaminants in Drinking Water

Several contaminants occur in nature that may present a health risk if they are found in drinking water. The various pollutant /contaminants are bacteria, viruses, uranium, radium, nitrate, arsenic, chromium and fluoride. Other sources of contamination are a result of human activity such as manufacturing or agriculture, or individual misuse. The following activities may cause harmful microorganisms and chemicals to enter the well water owner's water supply.

- Leakage from waste disposal, treatment, or storage sites.
- Discharges from factories, industrial sites, or sewage treatment facilities.
- Leaching from aerial or land application of pesticides and fertilizers on yards or fields.
- Accidental chemical spills.
- Leakage from underground storage tanks.

13.3.2 Contamination due to Harmful Microorganisms

The most common and widespread health risk associated with drinking water is microbial contamination, either directly or indirectly, by human or animal excreta and micro-organisms contained in faeces.

The pathogenic agents involved include bacteria, viruses and protozoa, which may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis or typhoid fever. Most of them are widely distributed through out the world. Fecal contamination of drinking water is only one of the several faeco-oral mechanisms by which they can be transmitted from one person to another, or in some cases, from animal to people. The human pathogens potentially transmitted in drinking water are Bacteria viz. *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio cholera*, *Yersinia enterocolitica*, *Campylobacter jejuni*; viruses viz, Adenoviruses, Enterovirus, Hepatitis A, Hepatitis E, Norwalk virus, Rotavirus, and small round viruses; The parasites, *Giardia*, *Cryptosporidium*, *Entamoeba histolytica* and *Dracunculus*. The removal of these agents from drinking water should be given top priority.

Apart from the above said **pathogens of high health significance**, there are some more organisms that are present in environment and **not normally regarded as pathogen, may caue disease opportunistically**. When such organisms are present in water they cause infection predominantly among people whose local or general defence mechanisms are impaired. Those most likely to be at risk include the very old, the very young and patients in the hospitals, e.g. those with burns or immunosuppressive therapy, and those suffering from acquired immunodeficiency syndrome (AIDS). Water used by such patients for drinking or bathing, if it contains excessive number of these agents, may produce a variety of infections involving the skin and mucous membrane of the eye, ear, nose and throat. *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, *Klebsiella* and *Serratia* are examples of such opportunistic pathogens. *Legionella* infects the lung. These organisms while clearly of medical importance, acquire public health significance only under certain conditions. Their removal from drinking water may therefore be given moderate priority.

13.3.3 Drinking Water Standards

Microorganisms, including pathogenic organisms, may enter water supplies at every stage of the collection and distribution cycle. Emphasis should be placed on the need for an active watershed protection program, including an emergency plan for responding to major pollution events such as spills or contamination. Major quality requirements for drinking water are listed in Table 13.3.

Table 13.3: Drinking water standards

Micro-organism	Requirement
<i>Cryptosporidium</i>	System must remove 99% of <i>Cryptosporidium</i>
Giardia lamblia	99.9% killed
Heterotrophic Plate count (HPC)	Not more than 500 colonies per ml.
Total Coliform	Must not be detectable in any 100 ml sample. In case of large supplies where sufficient samples are examined must not be present in 95% of the samples taken through out any 12 months period.
Fecal Coliform or <i>E.coli</i>	No fecal coliform is allowed.
Turbidity	At no time can turbidity go above 5 NTU (Nephelometric turbidity unit)
Viruses	99.99% killed / inactivated

Let us know in brief about the above contaminants.

Coliform bacteria

These are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease. **Fecal Coliform and *E.Coli*** are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. Microbes in these wastes can cause short-term effects, such as diarrhoea, cramps, nausea, headaches, or other symptoms.

Turbidity has no health effects. However, turbidity can interfere with disinfection and provide a medium for microbial growth. Turbidity may indicate the presence of disease causing organisms. These organisms include bacteria, viruses, and parasites that can cause symptoms such as nausea, cramps, diarrhoea, and associated headaches.

Cryptosporidium is a parasite that enters lakes and rivers through sewage and animal waste. It causes cryptosporidiosis, a mild gastrointestinal disease. However, the disease can be severe or fatal for people with severely weakened immune systems.

Giardia lamblia is a parasite that enters lakes and rivers through sewage and animal waste. It causes gastrointestinal illness (e.g. diarrhoea, vomiting, cramps).

