FOOD AND DAIRY BIOTECHNOLOGY

About the Author

She had completed Graduation in Industrial Microbiology from M.S.M College, Kayamkulam, Kerala, India and Post-Graduation in Biotechnology from Barkatullah Vishwavidyalaya, Bhopal, Madhya Pradesh, India. During the last semester of Post-Graduation, she had done her 6 months Dissertation cum Training in National Institute for Interdisciplinary Science and Technology (NIIST), Thiruvananthapuram under the guidance of Prof. Ashok Pandey, Chief Scientist & Head, Centre for Biofuels & Biotechnology Division, CSIR-National Institute for Interdisciplinary Science and Technology (NIIST), Trivandrum-695 019, Kerala, India. After Post-Graduation she worked one year as a Microbiologist in Quality Assurance Laboratory, Kollam, Kerala, India. She then joined in Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, India for Ph.D Degree under the guidance of Dr. C. Mohandas, Principal scientist (Rtd.), Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, India. She had successfully awarded Ph.D degree in Biotechnology from Kerala University in the year 2013. Later she got Level 3 Award in HACCP for Food Manufacturing conducted by Royal Society for Public Health, London (RSPH). At the moment she is working as a Guest Lecturer in the Department of Botany & Biotechnology, M.S.M College, Kayamkulam, Alappuzha, Kerala, India from 2016 onwards.

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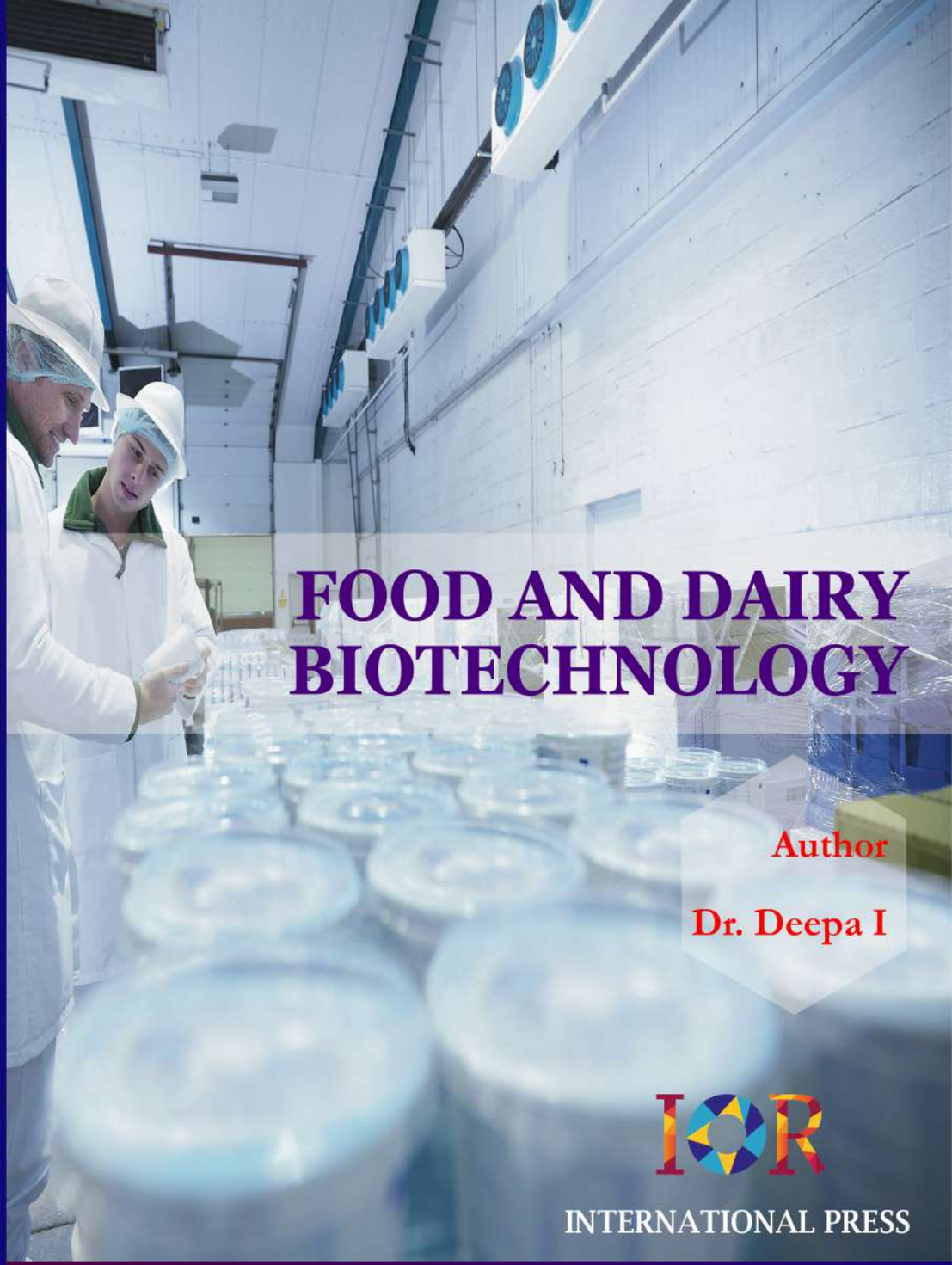
Dr. Deepa I



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Author

Dr. Deepa I

INTERNATIONAL PRESS

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Food and Dairy Biotechnology

Dr. Deepa I

Dr. Deepa I
Guest Lecturer
Department of Botany & Biotechnology,
M.S.M College, Kayamkulam,
Alappuzha, Kerala,
India.

ISBN 978-93-89631-09-8 ISBN 978-93-89631-10-4
https://doi.org/10.34256/ioriip201

This IOR INTERNATIONAL PRESS imprint is published by the registered company IOR PRESS.
The registered company address is: 5/11, Perumal Kovil St, Naickenpalayam, Coimbatore-641020 Tamil Nadu, India.

Preface

I am glad to present this book, especially designed to serve the needs of the students of Bachelor of Science (B.Sc) in Biotechnology as well as Biotechnology open course students. The contents of this book entirely based on the syllabus of the paper 'Food and Dairy biotechnology' of B.Sc. Biotechnology students.

First of all, I would like to thank the Founder of M.S.M College, Al- Haj P. K Kunju Sahib and Al-Haj P. A. Hilal Babu, Manager Cum Secretary, M.S.M Trust & Chairman, M.S.M College Governing Body, Kayamkulam, Kerala, India. Words are insufficient to express my deep gratefulness, indebtedness and sincere gratitude to you for giving me a chance to teach in his esteemed institution that inspired me to write this book.

I acknowledge special thanks to Mrs. Shabnam A (H.O.D, Department of Botany and Biotechnology, M.S.M College, Kayamkulam, Kerala, India) she is the main concept provider and also have been constant source of motivation throughout this endeavor.

In conclusion, I wish to express my deep sense of gratitude and indebtedness to my family, especially my brother Mr. Deepak M and my husband Mr. Rajesh G who helped me during the preparation of the text.

I look forward to receive valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book.

Dr. Deepa I

Synopsis

This text book has been written in simple, graspable language and is written to meet the requirements of B.Sc. Biotechnology and Biotechnology Open course students. The contents included in this book entirely based on the syllabus of the paper 'Food and Dairy Biotechnology'. So I assured that this book will be very helpful for these students to excel their knowledge in this topic.

Author

She had completed Graduation in Industrial Microbiology from M.S.M College, Kayamkulam, Kerala, India and Post-Graduation in Biotechnology from Barkatullah Vishwavidyalaya, Bhopal, Madhya Pradesh, India. During the last semester of Post-Graduation, she had done her 6 months Dissertation cum Training in National Institute for Interdisciplinary Science and Technology (NIIST), Thiruvananthapuram under the guidance of Prof. Ashok Pandey, Chief Scientist & Head, Centre for Biofuels & Biotechnology Division, CSIR-National Institute for Interdisciplinary Science and Technology (NIIST), Trivandrum-695 019, Kerala, India. After Post-Graduation she worked one year as a Microbiologist in Quality Assurance Laboratory, Kollam, Kerala, India. She then joined in Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, India for Ph.D Degree under the guidance of Dr. C. Mohandas, Principal scientist (Rtd.), Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, India. She had successfully awarded Ph.D degree in Biotechnology from Kerala University in the year 2013. Later she got Level 3 Award in HACCP for Food Manufacturing conducted by Royal Society for Public Health, London (RSPH). At the moment she is working as a Guest Lecturer in the Department of Botany & Biotechnology, M.S.M College, Kayamkulam, Alappuzha, Kerala, India from 2016 onwards.

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Chapter 1
Microbes of Food and Factors Affecting Microbial Growth in Food

A food microflora mainly depends on microbial type, characteristics of a food type, contamination, processing and storage conditions. The microbial groups associated with foods are bacteria, fungi, protozoa, algae and viruses. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in foods, the most important are aw, pH and temperature. aw: (Water Activity or Water Availability).

Types of Microorganism Associated with Food

Sources of Microorganisms in foods

The primary sources of microorganisms in the food are

I. Soil microbial flora

The soil contains the greatest variety of microorganisms of any source of contamination. The fertile soil contains large number of microorganisms which are contaminating the surfaces of plants growing on it and animals roaming over the land. Soil dust whipped by air current and soil particles are carried by running water to get into or on to foods.

The soil is important source of heat resistant spore forming bacteria. The most important types of organisms contaminating through soil are *Bacillus*, *Clostridium*, *E. coli*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, *Proteus*, *Leuconostoc*, *Acetobacter*, *Chromobacterium* etc.

II. Waterborne microorganisms

Water is an important source of microorganisms especially Coliforms which are indicator organisms for fecal contamination. Natural water contains the natural flora as well as the microorganisms from soil, animals and sewage. *Proteus*, *Micrococcus*, *Streptococcus*, *Pseudomonas*, *Bacillus*, *E. coli*, *Enterobacter* and *Chromobacterium* are the important kinds

Deepa I, *Food and Dairy Biotechnology*,
https://doi.org/10.34256/ioriip2011

of bacteria found in natural water. Coliforms are called the common waterborne bacteria. These are gram-negative, non-sporulating rods indicate water contamination. Hence they are called indicator organisms.

III. Microorganisms in Air

Air is another source of contamination in food. Because it contributes dust, droplets, droplet nuclei, aerosols and suspended particle. Disease organisms especially those causing respiratory infections may spread by air. The microorganisms in the air will not grow because of the lack of nutrients but they will suspend in air for very long time. Fungal spores and bacterial spores are predominant in air, mold spores are more resistant to dry and persisting for very long time. Among bacteria cocci are predominant than rods. Yeasts are also present in air. The number of microorganisms in the air may be depending on so many factors such as sunshine, location and the amount of suspended dust or spray. Dry air contents more organisms than moist air. Rain or snow removes the organisms from the air.

IV. Microorganisms in vegetation

The natural surface flora of plants varies with the plant but generally include the species of *Bacillus*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Coliforms*, *Micrococcus* and Lactic acid bacteria. The number of bacteria will depends on the plant and its environment and may range from a few hundred or thousand per square centimetre of surface to million. Exposed surface of plants become contaminated from soil, water, sewage, air and animal so that microorganisms from these sources are added to the natural flora. Plants are associated with the bacterial plant pathogens such as *Corynebacterium*, *Curtobacterium*, *Pectobacterium*, *Pseudomonas* and *Xanthomonas* and fungal pathogens among several genera of molds.

V. Microorganisms from Animal

The source of microorganisms from animals includes the surface flora, the flora of respiratory tract and the flora of the gastrointestinal tract. Hides, hooves and hair of animal contain large number of microorganisms from soil, manure feed and water. Similarly feathers and feet of poultry carry heavy contamination from soil and other sources. The skin of many meat animals contain *Micrococcus*, *Staphylococcus* and β- hemolytic *Streptococcus*. The feces and fecal contaminated products of animals can contain many enteric pathogens. Animal contributes their wastes and finally their bodies to the soil and water which contain large group of microbial flora. So many pathogenic organisms are present in animal and animal products including *Brucella* sps, *Mycobacterium tuberculosis*, *Coxiella burnetii*, *Salmonella typhi* and *paratyphi*, *Listeria monocytogens*, *Campylobacter jejuni*, enteropathogenic *E. coli*, *Clostridium perfringens* and *Clostridium botulinum*.

VI. Microorganisms from food handlers and food equipment

The microbial flora on the hands and outer garments of handlers generally reflect the environment and habits of individuals and the organisms may be those from soil, water, dust and other environmental sources, additional important sources are those that are common in nasal cavities, the mouth and on the skin and those from the gastrointestinal tract that may enter food through poor personal hygiene practices. When vegetables are harvested in containers and utensils, it is expected to find some of the surface organisms on the products to contaminate contact surfaces.

Common bacteria present in food

The bacteria which are important in food biotechnology are

- *Acetobacter*

  They are gram-negative bacteria oxidize ethanol to acetic acid. They are rod shaped motile bacteria found on fruits and vegetables.

- *Aeromonas*

  They are gram-negative, facultative anaerobic, psychrophilic rods and which are commonly found in fish, frog and other mammals.

- *Alcaligenes*

  They are gram-negative rod shaped bacteria present in feeds, soil, water and dust. The important species are *Alcaligenes viscolactis*, which produces ropiness in milk and *Alcaligenes metalcaligenes* gives a slimy growth on cottage cheese.

- *Alteromonas*

  These organisms are gram-negative aerobic rods. The important species is *Alteromonas putrefaciens*.

- *Arthrobacter*

  It is a gram-positive bacterium predominant in soil.

- *Bacillus*

  It is a gram-positive spore forming aerobic or facultative anaerobic organisms. The important species are *Bacillus subtilis* and *Bacillus stearotheromphilus* (these are hyper thermophilic and spore producing bacteria).

- *Brevibacterium*

  It is also a gram-positive bacteria produces orange red pigment and helps ripening.

- *Brothrothrix*

  These are gram-positive non spore forming bacteria found in many food items.

- *Campylobacter*

  It is gram-negative, spiral shaped rods. The species *Campylobacter jejuni* is associated with gastroenteritis in human.

- *Clostridium*

  There are gram-positive spore forming rods. There are obligate (strict) anaerobes. Many species are capable of fermenting carbohydrate and produce acids and gases. *Clostridium botulinum* (causes botulism) and *Clostridium perfringens* (causes gas of gangrene and food poisoning) are the most important species in food biotechnology.

- *Corynebacterium*

  There are gram-positive, rod shaped bacteria that are sometimes involved in the spoilage of vegetables and meat products. The important species, *Corynebacterium diphtheriae* causes diphtheria in humans.

- *Citrobacter*

  These enteric bacteria are slow lactose-fermenting, gram-negative rods that typically produce yellow colonies.

- *Desulfotomaculum*

  It is a gram-negative rod and inhabitants of the soil, fresh water and the rumen. It is a sulphur oxidizing bacteria.

- *Escherichia*

  It is a gram-negative, non-sporulating, motile and facultative anaerobic bacteria commonly referred as coliform and which are indicator organisms.

- *Enterobacter*

  These are enteric gram-negative coliform bacteria like *E. coli*.

- *Erwinia*

  These are gram-negative enteric rods especially associated with plants. *Erwinia carotovora* is the most important organism responsible for spoilage of food.

- *Flavobacterium*

  These are gram-negative rods characterized by their production of yellow to red pigments on agar and by their association with plants. Some are mesotrophs and others are psychrotrophs, where they participate in the

spoilage of refrigerated meats and vegetables.

- *Gluconobacter*

  These are gram-negative rod shaped bacterium which can oxidize ethanol to acetic acid and can cause ropiness in beer.

- *Hafnia*

  These are gram-negative enteric rods important in the spoilage of refrigerated meat and vegetable products.

- *Halobacterium*

  These are obligate halophiles and causes discoloration on the foods high in salt such as salted fish.

- *Klebsiella*

  These are gram-negative non-sporulating, non-motile and facultative anaerobic bacteria commonly referred as coilforms and which are indicator organisms. *Klebsiella pneumoniae* is the causative organism for bacterial pneumonia in human.

- *Lactobacillus*

  These are gram-positive, rod shaped, microaerophilic bacteria typically occur on most vegetables, along with some of the other lactic acid bacteria. Their occurrence in dairy products is common. It can ferment the carbohydrate lactose and can produce acids and gas. The important species are *Lactobacillus acidophilus, Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii* and *Lactobacillus pentosus*.

- *Leuconostoc*

  They are gram-positive bacterium. The most important species is *Leuconostoc mesenteroides*.

- *Listeria*

  They are gram-positive and non-sporing rods. The most important organism is *Listeria monocytogens* which causes the spoilage of fish and fish products.

- *Micrococcus*

  These are gram-positive cocci and are inhabitants of mammalian skin and can grow in the presence of high level of salt.

- *Moraxella*

  These are short gram-negative rods they do not form acid from glucose. The most important species is *Moraxella bovis*.

- *Mycobacterium*

  These are gram-positive rods. The most important species are *Mycobacterium tuberculosis* (causative agent of tuberculosis), *Mycobacterium leprae* (causative agent of leprosy) and *Mycobacterium bovis*.

- *Pediococcus*

  These are gram-positive homofermentative cocci. These are lactose fermenting bacteria.

- *Proteus*

  This enteric gram-negative rods are aerobes that often display pleomorphism. These organisms are motile and typically produce swarming growth on the surface of moist ager plates. They are typical of enteric bacteria. One important species is *Proteus vulgaris*.

- *Pseudomonas*

  These are gram-negative rod shaped motile bacteria. They are typical soil and water bacteria and they are widely distributed among fresh food especially vegetables, meats, poultry and sea food products. These are one of the largest foodborne bacteria.

- *Photobacterium*

  These are Coccobacilli and can cause phosphorescence of meat and fish.

- *Propionibacterium*

  These are gram-positive, small, non motile rod shaped bacteria can ferment carbohydrates. These bacteria are commonly present is cheese and cheese related products.

- *Salmonella*

  These are gram-negative enteric bacteria. They are considered to be human pathogens. The most important pathogen is *Salmonella typhi* and *Salmonella paratyphi*. These organism cause enteric fever.

- *Serratia*

  These are gram-negative rods that belong to the family Enterobacteriaceae are aerobic, proteolytic and produce red pigments on culture media and in certain food. *Serratia liquefaciens* and *Serratia marcescens* are the most prevalent of the foodborne species. It causes spoilage of refrigerated vegetables and meat products.

- *Shigella*

  These are gram-negative, non-sporulating motive and facultative anaerobic

bacteria and can cause bacillary dysentery in humans. *Shigella dysenteriae* is the causative organism other *Shigella* species are *Shigella flexneri*, *Shigella sonnei* and *Shigella boydii*.

- *Staphylococcus*

  These are gram-positive cocci occur in the form of grape like clusters and which include *Staphylococcus aureus*, causes several diseases in human including foodborne gastroenteritis.

- *Streptococcus*

  These are gram-positive cocci which occur in the form of long or short chains. It will cause pyogenic infection in human (*Streptococcus pyogens*).

- *Vibrio*

  These are gram-negative straight or curved rods. The most important organism is *Vibrio cholerae* which causes cholera disease.

- *Yesinia*

  These are gram-negative rods and include the causative agent of human plague, *Yersinia pestis*. Some species are causing foodborne gastroenteritis.

Molds and Yeast in Food

Fungi are eukaryotic spore producing organisms capable of reproducing both sexual and asexual manner. Fungi are commonly classified into two: unicellular fungi-yeast and multicellular fungi-mold. Fungi belong to plant Kingdom–Myceteae. These are multicellular, nonphotosynthetic organism with chemoheterotrophic mode of nutrition. Fungi lacks roots, stem or and devoid of chlorophyll.

Molds regarded as eumycetes or true fungi. Molds are filamentous fungi that grow in the form of a tangled mass that spreads rapidly and may cover several inches of area in 2-3 days. The total of the mass or any large portion of it is referred to as mycelium. Mycelium is composed of branches or filaments referred to as hyphae. Groups of hyphae are referred to as mycelium. Fungi are greatest importance in food and it multiply by ascospores, zygospores and conidiospores.

Major Molds Present in Food

Rhizopus, *Mucor*, *Aspergillus*, *Penicillium*, *Neurospora*, *Eupenicillium*, *Eurotium*, *Fusarium*, *Alternaria*, *Geortrichum*, *Helminthosporium*, *Trichothecium*, *Cephalosporium*, *Aureobasidium* (*Pullularia*) and *Botrytis* etc.

Common Foodborne Yeasts

Yeasts are unicellular fungus. It can be differentiated from bacteria by their larger size, oval, elongated-spherical cell shape. Yeasts are classified into two:

1. True yeast - Ascomycotina
2. False Yeast - Deuteromycetes or fungi imperfecti

Major Foodborne Yeasts

True Yeast

This type of yeast produces ascospores and arthrospores. Vegetative reproduction of such types of yeast is taking place by fission and budding.

eg:- *Pichia*, *Saccharomyces – S. cerevisiae*, *Debaryomyces*, *Torulospora – T. delbrueckii* and *Zygosaccharomyces*

False Yeast

Reproduction of false yeast is by budding. These are fungi imperfecti because their sexual stages of cell division are not yet identified.

eg:- *Candida – C. albicans*, *C. lipolytica* and *C. guilliermondii*

Rhodotorula (*R. mucilaginosa* - pigment producing yeast) and *R. glutinis* and *Trichospora*

Microbes of curd

Curd has lactic acid bacteria or *Lactobacillus*. This bacteria multiplies itself in the ambient temperature of 30-40^{0}C and in few hours ferments the milk to form curd. Curd is a rich source of calcium and protein and is suitable for lactose intolerant people. Curd is a probiotic, it makes the gut healthy. Curd contains a number of bacteria like *Lactococcus lactis*, *Lactococcus lactis cremoris*, *L. acidophilus* etc. Therefore, the exact knowledge about the different strains that exist in curd is lacking. A report was published in the European Journal of Clinical Nutrition that suggests that the type of needful bacteria present in curd vary considerably between places. It was also demonstrated that the Indians curd contains nearly 250 different strains of *Lactobacillus.*

Microbes of wheat

Wheat is naturally exposed to microbial organisms as it grows in the field and as it is stored and transported. The microbial content of wheat flour is lower than that of wheat itself as microorganisms reside on the outer bran layers of the wheat grain which are removed during the milling process.

Bacteria, fungi and actinomycetes were found in most wheat samples. Organisms common to both wheat and flour includes Psychrotrophic bacteria, fecal *Streptococci*, catalase negative bacteria, aerobic thermophilic spore-forming bacteria, Flat sour bacteria, *Aureobasidium pullulans* and *Streptomyces albus* survived the milling process.

