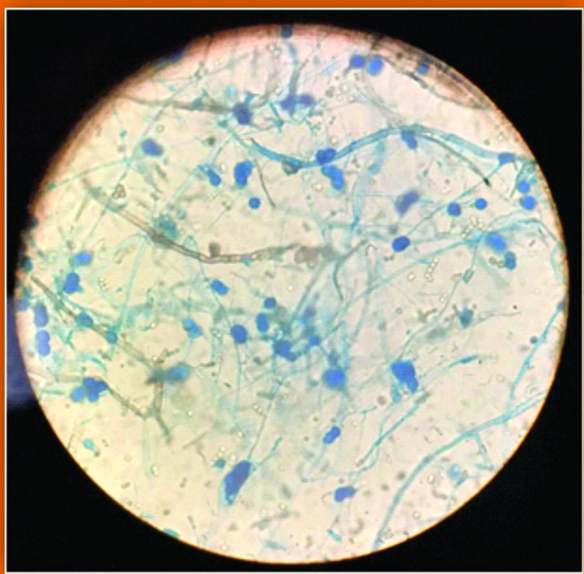
Women University Press
Jayoti Publication Desk
ISBN No. : 978-93-90892-83-9

A Book on Food Biotechnology Vol-II



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UGC Approved Under 2(f) & 12(b) | NAAC Accredited | Recognized by Statutory Councils

Printed by :
JAYOTI PUBLICATION DESK

Published by :
Women University Press
Jayoti Vidyapeeth Women's University, Jaipur

Faculty of Agriculture & Veterinary Science

Title: A Book on Food Biotechnology- Vol-II

Author Name Dr. Aziz Mohammad Khan

Published By: Women University Press

Publisher's Address: Jayoti Vidyapeeth Women's University, Jaipur
Vedaant Gyan Valley,
Village-Jharna, Mahala Jobner Link Road, NH-8
Jaipur Ajmer Express Way,
Jaipur-303122, Rajasthan (INDIA)

Printer's Detail: Jayoti Publication Desk

Edition Detail: I

ISBN: 978-93-90892-83-9

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Chapter -1 Historical background ofFood biotech

FOOD BIOTECHNOLOGY

Biotechnology has a long tradition of use in food production and manufacturing. For ten thousand years fermentation, a form of biotechnology, has been used to manufacture wine, beer and bread. For decades, selective breeding of animals like horses and dogs has been going on. Compared to their wild ancestors, selective breeding of important foods such as corn, maize and wheat has produced thousands of local varieties with increased yield.

The use of technologies for manipulating the genes of our food supplies is food biotechnology. Animals, herbs, and microorganisms are our sources of food. With food biotechnology, we develop new animal and plant organisms, precisely the animals and plants we consume, for example. Nutritional, development, and marketing properties have been desired by these new animals.With food biotechnology, we use what we know about science and genetics to improve the food we eat. We also use it to improve how we produce food.

By change, we mean either making the food cheaper, longer-lasting, more resistant to disease, or more nutritional to make.

The International Food Intelligence Council Foundation writes about the use of biotechnology to help generate the food we need:

"Food biotechnology tools include both traditional breeding techniques, such as cross-breeding, and more advanced techniques, which involve using what we know about genes or specific trait instructions to improve the quantity and quality of plant species."

We may transfer beneficial characteristics from one plant or animal to another with science techniques.

The origins of food fermentation

Pasteur took the more realistic view of an applied scientist, unlike Darwin and Mendel, who is identified as pure scientists. One of his main interests was the manufacture of vinegar, a method that had previously had mixed results due to infection by inappropriate bacteria. The first to recognize the type of bacteria necessary and isolate them in a pure form was Pasteur. From then on, under regulated conditions, vinegar could be produced in a reliable manner, facilitating large-scale, economical production of consistently high quality vinegar.

Now, commercial fermentation of micro-organisms produces multiple food products. In a procedure which is more cost-effective and convenient than the use of lemons, citric acid is extracted from the fungus, Aspergillus niger. The flavor enhancer, monosodium glutamate, is extracted from Corynebacterium glutamicum, a bacterium that produced more than 300,000 tons of this compound worldwide in 1993. Extracts of yeast for use as fruit flavouringYeast extracts for use as food flavourings are produced by fermentation; lactic acid is also made using this method.