Hence, it is important that our drinking water does not contain any concentration of microorganisms, parasites or any other substance which constitutes a potential human health risk and it meets the minimum requirements (microbiological and chemical parameters and those relating to radioactivity) laid down by the directives.



Check Your Progress Exercise 2

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

1. Name some common diseases caused due to contaminated drinking water?

.....
.....
.....
.....
.....

2. Name various pollutant/Contaminants of water?

.....
.....
.....
.....
.....

3. List some important human pathogen transmitted by drinking water?

.....
.....
.....
.....
.....

4. Name some opportunist pathogens in drinking water?

.....
.....
.....
.....
.....

13.4 MICROBIOLOGY OF CANNED FOODS

Foods are processed for convenience and safety. Food processing involves procedures such as: drying, canning, freezing, and pasteurization. For example, drying is a process by which water is removed from the product, causing the product to become dehydrated. Since microorganisms need water to grow, without moisture, they can't flourish. Canning is a process where foods are put into a container and given a high heat treatment to make the product sterile. The process of canning, be it vegetables, meat, or seafood, makes food safe because all dangerous microorganisms are destroyed. The canning process was developed to preserve food safely and for long periods of time.

13.4.1 History of Canning

The canning process dates back to the late 18th century in France when the Emperor Napoleon Bonaparte, concerned about keeping his armies fed, offered a cash prize to whoever could develop a reliable method of food preservation. Nicholas Appert conceived the idea of preserving food in bottles, like wine. After 15 years of experimentation, he realized if food is sufficiently heated and sealed in an airtight container, it will not spoil. An Englishman, Peter Durand, took the process one step farther and developed a method of sealing food into unbreakable tin containers, which was perfected by Bryan Dorkin and John Hall, who set up the first commercial canning factory in England in 1813. As more and more of the world was explored, and as provisioning armies took on greater importance, the demand for canned foods grew. Thomas Kensett, who emigrated to the United States, established the first U.S. canning facility for oysters, meats, fruits and vegetables in New York in 1812. More than 50 years later, Louis Pasteur provided the explanation for canning's effectiveness when he was able to demonstrate that the growth of microorganisms is the cause of food spoilage.

13.4.2 Basic Principal of Canning

The basic principles of canning have not changed dramatically since Nicholas Appert and Peter Durand developed the process. Heat sufficient to destroy microorganisms is applied to foods packed into sealed or "airtight" containers. The canned foods are then heated under steam pressure at temperatures of 116-121°C. The amount of time needed for processing is different for each food, depending on the food's acidity, density and ability to transfer heat. For example, tomatoes require less time than green beans, while corn and pumpkin require far more time. Processing conditions are chosen to be the minimum needed to ensure that foods are commercially sterile, but retain the greatest flavour and nutrition.

13.4.3 Spoilage of Canned Products

Heated canned foods may undergo spoilage either due to chemical or biological reasons. The most common spoilage of canned foods is the hydrogen swells produced as a result of action of food acid with the metal can. Such spoilage occurs mostly due to imperfect tinning and lacquering of interior of the can used for canning acidic foods. Biological spoilage of canned foods by the microorganism may result either from the survival of the organisms after the heat treatment or leakage of the container permitting entrance of the microorganisms, Surviving organisms may be vegetative cells or spore formers depending upon the heat treatment. Acid foods are processed at a temperature around 100°C which result in killing of all vegetative cells of bacteria yeast and molds.

13.4.4 *Clostridium botulinum* a Major Threat in Canned Products

Growth of the bacterium *Clostridium botulinum* in canned food may cause **botulism** – a deadly form of food poisoning. These bacteria exist either as spores or as vegetative cells. Botulism is an **intoxication** that is caused by the ingestion of a virulent nerve toxin produced by the growth of the gram positive, obligate anaerobe, spore-former *Clostridium botulinum*. This bacterium appears to be a normal inhabitant of the soil, hence is ready contamination of most foods. The spores can survive harmlessly in soil and water for many years. When ideal conditions exist for growth, the spores produce vegetative cells which multiply rapidly and may produce a deadly toxin within 3 to 4 days of growth in an environment consisting of:

- a moist, low-acid food
- a temperature between 4°C and 49°C
- less than 2 percent oxygen