Microbiology of Cereals and Cereal products

Cereals and cereal products are significant and important human food resources and livestock feeds worldwide. The main cereal grains used for foods include corn (maize), wheat, barley, rice, oats, rye, millet and sorghum. Because of their extensive use as human foods and livestock feeds, the microbiology and safety of cereal grains and cereal products is a very important area. The source of microbial contamination of cereals are many, but all traceable to the environment in which grains are grown, handled and processed. Microorganisms that contaminate cereal grains may come from air, dust, soil, water, insects, rodents, birds, animals, humans and processing equipment. Many factors that are a part of the environment influence microbial contamination of cereals including rainfall, drought, humidity, temperature, sunlight, frost, soil conditions, wind, insect, bird and rodent activity, harvesting equipment, use of chemicals in production versus organic production, storage and handling and moisture control.

The microflora of cereals and cereal products are varied and includes molds, yeasts, bacteria (Psychrotrophic, mesophilic, thermophilic, thermoduric), lactic acid bacteria, rope-forming bacteria (*Bacillus* spp.), bacterial pathogens, coliforms and *Enterococci*. Bacterial pathogens that contaminate cereal grains and cereal products and cause problems include *Bacillus cereus*, *Clostridium botulinum*, *C. perfringens*, *E. coli*, *Salmonella* and *Staphylococcus aureus*. Coliforms and *enterococci* also occur as indicators of unsanitary handling and processing conditions and possible fecal contamination.

Bacteria are frequent surface contaminants of cereal grains. For the bacteria to grow in cereal grains, they require high moisture or water activity (a_w) in equilibrium, with high relative humidity. Generally, bacteria are not significantly involved in the spoilage of dry grain and become a spoilage factor only after extensive deterioration of the grain has occurred and high moisture conditions exist. Lactic bacteria may also be present in the raw grain and carry over into flour and cornmeal and spoil dough prepared with them. Yeasts present on cereal grains may also carry through into processed products. The main spoilage organisms in cereal grains, however are molds.

There are more than 150 species of filamentous fungi and yeasts on cereal grain. The filamentous fungi that occur on cereal grains are divided into two groups, depending on when they predominate in grain in relation to available moisture in the grain. These groups have been referred to as field fungi and storage fungi. Field fungi invade grain in the field when the grain is high in moisture (18 to 30% i.e, at high a_w)

and at high relative humidities (90 to 100%). Field fungi include species of *Alternaria*, *Cladosporium*, *Fusarium* and *Helminthosporium*. Storage fungi invade grain in storage at lower moisture contents (14 to 16%), lower a_w and lower relative humidities (65 to 90%). These main storage fungi are species of *Eurotium*, *Aspergillus* and *Penicillium.*

Microbes of Meat

The growth of microbes in meat is governed by a number of intrinsic and extrinsic factors. Intrinsic properties of meat such as pH and moisture can promote microbial growth, whereas temperature is an extrinsic factor. Fresh meat has a high water content that is favorable for the growth of microorganisms. It also generally contains bacteria, including those that can cause diseases. The animals naturally carry bacterial species like *Salmonella* and *E. coli* in their intestines and raw meat can become contaminated during the slaughter process. Equipments and tools used in the processing of meat can also become contaminated with microbes and spread those to the raw meat. Raw meat should be cooked thoroughly before consumption.

Beef

The most common pathogenic bacteria found in beef is *E. coli*. The *E. coli* strain 0157:47 is a rare, dangerous bacterium that can cause severe damage to the intestinal lining. *Salmonella*, *Staphylococcus aureus* and *Listeria monocytogenes* are also common contaminants in beef. All these organisms can be destroyed by cooking.

Pork

In Pork, *E. coli*, *Salmonella*, *S. aureus* and *Yersinia enterocolitica* are the most common bacterial contaminants. Chitterlings (intestine of pig) can be contaminated with *Y. enterocolitica* leading to a diarrhoeal illness known as Yersiniosis. Microbial contaminants in pork can be destroyed by cooking to an internal temperature of 145^0F.

Chicken

Chicken is often contaminated with *Salmonella enteritidis*, *S. aureus*, *Campylobacter jejuni*, *L. monocytogenes* and *E. coli* can also be found in chicken. Chicken should be cooked to an internal temperature of 165^0F to kill the microbes.

Bacteria in cooked meat

Thorough cooking can generally destroy most bacteria on raw meat, including pathogenic ones. Nevertheless, if there are subsequent lapses in food safety practices, food poisoning may still occur. Raw meat may be contaminated with spores of certain pathogenic bacteria (eg:- *Clostridium perfringens*) and spores are not readily destroyed by normal cooking temperature. Heat of cooking can rather activate the spores to germinate and develop into vegetative cells which can multiply rapidly in

foods that are placed at ambient temperature for a long period. Consuming foods that contain high levels of *Clostridium perfringens* vegetative cells may lead to foodborne illness.

In addition, pathogenic bacteria may be introduced into the ready-to eat cooked meat through cross-contamination and multiply to larger amount as a result of time and temperature abuse of the food, causing foodborne illness in consumers.

Microbes of fish

The population of microorganisms associated with living fish reflects the microflora of the environment at the time of capture of harvest, but is modified by the ability of different microorganisms (mainly bacteria) to multiply in the sub-environments provided by the skin/shell surfaces, gill areas and the alimentary canal. Shell fish taken from water near human habitations will tend to have higher bacterial loads and a more diverse microflora compared with those taken from isolated areas. The muscle tissue and internal organs of freshly caught, healthy finish and molluscan shellfish are normally sterile, but bacteria may be found on the skin, chitinous shell, gills of fish, as well as in their intestinal tract. The circulatory system of some crustaceans is not "closed" and the hemolymph of crabs can harbor substantial levels of bacteria, particularly members of the genus *Vibrio*. Microbial levels vary depending on water conditions and temperature.

Typically, bacteria from skin and gills are predominantly aerobic although facultative bacteria, particularly *Vibrio* spp., may occur in high numbers on pelagic fish. Obligately anaerobic bacteria are uncommon on the surface of fish but can occur in significant numbers in the intestine. Lactic acid bacteria in particular *Carnobacteria* are also commonly isolated from fish gut.

The bacteria on finfish and shellfish are predominantly gram-negative for fish from temperate waters. A higher proportion of gram-positive cocci and bacillus spp. can be found on some fish from warm, tropical water and some studies report as much as 50-60% of the microflora being of these types. However, the microflora of fish from warm, tropical water may also be dominated by gram-negative bacteria. The microflora of living fish from temperature water is remarkably consistent and commonly includes members of the genera *Psychrobacter*, *Moraxella*, *Pseudomonas*, *Acinetobacter*, *Shewanella* (previously *Alteromonas*), *Flavobacterium*, *Cytophaga*, *Vibrio*, *Aeromonas*, *Corynebacterium* and *Micrococcus*.

In addition to bacteria, yeast such as *Rhodotorula*, *Torulopsis*, *Candida* spp. and occasionally fungi are reported from finish and shellfish.

Microbes of Egg

Freshly laid eggs are generally sterile particularly the inner contents. However the shells get contaminated from the environmental sources such as fecal

matter of the bird, beddings, by the handlers and wash water and also the packaging materials in which the eggs are packed. There are several extrinsic and intrinsic mechanisms through which the egg protects itself from the microbial invasion. Waxy shell membrane retards the entry of microorganisms. Further, the shell also prevents the entry of microorganisms. The membranes inside the shell behave as mechanical barriers to the entry of microorganisms. Further lysozymes present in the egg white is effective against gram-positive bacteria and avidin in the egg white forms a complex with biotin, thus making it unavailable for the microorganisms. Also high pH (pH 9-10) of albumin inhibits the microbial growth. Binding of riboflavin by the apo protein and chelation of iron by conalbumin further helps in hindering the growth of microorganisms that might have gained entry inside the egg.

Microflora in Rice Soils

Flooding of rice soils provides a favorable environment for anaerobic microbes and the biochemical changes are varied and numerous. However, a thin surface layer of lowland soil generally remains oxidized and sustains aerobic microbes. The main biochemical processes in flooded soil, however, can be regarded as a series of successive oxidation-reduction reactions mediated by different types of bacteria. Three major types of microbes are present in lowland rice soils in variable proportion.

- Obligate aerobes that grow only in the presence of molecular oxygen
- Obligate anaerobes that grow only in the absence of oxygen
- Facultative anaerobes that can grow either with molecular oxygen or an aerobically when supplied with a suitable electron acceptor other than molecular oxygen

Results of studies in Japan, India, Egypt and the Philippines suggest that bacteria predominate in flooded soils, whereas fungi and actinomycetes are more abundant in upland soils. Bacteria such as *Mycobacteria*, *Bacillus*, *Pseudomonas* and other biologically active bacteria are present in greater numbers in the rhizosphere than in the soil farther away. Some aerobic microbes including fungi, nematodes, yeast and protozoa have occasionally been found inside the root tissue of the rice plant. Flooding causes changes in the character of the microbial flora in soils.

There are many kinds of nitrogen fixing microbes in lowland rice soils. A study in Thailand identified *Azotobacter* (0-10^4/g soil), *Beijerinckia* (0-10/g soil), *Clostridia* (10^4 – 10^6/g soil), non sulphur purple bacteria (10^2-10^6/g soil) and blue green algae (10^2-10^3/g soil). The population density of these nitrogen fixers depends primarily on specific soil properties such as pH, organic matter content and available phosphorus.

Factors affecting microbial growth in food

Intrinsic factors

These are inherent in the food. They include pH, moisture content and nutrient content of the food, antimicrobial substances and biological structures.

pH (Hydrogen ion concentration)

The most of the bacteria grow best at neutral or weakly alkaline pH usually between 6.8 & 7.5. Some bacteria can grow within a narrow pH range of 4.5 and 9.0 eg: *Salmonella*. Other microorganisms especially yeast and molds and some bacteria grow within a wide pH range, eg: molds grow between 1.5 to 11.0, while yeasts grow between 1.5 and 8.5.

The microorganisms that are able to grow in acid environment are called acidophilic microorganisms. These microorganisms are able to grow at pH of around 2.0. Yeasts and molds grow under acid conditions. Other microorganisms such as *Vibrio cholerae* are sensitive to acids and prefer alkaline conditions. Most bacteria are killed in strong acid or strong alkaline environment except *Mycobacteria*.

The minimum and maximum pH for growth of some specific microorganisms are given below

Microorganisms	**Minimum**	**Maximum**
E. coli	4.4	9.0
Salmonella enterica serovar Typhi	4.5	8.8
All bacteria	4.0	9.0
Molds	1.5	11.0
Yeast	1.5	8.5

The pH values of some food products are given below

Food type	**Range of pH values**
Beef	5.1-6.2
Chicken	6.2-6.4
Milk	6.3-6.8
Cheese	4.9-5.9
Fish	6.6-6.8

Fruits	<4.5 (most <3.5)
Vegetables	3.0-6.1

Moisture Content

Water activity (a_w) is a term describing the availability of water to microorganisms. It is only roughly related to percent moisture. Pure water has an a_w of 1.00.

The water activity of some food products are given below

Food product	**a_w**
Raw meat and milk	0.99-1.0
Luncheon meat	0.95
Boiled ham, sliced bacon	0.90
Dried grains	0.80

The water activity limits for growth of principal food borne disease organisms are given below

Microorganisms	**Minimum a_w for growth**	**Reference**
Salmonella	0.945	Christian and Scott, 1953
C. botulinum	0.95	Scott, 1957
C. perfringens	0.93	Kang *et al*.,1969
Vibrio parahaemolyticus	0.94	Benchat, 1974

Certain molds and bacteria can grow on fish immersed in saturated salt solution where the a_w is about 0.75. Some molds can grow in foods with a_w 0.62 -0.65. At these lower limits, growths are very slow. The a_w of fully dried foods, such as crackers or sugar, is about 0.10 and such products are microbiologically stable because of this factor alone. The stability of intermediate moisture foods (a_w 0.75-0.90), such as dried fruits, jams and soft moist pet foods depends on combinations of factors, such as low a_w, low pH, pasteurization, chemical additives and impervious packaging.

Oxygen

The oxygen is essential for growth of some microorganism, these are called aerobes. Others can't grow in its presence and are called anaerobes. Still others can grow either with or without oxygen and are called microaerophilic. Strict aerobes grow

only on food surfaces and can't grow in foods stored in cans or in other evacuated, hermetically sealed containers. Anaerobes grow only beneath the surface of foods or inside containers. Aerobic growth is faster than anaerobic. Therefore, in products where both conditions exist, such as in fresh meat, the surface growth is promptly evident, whereas sub surface growth is not.

Lethal effects of Temperature

Heat is the most practical and effective means to destroy microorganisms. Microbial cell reduction occurs slowly just above maximal growth temperatures. However, the rate of death increases markedly as the temperature is raised. Pasteurization, the destruction of vegetative cells of disease producing microorganisms, consists of a temperature of 140^0F for 30 minutes, or about 161^0F for 16 seconds. Yeasts, molds and the vegetative cells of spoilage bacteria also die at pasteurization temperatures. To render long-acid foods commercially sterile requires a retort capable of operating at temperatures above 212^0F. The rate of thermal destruction is greater in foods with high a_w than in those with low a_w. Microbial contaminants in dry foods, such as chocolate or dried bone meal are hard to destroy with heat.

Chilling to temperature below the growth range, but above freezing, stops reproduction but kills few cells except for extremely sensitive organisms, such as vegetative cells of *C. perfringens*. Freeze kills part of a microbial population within a few hours and storage continues to be lethal at a much slower rate. The most rapid drop in aerobic plate count (total count) occurred in orange juice, which is an acid product. Bacterial spores die very slowly, if at all, during freezing and frozen storage. For example, the vegetative cells of *C. perfringens* generally all die, but the spores survive. *S. aureus* and related organisms survive well, but in most cases, there is wide variation of susceptibility among microorganisms, even among closely related species. In any case freezing is not a dependable means to destroy microorganisms since some cells of the original population almost always survive.

Some psychrotrophic microorganisms grow very slowly in foods below freezing, but usually not below 19^0F. There are a few reports of growth, usually of molds, at 14^0F, but no reliable reports of growth below that temperature. This means that the standard storage temperature for frozen foods, 0^0F does not permit microbial growth. However many microorganisms survive freezing. Most psychrotrophs have difficulty growing above 90^0F. Most foodborne disease organisms are mesophiles.

In the temperature range where both mesophilic and psychrotrophic organisms grow (about 41^0F to 90^0F), the psychrotrophs grow more rapidly causing spoilage and at the same time frequently interfering with the growth of foodborne disease organisms.

Nutrient content of the food

Microorganisms require proteins, carbohydrates, lipids, water, energy, nitrogen, sulphur, phosphorus, vitamins and minerals for growth. Various foods have specific nutrients that help in microbial growth. Foods such as milk, meat and eggs contain a number of nutrients that are required by microorganisms. These food are hence susceptible to microbial spoilage.

Antimicrobial substances

Antimicrobial substances in food inhibit microbial growth. Various foods have inherent antimicrobial substances that prevent microbial attack. Such inhibitors are like lactinin and anticoliform factors in milk and lysozyme in eggs.

Biological Structures

Some foods have biological structure that prevents microbial entry. For example, meat has fascia, skin and other membranes that prevent microbial entry. Eggs have shell and inner membranes that prevent yolk and egg white from infection.

Extrinsic factors

These are factors external to the food that affects microbial growth. They include

- Temperature of storage
- Presence and concentration of gases in the environment
- Relative humidity of food storage environment

Temperature

Temperature is the most efficient means to control microbial growth. The growth of microorganisms are affected by the environmental temperature. Various microorganisms are able to grow at certain temperature and not others. Bacteria can therefore be divided into the following groups depending upon their optimum temperature of growth.

Psychrophilic microorganism

These grow best in the temperature range of 0-15^{0}C but also down to -10^{0}C in unfrozen media. They can cause food spoilage at low temperature. Several of the microorganism found in the soil and water belong to this group. Whereas psychrotrophs thrive between 4^{0}C and 25^{0}C.

Mesophilic bacteria

These organisms grow between 25^0C and 40^0C, with an optimum growth temperature close to 37^0C. Some such as *Pseudomonas aeruginosa* may grow at even lower temperature between 5-43^0C. None of the mesophilic bacteria are able to grow below 5^0C or above 45^0C. Most pathogenic bacteria belong to this group.

Thermophilic bacteria

These grow at temperature above 45^0C. Often their optimum growth temperature is between 50^0C and 70^0C. Growth of some bacteria occurs at 80^0C. Bacteria in this group are mainly spore formers and are of importance in the food industry especially in processed foods.

Presence and Concentration of gases in the environment

This relates to the presence and concentration of gases in the food environment. Various microorganisms require for growth either high oxygen tension (aerobic), low oxygen tension (microaerobic) or absence of oxygen (anaerobic). Some microorganisms may grow either in high oxygen tension, or in the absence of oxygen (facultative anaerobes).

Foods affected by various groups

- Anaerobic or facultatively anaerobic spore formers are most likely to grow in canned foods.
- Microaerophilic bacteria are most likely to grow in vacuum packed foods since they have low oxygen tension.
- Aerobic bacteria are likely to grow on the surface of raw meat
- Aerobic molds will grow in insufficiently dried or salted products

Relative humidity

Relative humidity is the amount of moisture in the atmosphere or food environment. Foods with low water activity placed at high humidity environment take up water, increase their water activity and gets spoiled easily. For example, dry grains stored in an environment with high humidity will take up water and undergo mold spoilage.

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Chapter 2
Microbial Food Spoilage

The microbial food spoilage is one type of food spoilage that is caused by microorganisms. Food spoilage can define as the process in which the quality of the food deteriorates to some extent which is inconsumable for the person to eat. Food spoilage occurs as a result of the microbial attack, enzymatic digestion, chemical degradation, physical injury etc. The microbial food spoilage can be determined physically by the following method.

Change in appearance: The appearance of the food changes by the microbial attack, which forms cloudiness and liquid formation in the food.

Change texture: Texture changes occur as a result of slime formation due to an accumulation of microbial cells and tissue degradation.

Color change: Color changes due to the chlorophyll breakdown and by the growth of mycelia.

Change in taste in odor: The taste and odor of the food changes due to the oxidation of nitrogenous compounds, sulphides, organic acids etc.

Causes of microbial food spoilage

There are two common factors which favor the growth and multiplication of microorganisms, which include storage conditions of the food and chemical properties of the food.

Storage conditions of the food: The storage conditions basically involve two environmental factors like temperature, pH and oxygen that favors the microbial growth on food.

Temperature

Psychrophilic and psychrotrophic microorganisms have the ability to grow at 0°C. Psychrotrophic microorganisms have a maximum temperature for growth above

Deepa I, *Food and Dairy Biotechnology*,
https://doi.org/10.34256/ioriip2012

20°C and are widespread in natural environments and in foods. The temperature above this refers as "Mesophilic temperature" which is the most favorable for the microbial growth. A mesophile is an organism that grows best in moderate temperature, neither too hot nor too cold, typically between 20 and 45°C. Therefore the warm temperature is optional for microbial growth like mesophilic and thermophilic microorganisms. A thermophile is an organism—a type of extremophile—that thrives at relatively high temperatures, between 41 and 122°C.

Oxygen

There are aerobic and anaerobic microorganisms which attack the food in storage conditions either in presence or absence of O_2. Aerobic storage conditions favor the aerobic bacteria and molds. If there are anaerobic storage conditions then it will favor the growth of anaerobic bacteria like *Clostridium* sp.

Chemical properties of the food

Chemical properties are another major factor which causes spoilage due to the food's own chemical properties. The chemical properties of the food that influences the microbial growth.

Chemical composition of the food

In food, certain organic biomolecules like protein, carbohydrates, and fats are present which are necessary for the microbial growth.

- Protein-rich foods

  In protein-rich foods, the microorganisms which attack are "proteolytic microorganisms". The proteolytic enzyme causes the degradation of protein into simpler forms like amino acids, amines etc. The proteolytic microorganisms include gram-negative, spore forming bacteria.

- Carbohydrate-rich foods

  In carbohydrate-rich foods, the microorganisms which attack are "carbohydrate fermenting microorganisms". Carbohydrate fermenting microorganisms causes the degradation of carbohydrate into the fermentative products by producing acids, alcohols and gases. The carbohydrate fermenting microorganisms include yeast, molds and bacteria (*Micrococcus* sp, *streptococcus* sp.etc).

- Fat rich foods

  In fat-rich foods, the microorganisms which attack are "lipolytic microorganisms". Lipolytic microorganisms causes the degradation of fat into simple forms like fatty acids, glycerol etc. The lipolytic microorganisms includes molds and some gram-negative bacteria.

- Acidity of the food

  The pH below 4.5 doesn't allow the subsequent bacterial growth and are affected mostly by yeasts and molds like in citrus fruits and vegetables. The high pH allows bacterial growth occurs mainly in the non-acid foods.

- Moisture and osmotic concentration of food

  In food, 13% of the free water favors microbial growth. High sugar and salt concentration prevent microbial growth. For the growth of molds, the required sugar concentration is 65-70%. For the growth of yeasts and bacteria, the required sugar concentration is 50%.

Classification of Food

Based on the spoilage, the food can categorize into 3 types.

Non perishable food: It has no water content and can be stored for a long time. These are having a long shelf life.

Semi perishable food: It has less water content and can be stored for some time. These are having a medium shelf life.

Perishable food: It has high water content and can't store for a long period. These are having a short shelf life. The high water or moisture content is a factor which will directly influence the microbial growth, as water promotes the growth of all living beings. Therefore, the food which is susceptible to the spoilage process refers to the perishable food.

Microorganisms involved in Food spoilage

There are commonly 3 kinds of microorganisms which causes food spoilage are as follows.

Yeast: These are the type of fungi which are single-celled and cause "fermentation of food". Yeasts are of two types namely true yeasts and false yeasts. The favorable condition for the yeasts to cause food spoilage are low pH and low moisture. True yeasts convert sugar into alcohol and carbon dioxide. False yeasts grow on the food surface as a dry film.

Mold: These are also the type of fungi, which are multicellular and produce a tough visible mass on the food surface refers as mold growth. These are aerobic organisms which require oxygen to grow, slightly acidic conditions, moisture, a temperature of 20-40^{0}C. They affect mostly the bread, cheese, meat etc.

Bacteria: These are organisms which cause food spoilage at low moisture, warm environment (5-60^{0}C), neutral acidity and in the presence of oxygen.

Process of microbial food spoilage

Microbial food spoilage involves the following steps

Microbes first attack the food: As the food contain all the nutrients required by the microorganisms, at a favorable temperature, pH, moisture, oxygen etc.