The birth of gene technology

The food industry has benefited from the pharmaceutical sector's investment in biotechnology this century, as fermentation methods have been developed to manufacture antibiotics and the knowledge of genetics by scientists has improved. James Watson and Francis Crick lay the basis of genetic modification with the discovery in the 1950s of the replication mechanism of DNA (deoxyribonucleic acid). During the 1970s, developments led to processes becoming more predictable and consistent than ever before, due to increasing molecular level regulation.

Plant breeding has since been changed by genetic modification techniques. Plant breeding is historically aimed at matching the optimal traits of two types of plants. Tomato variety X, for instance, can yield high yields but is vulnerable to diseases, while variety Y is disease-resistant but produces low yields. It may take several years for high yield to be combined with disease resistance in a new variety. Gene technology now has the ability to recognize and spread the disease-resistance gene in variety Y directly to variety X, without the need for time-consuming breeding programs.

Gene technology will also allow genetic material to be mixed in a way that could not exist in nature, in addition to speeding up breeding programs and improving their chances of success. For starters, it is possible to inject copies of animal genes into plants and to incorporate copies of plant genes into bacteria. It is this capacity that increases the diversity of ethical and safety issues currently being addressed across Europe, a dialogue to which the food industry wishes to make a complete and transparent commitment.

Chapter -2 History of Food Microbiology

Food Microbiology does not have a precise start as a discipline. In the end, occurrences over many decades lead to the understanding of the importance and role of microorganisms in foods. From the dawn of our race, food-borne illness and food spoilage have become part of the human condition. While for thousands of years the true cause of these issues will remain a mystery, many early cultures found and applied successful methods to conserve and safeguard their food:

7000 BC- Evidence that beer was made by the Babylonians (fermentation). About 3500 BC, wine emerged. Alcoholic drinks such as beer and wine were far better to drink in early cultures (and still still in underdeveloped countries where modern hygiene is lacking) than the local water source, since the water was sometimes polluted with intestinal microorganisms that triggered cholera, dysentery and other severe illnesses.6000 BC – The first apparent reference to food spoilage in recorded history.

3000 BC-Cheese (fermentation) and butter produced by Egypt (fermentation, low aw). Again, fermented foods such as cheese and sour milk (yogurt) were safer to consume than their raw agricultural counterparts, and avoided spoilage better. In order to protect meat and other foods at this time, many cultures have learnt to use salt (low aw).

1000 BC-Romans used snow to sustain shrimp (low temp), as well as accounts of smoked and fermented meats.

Although early human civilizations found efficient ways to conserve food (fermentation, salt, ice, drying, and smoking), they did not understand how food spoilage or foodborne illness was inhibited by these activities. Their confusion was exacerbated by the assumption that living life arose from non-living matter naturally. (Theory of Spontaneous Generation).

1665-Francesco Redi's Italian physician proved that maggots on putrefying meat did not emerge naturally, but instead were the larval stages of flies (put meat in a jar filled with fine gauze such that flies could not have access to eggs). This was the first step away from the random generation doctrine.

1683-Bacteria through a microscope were studied and identified by Anton van Leeuwenhoek from the Netherlands. At around the same time, in order to interact and publish experimental work, the Royal Society was founded in England and they invited Leeuwenhoek to share his findings. Before his death in 1723, he did so for almost 50 years.As a result, Leeuwenhoek's reports were widely disseminated and he is justifiably regarded as the <u>person who discovered the microbial world.</u>

1765-The Italian Spallanzani sought to disprove the hypothesis of spontaneous life generation by showing that boiled and then sealed beef broth remained sterile. The theory's proponents dismissed his work because they believed that his treatment omitted O2, which they believed was necessary for spontaneous generation.

1795- 12,000 francs were given by the French government to anybody who could create a realistic method of storing food. The patent was given to a French confectioner named Nicholas Appert after proving that meat could be stored when put in glass bottles and cooked. This was the beginning of the conservation of food by canning.