It is able to grow in **absence of oxygen** in a wide variety of foods and in so doing produces a **protein neural toxin**, two to three grams (an amount equivalent to the quantity of salt in the average salt shaker on your table) of which would be sufficient to kill human being. However, the organism will not grow in the presence of oxygen or nitrate salts and it does not produce the toxin at a pH below 4.7. Only one strain, which is found associated with marine organisms, is able to produce the toxin at refrigerator temperature. The toxin is destroyed by boiling it at 100°C for 10 to 15 min. However, the spore requires a temperature of 121°C for 15 min to kill it. The toxin acts by binding to nerve junctions and destroying the nerve. The symptoms, which occur usually within 12 to 36 hours, but which can take up to 8 days to appear, classically consist of double vision, dizziness, inability to speak, breathe or swallow. Death often occurs due to the inability to breath. The only treatment is the injection of *antitoxin* to the several varieties of the toxin. This treatment is only effective against free toxin, as once the toxin has bound to the nerves the damage is irreversible. **The entire canning process is built around ensuring that all spores of this bacterium contaminating any canned food are destroyed in the sterilization process.** Industry has a sterling record in that deaths from commercial-botulism are very rare. This is influenced by the fact that once a product is known to contain botulism toxin none of that product is ever again purchased by a customer. **The majority of botulism poisonings occur in HOME-CANNED FOODS** prepared by grandma or your favourite aunt. A rule of thumb is “READ THE CANNING DIRECTIONS” and if you think a food might contain the botulism toxin never tastes even the smallest drop of it!

Some interesting additional information about this disease is:

- Never feed **raw honey** to a child under the age of two because the botulism spores can grow in the immature gut and produce the toxin. This can not occur in the adult due to our gut micro flora which is absent in infants.
- The botulism toxin is being used to treat certain neurological conditions where nerves that shouldn't fire do. In these cases tiny quantities of the botulism toxin is injected into the nerve, which the toxin kills and cures the condition.
- Ducks and chickens often die from botulism poisoning by eating rotting material in which the bacterium has grown. However, vultures, which as

you know, eat disgusting rotten, stinking carrion, are immune to the toxin through evolution.

Botulinum spores are on most fresh food surfaces because they grow only in the absence of air, they are harmless on fresh foods. Botulinum spores are very hard to destroy at boiling-water temperatures; the higher the canner temperature, the more easily they are destroyed. Therefore, all low-acid foods should be sterilized at temperatures of 115°C to 121°C, attainable with pressure canners operated at 10 to 15 PSIG. PSIG means pounds per square inch of pressure as measured by gauge. At temperatures of 115°C to 121°C, the time needed to destroy bacteria in low-acid canned food ranges from 20 to 100 minutes. The exact time depends on the kind of food being canned, the way it is packed into jars, and the size of jars. The time needed to safely process low-acid foods in boiling-water canner ranges from 7 to 11 hours; the time needed to process acid foods in boiling water varies from 5 to 85 minutes.

Check Your Progress Exercise 3



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

1. What is Canning?

.....

.....

.....

2. Name the person who for the first time conceived the idea of canning?

.....

.....

.....

3. Name the factor important in deciding canning time?

.....

.....

.....

4. Why is *Closteridium botulinum* a major threat in canned products?

.....

13.5 MICROBIOLOGICAL STANDARDS FOR PROCESSED FOODS

By now you know that microbiological hazards are one of the biggest threats to food safety. With better understanding in microbiology and food safety, safety limits have been set for a range of pathogenic microorganisms in foods. This subunit presents the recommended microbiological guidelines for some ready-to-eat food. According to the Codex Alimentarius Commission of the United Nations, an international food standard setting authority, the functions of

microbiological guidelines include formulation of design requirements, indication of required and expected microbiological status of the food commodities, and the verification of efficacy of hygienic practice. These guidelines stipulate the safety limits of nine major food borne pathogens such as *Salmonella* species, *Listeria monocytogenes*, *E coli O157* and *Vibrio cholerae*, as well as providing a classification of microbiological quality of ready-to-eat food for reflecting the hygienic status of the food concerned.

13.5.1 Purpose of Microbiological Standards

Microbiological Guidelines are criteria indicating the microbiological condition of the food concerned so as to reflect its safety and quality. These standard lists the maximum permissible levels of food borne microorganisms that pose a risk to human health in nominated foods, or classes of foods. They can be introduced to the food industry to observe voluntarily or stipulated in legislation for compliance.