Food degradation: Microorganisms not only degrades the food material by utilizing the nutrients available in the food but also decompose the food material.

Decomposition: The enzymatic reaction occurs between the food components like protein, lipid, fat, carbohydrates etc. and the microbial enzymes which carry out some chemical changes.

Changes as a result of food decomposition

The changes appear in the form of appearance, texture, color, taste, odor etc as a result of spoilage.

Spoiled Food

Damages or injuries that make food undesirable for human consumption. Spoilage of food can be the result of

a) insect damage
b) physical injury
c) enzymatic degradation
d) microbial activity

Basic types of food spoilage

- **Appearance**
  - **Microbial Growth**
    - Mycelia or colonies visible on surface
    - Development of cloudiness in liquids
  - **Changes in food color due to heme or chlorophyll breakdown.**
    - Colony pigments, growth of mycelia etc.
- **Textural Changes**
  - Slime formation
  - Tissue softening due to enzymatic degradation
- **Changes in Taste and odor**
  - Development of nitrogenous compounds (Ammonia, amines etc),

sulphides and organic acid

The numbers and types of microorganism in a food are largely determined by

- Environment from which the food was obtained
- Microbiological quality of the food in its raw or unprocessed state (intrinsic factors)
- Handling and processing sanitation
- Effectiveness of packaging, handling and storage conditions in restricting microbial growth (extrinsic factors)

Fresh Meat

Chemical composition includes 75% water, 18% protein, 3% fat, 1% ash, traces of carbohydrate, vitamins etc.

1. Whole meats

The microflora of fresh meat is composed primarily of

1. Gram-negative aerobic rods such as *Pseudomonas*, *Acinetobacter* and *Moraxella*.
2. *Bacillus* and *Clostridia* (Eg:- *C. perfringens*) are also common on all types of meat.

   Although subsurface portions of meat are generally sterile, some parts such as lymph nodes may be heavily contaminated. Mechanic disruption of the tissue during processing can distribute microorganisms from the meat surface throughout the product. Fresh meats are among the most perishable foods. Several genera of molds grow on the surface of meat and can cause spoilage, but can't grow on meat stored below 5^0C. Usually fresh cut meats in the refrigerator at high humidity undergo bacterial spoilage by gram-negative aerobes like *Pseudomonas*, *Acinetobacter* and *Moraxella* spp. The intrinsic and extrinsic parameters of ground beef favor these bacteria so strongly that they are almost exclusive spoilage agents. Meat spoilage is characterized by the appearance of off odors and slime which are manifest when surface loads exceeds 10^7 (CFU/cm^2).

2. Ground Meats

Same microorganisms as whole meats, but always have higher microbial loads. Greater surface area which gives microbes better access to the food and also trap air to favor the growth of gram-negative aerobic bacteria like *Pseudomonas* spp. Every handling or processing (storage utensils, cutting knives, grinders) step can contribute additional contamination to the final product. One heavily contaminated

piece (eg: lymph node) can contaminate an entire lot when they are ground together.

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1. Slime development
2. Greening caused by microbial production of H_2O_2 or H_2S

H_2O_2 production in meat has been associated with several types of lactic acid bacteria (primarily *Lactobacillus*). The oxidant (H_2O_2) react with nitrosohemochrome (cured meat color compound) to form a green porphyrin compound. H_2S greening occurs in fresh meats that have been vacuum packaged and stored between 1-5^{0}C. H_2S react with myoglobin to form sulphmyoglobin in meats with a pH above 6.0. H_2S is produced by *Shewanella putrefaciens* and *Pseudomonas* spp. and some *Lactobacilli*. Off odors which result from the release of short chain fatty acids and the production of volatile compounds like acetoin, diacetyl and H_2S.

The type of spoilage bacteria that will dominate is influenced by several factors that include

1. Is the meat product raw or cooked

Cooked products have a higher pH (>6.0) which may allow growth of gram-negative facultative anaerobic pathogens like *Yersenia enterolitica*. Raw products have a pH of about 5.6 which favors the lactic acid bacteria, especially *Lactobacillus*, *Carnobacterium* and *Leuconostoc*.

2. Nitrite concentration in meat

High nitrite concentration favors lactic acid bacteria. Low nitrite levels may allow growth of *Brochothrix thermosphacta* (gram-positive rod, facultative anaerobe, growth at 0-30^{0}C from pH 5.0-9.0, catalase positive). *B. thermosphacta* is an important spoilage bacterium in anaerobically stored meats kept at low temperature, but the bacterium is inhibited by nitrite.

3. Processed meats (hot dogs, sausage and luncheon meats)

These products are composed of a variety of blended ingredients, any of which can contribute microorganisms to the food. Yeast and bacteria are the most common causes of spoilage, which is usually manifest in 3 ways.

a) **Slimy spoilage**: - Like other meat products, this occurs on the surface and is caused by the build up of cells of yeasts. *Lactobacilli*, *Enterococci* or *Brochothrix thermosphacta*. Washing the slime off with hot water can

restore the product quality.

b) **Sour spoilage**: - Results from growth of lactic acid bacteria (which originate from contaminated ingredients like milk solids) under the casing. These organisms ferment lactose and other carbohydrates in the product and produce organic acids. Taste is adversely affected but the product is not harmful if eaten.

c) **Greening due to H_2O_2 or H_2S production**: - Greening indicates more extensive product breakdown.

Cured meats (bacon, hams) are resistant to spoilage due to the

1. Use of nitrite/nitrate
2. Smoking or brining of hams
3. The high fat content (thus low a_w) of bacon

Instead spoilage of these products is often caused by molds from several genera including *Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus and Botrytis*.

Poultry Meat

Poultry meat like meat of other animals are also susceptible for contamination by various sources. Contamination of skin and lining of the body cavity take place during various processing operations. The organisms of great importance in poultry are *Salmonella* spp. and *Campylobacter jejuni*. Several gram-negative Psychrotropic bacteria viz; *Pseudomonas*, *Acinetobacter* and *Flavobacterium* have also been isolated from poultry carcasses. 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 (-18^0C or below).

Fish Spoilage

The spoilage in fish is accompanied by the changes in physical characteristics. Change in color, texture, odor, color of eyes, color of gills, softness of muscle, belly bursting are some of the characteristic of spoiled fish. The spoilage of fish is caused by enzymatic, bacterial and chemical action. The following factors contribute to spoilage of fish.

- High moisture content
- High fat content
- High protein content
- Weak muscle tissue
- Ambient temperature

- Unhygienic handling

Process of Spoilage

Fish is highly nutritive. It is tasty because of its constituents. The main components of fish are water, protein and fat. The spoilage process starts immediately after the death of fish. The process involves three stages.

1. Rigor mortis
2. Autolysis
3. Bacterial invasion and putrefaction

The rigor mortis is a physical effect on the muscle tissue of fish caused by chemical changes following the death. In live fish, its movements are controlled by chemical signals which cause the rhythmic contraction (stiffing) and relaxation of the muscles. This produces swimming action. After the death, the normal circulatory system breaks down and chemical signals leak into the muscle causing them to stiffen. This process is known as Rigor mortis. After the completion of rigor mortis, muscle stiffness gradually decreases accompanied by increase in pH, ending up in softening of muscle. This is followed by breakdown of proteins by enzymes. This process is called as autolysis. Thus autolysis can be described as an internal breakdown of the structure of the protein and fats due to a complex series of reactions by enzymes. Autolysis of protein starts immediately after rigor mortis and creates favorable conditions for the growth of bacteria.

Action of the Bacteria

The freshly caught fish will be almost free from bacteria but the surface slime, gills and intestine may contain considerable load of bacteria. When the fish is dead, these bacteria start attacking the fish causing spoilage and produce undesirable compounds. The nature and type of bacteria present in a fish depends upon the water from where it is caught and methods used for handling of the fish after its catch. The important changes brought out by the action of the bacteria in fish are

1. Reduction of TMAO (Trimethylamine N-oxide) to TMA (trimethylamine):- Marine fish contains a small percentage of odorless TMAO which is reduced to an offensive smelling TMA by the action of bacteria.
2. Breakdown of amino acids and formation of primary Amines:- The bacterial action of amino acids present in the fish muscle leads to formation of primary amines. Examples are formation of histamine from histidine, arginine from glutamic acid etc. This bacterial action may cause food poisoning in extreme cases.
3. Breakdown of Urea:- The high concentration of urea in the flesh of some fishes are degraded to ammonia by the microorganisms. The formation of

ammonia is accompanied by an offensive odor.

Chemical action

The most common chemical action which causes spoilage is the oxidative rancidity in the fatty fishes. The levels of peroxide value and free fatty acid content both a measure of oxidative rancidity are considered an index of quality of fat fishes.

Bacteria causing spoilage

At chilling Temperature: *Pseudomonas*, *Achromobacter*, *Flavobacterium*

At ordinary atmospheric temperature: *Escherichia*, *Serratia*, *Proteus*, *Sarcina* and *Clostridium*

At higher temperature: *Micrococcus* and *Bacillus*

Discolorations of fish

Yellow to greenish - *Pseudomonas fluorescence, Micrococcus* and others

Red or Pink - *Sarcina*, *Micrococcus, Bacillus*, or by molds and yeast

Chocolate-brown - Asporogenous yeast

Spoilage of Egg

Breaks or cracks in egg shell taking place due to transportation or mechanical damage may allow microorganisms to enter into the egg yolk and causes spoilage on storage. Eggs on storage may lose moisture and hence lose weight. The white of the egg becomes thinner and more watery on storage. The major changes in the egg take place due to spoilage organisms. In general the spoilage of eggs are caused by bacteria as compared to molds and can be described as green rot due to the growth of *Pseudomonas fluorescens*, colorless rot due to the growth of *Pseudomonas*, *Acinetobacter* and other species, black rots due to *Proteus*, *Pseudomonas*, red rots due to *Serratia* spp. and custard rots due to *Proteus vulgaris* and *Pseudomonas intermedium*. Growth of *Aeromonas* in the egg yolk turns it to black colour and also there is strong putrid odour due to formation of hydrogen sulphide (H_2S). Storage of eggs in high humid atmosphere may help in growth of several molds on the surface of the egg shell. Molds causing spoilage of eggs include species of *Penicillium*, *Mucor*, *Alternaria* etc.

Microbial Spoilage of Cereals

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.

Cereal grains and Flours

At initial stages, the grains are contaminated by *Pseudomonas*, *Micrococci*, *Lactobacillus* and *Bacillus*. The initial bacterial population may vary from 10^3 to 10^6 per gram while mold population may be more than 10^4 spores per gram.

Due to low moisture content grains and flour usually have long shelf life if these are properly harvested or stored under proper conditions as microbial growth is not supported. If due to any reason they attain moisture, the microbial growth may occur with molds growing at initial stages of moisture while yeasts and bacteria may grow with increasing moisture.

Spoilage of stored grains by molds is attributed to the following factors.

1. Types 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*, *Muco*r, *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, milling reduce the microbial content. 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 are essential for spoilage by microbes.

Spoilage of Bread

Bread is a major product prepared using flours. Dough 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 viz. moldiness and ropiness.

During bread making, it is baked at very high temperature, thereby there are 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* (bread mold), *Penicillium expansum*, *Aspergillus niger*, *Mucor* and *Geotrichum* also develop.

Ropiness in bread is usually due to bacterial growth and is considered more prevalent in homemade breads. The chief causative organism is *Bacillus subtilis* or *B. licheniformis*. These are spore forming bacteria surviving baking temperatures. Thus

spores can germinate into vegetative cells, once they get suitable conditions as heat treatment activates them. In ropiness, the hydrolysis of bread flour protein (gluten) takes place by proteinases. Starch is also hydrolysed by amylases, which encourage ropiness. The manifestation of ropiness is development of yellow to brown color and soft and sticky surface. It is also accompanied by odor.

Another type of spoilage of bread is chalky bread which is caused by growth of yeast like fungi. *Endomycosis fibuligera* and *Trichosporon variable*. This spoilage is 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 bacteria *Serratia marcescens*. This organisms 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.

Spoilage of cooked Rice

The biggest problem with detecting food spoilage in cooked rice is that there are no signs at all. It might taste, look and smell like it would normally but still be spoiled. The spoilage is caused by *Bacillus cereus*. Only some strains are harmful to humans by causing foodborne illness, while other strains can be beneficial as probiotics for animals. The spoilage of rice is so common that it ever has a own syndrome, "Fried Rice Syndrome", named by Luke Fisher as the bacteria is classically contracted from fried rice dishes that have been sitting at room temperature for hours.

B. cereus bacteria are facultative anaerobes and are able to produce protective endospores. These spores can survive if the rice is cooked in temperatures less than 100^0C. The problem occurs if the rice is improperly refrigerated, since germination and growth generally occur between 10-50^0C, though some strains are psychrotrophic. Cooked rice not meant for either immediate consumption or rapid cooking and refrigeration should be kept at temperature above 60^0C, which causes a problem since everybody knows cooked rice quickly dries out when left on the stove.

Bacterial growth results in production of enterotoxins, one of which is highly resistant to heat and to pH between 2 and 11. Ingestion leads to two types of illness diarrhoeal and emetic (vomiting) syndrome.

Uncooked Rice

It is usually easy to spot that it is bad. When the rice is not perfectly dry it means moisture found its way into the package and discarding the package seems to be the right thing to do. Funny or "off" order is a sign of spoilage as well.

Dairy Products

Raw milk flora may include all microorganisms found on the cowhide (which

include soil and fecal bacteria), udder and milking utensils. It can also include gram-negative and gram-positive bacteria, yeasts and molds. When properly handled and stored, the flora of pasteurized milk is primarily gram-positive bacteria.

Psychrotrophic *Pseudomonads* are common in bulk stored raw milk. They produce heat stable enzymes that can reduce milk quality and shelf life. Pasteurization kills most gram-negative (including *Pseudomonads)*, yeasts and molds. Some gram-negative enzymes, thermotolerant gram-positive bacteria and spores survive. Psychrotrophic *Bacillus* spp. are also common in raw milk.

Pasteurized Fluid Milk

Pasteurized fluid milk is generally spoiled by a variety of bacteria, yeasts and molds. In the past, milk was usually soured by LAB such as *Enterococci*, *Lactococci*, or *Lactobacilli*, which dropped the pH to 4.5 where milk proteins coagulate (curdling). Today milk is more frequently spoiled by aerobic spore formers such as *Bacillus*, whose proteolytic enzymes cause curdling. Molds may grow on the surfaces of spoiled milk, but the product is usually discarded before this occurs.

Butter

High lipid content and low a_w make it more susceptible to surface mold growth than to bacterial spoilage. Some *Pseudomonads* can be a problem; "surface taint"- putrid smell caused by the production of organic acids from *P. putrefaciens*. Rancidity due to butterfat lipolysis caused by *P. fragi* are common.

Cottage Cheese

It can be spoiled by yeasts, molds and bacteria. The most common bacterial spoilage is "slimy curd" caused by *Alcaligenes* spp. (gram-negative aerobic rod found in soil, water and intestinal tract of vertebrates). *Penicillium*, *Mucor* and other fungi also grow well on cottage cheese and imparts stale or yeasty flavors.

Ripened cheese

Low pH, low a_w and high salt inhibit most spoilage microorganisms except surface mold growth. Spores of *C. butyricum*, *C. sporogenes* and others can germinate in cheeses (eg: Swiss) with intrinsic properties that are less inhibitory (eg: lower salt, higher pH). These organisms may metabolize citrate, lactose, pyruvate or lactic acid and produce butyrate or acetate plus CO_2 or H_2 gas which "blows" the cheese.

Bakery Goods

These products are characterized by a low a_w, which when stored properly

under low humidity, restricts all microorganisms except molds. *Rhizopus stolonifer* is the common bread mold, and other species from this genus spoils baked goods. Refrigerated frozen dough products have more water and can spoiled by Lactic acid bacteria.

Fermented Foods and Beverages

The low pH or ethanol content of these products does not allow growth of pathogens, but spoilage can occur. Beer and wine (pH 4-5) can be spoiled by yeasts and bacteria. Bacteria involved are primarily lactic acid bacteria like *Lactobacilli* and *Pediococcus* spp; and acetic acid bacteria like *Acetobacter* and *Gluconobacter* spp. Acetic acid bacteria convert ethanol to acetic acid in the presence of oxygen. The anaerobic bacterium *Megasphaera cerevisiae* can also spoil beer by producing isovaleric acid and H_2S.

Spoilage in packaged beer is often due to growth of the yeast *Saccharomyces diastaticus*, which grows on dextrins that brewer's yeast can't utilize. *Candida valida* is the most important spoilage yeast in wine. In either case, spoilage by yeasts results in the development of turbidity, off- flavors and odors.

Wines can also be spoiled by lactic acid bacteria which are able to convert malic acid to lactic acid (malo-lactic fermentation). This reduces the acidity of the wine and adversely affect wine flavor.

Microbial Spoilage of Canned Foods

Canning is one of the important methods of packaging food for long term storage. Normally food is stored in metallic containers along with heat treatment. The heat treatment varies depending upon the type of food. There is always a chance that microorganisms may survive if the heat treatment is not proper thereby leading to spoilage of food. Usually the incidences of food spoilage in cans are low. The spoilage of can could be due to biological or chemical reasons or combination of both. The biological spoilage is primarily due to microbial growth while chemical spoilage is due to hydrogen produced due to reaction of acid in food and iron on can. The degree of swelling can also be increased by high summer temperature and high altitudes.

Causes of Spoilage in Cans

Chemical Spoilage

The chemical spoilage in most cases is due to the production of hydrogen gas produced in the can because of action of acid of food on iron of can. This spoilage is termed as hydrogen swell. Increased storage temperature, increased acidity of food, improper exhaust, presence of soluble sulfur and phosphorous compound are some of the factors responsible for the hydrogen swell.

Biological Spoilage

The causes of biological spoilage is the microbial activity. In heat treated cans, the growth of microorganisms occur due to leakage of cans and under processing.

Microbial spoilage of canned foods are classified as caused by thermophilic bacteria and mesophilic organisms. Most common spoilage of microbial origin are known as flat sour spoilage, putrefaction and Thermophilic Anaerobic spoilage (TA spoilage).

Spoilage by thermophilic spore forming bacteria

Spoilage by thermophilic spore forming bacteria is most prevalent in under processed heat treated canned foods. Their spores survive the heat treatment and undergo vegetative cell formation and subsequent growth in canned conditions. Major spoilage by these organisms are

Flat sour spoilage

This is caused by souring bacteria. One characteristic of this spoilage is that ends of can remain flat during souring. Because of this condition, the detection of spoilage from outside is not possible thereby culturing of contents become necessary to detect the type of organisms. Main organisms involved are *Bacillus*, while it occurs more frequently in low acid foods. *Bacillus* spp. has ability to produce acid without gas formation.

T A spoilage

This types of spoilage is caused by thermophilic anaerobe not producing hydrogen sulfide. *Clostridium thermosaccharolyticum* is the main organism involved. It produces acid and gas in foods. Spoiled food produces sour or cheesy smell.

Sulfur stinker spoilage

This type of spoilage occurs in low acid foods and primarily *Desulfotomaculum nigricans* is involved. The spores of these organisms are destroyed at optimal heat treatment, thus presence of this organism usually indicates under processing in terms of heat treatment. It produces H_2S which produce typical odor.

Spoilage by mesophilic spore formers

Bacillus and *Clostridium* are involved in this type of spoilage which is usually indicative of spoilage.

Spoilage by non-spore formers

The presence of non-spore formers in cans indicate post processing contamination. The organism whose vegetative cells are heat resistant are more readily found. *Enterococcus*, *Streptococcus thermophilus*, *Micrococcus*, *Lactobacillus*, *Leuconostoc*, *Microbacterium* are the more prominent organisms. Presence of these organisms indicates leakage of container. Cooling water is one of the important sources of contamination, thus Coliforms also gain entry into the can through leakage.

Spoilage by yeasts and fungi

Yeasts: Yeasts and their spores are not thermotolerant, thus they are not found in suitably heat treated cans. Their presence indicates under processing or post pasteurization contamination through leakage. Fermentative yeasts are more prominent and they produce CO_2, thus causing swelling of cans. Film yeasts too can grow on the surface of the food products.

Molds: Among mold, *Aspergillus* and *Penicillium* are the most spoiling organisms. These can grow at high sugar concentration. Acidification is considered method for the prevention of the growth of molds. Some of the molds are resistant to heat. Molds are more common in home canned foods where heating as well as sealing is not under total aseptic conditions.

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Chapter 3
Food Poisoning and Foodborne Diseases

Microorganisms that Causes Food Poisoning

Food poisoning actually is caused by microorganisms that are in the food that we eat and may not be destroyed by cooking. There are actually three different categories of these microorganisms that could cause food poisoning if proper food safety precautions are not followed.

The three types are bacteria, virus and parasite.

Bacteria

This is the most common type of food poisoning. This type of problem usually comes along when food has gone bad because it was not stored properly. The most common types of bacteria that cause food poisoning includes *Staphylococcus aureus, E. coli, Listeria monocytogenes, Salmonella*, *Campylobacter jejuni*. If foods are kept properly refrigerated, then these types of bacteria can usually be avoided unless there is a problem of contamination.

Virus

Virus are not as common as bacteria when it comes to food poisoning, but it is still a possibility. The main two types of foodborne viruses are Hepatitis A and Norovirus. Generally, these contaminate foods that have been grown in or near sewage. Food poisoning of this type usually results in a recall of the contaminated foods for the protection of the consumers.

Parasite

Generally, parasites are not as big of a problem for food poisoning, but they could happen. If an animal is contaminated when it is butchered for food, then the result could be contaminated meats. The two main types of parasite food poisoning would be *Cryptosporidium parvum* and *Giardia lamblia*. If meats are cooked properly, then there should be no issue of food poisoning from parasites.

Microbial toxins

Microbial toxins are toxins produced by microorganisms including bacteria and fungi. Microbial toxins promote infection and disease by directly damaging host

Deepa I, *Food and Dairy Biotechnology*,
https://doi.org/10.34256/ioriip2013

tissues and by disabling the immune system. Bacterial toxins are classified as exotoxins and endotoxins.

Endotoxins

Endotoxins are cell-associated substances that are structural components of bacteria. Most endotoxins are located in the cell envelope. Endotoxin refers specifically to the lipopolysaccharide (LPS) or lipooligosaccharide (LOS) located in the outer membrane of gram-negative bacteria. Although structural components of cells, soluble endotoxins may be released from growing bacteria or from cells that are lysed as a result of effective host defense mechanisms or by the activities of certain antibiotics. Endotoxins generally act in the vicinity of bacterial growth or presence.