1837-Schwann reveals that in the presence of air (which he passed in by heated coils), healed infusions remain sterile again to disprove spontaneous generation. It is important to note that while Spallanzani and Schwann both used heat to preserve food, the importance of making these findings into a commercial tool for food preservation was obviously not understood by either individual. (Critics say that heating somehow changed the influence of air as spontaneous generation required it.)

Louis Pasteur was the first human to fully appreciate and understand the causal relationship between microorganisms in infusions and the chemical changes that resulted in those infusions. Via his experiments, Pasteur persuaded the science community that microorganisms were responsible for all fermentative processes and that particular forms of fermentation (e.g. alcoholic, lactic or butyric) were the product of specific microorganism types.

Pasteur showed that souring milk was caused by microbes in 1857, and he showed that heat in wine and beer killed undesirable microbes in 1860. For a variety of foods, the latter method is still used and is called pasteurization. Pasteur is regarded as the father of food microbiology and microbiological research because of the relevance of his work. Using his famed swan-necked flasks that finally disproved spontaneous generation, he showed that air doesn't have to be heated to stay sterile.Some of Pasteur's most notable achievements include:

- Fermentation has been found to be a result of microbial action and different forms of fermentation (i.e. lactic, butyric, etc.) have been induced by various types of microorganisms. The knowledge that fermentation and putrefaction were the responsibility of microbes led Pasteur to argue that microbes were also causative agents of disease. Eventually, these claims hit Joseph Lister, an English surgeon who used them to establish the first aseptic surgical procedures.

- Developed a vaccine to protect sheep from anthrax by isolating Bacillus anthracis, the attenuated (virulent) form of the causative bacterium. By developing them at higher temperatures, Pasteur isolated the attenuated organisms (42oC). Sheep is resistant to virulent strains exposed to the attenuated bacterium. While the basis for attenuation was not known by Pasteur, we now know that in this bacterium, virulence relies on the existence of a plasmid that does not reproduce at 42oC.

- A method to make chickens resistant to cholera caused by Pasteurella septica was also developed by Pasteur, again using an attenuated bacterium which he had isolated in his laboratory.

Microbiological discoveries and inventions started to progress more quickly from the time of Pasteur. In many pathogens, bacteria were active, heat-resistant spores were detected, toxins were detected, and by the late 1800s, legislatures started enacting laws to protect food safety.

Many food industries in the U.S. refused to follow broad microbiological standards in the sector until they were economically challenged by the ads surrounding foodborne disease outbreaks. In the early 1920s, many unpleasant outbreaks of botulism gradually forced the U.S. canning industry to introduce a rather restrictive heat treatment, known as the 12D system, which decreases the likelihood of the most heat tolerant C. survival. Up to one in a billion botulinum spores (10-12). This tradition continues today, and since 1925, with just 5-6 documented cases of botulism, the canning field has created more than a trillion containers. Faulty containers were involved in most of these cases, not under packaging.

At the the same time, because of several infamous outbreaks of milk-borne typhoid fever, diphtheria, tuberculosis and brucellosis, the dairy industry was forced to introduce microbiological regulation on milk. Requirements covering animal welfare, hygiene, pasteurization (which had an immediate and very successful impact on the problems) and refrigeration were developed by the public health authorities, both of which were strengthened by bacterial requirements. As a consequence, by the mid-1900s, pasteurized milk was among our safest foods.

"The New York state government institutionalized a woman who came to be known as "Typhoid Mary" in one of the most peculiar episodes of early food microbiology. Mary was an asymptomatic typhoid carrier who served at the turn of the century as a cook for many families. Seven typhoid infections have been specifically attributed to her for more than ten years, and reports indicate that she could have been responsible for 51 cases of typhoid fever. New York police arrested her and threatened to remove her gall bladder, but finally released her after she decided that she would never function again as a chef. A few years after, after another epidemic was linked to her, she was imprisoned as a public safety threat and institutionalized until her death in 1938.

We establish an environment free of competition when we extract microbes from food, which could encourage other microorganisms to develop and cause disease. For this cause, there is great interest in finding healthy bacteria (e.g. lactic acid bacteria) that would prevent the growth of pathogens when purposely applied to food but would not easily ruin the substance itself (though some lost shelf life seems inevitable).