13.5.2 Sampling

The statistical validity of a microbiological examination increases with the number of field samples analysed. For regulatory purposes, **a minimum of 5 sample units** from a lot is generally specified for examination. The size of the samples taken should also be adequate to enable appropriate microbiological analyses to be undertaken. A minimum sample size of 100g or ml is commonly required. **A lot** is defined as a quantity of food or food units produced and handled under uniform conditions. This may be restricted to a food item produced from a particular production line or piece of equipment within a certain time period (not exceeding 24 hours).

13.5.3 Microbiological Assessment

There are three major components under microbiological assessment of any food.

Aerobic colony count is a count of viable bacteria based on counting of colonies grown in nutrient agar plate. This is commonly employed to indicate the sanitary quality of foods. The incubation condition of ACC used in this guideline is **30° C for 48 hours**.

Indicator organism Counts refers to the selected surrogate markers. The main objective of using bacteria as indicators is to reflect the hygienic quality of food. *E. coli* is commonly used as surrogate indicator. Its presence in food generally indicates direct or indirect fecal contamination. Substantial number of *E. coli* in food suggests a general lack of cleanliness in handling and improper storage. **Specific pathogens Counts** refer to bacteria that may cause food poisoning. Mechanisms involved may be toxins produced in food or intestinal infection. Nine specific bacterial pathogens are included in this set of guidelines. The symptoms of food poisoning vary from nausea and vomiting (e.g. caused by *S. aureus*), through diarrhoea and dehydration (*Salmonella* spp. and *Campylobacter* spp.) to paralysis and death in the rare cases of botulism. The infectious doses vary from less than 10 to more than 10^6 organisms.

13.5.4 Categories of Food based on Microbial Quality

For assessment of hygienic quality, food items are grouped into five categories taking into account the raw ingredients used, and the nature and degree of processing before sale. The microbiological assessment of ready-to-eat food on the above three components will lead to the classification of the food quality into one of the following four classes:

- Class A:** the microbiological status of the food sample is **satisfactory**.
- Class B:** the microbiological status of the food sample is **less than satisfactory but still acceptable for consumption**.
- Class C:** the microbiological status of the food sample is **unsatisfactory**. This may indicate a sub-optimal hygienic conditions and microbiological safety levels. Licensees of food premises should be advised to investigate and find out the causes and to adopt measures to improve the hygienic conditions. Taking of follow-up samples to verify the improvement may be required.
- Class D:** the microbiological status of the food sample is unacceptable. The food sample contains unacceptable levels of specific pathogens that is **potentially hazardous to the consumer**. In addition to giving advice to the licensee of the food premises as stated in (c) above, warning letters as well as other enforcement actions should be considered. Microbiological limits in respect of the above components are summarized in the table 13.4.

Table 13.4: Guideline levels for determining the microbiological quality of ready-to-eat foods

Criteria	Microbiological Quality (CFU per gram)				
	Class A Satisfactory	Class B Marginal	Class C Unsatisfactory	Class D Potentially Hazardous	
Aerobic colony count (ACC)[30⁰C/48 hr]					
Food Category	1	<10 ³	10 ³ -<10 ⁴	≥10 ⁴	N/A
	2	<10 ⁴	10 ⁴ -<10 ⁵	≥10 ⁵	N/A

**Safe Chemicals and
Microbial Limits for
Different Foods**

(Food items detailed in Table 5)	3	$<10^5$	$10^5- <10^6$	$\geq 10^6$	N/A
	4	$<10^6$	$10^6- <10^7$	$\geq 10^7$	N/A
	5	N/A	N/A	N/A	N/A
Indicator organisms (Apply to all food categories)					
<i>E.coli</i> (Total)		<20	$20 - < 100$	≥ 100	N/A
Pathogens (Apply to all food categories)					
<i>Campylobacter</i> spp		not detected in 25g	N/A	N/A	Present in 25 g
<i>Escherichia coli</i> 0157		not detected in 25g	N/A	N/A	Present in 25 g
<i>Listeria monocytogenes</i>		not detected in 25g	N/A	N/A	Present in 25 g
<i>Salmonella</i> spp		not detected in 25g	N/A	N/A	Present in 25 g
<i>Vibrio cholerae</i>		not detected in 25g	N/A	N/A	Present in 25 g
<i>Clostridium perfringens</i>		<20	$20 < 100$	$100 < 10^3$	$\geq 10^3$
<i>Staphylococcus aureus</i>		<20	$20 < 100$	$100 < 10^4$	$\geq 10^4$
<i>Vibrio parahaemolyticus</i>		<20	$20 < 100$	$100 < 10^4$	$\geq 10^4$
<i>Bacillus cereus</i>		$<10^3$	$10^3 - < 10^4$	$10^4 - < 10^5$	$\geq 10^5$

N/A Not applicable

The desired microbiological quality of the some food samples is summarized in Table 13.5.