Exotoxins

Exotoxins are usually secreted by bacteria and act at a site removed from bacterial growth. However, in some cases, exotoxins are only released by lysis of the bacterial cell. Exotoxins are usually proteins, minimally polypeptides that act enzymatically or through direct action with host cells and stimulate a variety of host responses. Most exotoxins act at tissue sites remote from the original point of bacterial invasion or growth. However some bacterial exotoxins act at the site of pathogen colonization and may play a role in invasion.

Bacterial protein toxins

Exotoxins are usually secreted by living bacteria during exponential growth. The production of the toxin is generally specific to a particular bacterial species that produces the disease associated with the toxin. (eg:- only *Clostridium tetani* produces tetanus toxin, only *Corynebacterium diphtheriae* produces disphtheria toxin). Usually virulent strains of the bacterium produce the toxin while non virulent strains do not, and the toxin is the major determinant of virulence (eg:- tetanus and disphtheria). At one time, it was thought that exotoxin production was limited mainly to gram-positive bacteria, but clearly both gram-positive and gram-negative bacteria produce soluble protein toxins. Bacterial protein toxins are the most powerful human poison known and retain high activity at very high dilutions.

Usually the site of damage caused by an exotoxin indicates the location for activity of that toxin. Terms such as enterotoxin, neurotoxin, leukocidin or hemolysin are descriptive terms that indicate the target site of some well-defined protein toxins. A few bacterial toxins that obviously bring about the death of an animal are known simply as lethal toxins. Some bacterial toxins are utilized as invasins because they act locally to promote bacterial invasion. Examples are extracellular enzymes that degrade tissue matrices of fibrin, allowing the bacteria to spread. This includes collagenase, hyaluronidase and streptokinase.

Some protein toxins have very specific cytotoxic activity. (ie, they attack specific types of cells). For examples, tetanus and botulinum toxins attack only neurons. But some toxins (as produced by *Staphylococcus*, *Streptococcus*, *Clostridia* etc) have fairly, broad cytotoxic activity and causes non specific death of various types

of cells or damage to tissues, eventually resulting in necrosis.

Protein exotoxins are inherently unstable. In time they lose their toxic properties but retain their antigenic one. This was first discovered by Ehrlich who coined the term "toxoid" for this product. Toxoids are detoxified toxins which retain their antigenicity and their immunizing capacity. The formation of toxoids can be accelerated by treating toxins with a variety of reagents including formaline, iodine, pepsin, ascorbic acid, ketones etc. The mixture is maintained at 37^0C at pH range of 6-9 for several weeks. The resulting toxoids can be used for artificial immunization against disease caused by pathogens. Toxoids are effective immunizing agents against diphtheria and tetanus that are part of the DPT (DTP). Bacterial protein toxins are strongly antigenic.

Foodborne intoxications or food poisoning is caused by ingestion of toxicants found as toxins of certain plants or animals, toxin formed by microbes while they multiply in the foods or after entering the intestine and poisonous substances that may be intentionally or incidentally added to foods during production, processing and transportation or storage. Toxicants or toxic substances in food are substances that are found in food that can produce harmful effects on ingestion by human and animals.

Bacterial intoxications

Staphylococcal poisoning

- Most common infection caused by *Staphylococcus aureus*
- Enterotoxins produced are heat stable
- Toxin causes gastroenteritis, symptoms appear within 1-6 hours of consuming contaminated food.
- The symptoms of Staphylococcal food poisoning includes nausea, vomiting and moderate diarrhoea. But usually no fever occurs.
- The disease usually lasts for less than 12 hours and never fatal
- The foods likely to be involved are Milk products, Custards, Processed meat, Creampuff, Sandwich, Poultry stuffing, Potato salad etc.
- The best preventive measures are to use sanitary precautions when preparing all perishable foods and refrigerate the food at temperature below 6-7^0C and food should not be allowed to stand for several hours at room temperature before serving.

Botulism

- Produced by the bacterium *Clostridium botulinum*
- It produces neurotoxin which is heat sensitive

- It is a food intoxication caused by the toxin secreted by the bacteria and not by the bacteria themselves.
- *C. botulinum* grows only in anaerobic condition, it grows in canned, smoked or cured food. It doesn't grow in fresh food.
- The food is easily contaminated by the spores of *Clostridium*. In improperly canned or preserved food, the spores grow and produce neurotoxin.
- While multiplying, it releases a powerful exotoxin called botulinum. It affects the nervous system. Hence it is called a neurotoxin.
- This toxin is a protein and is easily destroyed by heat (70^0C)
- Common foods implicated are fermented or smoked marine products, home cured ham, meat products etc.
- Disease starts within 2 hours to 14 days after ingestion of contaminated food
- The first sign of the disease is the paralysis of muscles of eye lid. This symptom appears in a few hours of eating the food.
- Next the paralysis affects the muscles of speech, swallowing becomes difficult. It is a neuroparalytic disease. Other symptom are blurred vision or double vision, dilated pupils, nausea, vomiting, headache, persistent constipation etc.
- Botulism can be prevented by killing *C. botulium* spores in the foods
    - During processing
    - Eliminating recontamination of processed food
    - Destroying the toxin by proper heating of processed food
    - By proper storage
    - By discarding the product that has developed signs of spoilage such as off odor, bulging of cans and gas bubbles on opening the can.

Bacillus cereus Poisoning

- Caused by *Bacillus cereus* infection
- Symptoms may appear within 15 minutes to 11 hours
- May cause nausea, abdominal pain or diarrhoea
- Common foods attributed are cereal dishes like rice, pudding, mashed potatoes, sauces, vegetable soups etc.
- Most of the out breaks are due to contamination of cooked rice

Preventive measures

- Proper hygiene before and after cooking
- Proper storage of food till usage. Rice and other hot foods have to be maintained at 65^0C and above till consumption. Other foods like milk and its products are to be stored below 7^0C.
- Avoid holding rice and other cooked preparation at room temperature for long periods
- Avoid frequent handling

Infantile gastroenteritis and Traveller's diarrhoea

These are caused by the enterotoxin produced by *E. coli* and *B. cereus*.

Mycotoxicosis

Food poisoning caused by the ingestion of fungal toxin. The fungal toxin is called mycotoxin.

- It is produced in the food in which the fungus lives
- The fungus *Aspergillus flavus* produces a toxin called aflatoxin. It causes hepatoma and carcinoma (cancer).
- *Penicillium rubrum* produced rubratoxin which affects the liver
- The mushroom *Amanita phalloides* produce amatoxin which causes liver damage and hypoglycemia.

Food infections

It is caused by ingestion of pathogenic microbes that penetrate the intestinal mucosa and multiply or migrate into other tissues where they multiply.

Salmonellosis (Typhoid)

- Disease caused by *Salmonella* food injection
- *Salmonella* is a gram-negative rod-shaped bacterium
- Infection occurs through contaminated food or domestic animals
- Salmonellosis is of two types namely enteritis and typhoid fever
- Enteritis is due to the existence of *Salmonella enteritidis* in the intestine. It produces a toxin called enterotoxin.
- The symptoms of enteritis include chills, head ache, nausea, vomiting, abdominal pain and several diarrhoea. Symptoms persists for 2-3 days. Mortality is low.

- Typhoid fever is also a kind of salmonellosis caused by *S. typhi* and *S. paratyphi*
- The symptoms of typhoid fever include fever, headache, abdominal tenderness, constipation and appearance of rose red spots on the body.
- In later stages, diarrhoea with 'pea soup' stools appear. In severe cases, there is haemorrhage in the intestine and perforation of the intestine leading to peritonitis.
- Salmonellosis can be prevented by avoiding consumption of contaminated food, destruction of *Salmonella* by heat, prevention of *Salmonella* growth by refrigeration etc.
- Salmonellosis can be treated by antibiotics like chloramphenicol, ampicillin, amoxycillin etc.

Shigellosis (Bacillary Dysentery)

- Caused by bacteria of genus *Shigella*
- It can be destroyed by heating
- Mostly caused by human to human to human transmission, contaminated water, milk and salad preparation.
- Incubation period ranges from 1-7 days
- Symptoms are bloody diarrhoea, fever, nausea and cramps
- *Shigella* infection in an intestinal disease caused by a family of bacteria known as *shigella*
- Children under age 5 are most likely to get *shigella* infection, but it can occur at any age

Vibrio parahaemoliticus Gastroenteritis

- Curved, rod-shaped, gram-negative bacterium found in brackish, salt water, which when ingested causes gastro intestinal illness in humans.
- Incubation period is 12-24 hrs
- Major symptoms are severe abdominal pain, vomiting and diarrhoea
- Sources of contamination are fish, shellfish, crab and shrimp
- Organisms is easily destroyed by heat

Enteropathogeic *E. coli* diarrhoea

- Enteropathogeic *E. coli* (EPEC) is a special kind of *E. coli* that lets it

attach to intestinal cells. Some types of EPEC may cause diarrhoea.

- Presence of *E. coli* in foods indicates faecal contamination
- It is a heat sensitive organism
- Pasteurization and normal cooking temperature are effective in destroying the organism
- Symptoms appear within 12-72 hrs
- Abdominal pain, diarrhoea, vomiting and fever are common
- Foods implicated are poultry, meat and dairy products
- It can be prevented by adopting strict personal hygiene and good sanitary practices

Hepatitis A

- It is a viral liver disease that can cause mild to severe illness
- It is caused by faecal contamination and spreads from an to man
- The hepatitis A virus (HAV) is transmitted through ingestion of contaminated food or water or through direct contact with an infected person.
- It has long incubation period 15-50 days
- Symptoms are fever, abdominal pain, headache and jaundice
- Prevention includes ensuring good personal hygiene of food handlers and avoiding eating foods if hygiene practised is doubtful.

Shellfish Poisoning

- Shellfish like Oysters, Mussels and Clams are generally bred in sewage polluted beds or brackish water.
- Poisoning occurs due to accumulated toxins produced by a dino-flagellate algae *Gonyaulax catenella* in the shellfish.
- Shellfish is also usually consumed under cooked or uncooked hence may have other pathogenic organisms. Poisoning is usually an emergency and needs medical advice at the earliest.

Other toxic infection

Some foodborne toxic infections are caused by ingestion of large number of enterotoxigenic bacteria which while multiplying in the intestine produce and release enterotoxins in the intestine which are responsible for the symptoms.

Clostridium perfringens Gastroenteritis

- Common in places where large number of people eat like in restaurants, institutional canteens, hospitals etc.
- *C. perfrigens* is a gram-positive, spore forming, anaerobic bacteria that is normally found in the intestine of human and animals.
- It is also a common cause of food poisoning when ingested in sufficient numbers
- *C. perfringens* is also known to cause other diseases, such as infections of the skin and deeper tissues. This is known as "Clostridial myonecrosis or gas gangrene" and also results from toxins produced by *C. perfringens*. Gas gangrene can occur when deep wounds are contaminated with foreign objects containing the bacteria.

Enterotoxigenic *E. coli* Gastroenteritis

- It is one of the chief causes of traveller's diarrhoea
- Occurs due to contaminated water and improper food handling

Cholera

- It is an infectious disease that causes severe watery diarrhoea; which can lead to dehydration and even death if untreated.
- It is caused by eating food or drinking water contaminated with *Vibrio cholerae*
- It is characterized by watery diarrhoea, extreme loss of fluid and electrolytes and severe dehydration. It can be fatal. Incubation period for cholera is few hours to 5 days.
- Symptoms include abrupt onset of vomiting and watery diarrhoea and dehydration. It may turn fatal if not promptly rehydrated.
- Cholera can be prevented by proper and safe disposal of sewage and supply of protected water.

Listeriosis

- Listeriosis is a serious infection usually caused by eating food contaminated with the bacterium *L. monocytogenes*.
- *L. monocytogenes* is commonly found in soil, stream water, sewage, plants and food
- It mostly occur in pregnant women, newborn children and in people whose immune system is compromised. The disease could be fatal.
- Milk and dairy products, contaminated sea foods, vegetables and salads

are implicated

- Proper cooking and hygienic food handling can prevent the infection

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Chapter 4
Food preservation

Food preservation refers to any one of a number of techniques used to prevent food from spoiling. The oldest methods of preservation are drying, refrigeration and fermentation. Modern methods include canning, pasteurization, freezing, irradiation and the addition of chemicals. Advances in packaging materials have played an important role in modern food preservation.

Methods of preservation used to extend shelf life includes removal of moisture, temperature control, pH control, use of chemical preservatives, irradiation etc.

Historical methods of food preservation

Primitive and tedious methods used for food preservation are

Drying

Used to preserve fruit, vegetables, meat and fish. Mainly used in the South-warmer climate. Causes the loss of many natural vitamins and texture.

Salting

Used extensively for pork, beef, and fish. Done mainly in cool weather followed by smoking.

Sugaring

Used to preserve fruits for the winter. Jams and Jellies. Expensive because sugar was scarce commodity in early America.

Pickling

Fermenting, used to preserve vegetables. Use of mild salt and vinegar brine. It increases the salt content and reduces the vitamin content of the food. Oldest form of food preservation.

Cold storage

Used extensively in the northern U.S. Root cellars were used to store vegetables at 30-40^{0}F. Root cellars were replaced by ice boxes in the mid 1800's.

Deepa I, *Food and Dairy Biotechnology*,
https://doi.org/10.34256/ioriip2014

Current preservation methods

Physical	Chemical	Biological
Chilling and cooling	Preservatives	
Freezing	eg: benzoates	Fermentation:
Blanching/cooking	Nitrites	Alcoholic
Pasteurization	Sugar	Acetic
Canning	Salt	Lactic
Freezing	Spices	
Drying/Dehydration	Additives	
Separation/Filtration	eg: antioxidants	
Concentration		
Irradiation		
Modified/Controlled Atmosphere packaging		

Physical methods of preservation

Methods of preserving an array of local products for use throughout the year have been based on traditional methods. More sophisticated techniques such as irradiation may also extend shelf life mainly by the destruction of enzymes and the inactivation of microorganisms.

Chilling and Cooling

Chilling may be referred to as the process that lowers the food temperature to a safe storage temperature between 0^0Cand 5^0C, whereas cooling is a more general term applied to the lowering of a food temperature. Chilled foods can potentially present a greater risk to public safety than frozen foods. Keeping products at a low temperature reduces the rate of microbiological and chemical deterioration of the food. In most processed chilled foods, it is the microbial growth that limits the shelf life, even the slow growth rates that occur under chilled conditions will eventually result in microbial levels that can affect the food or present a potential hazard. This microbial growth can result in the spoilage of the food (it may go putrid or cloudy or show the effects of fermentation), but pathogens if present, may have the potential to grow and may show no noticeable signs of change in the food.

Freezing

Freezing of food doesn't render it sterile, although it can reduce the levels of some susceptible microorganisms that is not significant in the context of the overall microbial equality of the food. Once a frozen food is defrosted, those viable microorganisms present will grow and multiply.

Rapid freezing in blast freezers is desirable to prevent the formation of large ice crystals that will tend to adversely affect the texture of the food by disrupting cell integrity in fruits and vegetables or degrading the muscle proteins of meat, fish and poultry. Apart from enzymatic activity, there are many other chemical and physical changes which may limit the shelf life of frozen food, examples include fat oxidation and surface drying both of which may occur over a period of months depending on the food.

Damage to tissue may also result from ice crystals, particularly in the case where slow freezing occurred. For example, in a domestic freezer. In commercial freezers, where temperature of -40^0C and below are maintained, freezing of the product takes place quickly and the shelf-life is even longer.

Heat Preservation

Microorganism and enzymes are the major causes of undesirable changes in foodstuffs. Both of them are susceptible to heat, and appropriate heating regimes can reduce, inhibit or destroy their activity. The degree of heat treatment required to produce a product of acceptable stability will depend on the nature of the food, its associated enzymes, the number and types of microorganisms, the conditions under which the processed food is stored and other preservation techniques used.

Blanching

Blanching is a process designed to inactivate enzymes and is usually applied immediately prior to other thermal preservation processes either using high temperatures (eg: thermal processing) or low temperatures (eg: freezing). It does not reduce the microbial population on the surface of foods, but it reduces the number of organisms of lower heat resistance such as yeasts, molds and certain bacteria (eg: *Listeria*, *Salmonella*, *E. coli*). Without a blanching step, the shelf life of frozen vegetables would be substantially reduced as a results of chemical break down during storage.

In thermal processing of fruits and vegetables, the objective of blanching is to prevent further enzymatic break down of the foods if delays occur prior to processing the foods. It is mainly used for vegetables by heating the food with steam or hot water to 180-190^{o}F and cooling in ice water, which prevents bacteria from growing.

During hot water blanching, some soluble constituents are leached out: water-soluble flavors, vitamins (vitamin C) and sugars. With potatoes this may be an advantage as leaching out of sugars makes the potatoes less prone to turning brown.

Blanching is a delicate processing step. Time, temperature and the other conditions must be carefully monitored. Sodium bicarbonate is added to the blanching water when okra, green peas and some other green vegetables are blanched. The chemical raises the pH of the blanching water and prevents the fresh green color of chlorophyll being changed into pheophytin which is unattractive brownish green.

If products are over-blanched (boiled for too long) they will stick together on the drying trays and they are likely to have a poor flavor. Green beans, carrots, okra, turnip and cabbage should always be blanched. The producer can choose whether or not potatoes need blanching. Blanching is not needed for onions, leeks, tomatoes and sweet peppers. Tomatoes are dipped into hot water for one minute when they need to be peeled but this not blanching. As a rule fruit is not blanched.

Benefits of blanching

1. It helps clean the material and reduce the amount of micro organisms present on the surface
2. It preserves the natural color in the dried products
3. It shortens the soaking and/or cooking time during reconstitution
4. Destroys enzymes in the food.

Pasteurization

Pasteurization is a process in which water and certain packaged and non packaged foods (such as milk and fruit juice) are treated with mild heat, usually to less than 100°C (212°F) to eliminate pathogens and extend shelf life.

The actual degree of heat process required for an effective pasteurization will vary depending on the nature of the food and the types and numbers of microorganisms present. Milk is the most widely consumed pasteurized food, and the process was first introduced commercially in the UK during the 1930s, when a treatment of 63°C for 30 minutes was used. Modern milk pasteurization uses an equivalent process of 72°C for 15 seconds.

Pasteurization is used extensively in the production of many different types of food, including fruit products, pickled vegetables, jams and chilled ready meals. Food may be pasteurized in a sealed container (analogous to a canned food) or in a continuous process (analogous to an aseptic filling operation). It is important to note that pasteurized foods are not sterile and will usually rely on other preservative mechanisms to ensure their extended stability for the desired length of time. Once the food product is exposed to temperatures of 60-70°C, microbial growth stops and enzyme inactivation starts. As the temperature is increased (80-90°C), the vegetative forms of microorganisms are destroyed and the rate of enzyme inactivation increases. Heat processing of acid products, such as fruits and fruit juices is usually done at higher temperature (100°C), for short times (10-15 seconds).

Heat processing requirements - dependent on product acidity

Acidity class	pH value	Food item	Heat and processing requirements
Low acid	6.0	Peas, carrots, beets, potatoes, asparagus, poultry, meat, sea foods, milk etc.	High temperature processing 116-121°C (240-250°F)
	5.0	Tomato soup	
Medium acid	4.5	Tomatoes, pears, apricots, peaches	Boiling water processing 100°C (212°F)
Acid	3.7	Jams, sauces, fruits, Sauerkraut, apple,	Temperature of 93-100^{o} C, (200-212^{o} F)
High acid	3.0	Pickles	

Canning

Frenchman Nicholas Appert is credited as the further of modern-day canning. It is a preservation method involves placing foods in jars or similar containers and heating them to a temperature that destroys microorganisms that cause food to spoil. During this heating process air is driven out of the jar and as it cools a vacuum seal is formed. This vacuum seal prevents the air from getting back into the product bringing with it contaminating microorganisms. Canning is suitable for low and high acidic foods, prevents contamination and it also enhances the shelf life. Canning process involves the following steps.

- Cleaning usually involves passing the raw food through tanks of water or under high pressure water sprays, after which vegetable or other product is cut, peeled, cored, sliced, graded, soaked and pureed and so on.
- Almost all vegetables and some fruits require blanching by immersion in hot water or steam.
- The filling of cans are done automatically by machines
- The filled cans are then passed through a hot-water or steam bath in an exhaust box, this heating expands the food and drives out the remaining air.
- The exhausted cans are then immediately closed and sealed

- Sealed cans are then sterilized i.e., they are heated at temperatures high enough and for a long enough time to destroy all microorganisms.
- The cans are than cooled in cold water or air, after which they are labelled.

Canning methods

Hot water canning methods

Here the food jars are submerged in boiling water (212^0F at sea level) and cooked for a specified amount of time. Hot water canning can be used for high acidic foods such as fruits, pickled vegetables etc.

Pressure canning method

In pressure canning method, food jars are placed in 2-3 inches of water in a special pressure cooker. Which is heated to a temperature of at least 240^0F. This temperature can only be reached using the pressure method. Pressure canning can be used for low acidic foods such as metals, sea food, poultry, dairy products, vegetables etc.

Use of ionizing radiation

Food irradiation (the application of ionizing radiation to food) is a technology that improves the safety and extends the shelf life of foods by reducing or eliminating microorganisms and insects. There are 3 sources of radiation approved for use on foods. They are gamma rays, X-rays and electron beam.

Mode of action

- Affects on bacteria, yeasts & molds
- Main sites of damages: nucleic acids & lipids of the cell membrane
- Membrane lipid degradation
- Change the permeability of the cell membrane
- Inhibition of DNA replication
- Leach out of cell components.

Use of Non ionizing radiation

Microwave radiation

- Two frequencies used in food processing are 2450 MHz and 915 MHz
- Domestic microwave ovens use 2450 MHz which is less penetrating than the lower frequency.

Mode of action

- Microwave act indirectly on microorganism through the generation of heat
- Destruction of microorganism is accomplished through the denaturation of protein & nucleic acids.

Light energy in food preservation

UV radiation

- Used to inactivate microorganisms on the surface of foods and thin films of liquid
- Used extensively in disinfection of equipment, glassware & air
- The optimum wavelength is 260 nm

Mode of action

- UV light is absorbed by proteins and nucleic acids, in which photochemical changes are produced.
- It disrupts the DNA molecules, produce lethal mutations and thereby prevent cell replication.
- Degradation of bacterial cell wall also cause the germicidal effect

Physical methods of food preservation also involves Pulse electric fields and Modified atmosphere.

Pulse electric fields

- Involves the application of short pulses of high electric fields to foods placed between two electrodes.
- No significant detrimental effect on heat labile components present in foods such as vitamins.
- High initial investment is the major disadvantage of this method
- Gram-negative bacterial cells more sensitive than gram-positive or yeasts
- By increasing the intensity of electric field and number of pulses, greater microbial destruction can be achieved.
- Destruction of bacterial & fungal spores require a higher voltage & longer period of time.