Chapter -3 -Types of Microorganisms in food

Microorganisms

Microorganisms in the food industry play a significant part. Microorganisms are used in the manufacturing of various agricultural products and are also responsible for the spoilage of food, causing poisoning and disease.

Microbial infection of food products typically happens on the way to the processing plant from the farm, or during processing, packaging, transportation and delivery, or prior to use. Bacteria, molds and yeasts are predominantly the microorganisms that cause food spoilage and also find optimum exploitation in the processing of food and food products.

Bacteria

The largest community of unicellular microorganisms is bacteria. In-cocci or circular cells; bacilli or cylindrical or rod-shaped cells; and spiral or curved forms are known as shapes of medically important bacteria. Pathogenic or disease-causing bacteria are normally gram-negative, although it is recognized that three gram-positive rods cause food poisoning: Clostridium botulinum,C.Bacillus cereus, perfringens, and

Acinetobacter, Aeromonas, Escherichia, Proteus, Alcaligenes, Flavobacterium, Pseudomonas, Arcobacter, Salmonella, Lactococcus, Serratia, Campylobacter, Shigella, Citrobacter, Listeria, Staphylococcus, Micrococcus, Corynebacterium, Vibrio Enterobacter, Paenibacillus, Weissella, Enterococcus, Yersinia are some of the other most common bacteria causing food spoilage, infection and disease.

In the processing of various food and dairy products, separate types of bacteria are often used. Streptococcus strains, Bifidobacterium Lactobacillus, Erwiniaetc. They are used in the manufacturing of fermented food and milk products. The processing of yogurt is carried out by Streptococcus thermophilus and Lactobacillusbulgaricus.

Molds:

Molds are multicellular filamentous fungi and are typically easily identified by their fuzzy or cottony appearance for food growth. They are largely responsible for the spoilage of food at room temperature of 25-30oC and low pH, and have minimal requirements for moisture. When these goods are processed under wet conditions, moulds can grow rapidly on grains and maize. For growth, molds need free oxygen and thus grow on the surface of polluted food.

Molds are also found to be used in the manufacture of various foods and dairy items. They are used to ripen different kinds of food items, such as cheese (e.g. Roquefort,Camembert). Molds are also cultivated as feed and food and are used in soft drinks to produce ingredients such as enzymes such as amylase used in the manufacturing of bread or citric acid. Molds play a significant role in the ripening of many Oriental foods. The *Bothrytiscinerea* is used in the rotting of grapes for wine processing. The product of lactic fermentation using molds is a distinctive Finnish.

Yeasts:

Yeasts are capable of fermenting ethanol and carbon dioxide sugars, and are thus commonly used in the food industry. The yeast most widely used, the baker's yeast, is produced industrially. Most commonly, Saccharomyces carlsbergensis is used in the fermentation of most beers. *Brettanomyces, Schizosaccharomyce,, Candida, Cryptococcus, Debaryomyces, Zygosaccharomyces, Hanseniaspora, Saccharomyces* are the other yeast strains of significance.

Points to remember

- The most significant microorganisms that cause food spoilage and also find optimum exploitation in the processing of food and food products are bacteria, molds and yeast.

- For the fermentation of dairy products, different types of bacteria and fungi are used for the processing of a wide range of cultured milk products. Both bacteria and fungi are used in these cheese processing processes.
- For milk coagulation, lactic acid bacteria are used and can be processed to produce a wide range of cheeses, including soft unripened, soft ripened, semisoft, strong, and very hard forms.
- As in the food and health sector, microorganisms such as Lactobacillus and Bifidobacterium are included.
- For the development of various wine varieties, molds are used to rot grapes.
- Spirulina, a cyanobacterium, is a common source of food sold in specialty stores as well.
- Mushrooms are one of the most crucial fungi used as a food source (Agaricusbisporus).
- One of the most important fungi used as a food source is mushrooms (Agaricusbisporus).
- Ferme manufactures soft beverages such as beer. For the processing of various types of wines, moulds are used for the rotting of grapes.
- By fermenting cereals and grains using various strains of yeasts, alcoholic drinks are produced as beer.