Table 13.5: Food category table for aerobic colony count assessment

Food group	Food item	Category
Meat	Beefburgers and kebabs	1
	Dim sum	2
	Pate (meat, seafood or vegetable)	3
	Poultry (unsliced)	2
	Preserved meat	4
	Salami and fermented meat products	5
	Sausages	2
	Smoked meat	5
	Siu-mei & lo-mei	3
	Sliced meat (ham and tongue) (cold)	4
	Sliced meat (beef, haslet, pork, poultry, etc.) (dried)	3
	Steak and kidney / meat pies	2
	Tripe and other offal	4
Seafood	Crustaceans	3
	Pickled fish	1
	Other fish (cooked)	3
	Oysters (raw)	5
	Seafood meals	3
	Shellfish (cooked)	4
	Smoked fish	4
Dessert	Cakes, pastries, slices and desserts – with dairy cream	3
	Cakes, pastries, slices and desserts – without dairy cream	2
	Cheesecake	5
	Mousse / dessert	1
	Tarts, flans and pies	2
	Trifle	3
Savoury	Bean curd	5
	Cheese-based bakery products	2
	Fermented foods	5
	Flan / quiche	2
	Dips	4
	Mayonnaise / dressings	2
	Samosa	2
	Satay	3
	Spring rolls	3
Vegetable	Coleslaw / salads (with or without meat)	3
	Fruit and vegetables (dried)	3
	Fruit and vegetables (fresh)	5
	Rice	3

	Vegetables and vegetable meals (cooked)	2
Dairy	Cheese	5
	Yoghurt	5
Ready-to-eat meals	Pasta / pizza	2
	Meals (others)	2
Sandwiches and filled rolls	With salad	4
	Without salad	3
Sushi & sashimi	Fish fillet and fish roe sashimi / sushi	3
	Sashimi other than fish fillet and fish roe	4

Controlling microbes

Control of microbes in processed products primarily depend upon good manufacturing practices and one of the most effective way to ensure this is by application of HACCP. HACCP stands for Hazard Analysis of Critical Control Point. HACCP is a preventive system for assuring production of safe food. It is a process that identifies food safety hazards associated with a product and process and strictly manages and monitors the Critical Control Points (CCP's) designed to control the hazard as a way of ensuring the process is in control and that the safest product possible is being produced. It requires establishment of hazard, identification of critical control points, effective monitoring follow up and evaluation. For a food processor it is necessary to know the microbial quality of the raw material, the processing environment, and the packaging component. This also requires validation of all processing stages designed to destroy both the pathogens and the spoilage agents and the efficacy of preservative system.



Check Your Progress Exercise 4

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

1. What is codex Alimentarius?

.....

.....

.....

.....

.....

.....

.....

.....

2. Why do we need microbial standards?

.....

.....

.....

.....

3. How many sample units from a lot are generally specified for examination?

.....

.....

.....

.....

.....

4. What are the major components under microbiological assessment of any food?

.....

.....

.....

.....

5. Define Aerobic colony count?

.....

.....

.....

.....

6. What does the presence of indicator organism in food reflect?

.....

.....

.....

.....

7. Name the specific pathogens to be observed for microbial assessment of any food?

.....

.....

.....

.....



13.6 LET US SUM UP

In this unit you have learnt about the microbial quality limits for various foods (raw and processed). Herein we have discussed the sources of microorganisms on fresh fruits and vegetables and in drinking water. Various Food and water borne harmful microorganisms including indicator bacteria, viruses and pathogens have been discussed briefly. *Clostridium botulinum* is an important organism for canning point of view. Hence, it has been dealt in more detail. WE HOPE that after reading this unit you will become more conscious about the microbial quality of food that you are going to eat.