Mode of action

- Pulse electric field causes the cell death by the disruption of cell

membrane function

- When microbial cells are exposed to pulse electric field, a potential difference occurs between outside &inside cell membrane. Because of this difference, pore formation occurs in the membrane, causes the destruction of membrane function & cell death.

Modified atmosphere

Three different procedures are used:

Modified Atmosphere Packaging (MAP)

Bulk or retail pack is flushed with a gas mixture usually containing a combination of carbon dioxide, oxygen and nitrogen. It does not require a control of gaseous environment during the entire storage period. The composition of the gaseous atmosphere changes during storage as a result of product & microbial respiration.

Controlled Atmosphere Packaging (CAP)

Here the atmosphere in the storage facility is altered and continually monitored the gas levels. A constant product environment is maintained throughout storage. It is used for long term storage of fruits & vegetables for maintaining their freshness.

Vacuum packaging

Air is removed from the packages and the packages are then sealed hermetically.

Mode of action

Growth of aerobes (mold, yeast, aerobic bacteria) are prevented in products. However anaerobic & facultative anaerobic bacteria can grow unless other techniques are used to control their growth.

Natural Food Preservatives

In the category of natural food preservatives comes the salt, sugar, alcohol, vinegar etc. These are the traditional preservatives in food that are also used at home while making pickles, jams and juices etc. Also the freezing, boiling, smoking, salting are considered to be the natural ways of preserving food. Coffee powder and soup are dehydrated and freeze-dried for preservation. In this section the citrus food preservatives like citrus acid and ascorbic acid work on enzymes and disrupt their metabolism leading to the preservation.

Sugar and salt are the earliest natural food preservatives that very efficiently drops the growth of bacteria in food. To preserve meat and fish, salt is still used as a natural food preservative.

Chemical Food Preservative

Chemical food preservatives are also being used for quite some time now. They seem to be the best and the most effective for a longer shelf life and are generally fool proof for the preservation purpose. Examples of chemical food preservatives are:

- Benzoates (such as sodium benzoate, benzoic acid)
- Nitrites (such as sodium nitrite)
- Sulphites (such as sulphur dioxide)
- Sorbates (such as sodium sorbate, potassium sorbate)

Antioxidants are also the chemical food preservatives that act as free radical scavengers. In this category of preservatives in food comes the vitamin C, BHA (butylated hydroxyanisole), bacterial growth inhibitors like sodium nitrite, sulfur dioxide and benzoic acid.

Then there is ethanol that is a one of the chemical preservatives in food, wine and food stored in brandy. Unlike natural food preservatives some of the chemical food preservatives are harmful. Sulfur dioxide and nitrites are the examples. Sulfur dioxide causes irritation in bronchial tubes and nitrites are carcinogenic.

Artificial Preservatives

Artificial preservatives are the chemical substances that stops of delayed the growth of bacteria, spoilage and its discoloration. These artificial preservatives can be added to the food or sprayed on the food.

Types of Artificial Preservatives Food

- Antimicrobial agents
- Antioxidants
- Chelating agent

In antimicrobial comes the Benzoates, Sodium benzoate, Sorbates and Nitrites. Antioxidants include the Sulfites, Vitamin E, Vitamin C and Butylated hydroxytoluene (BHT) Chelating agent has the Disodium ethylenediaminetetraacetic acid (EDTA), Polyphosphates and Citric acid

Harmful Food Preservatives

Although preservatives food additives are used to keep the food fresh and to stop the bacterial growth. But still there are certain preservatives in food that are harmful if taken in more than the prescribed limits.

Certain harmful food preservatives are:

Benzoates

This group of chemical food preservative has been banned in Russia because of its role in triggering allergies, asthma and skin rashes. It is also considered to cause the brain damage. This food preservative is used in fruit juices, tea, coffee etc.

Butylates

This chemical food preservative is expected to cause high blood pressure and cholestrol level. This can affect the kidney and live function. It is found in butter, vegetable oils and margarine.

BHA (butylated hydroxyanisole)

BHA is expected to cause the live diseases and cancer. This food preservative is used to preserve the fresh pork and pork sausages, potato chips, instant teas, cake mixes and many more.

Caramel

Caramel is the coloring agent that causes the vitamin B6 deficiencies, genetic effects and cancer. It is found in candies, bread, brown colored food and frozen pizza.

In addition to this there are many other harmful food preservatives. These are Bromates, Caffeine, Carrageenan, Chlorines, Coal Tar AZO Dies, Gallates, Glutamates, Mono- and Di-glycerides, Nitrates/Nitrites, Saccharin, Sodium Erythrobate, Sulphites and Tannin.

Preservatives Food Additives

All of these chemicals act as either antimicrobials or antioxidants or both. They either inhibit the activity of or kill the bacteria, molds, insects and other microorganisms. Antimicrobials, prevent the growth of molds, yeasts and bacteria and antioxidants keep foods from becoming rancid or developing black spots. They suppress the reaction when foods comes in contact with oxygen, heat, and some metals. They also prevent the loss of some essential amino acids some vitamins.

Some common preservatives and their primary activity

Chemical Affected	Organism(s)	Action	Use in Foods
Sulfites	Insects & Microorganisms	Antioxidant	Dried Fruits, Wine, Juice
Sodium Nitrite	*Clostridia*	Antimicrobial	Cured Meats
Propionic Acid	Molds	Antimicrobial	Bread, Cakes, Cheeses
Sorbic Acid	Molds	Antimicrobial	Cheeses, Cakes, Salad Dressing
Benzoic Acid	Yeasts & Molds	Antimicrobial	Soft Drinks, Ketchup, Salad Dressings

Biological preservation

Biopreservation is the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life. Beneficial bacteria or the fermentation products produced by these bacteria are used in biopreservation to control spoilage and render pathogens inactive in food.

Lactic acid bacteria

Lactic acid bacteria have antagonistic properties which makes them particularly useful as biopreservatives. When lactic acid bacteria compete for nutrients, their metabolites often include active antimicrobials such as lactic acid and acetic acid, H_2O_2 & peptide bacteriocins. Some lactic acid bacteria produce the anti microbial nisin which is a particularly effective preservative. Lactic acid bacteria (LAB) & propionibacteria have been extensively studied for their efficacy against spoilage causing yeasts & molds in food spoilage.

Yeasts

In addition to LAB, yeasts also have been reported to have biopreservation effect due to their antagonistic activities relying on the competition for nutrients, production and tolerance of high concentration of ethanol, as well as the synthesis of a large class of antimicrobial compounds exhibiting large spectrum of activity against food spoilage microorganisms but also against plant, animal & human pathogen.

Bacteriophage

Bacteriophages are viruses which infect bacteria. Bacteriophages have recently received a Generally Recognised As Safe (GRAS) status because of their lack of toxicity & other detrimental effects to human health for application in meat products in U.S.A. Phage preparations specific for *Listeria monocytogenes*, *E. coli* 0157:H7 & *Salmonella enterica* serotypes have been commercialized and approved for

application in foods.

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Chapter 5
Microbes of Dairy Industry and Major Dairy Products

Products

The products synthesized from milk are called dairy products. Microorganism plays an important role in the manufacture of dairy products. Each dairy product is produced with the help of selected microorganisms. These microorganisms are known as starter.

Starter culture

A starter culture is a microbial preparation used in the initiation of fermentation for the preparation of fermented food and drinks. It consists of culture medium and microorganisms. A small amount of curd added to the milk for converting it into curd is an example for starter culture. The microorganisms include bacteria, yeast and mold. Starter culture is of two types. They are simple starter and mixed starter. A simple starter contains a single strain of microorganisms. Mixed starter contains more than one strain of microorganism. Based on the optimum temperature, the starter is classified into 2 types. They are mesophilic starter and thermophilic starter. Mesophilic starter contains mesophilic bacteria. Mesophilic bacteria grow in moderate temperature around 30^0C Examples of mesophilic bacteria are *Lactococcus lactis* subsp. *cremoris*, *Lactococcus delbrueckii* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris*. Thermophilic starter contains thermophilic bacteria. They prefer warmer conditions around 42^0C. *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *lacti*s, *Lactobacillus delbrueckii* subsp. *bulgaricus* are the examples of thermophilic bacteria.

Different strains of fungi and bacteria are used for the fermentation of milk in order to produce a wide variety of dairy products viz. curd, yogurt, cheese, kumiss, kefir etc. The main bacteria are lactic acid bacteria that are used for milk coagulation and these can be processed for diverse products.

Fermented milk products are classified into viscous products, beverage products and carbonated products. The primary function of fermenting milk was to extend its shelf life. Some of the fermented milk products are acidophilus milk,

Deepa I, *Food and Dairy Biotechnology*,
https://doi.org/10.34256/ioriip2015

cultured buttermilk, kefir, kumiss, sour cream, villi etc. Yogurt and cheese are also fermented milk products.

Kefir

Kefir is a fermented milk drink, traditionally made using cow's milk or goat's milk. By the addition of kefir grains to milk kefir can be prepared. These are not cereal grains, but grain-like colonies of yeast and lactic acid bacteria that resembles a cauliflower in appearance.

This drink originated in the Caucasus, Eastern Europe and Russia. During the fermentation process changes in the composition of ingredients occur. Lactose, the sugar present in milk is broken down mostly to lactic acid (25%) by the lactic acid bacteria, which results in acidification of the product. *Propionibacteria* further break down some of the lactic acid into propionic acid. Other substances that contribute to the flavor of kefir are pyruvic acid, acetic acid, diacetyl and acetoin, citric acid, acetaldehyde and amino acids resulting from protein break down. The slow-acting yeasts late in the fermentation process, break down lactose into ethanol and carbon dioxide. Depending on the process, ethanol concentration can be as high as 1-2% with the kefir having a bubbly appearance and carbonated taste. This makes kefir different from yogurt and most other sour milk products where only bacteria ferment the lactose into acids.

Kumiss

It is a fermented dairy product traditionally made from Mare's milk. Mare's milk has higher sugar content than cow's and goat's milk and as a result kumiss has a slightly higher alcohol content than kefir. Nowadays, cow's milk is generally used for kumiss, with the addition of sugar to better approximate the composition of mare's milk.

Sour Cream

Sour cream or soured cream is a dairy products obtained by fermenting regular cream with lactic acid bacteria. The bacterial culture which is introduced either naturally or deliberately, sours and thickens the cream. Its name comes from the production of lactic acid by bacterial fermentation, which is called souring.

Buttermilk

Buttermilk is a fermented dairy drink. Traditionally buttermilk is the liquid that is leftover after churning butter. True buttermilk ferments naturally into a thick, tangy cream. These days, buttermilk is usually made by introducing a bacterial culture

to low-fat milk and then heating the mixture.

The starting ingredient for buttermilk is skim or low-fat milk. The milk is pasteurized at 82-88^{0}C for 30 minutes or at 90^{0}C for 2-3 minutes. This heating process is done to destroy all naturally occurring bacteria and to denature the protein in order to minimize wheying off. The milk is then cooled to 22^{0}C and starter cultures of desirable bacteria such as *streptococcus lactis*, *S. cremoris*, *Leuconostoc citrovorum*, and *L. dextranicum* are added to develop buttermilk's acidity and unique flavor. These organisms may be used singly or in combination to obtain the desired flavor. The ripening process takes about 12-14 hrs.

Cultured Buttermilk

Cultured buttermilk was first commercially introduced in the United States in the 1920s. Commercially available cultured buttermilk is milk that has been pasteurized and homogenized and then inoculated with a culture of *Lactococcus lactis* or *Lactobacillus bulgaricus* plus *Leuconostoc citrovorum* to stimulate the naturally occurring bacteria in the old-fashioned product. The tartness of cultured buttermilk is primarily due to lactic acid produced by lactic acid bacteria while fermenting lactose, the primary sugar in milk. As the bacteria produce lactic acid, the pH of the milk decreases and casein, the primary milk protein precipitates causing curdling or clabbering of milk. This process makes buttermilk thicker than plain milk. While both traditional and cultured buttermilk contain lactic acid, traditional buttermilk tends to be less viscous, whereas cultured buttermilk is more viscous. Condensed buttermilk and dried buttermilk remain important in the food industry.

Acidified Buttermilk

It is a substitute made by adding a food-grade acid such as vinegar or lemon juice to milk. It can be produced by mixing 1 table spoon of acid with 1 cup of milk and letting it sit until it curdles, about 10 minutes. In the process used to produce paneer, such acidification is done in the presence of heat.

Curd (Dahi)

Indian curd is a fermented milk product. It is a product obtained from pasteurized or boiled milk fermented by a harmless lactic acid bacteria or other bacterial culture which is retained from the previous days curd.

Lassie

Lassie is prepared by churning curd with water and ice. Ice facilitates the separation of fat globules during churning. Spices, salt or sugar may be added during

consumption. Lassie contains protein and phospholipids.

Butter

Butter may be defined as a fat concentrate obtained by churning cream or curd with or without salt. There are 5 types of butter. On the basis of presence or absence of salt, butter is of 2 types

- Salted butter
- Unsalted butter

On the basis of types of cream used for the manufacture of butter, it is classified into

- Sweet cream butter
- Sour cream butter
- Desi butter or Indigenous type of butter

Desi butter

It is prepared by churning sour cream (curd) to separate fat globules from the liquid portion. The liquid portion after separation of butter is called butter milk which is drained off. This butter has about 80% fat and small quantity of lactose and protein. This is mainly used for the preparation of ghee.

Ghee

It is a clarified butter fat derived from desi butter with no coloring matter. It contains 99.5% fat and 0.5% moisture.

Whey

The watery part of milk that remains after the formation of curds. Whey is full of probiotics and has a bracing acidity. Whey is the byproduct of the manufacture of cheese or casein. Sweet whey is a byproduct produced during the manufacture of rennet types of hard cheese like cheddar or Swiss cheese. Acid whey is a byproduct produced during the making of acid types of dairy products such as cottage cheese or strained yogurt.

Yogurt

It is produced by the bacterial fermentation of milk. Yogurt cultures are the

bacteria used to make yogurt. Lactose fermentation by these bacteria produces lactic acid, which acts on milk protein to give yogurt its characteristic tart flavor and texture. Cow's milk is most commonly used to make yogurt. The milk used may be homogenized or not, even pasteurized or raw. Yogurt is usually produced by using a culture of *Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus thermophilus* bacteria. Other *lactobacilli* and *bifidobacteria* are sometimes added during or after culturing yogurt. For producing yogurt, first milk is heated usually at about 85^0C to denature the milk proteins so that they do not form curds. After heating the milk, it is cooled to about 45^0C. The bacterial culture is mixed in, and that temperature of 45^0C is maintained for 4-12hrs to allow fermentation to occur.

Cheese

Cheese is also a dairy product produced in a wide range of flavors, textures and forms by the coagulation of milk protein casein. During the cheese production, the milk is usually acidified and coagulated by adding the enzyme rennet. The solids are separated and pressed into final form. Most cheeses melt at cooking temperature.

The basic steps in cheese making are

- Setting milk (adding starter cultures and coagulant to pre-warmed milk)
- Cutting the coagulum
- Cooking the cut coagulum (curd)
- Removing whey from the curd
- Allowing curd particles to "knit"
- Salting
- Pressing
- Ripening of the finished cheese

Fresh milk obtained from healthy cows or other animals should be cooled rapidly and then promptly delivered to the cheese factory. After the milk is commonly clarified with a centrifuge to remove small extraneous particles and somatic cells. The fat content of the clarified milk may be adjusted depending on the variety of cheese that is to be made. Some cheese is made from raw milk but it is more common to use heat treated or pasteurized milk. Heat treated milk is sometimes preferred because the resultant cheese tends to be more flavorful than that made from pasteurized milk.

Starter culture

One or several species of lactic acid bacteria are commonly added to pre-warmed milk. The small amount of acid produced by these bacteria early in the cheese making process (fermentation) facilitates subsequent clotting of milk by the coagulant. The kind of cheese to be made determines which microorganisms to add to milk. For

eg:- to make cheddar cheese one would use *Streptococcus cremoris* and/or *Streptococcus lactis*, the so-called mesophilic lactic acid bacteria. In contrast, to make Swiss cheese one would use *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, the so-called thermophilic lactic acid bacteria. microorganisms other than lactic acid bacteria sometimes are added together with then when cheese is made. Examples are *Propionibacterium shermanii* for Swiss cheese or molds for blue or camembert cheese. Currently concentrated frozen starter cultures can be purchased and added directly to milk in preparation for cheese making.

Coagulant

After the addition of starter culture, a suitable coagulant is added to milk usually a short time. The coagulant is an enzyme that splits colloidal casein into a carbohydrate-rich peptide fraction and the insoluble paracasein that precipitates in the presence of calcium ions. Traditionally, rennet extract obtained from the fourth stomach of young calves has been used as the coagulant. Recently rennet extract from mature cows and coagulants from fungi have also been used. The fungi *Mucor miehei*, *Mucor pusilus* and *Endothia parasitica* produce coagulants and are used in cheese making.

Cutting the coagulum

Rectangular frames with thin wires, horizontal on some and vertical on others are used to cut the coagulated milk into cubes. Such cutting increases the surface area of the coagulum which facilitates its loss of whey. Cubes of coagulum also can be heated uniformly during the cooking process. Small cubes lead to low-moisture cheese whereas large cubes lead to high moisture cheese.

Cooking the cut coagulum

After the coagulum cutting, the coagulum (curds) cubes suspended in whey are heated at a temperature in a specified time. (eg: 37-38^{0}C in 30 min for cheddar cheese). This heating is accompanied by stirring of the curd- whey mixture and causes the curd cubes to contract and thus express free whey. Cooking also serves to control acid production by lactic starter culture, to suppress growth of some spoilage bacteria, to influence texture of curd and to aid in control of acids in control of the amount of moisture in the finished cheese.

Draining whey

After the completion of cooking, whey is removed from the curd. This can be accomplished by draining whey from a vat that contains the whey-curd mixture using appropriate precautions to prevent the loss of curd. Some additional lactic acid is produced by the starter bacteria during the time needed for removed of curd from the whey.

Knitting of curds

This step allows further production of lactic acid and modification of curd

particles. So they will adhere to each other and form a single mass of cheese. The characteristic texture of a given variety of cheese is partially determined by this process.

Salting of curds

Sodium chloride is applied to curds in one of several ways. Dry salt may be sprinkled on loose curds as in the manufacture of cheddar cheese or it may be rubbed onto the surface of freshly made cheese. Alternatively, freshly made cheese can be immersed in a nearly saturated aqueous solution of salt. Addition of salt contributes to the flavor, texture and appearance of cheese, controls production of lactic acid, suppresses growth of spoilage microorganisms and further reduces the amount of moisture in finished cheese.

Pressing of curds

This step sometimes comes before salting or afterward. Curds are placed into a form, sometimes called a hoop and pressure is applied hydraulically or through use of weights. If cheese with an open texture is desired, external pressure may not be applied. Pressing gives the cheese its characteristic shape and contributes to its compactness. Free whey is expressed and knitting of curd particles are completed during pressing. Use of vacuum chambers during or after pressing can aid in removal of occluded air from cheese and thus gives the product a closely knit body.

Ripening of cheese

The finished cheese is placed in a room with controlled temperature and relative humidity (eg: 4^0C and 85% for cheddar cheese) and is held therefore several months to several years, depending on the variety of cheese and the extent of ripening that is desired.

Ripening allows for enzymatically induced changes to occur in the protein and fat fractions of the cheese. These changes transform the freshly made cheese into one with desired and characteristic flavor, texture, aroma and appearance.

Villi

Villi is a mesophilic fermented dairy product found in Finland that originated in Scandinavia. This cultured milk beverage is the results of microbial action of lactic acid bacteria (LAB) and a surface growing yeast like fungus *Geotrichum candidum* present in milk, which forms a velvet like surface on villi. The LAB identified in villi includes *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis biovar. diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*.

Film jolk (Filmjolk)

It is also known as fil. It is a traditional fermented milk product from Sweden.

It is made by fermenting cow's milk with a variety of bacteria *Lactococcus lactis* and *Leuconostoc mesenteriodes*. The bacteria metabolize lactose into lactic acid. The acid gives filmjolk a sour taste and causes casein to coagulate, thus thickening of the final product. The bacteria also produce a limited amount of diacetyl, a compound with a buttery flavor, which gives filmjolk its characteristic taste.

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Chapter 6
Fermented Foods

Fermentation in food processing is the process of converting carbohydrate to alcohol or organic acids using microorganisms, yeasts or bacteria-under anaerobic conditions. Fermented foods are rich in probiotic bacteria so by consuming fermented foods the health of gut microbiome and digestive system can increase and also can enhances the immune system.

Sauerkraut

Sauerkraut is a finely cut raw cabbage that has been fermented by lactic acid bacteria. It is made by a process of pickling called lactic acid fermentation. The cabbage is finely shredded, layered with salt and left to ferment. Fully cured sauerkraut keeps for several months in an airtight container stored at 15^0C or below. The fermentation process involves three phases. In the first phase, anaerobic bacteria such as *Klebsiella* and *Enterobacter* lead the fermentation and beginning to produce an acidic environment that favors later bacteria.

The second phase starks as the acid levels becomes too high for many bacteria and *Leuconostoc mesenteroides* and other *Leuconostoc* spp. take dominance.

In the third phase, various *Lactobacillus* species including *L. brevis* and *L. plantarum* ferment any remaining sugars, further lowering the pH. Properly cured sauerkraut is sufficiently acidic to prevent a favorable environment for the growth of *Clostridium botulinum*, the toxins of which cause botulism.

Tempeh

Tempeh is a traditional Indonesian Soy product made from fermented Soybeans. The principal step in making tempeh is the fermentation of soybeans which undergo inoculation with *Rhizopus* spp. mold *Rhizopus oligosporus*. The beans are spread into a thin layer and are allowed to ferment for 24-36 hours at a temperature around 30^0C. The soybeans have to cool down to allow spore germination and abundant growth of mycelium. Later, the temperature of the beans will naturally rise and rapid mold growth happens for about 4 hours. As mold growth declines, the soybeans should be bound into a solid mass by the mycelium. In good tempeh, the

Deepa I, *Food and Dairy Biotechnology*,
https://doi.org/10.34256/ioriip2016

beans are knitted together by a mat of white mycelium. Typically tempeh is harvested after 48 hours of fermentation with its distinguishable whitish color, firm texture and nutty flavor. During the fermentation process, optimal time of fermentation, temperature, oxygen, humidity and pH levels are required to encourage the growth of *Rhizopus* mold, while discouraging the growth of undesired microorganisms.