13.7 KEY WORDS

Spoilage

microorganism : Microorganism which spoil the product by developing undesirable flavours, odours and changing food appearances or textures via microbial action.

Pathogenic : Microorganism which may infect plants, animals and man and make them sick.

Hazardous : Harmful

Epidemic : Out break of infectious disease

Gastroenteritis : Inflammatory change of lining of stomach caused by microorganism ingested with food and water.

Hepatitis : Virus Hepatitis A

Parasites : Life on, within or at the expense of other organisms.

Toxin : Poison

Intoxication : Toxin production

13.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following point:

- Sources of microorganisms on fresh fruit are air, orchard soil, irrigation water, harvesting device, storing and packaging containers, handling personnel etc.

2. Your answer should include the following points:

- The survival or growth of contaminating microorganisms is affected by intrinsic, extrinsic and processing factors.
- Factors of importance are nutrient composition, pH, presence of scales and fibers, redox potential, temperature and gaseous atmosphere.
- Mechanical shredding, cutting and slicing of the produce open the plant surfaces to microbial attack.

3. Your answer should include the following point:

- Members of the genera *Penicillium*, *Aspergillus*, *Sclerotinia*, *Botrytis* and *Rhizopus* are commonly involved in spoilage of fresh fruits and vegetables.

4. Your answer should include the following points:

- Most of the reported outbreaks have been associated with bacterial contamination.
- Members of the *Enterobacteriaceae*. Of these, *Salmonella* and *Escherichia coli* O157 are of particular concern.

Check Your Progress Exercise 2

1. Your answer should include the following points:

- Gastroenteritis
- Diarrhoea
- Dysentery
- Hepatitis
- Typhoid fever

2. Your answer should include the following points:

- Microbes
- Radionuclide
- Inorganics
- Volatile organics
- Disinfectants
- Disinfection by products etc.

3. Your answer should include the following points:

- The human pathogens potentially transmitted in drinking water are Bacteria viz. *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio cholera*, *Yersinia enterocolitica*, *Campylobacter jejuni*.
- Viruses viz. Adenoviruses, Enterovirus, Hepatitis A, Hepatitis E, Norwalk virus, Rotavirus, and small round viruses
- Parasites viz. *Giardia*, *Cryptosporidium*, *Entamoeba histolytica* and *Dracunculus*.

4. Your answer should include the following points:

- *Pseudomonas*

- *Flavobacterium*
- *Acinetobacter*
- *Klebsiella*
- *Serratia*
- *Legionella*.

Check Your Progress Exercise 3

1. Your answer should include the following point:

- Process where foods are put into a container and given a high heat treatment to make the product sterile.

2. Your answer should include the following point:

- Nicholas Appert

3. Your answer should include the following points:

- Food's acidity
- Density
- Ability to transfer heat.

4. Your answer should include the following point:

- Growth of the bacterium *Clostridium botulinum* in canned food may cause **botulism** – a deadly form of food poisoning.

Check Your Progress Exercise 4

1. Your answer should include the following point:

- Codex Alimentarius is an international food standard setting authority.

2. Your answer should include the following points:

- Lists the maximum permissible levels of food borne micro-organisms that pose a risk to human health in nominated foods, or classes of foods.
- Indicate the microbiological condition of the food concerned so as to reflect its safety and quality.

3. Your answer should include the following point:

- Minimum of 5 sample units from a lot is generally specified for examination.

4. Your answer should include the following points:

- Aerobic colony count
- Indicator organism Counts
- Specific pathogens Counts

5. Your answer should include the following points:

- Count of viable bacteria based on counting of colonies grown in nutrient agar plate.
 - Commonly employed to indicate the sanitary quality of foods.
6. Your answer should include the following point:
- The presence of indicator organisms in food reflect the hygienic quality of food.
7. Your answer should include the following points:
- *Campylobacter* spp.
 - *Escherichia coli* 0157
 - *Listeria monocytogenes*
 - *Salmonella* spp
 - *Vibrio cholerae*
 - *Clostridium perfringens*
 - *Staphylococcus aureus*
 - *Vibrio parahaemolyticus*
 - *Bacillus cereus*

13.9 SOME USEFUL BOOKS

1. Adams, M.R. and Moss, M.O. (2000) Food Microbiology. Royal Society of Chemistry, Cambridge, U.K.
2. Jay, J.M. (2000) Modern Food Microbiology, Van Nostrand Company, New York.