Miso

Miso is a Japanese traditional paste produced by fermenting soybean with fungus *Aspergillus oryzae* and salt, and sometimes with rice, wheat or oats. Miso is rich in essential minerals and a good source of various B vitamins, vitamin E, K and folic acid. As a fermented food, Miso provides the gut with beneficial bacteria that help us to stay healthy, vibrant and happy.

Different types of Miso

- White Miso
- Yellow Miso
- Red Miso

Typically Miso is salty, but its flavor and aroma depend on various factors in the ingredients and fermentation process. Miso's unique properties and flavor profile can be attributed to the compounds produced during the fermentation process. Miso, depending on the variety, consists of a starter culture called koji, soybeans, and usually a grain (either rice, barley or rye). The Miso goes through a two step process, first creating the Koji and second the Koji is combined with other components and the mixture is left to be enzymatically digested, fermented and aged.

Creating Koji

Koji is produced by introducing the mold *Aspergillus oryzae* on to steamed white rice. This mold culture comes from dried *A. oryzae* spores called starter Koji. Although other strains of fungi have been used to produce Koji, *A. oryzae* is the most desirable because of a number of properties including the fact that it doen't produce aflatoxin.

A small portion of wood ash added to the mixture which gives important nutrients to the fungus as well as promotes sporulation. *A. oryzae* is an aerobic fungus and is the most active fermenting agents in koji as it produces amylolytic, and proteolytic enzymes which are essential for creating the final Miso product. To create optional conditions for enzymatic production and the growth of *A. oryzae*, the Koji's environment must be carefully regulated. Temperature, humidity and oxygen content are all important factors in not only maximizing mold growth and enzyme production, but to prevent other harmful bacteria from producing. Once the Koji has reached a

desirable flavor profile it is usually mixed with salt to prevent further fermentation.

Natto

Natto is made from soybeans, typically natto soybeans. It is popular in Japan. Smaller beans are commonly used, as the fermentation process will be able to reach the center of the bean more easily. The beans are washed and soaked in water for 12-20 hours to increase their size. The soybeans are then steamed for 6 hours. The pressure cooker may be used to reduce the time. The beans are then mixed with the bacterium *Bacillus subtilis*, known as natto kin in Japanese. The mixture is fermented at 40^0C for up to 24 hours. Afterward the natto is cooled, then aged in a refrigerator for up to one week to allow the development of stringiness. When *B. subtilis natto* breaks up soy protein, the bacteria create chains of poly glutamic acid, gamma polyglutamic acid. Natto gets its stringiness from the gamma polygultamic acid. Its odor comes from diacetyl and pyrazines, but if it is allowed to ferment too long, then ammonia is released.

Kombucha

Kombucha is a fermented tea that has been consumed for thousands of years. It is also rich in beneficial probiotics. It also contains antioxidants, can kill harmful bacteria and may help fight several diseases. Kombucha is produced by fermenting sugared tea using a symbiotic culture of bacteria and yeast (SCOBY) commonly called a "mother" or "mushroom". The microbial populations in a SCOBY vary, the yeast component generally includes *Saccharomyces cerevisiae* along with other species. The bacterial component includes *Gluconacetobacter xylinus* to oxidize yeast produced alcohols to acetic acid and other acids. Although the SCOBY is commonly called "tea fungus" or mushroom" it is actually a symbiotic growth of acetic acid bacteria and osmophilic yeast species in a zoogleal mat.

Kimchi

Kimchi is a national dish of both North and South Korea. Cabbages and radishes are the most commonly need kimchi vegetables. Brining salt (large grain size compared to kitchen salt) is used mainly for initial salting of kimchi vegetables. Salt, Scallions, garlic, fish sauce and sugar are commonly added to flavor the Kimchi. Microorganisms present in Kimchi include *Bacillus mycoides*, *B. pseudomycoides*, *B. subtilis*, *Lactobacillus brevis*, *Lactobacillus kimchii*, *L. plantarum*, *L. pentosus*, *Lactococcus gelidum*, *Lactococcus lactis*, *Leuconostoc carnosum*, *Serratia marcescens*, *Weissella cibaria*, *W. confusa* etc.

The first step in the making of any Kimchi is to slice the cabbage or daiken into smaller, uniform pieces to increase the surface area. The pieces are then coated as

a preservative method, as this draws out the water to lower the free water activity. This inhibits the growth of undesirable microorganisms. The salting stage can use 5-7% salinity for 12 hours or 15% for 3-7 hours. The excess water is then drained away, and seasoning ingredients are added. The sugar that is sometimes added also acts to bind free water that still remains, further reducing free water activity. Finally the brined vegetables are placed in to an airtight canning jars and left to sit for 24-48 hrs at room temperature. The ideal salt concentration for the fermentation process is about 3% since the fermentation process results in the production of carbon dioxide, the jars should be "burped" daily to release the gas.

Bread

The cereal grain most commonly used for making bread is wheat flour. Other grains used are rye, maize, but the use of its are limited. For the preparation of bread dough, the ingredients required are refined flour, water and yeast. Salt and fat are also used for dough making. Flour is the bulking ingredient of bread, it forms the structure of the product. It contains gluten. Gluten helps to form an elastic stretchy dough.

Yeast is a raising agent. Yeast produces gases to make the bread rise. Salt is used to bring out flavor in the bread. It must be used in small quantities. Too much of this will stop the yeast growth.

Starter culture

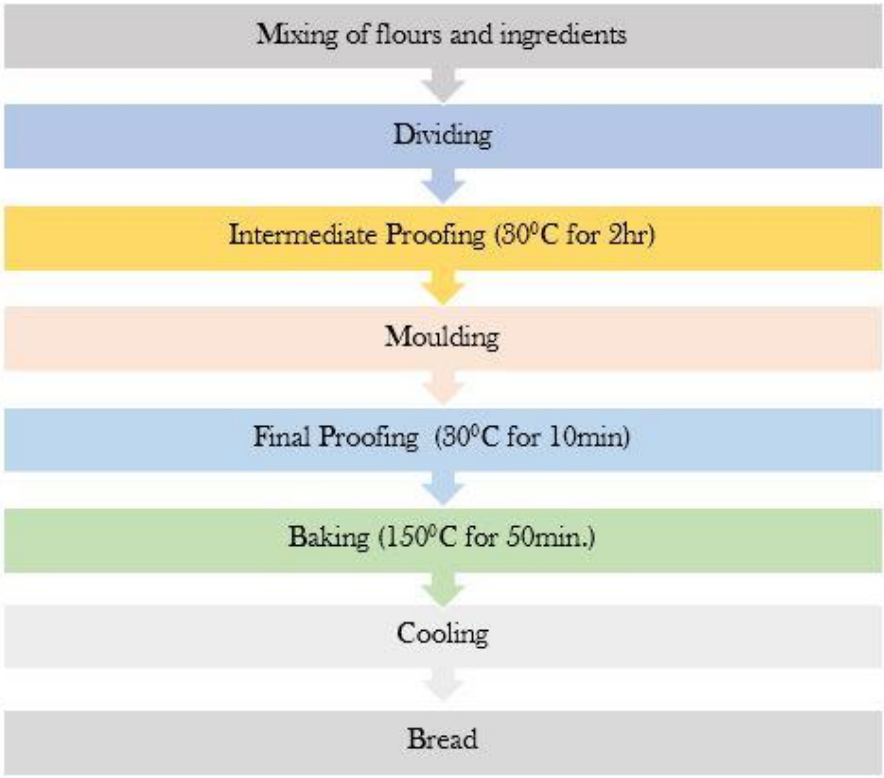
Baker's yeast is the common name for the strains of yeast commonly used as a leavening agent in baking bread and bakery products, where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol. Baker's yeast is of the species *Saccharomyces cerevisiae*, which is commonly need in alcoholic fermentation and is called **brewer's yeast**.

Leavening. It is the production or incorporation of gases in the baked product to increase volume and to produce shape and texture.

Methods of bread making

Straight dough method (Direct dough method)

All the ingredients are mixed together and the dough is fermented for pre-determined time. The fermentation time of the straight dough depends on the strength of the flour. Strong flour requires more fermentation time to mature adequately. Flours which require 2 to 3 hours for maturing should be used for making bread by straight method. Flours that take very long period for maturing should not be used because during prolonged fermentation periods it is very difficult to control the temperature of the dough and rise in temperature will cause acid taste and favor in bread.



Flow chart for straight dough method of bread making.

Sourdough method

Sourdough bread is made by the fermentation of dough using naturally occurring *Lactobacilli* and yeast. Sourdough bread has a more sour taste and better inherent keeping qualities than breads made with baker's yeast due to the lactic acid produced by the *Lactobacilli*. Sourdough is a dough containing a *Lactobacillus* culture in symbiotic combination with yeasts. Flour naturally contains a variety of yeasts and bacterial spores. When wheat flour comes into contact with water, the naturally occurring enzyme amylase breaks down the starch into the glucose and maltose, which sour dough's natural yeast can metabolize. The bacteria ferment starches that the yeast cannot metabolize and the by-products, chiefly maltose are metabolized by the yeast which produces carbon dioxide gas and leavening the dough. A satisfactory rise from sourdough takes longer time than a dough leavened with baker's yeast because the yeast in a sourdough is less vigorous. In the presence of lactic acid bacteria however some sourdough yeasts have been observed to produce twice the gas of baker's yeast. The acidic conditions in sourdough, along with the bacteria also producing enzymes that break down proteins, result in weaker gluten and may produce a denser finished

product.

Sponge-dough method

The sponge-dough method is a two step bread making process. In the first step a sponge is made and allowed to ferment for a period of time and in the second step the sponge is added to the remaining dough's ingredients to form a final dough. In the first stage, a certain amount of flour (around 50-70% of total dough flour), water and yeast are mixed and fermented for a period of time (2.5-4.5 hours) to produce a sponge. In the second stage the sponge is added to the other remaining ingredients to form a final dough. The final dough can be processed by a rapid processing method involves kneading, rolling and moulding.

Modified straight dough method

Modified straight dough method involves the following steps

- Dissolve the yeast in part of the water
- Combine the fat, sugar, salt, milk solids and flavorings
- Mix well, but do not whip
- Add eggs one at a time as they are absorbed into the mixture
- Add the rest of the liquids and mix briefly
- Add flour and the dissolved yeast last
- Mix until a smooth dough forms

The modified mixing method is basically for rich sweet dough. This is basically the modification of the straight dough method to ensure that the fat and sugar are evenly distributed in the dough. This method is very simple but requires a few more steps compared to straight dough method.

No- time dough method

- Dough is fermented. It is allowed for a brief period (abut 30mintes).
- Since dough is not fermented the two functions of fermentation (production of gas and conditioning of gluten) are achieved to some extent by increasing the quantity of yeast (2-3 times of original quantity) and by making the dough little slacker and warmer.
- Although it is possible to make fairly acceptable bread during emergency by using this method the product has poor keeping quality. Due to the absence of fermentation, the gluten and starch are not conditioned sufficiently to retain the moisture.

Delayed salt method

It is also referred to as the 'autolyse'. It is particularly useful when making

bread by hand, but commonly used when a mixer is employed. This method is very simple. Delayed salt allows natural or biological development of a part of the amino acids in gluten called cystein, to occur, which can't happen in the presence of salt.

This is a slight variation of straight method, where all the ingredients are mixed except salt and fat.

- As the salt has a controlling effect on enzymatic action on yeast, the speed of fermentation of a salt less dough will be faster, and a reduction in total fermentation time will be faster.
- The salt is added at a knock back stage. The method of adding salt at the later stage may be according to the convenience of individual baker. It may be sifted on the dough and mixed or it may be creamed with fat and salt.
- Whatever way is chosen for mixing the salt, only three-fourths mixing should be given initially and one-fourth mixing at the time of adding salt.

Biological leavening agent

- *Saccharomyces cerevisiae*: - producing carbon dioxide
- *Clostridium perfringens*: - producing hydrogen found in salt-rising bread, which leavens dough the same way carbon dioxide from yeast does.

Chemical leavens

Chemical leavens are mixtures or compounds that release gases when they react with each other, with moisture or with heat. Baking soda, bases, acids are used for chemical leavening in the baking industry. Bases such as sodium bicarbonate, ammonium bicarbonate and potassium bicarbonate and acids such as potassium acid tartrate, fumaric acid, sodium acid pyrophosphate, monocalcium phosphate, sodium aluminium phosphate, sodium aluminium sulfate, glucono delta-lactone etc are used as leavening chemicals.

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Chapter 7
Microalgae, SCP And Edible Mushroom

This chapter includes Microalgae, SCP and Edible mushroom. The importance of microalgae are they have a high protein and oil content, for example, which can be used to produce either biofuels or animal feeds, or both. In addition, microalgal biomass, which is rich in micronutrients, is already used for dietary supplements to advance human health. Single-cell protein (SCP) refers to protein derived from cells of microorganisms such as yeast, fungi, algae, and bacteria, which are grown on various carbon sources for synthesis. It is a protein source for human food supplements and animal feeds. Mushrooms are also very important they are rich in the B vitamins: riboflavin, niacin, and pantothenic acid. The combination helps protect heart health. Riboflavin is good for red blood cells. Niacin is good for the digestive system and for maintaining healthy skin.

Microalgae

Microalgae also called microphytes are microscopic algae, typically found in marine and fresh water systems living in water column and sediment. They are unicellular and exist individually or in groups or chains. Microalgae can be cultivated and are used to produce either biofuels or animal feeds. Microalgal biomass is rich in micronutrients is used as dietary supplement to improve human health. Apart from being a source of protein, presence of various bioactive components in microalgae provide an added health benefit. Compared to various plant and floral species, microalgae contain higher amounts of pigments. These pigments have anticarcinogenic, antioxidative and antihypertensive properties.

Microalgal derived proteins have complete Essential Amino Acids (EAA) profiles and their protein content is higher than conventional sources such as meat, poultry and dairy products. Numerous species of microalgae are reported to be rich in proteins, carbohydrates, lipids and other bioactive compounds. The Chinese people consumed *Nostoc* species of microalgae around 2000 years ago as food and later *Chlorella* and *Spirulina* species were consumed as functional healthy foods in Taiwan, Japan and Mexico. Currently the microalgae derived foods are marketed as healthy foods and are available in industry as capsules, tablets, powders and liquids. They are also mixed with candies, gums, snacks, pastes, noodles, breakfast cereals, wine and other beverages. The microalgae species widely used include *Spirulina plantesis*,

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https://doi.org/10.34256/ioriip2017

Chlorella sp; *Dunaliella terticola, Dunaliella saline* due to their high protein content and nutritional value. However, in recent years, *Chlorella* and *Spirulina* species are dominating the global microalgae market as they are gaining popularity in the health-food supermarkets and stores. Similar to human supplementation, microalgae is also a source of food for many aquatic species, ruminants, pigs, poultry and other animals. *Chlorella* species are often marketed as 'healthy foods' and are being promoted as a functional foods to prevent, cure or help common diseases or acute diseases like Alzheimer's disease, cancer etc. *Chlorella* sp. are excellent hepatoprotective and hypocholersterolemic agents during malnutrition and ethionine intoxication, they lower blood sugar concentration and increase haemoglobin concentration. *Chlorella* also contains an active immunostimulator-β- 1,3- glucan, which reduces blood lipids and act as a free radical scavenger.

Spirulina sp; also known as **"Superfood**", a label given by World Health Organiszation belongs to the blue-green photoautotrophic genus of unicellular microalgae. It is an excellent natural source of vitamin A, B1, B2 and B12, essential fatty acids and useful pigments such as Xanthophyll and Carotenoids. Additionally various other minerals such as magnesium, manganese, and potassium are reported in small amounts. *Spirulina* is reported to lower LDL cholesterol and triglyceride levels, lower blood pressure and control blood sugar. Supplementation with *spirulina* is also reported to increase haemoglobin levels of red blood cells in older people and improve their immune system. The WHO recommended *spirulina* spp. to be added in diet of National Aeronautics and Space Administration (NASA) astronauts in Space as it is an ideal and compact food for Space Travel. It contains wide range of nutrients even when consumed in small amount.

Single cell Protein (SCP)

SCP also known as microbial protein. The term single cell protein refers to the total protein extracted from the pure cultures of microorganisms (yeast, algae, filamentous fungi, bacteria) and can be used as a protein-rich food supplements by humans and animals.

Production of SCP

Production of SCP involves the following steps

- Selection of strain of microbe and substrate
- Fermentation
- Harvesting
- Post harvest treatment
- Processing of SCP

Selection of strain of microbe and substrate

It is the very crucial step. The microbe selected for the production of SCP shouldn't produce toxicity in its biomass, should not harmful for a consumer to consume and should produce a large quality of protein.. The substrate used should be cheap, effective, allow favorable growth and should be ease of isolation.

Fermentation

Fermentation is done in a large chamber of either glass or stainless steel called 'fermentor'. Fermentation should be done under sterilized conditions and under controlled conditions (Temperature, pressure, pH, humidity etc). Fed-batch cultures are usually used for the fermentation of microbes.

Harvesting

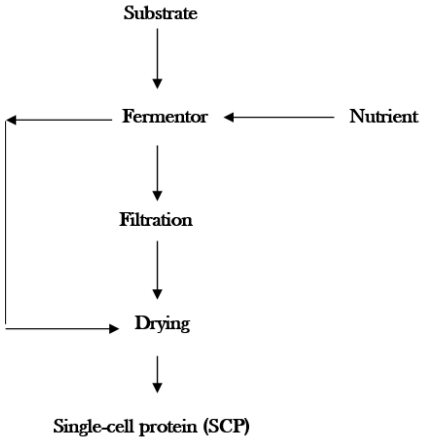
Fermentation yields larger number of microbial colonies from single cell. These colonies are isolated from individual cell by the method of "Decantation".

Post – harvesting fermentation

Isolated microbial colonies are subjected to various differential techniques namely centrifugation, washing, drying etc.

Processing of SCP

The protein produced may contain impurities in it (carbohydrates, nucleic acids, lipid contents, salts etc.). The isolation of pure protein can be achieved by disrupting the cell wall through crushing, crumbling, cycles of freezing and thawing, grinding and thermal shocks. The removal of nucleic acids are done by treatments with 10% NaCl, by chemicals eg: NaOH, by thermal shocks or by enzyme treatment eg: ribonucleases.



Flowchart for the production of SCP

Advantages of SCP

- Microbes can use a variety of raw materials as their source of carbon and thus can cause the removal of pollutants from the environment.
- They contain 43-85% of protein contents in their dry mass
- They can be easily modified genetically for varying the amino acid composition
- Microbes have rapid succession of generation thus number of generation can be obtained in a very short interval of time (algae 2-6 hrs, yeast 1-3 hrs, bacteria 0.5-2 hrs).

Disadvantages of SCP

- Some microbes are harmful for both humans and animals and can produce toxins in their biomass which may cause diseases in humans and animals.
- Microbial biomass may lead to some allergic reactions and indigestion
- The higher nucleic acid contents in SCPs may lead to human kidney stone
- Taste may change and some unacceptable coloration may produce
- Production of SCP is very expensive method and requires highly sterilized conditions

Microorganisms used for SCP

Microorganisms that can be used for the production of SCPs are yeast, filamentous fungi, algae and bacteria.

Yeast

Saccharomyces cerevisiae

Pichia pastoris

Candida utilis

Torulopsis

Geortrichum candidum

Fungi (Mycoprotein)

Aspergillus oryzae

Fusarium venenatum

Sclerotium rolfsii

Polyporus

Trichoderma

Scytalidium acidophilum

Bacteria

Rhodobacter capsulatus

Pseudomonas sps.

Achromobacter deluacuate

Algue

Spirulina (Dietary Supplement)

Chlorella

Mushrooms

- Fast growing basidiomycetous fungi
- Produces fleshy fruit bodies
- May be button like, fan or umbrella shaped
- They are characterized by having heterotrophic mode of nutrition
- Rich in protein and other accessory compounds

Nutrients in Mushroom

Generally all mushroom are

- Low in sodium, fat and calories, just about 20 calories in a cup
- High in fiber and protein: 20-30% protein by dry weight
- Rich in the minerals potassium, selenium, copper, zinc and magnesium; Oyster mushroom is rich in iron too.
- Rich in B complex vitamins, riboflavin, niacin and pantothenic acid
- The only vegetable or fruit for that matter that produces vitamin D when in sunlight and is a source of natural vitamin D.
- Rich in L- ergothioneine, a powerful antioxidant
- Rich in cancer fighting nutrients like polysaccharides and linoleic acids
- Free of cholesterol
- Contain triterpenes which inhibit histamine release and are anti-inflammatory

Edible Mushroom

Edible mushrooms are the fleshy and edible fruit bodies of several species of macrofungi. According to Chang and Hayes:

- They can appear either below ground (hypogeous) or above ground (epigeous) where they may be picked by hand.
- Edible mushroom are consumed for their nutritional value and they are occasionally consumed for their supposed medicinal value.

Agaricus bisporus

- Edible basidiomycete mushroom native to grasslands in Europe and North America
- They may grow on the soil or on another food source
- China is the largest producer of edible mushrooms accounting for over 50% of the world's edible mushroom production.
- This mushroom may be known as common mushroom, button mushroom, white mushroom, cultivated mushroom, table mushroom and champignon mushroom.

Pleurotus citrinopileatus

- Golden oyster mushroom is an edible gilled fungus
- Is one of the most popular wild edible mushroom
- Grow in clusters of bright yellow to golden brown caps with a velvety, dry surface texture
- Caps range from 20-65 mm in diameter
- The flesh is thin and white, with a mild taste and without a strong smell
- The gills are white, closely spaced and run down the stem
- The spores are cylindrical or elliptical in shape, smooth, hyaline, amyloid and measure 6-9 by 2-3.5 micrometre.

Volvariella volvacea

- Also known as Paddy straw mushroom or straw mushroom
- It is a species of edible mushroom cultivated throughout East and Southeast Asia and used extensively Asian cuisines.
- They are often available fresh in Asia, but are most frequently found in canned or dried forms outside their nations of cultivation.
- Straw mushrooms are grown on rice straw beds

- They are adaptable and take four-five days to mature and are most successfully grown in subtropical climates with high annual rainfall.

Common edible mushrooms

White or Button (*Agaricus bisporus*)

A creamy white to pale tan color, these mushrooms have a firm texture and delicate flavor. They are juicy, tasty and inexpensive. They can be grilled or mixed with other mushrooms, can also be stuffed and baked.

Chanterelle (*Cantharellus cibaris, C. formosus etc.*)

A medium textured mushroom with fruity aroma. The color ranges from pale white to yellow to orange and brown to black. It has wrinkles on the underside instead of gills.

Oyster (*Pleurotus ostreatus*)

It has a velvet like texture and is trumpet shaped with colors ranging from grey to pale brown to reddish caps on grey white stems. It has a mild seafood taste.

Portobello (*Agaricus bisporus*)

It has a big, large, umbrella like cap. The texture and taste is steak like yet is butterfly soft.

Shiitake (*Lentinula edodes*)

The color of its cap ranges from tan to dark brown. It has an earthy, smoky flavor and tastes best when cooked. It is low in water content.

Cremini (*Agaricus bisporus*)

This is actually the immature Portobello, resembling the white mushroom but with a firmer texture and deeper flavor. The cap can be from a pale tan to rich brown color.

Enokitake (*Flammulina velutipes*)

A mild flavored, crunchy textured mushroom with a fruity taste.

Porcini (*Boletus edulis*)

It has a rich woody flavor, the cap can be roasted like the Portobello or it can be diced and cooked. It can be also added raw to salad.

Morel (*Morchella angusticeps, M. esculenta etc*)

It is considered a delicacy. It has a deep and clean flavor. It can be toxic if eaten raw and should therefore be cooked. It can be cooked with non fat cream sauces and tastes best with just a little amount of butter.

Black Truffles (*Tuber melanosporum, T. magnatum etc.*)

It has a sweet, musky and pungent flavor.

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Chapter 8
Industrial Production of Antibiotics and Organic Acids

Production of Antibiotics

Antibiotics are chemicals that kill or inhibit the growth of bacteria and are used to treat bacterial infections. They are produced in nature by soil bacteria and fungi. Antibiotics are the most widely used chemotherapeutic agents. Most commonly used types of antibiotic are Aminoglycosides, Penicillins, Fluoroquinolones, Cephalosporins, Macrolides and Tetracyclines. Five basic mechanisms of antibiotic action against bacterial cells are inhibition of nucleic acid synthesis, inhibition of protein synthesis, inhibition of cell wall synthesis, alteration of cell membranes and antimetabolite activity.

Penicillin

Penicillins are a group of antibacterial antibiotics produced by the fungus *Penicillium notatum* and *Penicillium chrysogenum*. The penicillin producing bacteria were firs identified by Alexander Fleming in 1926. Penicillin was the first important commercial product produced by an aerobic, submerged fermentation. Like all antibiotics, penicillin is a secondary metabolite, so is only produced in the stationary phase. Penicillin are effective against actively growing gram-positive bacteria. Some penicillin like amoxicillin are also effective against gram-negative bacteria except *Pseudomonas aerugionsa.*

Industrial Production Techniques

Industrial microbiology can be used to produce antibiotics via the process of fermentation, where the source microorganism is grown in large containers (100,000-150,000 liters or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient levels must be optimal and are closely monitored and adjusted if necessary. As antibiotics are secondary metabolites the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product.

Deepa I, *Food and Dairy Biotechnology*,
https://doi.org/10.34256/ioriip2018

Penicillin Production Process

Penicillin is commercially produced in the industry by culturing the fungus *Penicillium chrysogenum* or *Penicillium notatum*. Penicillin production is previously achieved by surface process i.e, solid state fermentation and surface liquid fermentation. Nowadays commercial production is carried out by fed batch process.

Inoculum Preparation

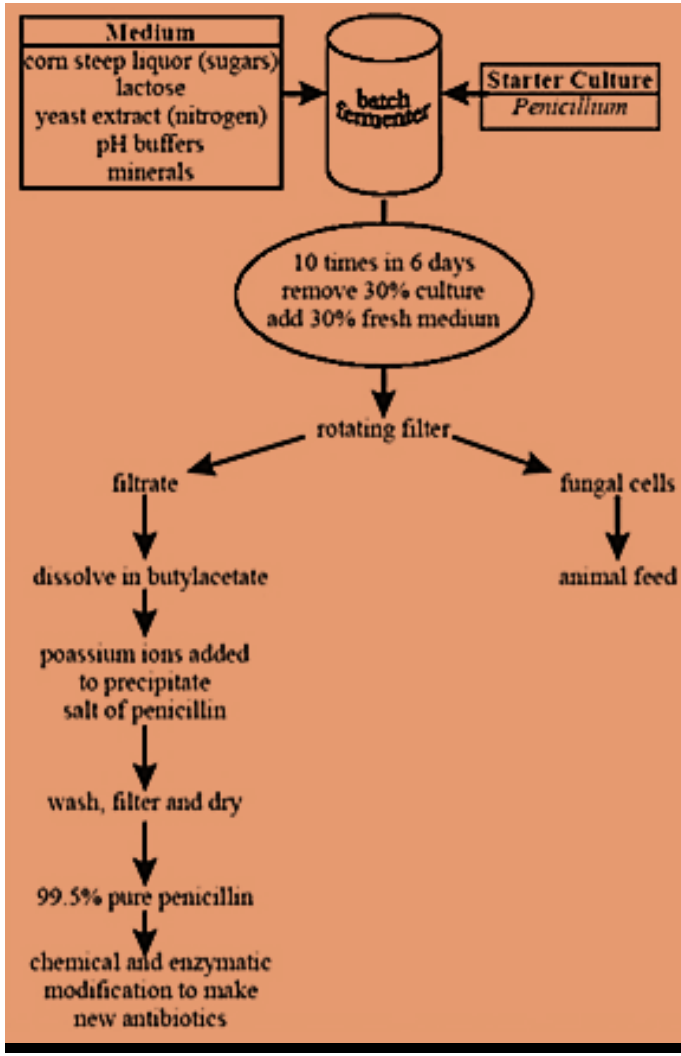
For inoculum preparation, spore from heavily sporulated working stocks are suspended in water or non-toxic wetting agents (Sodium sulfonate 1:10000). These spores are then added to flask containing wheat bran and nutrient solution for heavy sporulation. Incubated at 24^0C for 5-7 days. Spores are then transferred to seed tank and incubated for 24-48 hrs at 24^0C with aeration and agitation for sufficient mycelial growth. These mycelia can be used for production fermenter.

Fermentation

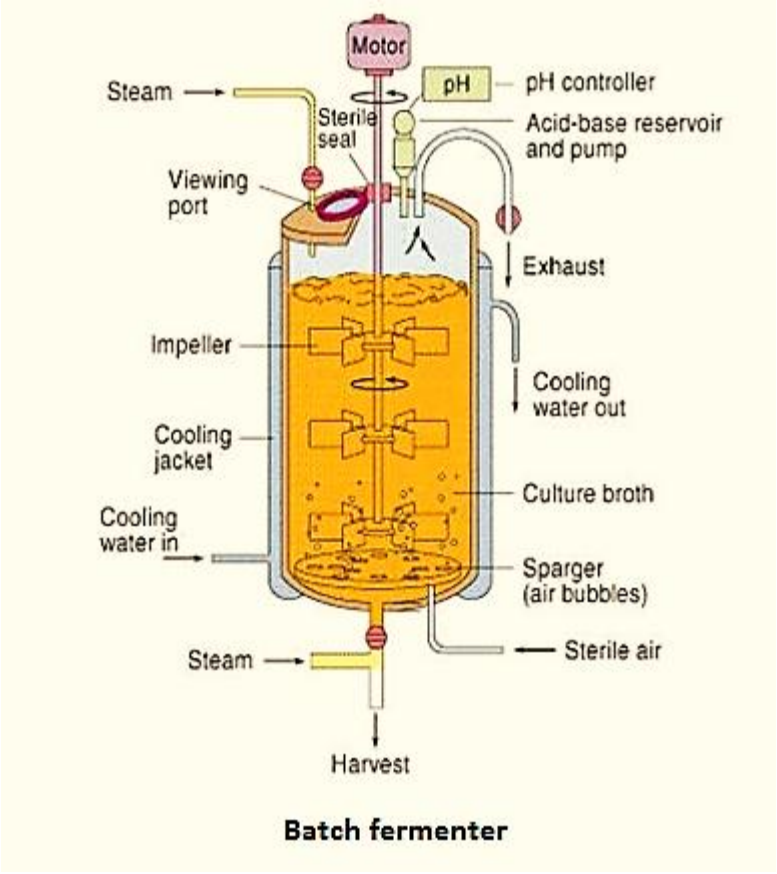
The method used is fed-batch or batch fermentation. Jackson in 1958 prepared a media for penicillin production. The major constituent of typical medium includes,

1) Fermentable carbohydrate	-	Corn steep liquor (3.5%), lactose (3.5%), glucose (1%).
2) Potassium dihydrogen phosphate	-	0.4%
3) Organic nitrogen source		
4) Phenyl acetic acid precursor		
5) Edible oil	-	0.25%
6) Calcium carbonate (act as buffer)	-	1%

Stirred tank or air lift tank fermenter is used. pH is adjusted to 5.5-6.0 which increased up to 7-7.5 (optimum) due to liberation of ammonia gas and consumption of lactic acid. If pH is 8 or more, $CaCO_3$ or $MgCO_3$ or phosphate buffer is added. Temperature employed for the penicillin production is 28^0C. Supply of oxygen in a bioreactor is the limiting factor in penicillin biosynthesis. Aeration speed is between 3.0-1.5m^3. Production of penicillin depends up on biomass production, therefore it is desirable to have a high biomass concentration in the vessel. It is achieved by increasing the agitation rate and power.



Industrial production of penicillin



Recovery and Purification

Harvested culture broth includes penicillin G along with a variety of other metabolites. Vacuum filter is used for separation of mycelium from the broth. Conversion of penicillin to the anionic form occurs at low pH (2.0-2.5). The lowering of pH is done by adding phosphoric acid or sulphuric acid. For the removal of pigment and other impurities from solvent containing penicillin it is treated with active charcoal. The product is back extracted into water from solvent by adding sodium hydroxide. The product of penicillin is then crystallized into sodium or potassium penicillin.

Streptomycin

Streptomycin is an antibiotic produced by the bacteria, *Streptomyces griseus*, is active against gram-negative bacteria and against tuberculosis bacterium,

Mycobacterium tuberculosis. However, it is proved to be useful in the treatment of infections caused by gram-positive specially resistant to penicillin. It is also useful in the control of plant disease caused by bacteria as it acts systemically in plants.

Industrial production

Industrially streptomycin is produced by submerged culture method.

Inoculum Production

Spores of *S. griseus* maintained as soil stocks or lyophilized in a carrier such as sterile skimmed milk, is employed as stock culture. The spores from the stock cultures are then transferred to a sporulation medium to provide enough sporulated growth to initiate liquid culture build-up of mycelial inoculum in flasks or inoculum tanks. After sufficient mycelilal growth, it is fed to production fermenter.

Preparation of Medium

A production medium contains carbon source and nitrogen source. Glucose is one of the best carbon sources which helps in increased yield of streptomycin. Apart from glucose, fructose, maltose, lactose, galactose, mannitol, xylose, and starch can also are used as carbon source. Peptone, soya extracts, meat extract, residue from alcohol distillation, ammonium salts, nitrates and glycine may be used as nitrogen source. Phenylacetic acid, L-naphthalene acetic acid may be added as growth stimulating compounds. It is also better to add proline into the medium which helps in high streptomycin production. Fats, oils and fatty acids may also be used along with glucose.

Fermentation

Sterilized liquid medium is fed to the fermenter. Inoculum of appropriate volume is introduced into the fermenter. The optimal fermentation temperature is in range of 25 to 30^0C and optimum pH range is between 7.0 - 8.0. The fermentation is highly aerobic and lasts 5-7 days and passes through 3 phases.

The first Phase

It takes about 24 hours to 48 hours. Rapid growth and formation of abundant mycelium occurs. Here the pH rises to 8.0 due to the release of ammonia into medium and due to the proteolytic activity of *S. griseus*. Glucose is utilized slowly and little production of streptomycin occurs.

The second phase

It lasts for 2 days. Streptomycin production occurs at a rapid rate without increase in the mycelial growth. Ammonia released in the first phase is utilized, which results in the decrease of pH to 7.6-8.0. Glucose and oxygen are required in large quantity during this phase.

The third phase

Cells here undergo lysis, releases ammonia and pH increases which falls again after a period of continuous streptomycin production. Oxygen requirement decreases and the contents of the medium including sugar get exhausted. Finally streptomycin production stops. The yield of 1200 micrograms per milliliter of streptomycin is obtained.

Harvest and Recovery

After completion of fermentation the mycelium is separated from the broth by filtration. Streptomycin is recovered by several methods. Generally employed method is the fermentation broth is acidified, filtered and neutralized. It is then passed through a column containing a cation exchange resin to adsorb the streptomycin from the broth. The column is than washed with water and the antibiotic is eluted with hydrochloric acid or cyclohexanol or phosphoric acid. It is then concentrated at 60^0C under vacuum.

The streptomycin is then dissolved in methanol and filtered and acetone is added to the filtrate to precipitate the antibiotic. The precipitate is again washed with acetone and vacuum dried. It is purified further by dissolving in methanol. The streptomycin in pure form is extracted as calcium chloride complex.

By-product Vitamin B12

Vitamin B12 is produced as a by-product which will not affect adversely the yield of streptomycin.

Acetic acid

Industrial production of Acetic acid

Acetic acid is systematically known as ethanoic acid.

- It is a colourless liquid organic compound
- It has a pungent smell
- It is the principle constituent of Vinegar
- The first Vinegar was Spoiled wine
- Glacial acetic acid is the pure form of acetic acid (99.98%)
- Vinegar is product of Acetic acid

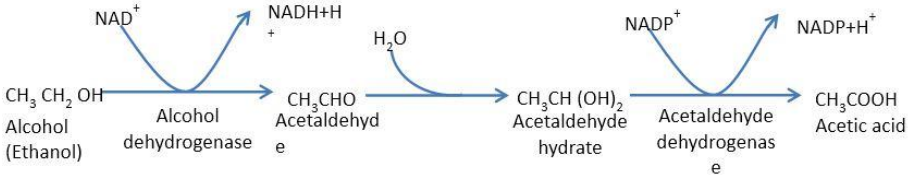
Microorganisms used for the production of Acetic Acid

The commercial production of acetic acid is carried out by a special group of

acetic acid bacteria, which are divided into two genera *Gluconobacter* that oxidizes ethanol exclusively to acetic acid. *Acetobacter* that oxidizes ethanol first to acetic acid, and then to CO_2 and H_2O. Acetic acid bacteria are gram-negative bacteria. Eg: *A. aceti* , *A. peroxidans*, *A. pasteurianus*.

Biosynthesis of acetic acid

Acetic acid is a product of incomplete oxidation of ethanol. Ethanol is first oxidized by alcohol dehydrogenase to acetaldehyde which then gets hydrated to form acetaldehyde hydrate. The latter is then acted upon by acetaldehyde dehydrogenase to form acetic acid.



Biosynthesis of acetic acid from ethanol

Production process for Acetic acid

For every molecule of ethanol oxidized, one molecule of acetic acid is produced. Thus high yielding strains can produce 11-12% acetic acid from 12% alcohol. For optimal production, adequate supply of oxygen is very essential. Insufficient oxygen, coupled with high concentration of alcohol and acetic acid result in the death of microorganism. Surface fermentation or submerged fermentation process can be carried out to produce acetic acid.

Recovery

The acetic acid produced is clarified by filtration and then subjected to decolorization by $K_4[Fe(CN)_6]$.

Vinegar

Vinegar is an aqueous solution containing about 4% by volume acetic acid and small quantities of alcohol, salts, sugars and esters. It is widely used as a flavoring agent for processed liquid foods such as Sauces and Ketchup. The starting material for vinegar production are wine, whey, malt. Vinegar production can be carried out either by surface process (trickling generator) or by submerged process.

Surface process

The fermentation material is sprayed over the surface which trickles through the shavings that contain the acetic acid producing bacteria. The temperature is around 30^0C on the upper part while it is around 35^0C on the lower part. Vinegar is produced in about 3 days.

Submerged process

The fermentation bioreactors are made up of stainless steel. Aeration is done by a suction pump from the top. The production rate in the submerged process is about 10 times higher than the surface process.

Raw materials

- Biomass feedstocks (beach wood, silicon tube, sieving, wood chips)
- Ethanol is used as substrate

Microbes

Acetic acid bacteria (*Acetobacter aceti*) used as bioreactor. The production of Vinegar actually involves two fermentation processes – the first utilizing yeast to produce alcohol from sugar and the second utilizing acetic acid bacteria to oxidize ethanol to acetic acid through acetaldehyde. The microbial oxidation of ethanol to acetic acid is an aerobic fermentation that has high oxygen requirement. *Acetobacter* bacteria are employed for the industrial production of vinegar. *Acetobacter* bacteria can be divided into 2 groups – *Gluconobacte*r and *Acetobacter*. *Gluconobacter* oxidizes ethanol to acetic acid while *Acetobacter* oxidizes ethanol first to acetic acid and then to CO_2 and H_2O. Species of the *Acetobacter* used commercially are *Acetobacter aceti* and *A. pasteurianum*. Similarly, *Gluconobacter oxydans* and its subspecies are employed in the commercial production of vinegar. Two oxidation steps occur during the conversion of ethanol to acetic acid. In the first step ethanol is oxidized to acetaldehyde in the presence of NAD or NADP and in the second step acetaldehyde is changed to acetic acid under the catalytic action of enzyme alcohol dehydrogenase.

Fermentation

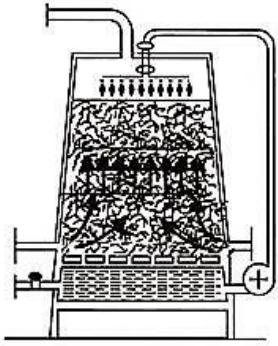
Commercially acetic acid is produced by two methods namely surface fermentation process and submerged fermentation process. Nutrient concentration that is used in submerged fermentation is generally five times greater than surface fermentation.

Surface fermentation process

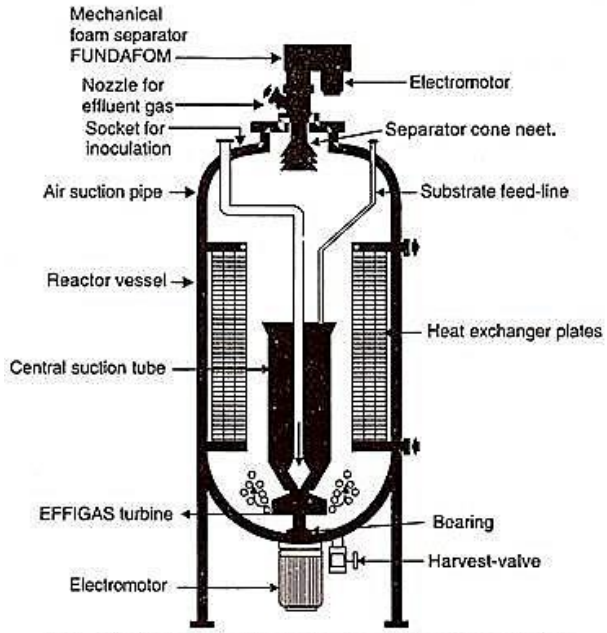
Tricking generator is generally needed in this process.

It is made up of wood and has a total volume up to $60m^3$ and its inner surface is lined with birch wood shavings. The starting material that is ethanol is passed into the generator from top which trickles through the birch wood shavings containing

bacteria into bottom basin where the partially converted solution is cooled and pumped back again to the top of the generator and passed again through it. This process is repeated again and again until 88-90% of alcohol is changed to acetic acid. The starting material should contain both acetic acid and ethanol for optimal growth of *Acetobacter*. Presently higher yielding strains are employed in vinegar fermentation which are able to yield 13-14% of acetic acid.



Trickling generator for acetic acid production



Submerged fermenter for acetic acid production

Submerged fermentation process

Material with low alcohol concentration such as fruits, wines and special

mashes were first used in the initial stages of submerged fermentation process, which generally do not require aeration. But presently high yielding materials are employed which are capable of yielding 13% acetic acid. However the process with such high yielding material requires high aeration up to $50m^3$ oxygen. Fermenter constructed with stainless steel are employed and they are stirred from bottom. Aeration is provided with a suction rotor, with the incoming air coming down through a pipe from the top of the vessel. Heat exchanger is provided to control the temperature along with foam eliminators. The fermentation process is carried up to 35 hours at 40^0C temperature. The yield of acetic acid is about 98% in fully continuous process.

Recovery

The Vinegar produced in a submerged fermentation process is turbid due to the presence of bacteria. It is clarified by filtration. Plate filters and filter aids are generally used. After filtration K_4 [Fe (CN)6] is used to decolorize the final product, if required.

Citric acid

Production of citric acid

Citric acid can define as the most common weak organic acid found naturally in lemon fruits. The production of citric acid is an industrial process, which makes the use of raw materials like substrates, citric acid growth promoting microorganisms and enzymes etc for the commercial production of citric acid. Generally, their commercial production are achieved by employing the method of fermentation. The commercial production of citric acid yields significant importance because its use has been constantly increased the human consumption by 4% each year and it is having high demand in pharmaceuticals, food and other industries like cosmetic, toiletries etc. In the year 1826, the commercial production of citric acid was firstly achieved by the John and Edmund Sturage Company, U K. Citric acid production involves natural process and synthetic process. In natural process, citric acid is naturally produced from citrus plants like lemon, orange etc. In synthetic process citric acid is chemically synthesized by the enzymes and biological fermentation by microorganisms.

Production process

- Biochemical method by fermentation
- Biological method by chemical reactions

Biochemical method

Citric acid produces as a primary metabolite by the microorganisms. The citric acid produces at the time of tropophase cell growth as a result of defective citric acid or krebs cycle. In defective krebs cycle, a high amount of sugar is transported through EMP pathway that forms acetyl-CoA. The acetyl-CoA condenses with oxaloacetic acid to yield "citric acid" by the help of citrate synthase enzyme. Therefore

for the production of citric acid, the enzymes of the Krebs cycle must be deactivated like enzyme Aconitase/Isocitrate dehydrogenase which can further break down the citric acid.

Biological method

Biological method involves the fermentation of citric acid by the use of microorganisms.

Fermentation of citric acid

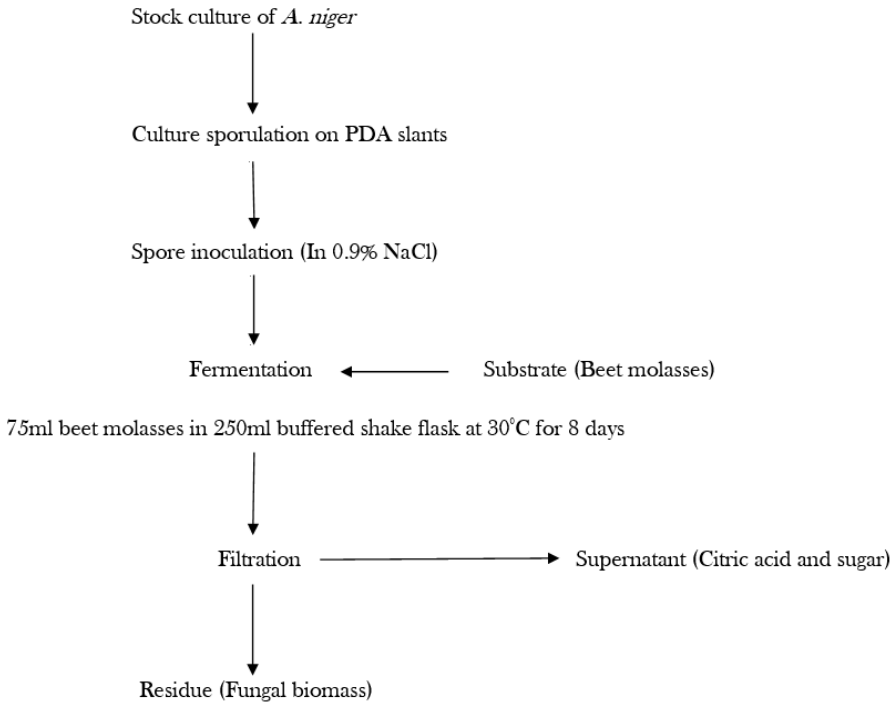
- Koji process
- Surface culture process
- Submerged process

Koji Process

Koji process also known as **"Solid- State Fermentation"**. This process was first introduced in Japan. It is related to the use of agro-industrial residues for citric acid production. Raw materials such as apple, pomace, sugar cane, beet molasses etc can be used. *Aspergillus niger* utilized these raw materials. The pH and moisture content of the raw material is adjusted to 4-5 and 70% respectively. The raw materials are than cooled at 30-60^{0}C and inoculated with *A. niger*. After inoculation, the medium is transferred into large trays of 3-5 cm depth and incubated at 25-30^{0}C for 3-7 days. Finally extracted the citric acid from the fermentation tank. The starch content of the raw material is degraded into citric acid by amylase enzyme of *Aspergillus niger*. Pre-treatment of substrate is not required in Koji process because the trace elements do not affect the citric acid production.

Submerged culture process

Submerged culture process also known as **"Submerged Culture Fermentation"**. By using this submerged fermentation method about 80% of citric acid production is carried out. Submerged fermentation makes the use of black *Aspergillus japonicus*. It is performed in a bioreactor made of stainless steel compiled with cooling system, impellers, proper aeration etc. Substrates like beet molasses, corn starch etc used as carbon source. For the nitrogen source, ammonia is used. Pre-treatment is required for the substrate used like the addition of nutrients, sterilization etc. Inoculated the culture medium with *A. japonicus* and maintained at 30^{0}C. Submerged fermentation is mostly carried out in a batch bioreactor in which 1500 kg of citric acid and 500 kg of biomass can be produced from the 2500 kg glucose and 860 kg of oxygen.



Citric acid production by biological pathway

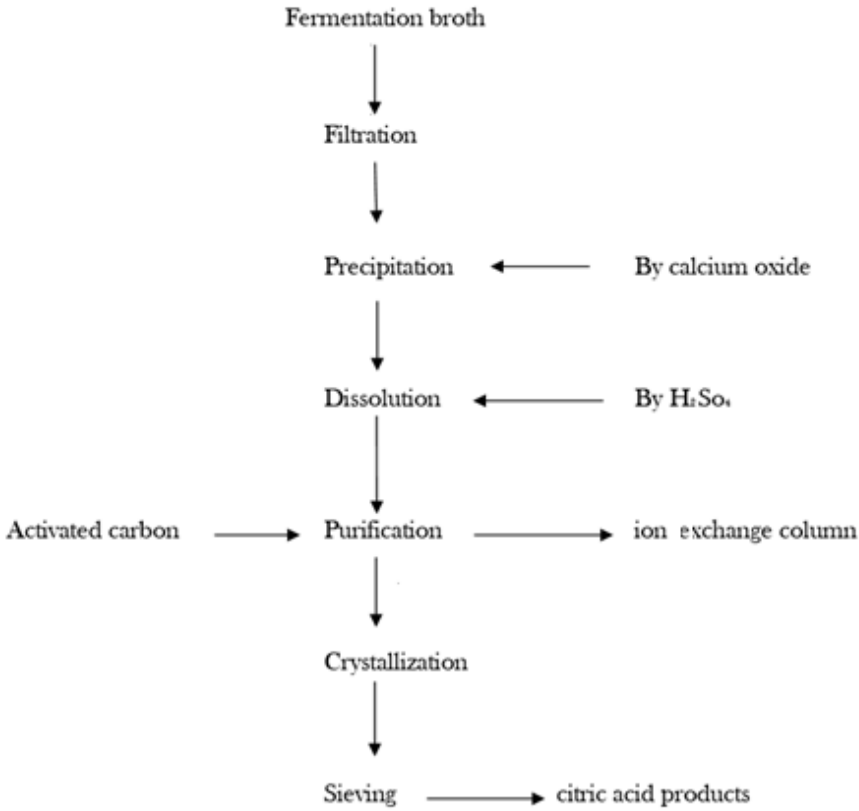
Surface culture process

It is also known as **"Liquid Surface Fermentation"**. Surface culture fermentation was the first method introduced for citric acid production in 1919. In liquid surface fermentation, the culture medium of 5-6 pH is added to the aluminium shallow trays up to 5-20 cm depth. This process is carried out in fermentation chamber which provides uniform air circulation, maintains relative temperature and humidity. The spores of *A. niger* is firstly blown on to the surface of the culture medium for about 5-6 days and then dry air is passed. The pH of the medium is adjusted to 1.5-2.0. After 24 hrs, the spores started to germinate and the growth of white mycelium is observed on the surface of culture medium. After the utilization of sugar content by the mold, the remaining liquid is separated from the mycelial mat. In the surface culture process, a small amount of citric acid is produced as the primary metabolites by the *A. niger*.

Recovery of citric acid

The product formed after fermentation is the fermented liquor looks hazy due

to the presence of antifoaming agents, mycelia etc. For the separation of these things, a slurry of Calcium hydroxide ie, $Ca(OH)_2$ is used and it form a precipitate of calcium citrate. The calcium citrate precipitate is filtered and washed. After filtration, it is treated with H_2So_4 for the precipitation of calcium as $CaSo_4$. Calcium sulphate is then treated with the activated carbon by which it gets demineralized after passing it consecutively from the ion exchange bed. The solution obtained is subjected to circulating crystallizers. Crystals are formed as a result of crystallization are then removed by centrifugation. The remaining solvent is then dried, sieved and then packed.



Recovery of Citric acid

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Chapter 9
Indicator Organisms, Detection of Indicator Organisms, Microbiological Culture Methods and Immunological Methods Employed in Food Industry

Microbiological indicator organisms can be used to monitor hygienic conditions in food production. The presence of specific bacteria, yeasts or molds is an indicator of poor hygiene and a potential microbiological contamination.

Total aerobic count

The total viable count on surface describes the number of colony forming units (cfu) which exist on a defined area (eg. $1cm^2$) of the analyzed surface. Normally it will be determined using a total plate count agar by growing the colonies after incubation at 30-35^{0}C for approximately 48 hours. Counted the colonies. The total viable count is an indicator for the hygienic status of the food production and shows possible microbial loads and contamination sources. The aerobic mesophilic count indicates the number of colony forming units (cfu) formed on a plate count medium during a specified incubation time at mesophilic temperatures (30-37^{0}C). The aerobic count is an indicator for the microbial status of the production and environmental conditions.

Coliform bacteria

Coliform bacteria are considered to be indicators of fecal contaminations and are often used for monitoring water quality. Detection of coliform bacteria on surfaces in the production environment or solid foods indicates that the hygienic conditions in the food production process needs to be optimised. These bacteria can be easily identified using nutrient media which contain chromogenic substrates for their enzyme β- galactosidase (eg: X-GAL).

Enterobacteria

The Enterobacteriaceae are gram-negative, rod shaped bacteria which are

typically 1-5 μm in length. They are facultative anaerobes and most are motile, but non motile genera exist as well. Enterobacteriaceae cannot produce oxidase and can be distinguished from similar genera by this criterion. Enterobacteriaceae are a normal part of the gut flora which is found in the intestinal tract of humans and animals. They are also spread widely in the environment (eg: soil, water). Some genera are pathogenic and can cause serious diseases. Genera of Enterobacteriaceae are *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia*, *Shigella*, *Yersinia*, *Morganella*, *Hafnia*, *Citrobacter* etc.

Enterococcus

Enterococci are gram-positive organisms which belong to the intestinal bacteria as well as the gram-negative Enteriobacteriacae. Enterococci may appear as contaminations in a variety of fermented foods. Their presence in food products has been considered as an indication of poor sanitary conditions during production and processing. On the other hand enterococci are specifically used as starter cultures for the fermentation processes of a variety of foods. It is claimed that enterococci play an important role in the development of the organoleptic properties of the fermented foods. For water, the presence of enterococci serves as an indicator of fecal contamination. Enterococci will only appear in water if they are inserted by contamination with human or animal feces.

Yeast and molds

Yeast and molds are able to contaminate foods and are responsible for quick spoilage of the infested food stuff. Due to their ability to produce toxic or allergenic substances molds are especially predestinated to be a potential health risk. As these organisms might be rapidly spread by dusts and aerosols, surfaces in the production environment will be consistently contaminated.

Yeasts are facultative anaerobe, mono cellular fungi fermenting sugar substrates to CO_2 and H_2O. Under anaerobic conditions yeasts ferment sugar to alcohol and CO_2. In terms of food spoilage genera of *candida* play an important role. This is located on the human and animal mucosa (nose, throat). The term ‘mold’ is commonly used for the visible part of the fungi present on the surface of contaminated food. Under the surface, the fungi forms mycelium which can’t be recognized with the naked eye. Specific molds as well as yeast are used for industrial purposes (eg: cheese production). Harmful genera of molds exist are able to produce toxins (mycotoxins). Almost all molds have an allergenic potential related to their spore form capabilities.

Culture methods

Indicator bacteria can be cultured on media which are specifically formulated to allow the growth of the species of interest and inhibit growth of other organisms. Typically, environmental water samples are filtered through membranes with small pore sizes and then the membrane is placed on to a selective agar. It is often necessary to vary the volume of water sample filtered in order to prevent too few or too many colonies from forming on a plate. Bacterial colonies can be counted after the 24-48 hours depending on the type of bacteria. Counts are reported as colony forming units per 100 ml (cfu /100 ml).

Fast detections using chromogenic substances

One technique for detecting indicator organisms is the use of chromogenic compounds, which are added to conventional or newly devised media used for isolation of the indicator bacteria. These chromogenic compounds are modified to change color or fluorescence by the addition of either enzymes or specific bacterial metabolites. This enables for easy detection for isolation of pure cultures and confirmatory tests.

Application of Antibodies

Immunological methods using Monoclonal Antibodies can be used to detect indicator bacteria in water samples. ELISA antibody technology has been developed to allow for readable detection by the naked eye for rapid identification of coliform micro colonies

Gene Sequence – based methods

Gene sequence based methods depend on the recognition of exclusive gene sequences particular to specific strains of organisms. Polymerase Chain Reaction (PCR) and Fluorescence In Situ Hybridization (FISH) are gene sequence – based methods currently being used to detect specific strains of indicator bacteria.

Test for metabolic products of Pathogens that indicate the health hazard

In certain cases, tests for metabolic products of pathogens are preferred to indicate the presence of pathogens or their toxins. Thermonuclease test for evidence of growth of *Staphylococci* and presence of enterotoxins. *S. aureus* produces thermostable deoxyribounclease (TNase), which has been used as a rapid and

inexpensive procedure for screening foods for indication of extensive staphylococcal growth and presence of enterotoxin. The TNase test has been recommended for testing foods such as cheeses and sausages. TNase can be a useful indicator because it can almost always be detected in foods whenever enterotoxins can be detected.

Aflatoxin detection by ultraviolet light

Long-wave ultraviolet (black) light has been used to detect the presence of *Aspergillus flavus* and *Aspergillus parasiticus* in corn. When corn viewed under U-V light displays a bright greenish- yellow fluorescence (BGYF). The examination of corn and other grains with U-V light as a rapid screening procedure has been adopted by industry.

Test for phosphatase

The phosphatase test is used for certain milk and milk products to determine whether the product was pasteurized properly and also to detect the possible addition of raw milk to pasteurized milk.

Microbial culture methods

The food material on which microorganisms are grown in the laboratory is known as a culture medium and the growth itself is called a culture. The most common growth media for microorganisms are nutrient broth and nutrient agar. But some microorganisms needs specialized media. Fastidious organisms require specialized environments due to complex nutritional requirements.

Classification of bacterial culture media on the basis of consistency

Solid medium:

Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for **isolating bacteria** or for determining the colony characteristics of the isolate.

Semisolid medium:

Semisolid media are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

Liquid (Broth) medium:

These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests, MR-VR broth**.**

Classification of culture media on the basis of composition

Synthetic or chemically defined medium

A chemically defined medium is one prepared from purified ingredients and therefore its exact composition is known.

Non synthetic or chemically undefined medium

Non-synthetic medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Nutrient broth, for example, is derived from cultures of yeasts.

Synthetic medium may be simple or complex depending up on the supplement incorporated in it. A simple non-synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors whereas complex non-synthetic medium support the growth of more fastidious microorganisms.

Classification of Bacterial Culture media on the basis of purpose/ functional use/ application

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

1. General purpose media/ Basic media: Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar (NA) are considered as basal medium. These media are generally used for the primary isolation of microorganisms.
2. Enriched medium (Added growth factors): Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media. Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base. Chocolate agar is also known as heated blood

agar or lysed blood agar**.**

3. Selective and enrichment media: Are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

a. Selective medium: Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium are agar based (solid) medium so that individual colonies may be isolated. Examples of selective media include:

1. Thayer Martin Agar used to recover *Neisseria gonorrhoeae* contains antibiotics; vancomycin, colistin and nystatin.
2. Mannitol Salt Agar and Salt Milk Agar used to recover *S.aureus* contains 10% NaCl.
3. Potassium tellurite medium used to recover *C.diphtheriae* contains 0.04% potassium tellurite.
4. Mac Conkey Agar used for Enterobacteriaceae members contains bile salt that inhibits most gram positive bacteria.
5. Pseudosel Agar (Cetrimide Agar) used to recover *P. aeruginosa* contains cetrimide (antiseptic agent).
6. Crystal Violet Blood Agar used to recover *S. pyogenes* contains 0.0002% crystal violet.
7. Lowenstein Jensen Medium used to recover *M.tuberculosis* is made selective by incorporating malachite green.
8. Wilson and Blair's Agar for recovering *S. typhi* is rendered selective by the addition of dye brilliant green.
9. Selective media such as TCBS Agar used for isolating *V. cholerae* from faecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.

b. Enrichment culture medium: Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. Unlike selective media, enrichment culture is typically used as broth medium. Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water (APW) are used to

recover pathogens from faecal specimens.

4. Differential/indicator media: Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony color. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently colored colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies. Examples of differential media include

    1. Mannitol Salt Agar (Mannitol fermentation- yellow).
    2. Blood Agar (Various kinds of hemolysis i.e. α, β & γ hemolysis).
    3. Mac Conkey Agar (Lactose fermenters, pink colonies whereas non-lactose fermenter produces pale or colorless colonies).
    4. TCBS (*Vibrio cholerae* produces yellow colonies due to fermentation of sucrose).

5. Transport media: Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.

    1. **Cary Blair transport medium** and Venkatraman Ramakrishnan (VR) medium are used to transport feces from suspected cholera patients.
    2. Sach's buffered glycerol saline is used to transport feces from patients suspected to be suffering from bacillary dysentery.
    3. Pike's medium is used to transport *streptococci* from throat specimens.

6. Anaerobic media: Anaerobic bacteria needs special media for the growth because they need low oxygen content, reduced oxidation-reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to be reduced by physical or chemical means. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine can render a medium reduced. Before using the medium it must be boiled in waterbath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

    1. Robertson Cooked Meat (RCM) medium that is commomly used to grow *Clostridium* spp. contains a 2.5 cm column of bullock heart meat and 15

ml of Nutrient broth.

2. Thioglycollate broth contains sodium thioglycollate, glucose, cystine, yeast extract and casein hydrolysate. Methylene blue or resazurin is an oxidation-reduction potential indicator that is incorporated in the medium. Under reduced condition, methylene blue is colorless.

7. Assay media: These media are used for the assay of vitamins, aminoacids and antibiotics. Example- antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique.

   Other types of media include:

   - Media for enumeration of Bacteria
   - Media for Characterization of Bacteria
   - Maintenance media etc.

Culture Techniques

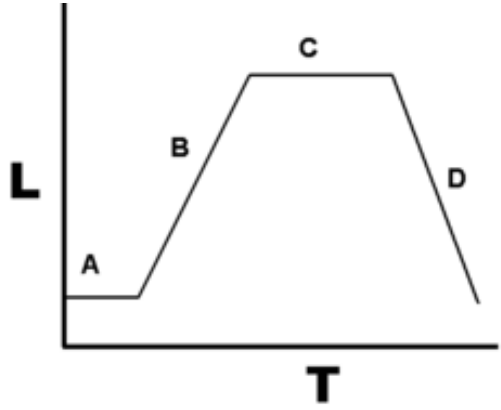
Batch culture is the most common laboratory-growth method in which bacterial growth is studied, but it is only one of many. The bacterial culture is incubated in a closed vessel with a single batch of medium.

In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a chemostat, also known as an open or continuous culture: a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related devices include turbidostats and auxostats. Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria.

In a synecological culture, a true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual.

Bacterial growth curve

The growth of bacteria in closed culture systems, such as a batch culture in LB broth, where no additional nutrients are added and waste products are not removed, the bacterial growth will follow a predicted growth curve and can be modeled. Growth is shown as L= log (numbers) where numbers is the number of colony forming units per ml, versus T (time).



Bacterial growth curve: Bacterial growth in batch culture can be modeled with four different phases: lag phase (A), exponential or log phase (B), stationary phase (C), and death phase (D).

Growth phases

During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.

Exponential phase (sometimes called the log or logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. Under controlled conditions, cyanobacteria can double their population four times a day. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

The stationary phase is due to a growth-limiting factor; this is mostly depletion of a nutrient, and/or the formation of inhibitory products such as organic acids.

At death phase, bacteria run out of nutrients and die.

Immunological Methods employed in food industry

Immunoassay

- Immunoassay means a method to measure any particular substance in a

mixture using its specific binding antibody.

- One of the merits of immunoassay is that we can measure a substance that is present in a mixture of various contaminants.
- Immunoassays have become very popular in view of their high sensitivity, safety, economy and simple instrument requirements.
- Immunoassay technique in their most simple forms provide excellent screening tools to detect adulteration and contaminations qualitatively.
- Immunoassay techniques using the highly specific and sensitive nature of immunological reactions have been developed and applied in the food industry for detecting the naturally occurring constituents, antibiotics, pesticide residues, microorganisms, fragments of microbial constituents related to food analysis.

Radio Immuno Assay (RIA)

- RIA is an immunoassay that uses radiolabelled molecules in a step wise formation of immune complexes.
- RIA is a very sensitive in vitro assay technique used to measure concentrations of substances, usually measuring antigen concentrations by use of antibodies.
- It combines the principle of radioactivity of isotopes and immunological reaction hence the name Radio immuno assay.
- It is highly sensitive and specific analytical tool
- RIA can be used in evaluating the quality and wholesomeness of food. The method is advantageous for its speed, specificity, high sensitivity, relative ease of performance and the possibility of performing a great number of parallel determinations using automation and computer evaluation.
- It can be used in determining macromolecules of proteins and enzymes. Other possibilities of the methods include the determination of microbial toxins of the peptide nature, vitamins, hormones, antibiotics, pesticides and their residues, alkaloids and carcinogenic materials.

RAST

Radioallergosorbent Test is a way of testing a person's blood to see if they have any allergies.

ELISA

- ELISA is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

- In this method the antigen or antibody is conjugated to an enzyme
- First screening test widely used for HIV because of its high sensitivity
- It is a plate based assays designed for detecting and quantifying substances such as peptides, proteins, antibodies, antigens and hormones.
- The test can be done in polystyrene tubes (Macro-ELISA) or polyvinyl microtiter plates (Micro-ELISA).
- It has found applications in the food industry in detecting potential food allergens such as milk, peanuts, walnuts, almonds and eggs.
- Detection of enterotoxin of *E. coli* in feces
- Detection of HIV antibody in blood samples
- Detection of rotavirus in feces

ELISA and PCR in Food Industry

In food industry, the two most common and preferred methods for the detection of allergens are ELISA and PCR. Testing methods have been developed that can now detect these allergens in finished products at very low levels.

Techniques such as ELISA and PCR can detect levels of these contaminants at concentrations in the low parts per million (ppm) range. These techniques detect the food allergen at the molecular level and provide a quick and definitive result that allows manufacturers to dispose of or re-label contaminated products before they are released. It also allerts them to areas of their processing facilities that need to be decontaminated or to production lines that need to be used for other products. The ELISA methods detect the actual allergen protein molecule by binding antibodies to the allergen and then using an enzyme-linked conjugate to create a colorimetric change that can be measured. There are certain instances though, that ELISA methods should not be used. Some matrices can interfere with the ELISA method, such as chocolate can cause cross reactivity as seen between different types of nuts. This method is also not the most suitable for cooked or heated products because the protein molecules are denatured or broken down and the allergen is no longer detectable, but may still cause problems to sensitive individuals.

The PCR methods, which are more sensitive and detect the DNA molecules of these allergens can be used in raw and cooked products and are not affected by the heating process because DNA typically remains intact after exposed to the cooking temperatures of most foods. PCR methods are also not subject to the typical interferences that inhibit ELISA-based methods because the DNA is purified away from these inhibitors before analysis begins. PCR, however can't be used on all products. Oils and other products such as milk or egg whites can't be tested by PCR

because they do not contain DNA. These products must instead be tested using ELISA- based methods for detection. Using a PCR, a single copy of a DNA sequence is exponentially amplified to generate thousands to millions of more copies of that particular DNA segment. PCR have been used in the detection of numerous foodborne pathogens like *Listeria monocytogenes*, *E.coli* 0157 : H7, *Staphylococcus aureus*, *Campylobacter jejuni*, *Salmonella*, *Shigella* spp. and other important targets in food.

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