Pathogenic micro-organisms

Pathogenic micro-organisms, including microbes, viruses, fungi and moulds, cause food-borne illnesses or intoxication. It is important to remember that pathogenic bacteria and viruses typically do not cause food spoilage, and it is difficult to see or taste their infection.

The major contributing factors to the outbreak of foodborne diseases are:

1. Usage of raw produce and products coming from unhealthy sources
2. Insufficient cooking or heat processing
3. Improper cooling and drying, such as holding cooking food for extended periods of time at room temperature or storing food in large containers in the refrigerator.
4. Allowing many hours to pass between food preparation and feeding
5. Inadequate reheating inadequate

6. Improper warm keeping, which means below 65 ° C
7. Handling of food by sick people or carriers of infection
8. From raw to prepared food, cross contamination. For example, you cut vegetables for salad on a cutting board where you cut raw meat until you cut it.
9. Improper washing of equipment and utensils

Bacteria

- Campylobacter jejuni: is a frequent cause of human diarrhea as well as of some species of animals. Transmission may occur by direct human interaction with infected animals or their waste. More frequently, it is transmitted by the ingestion of infected food or drink, passing from person to person. Symptoms vary from moderate diarrhea to extreme invasive illness, including stomach pain, fever, and stool blood and mucus.
- Non-typhi salmonellosis: Salmonella spp has more than 2000 serotypes, of which only a handful cause human Salmonella gasteroenteritis. Acute watery diarrhea followed by nausea, cramps and fever are the symptoms. Blood can be found in the stools. Animals are the primary reservoir, and infection happens by absorption of infected materials. Poultry, fruit, eggs and milk are foods which are mainly at risk.
- Salmonella typhi and paratyphi, respectively, cause typhoid fever and paratyphoid fever. Transmission happens primarily by person-to-person contact or food contamination by food handlers, as the source for all of these bacteria is generally human.
- **Staphylococcus aureus**: Humans are the cause of this infection. In the nose and on the skin of genetically healthy individuals, bacteria are also present in smaller concentrations. In skin lesions, such as contaminated eczema, psoriasis or some other pus drainage lesion, higher levels can be detected. Thus, these persons should not be handling fruit. Food poisoning caused by this bacteria is caused by staphylotoxin, which is immune to heat, leading to diarrhea, vomiting, cramps and fever. The symptoms unexpectedly start and usually go away within 24 hours.

- **Escherichia coli**: There are many serotypes, several of which can cause gastroenteritis, while others are harmless to humans. The most frequent cause of traveller's diarrhea is Enterotoxigenic E.coli. Humans are the root, and transmission typically takes place by polluted food and water.
- **Listeria monocytogenes**: This bacterium is strongly correlated with food kept in the refrigerator for long periods of time because it is omnipresent and is capable of developing slowly, even at low temperatures. In immunocompromised cases, where it can cause septicemia and meningitis, it can be fatal.
- **Shigella**: Humans and primates are the cause. Since it has a low contagious dose, contact from person to person is the main mode of transmission. It can also be spread by food and drink that is contaminated. Fever and watery diarrhea are the symptoms of shigellosis. The infection may also present itself as a dysenteric condition involving fever, stomach cramps and tenesmus, as well as regular, limited amount, bloody stools.
- **Vibrio Cholerae 01**:People are the cause of this infection. In overcrowded, unhygienic conditions, the primary mode of transmission is by polluted water and food, or person-to-person diffusion. Extreme watery diarrhea, which can reach up to 20 liters a day, is induced.
- **Clostridium Botulinum**: The digestive tract of fish, birds, and mammals is its source. It is also spread extensively in nature. The bacterium is an anaerobic spore with a very strong heat-labile toxin that affects the nervous system.

Viruses

Viruses do not replicate in foods, unlike bacteria. Therefore, the predominant mode of transmission by food handlers and the use of filthy utensils that spread the virus to food is eaten by humans.

- The main causes of gastroenteritis are Rotaviruses and Norwalk viruses.
- Viral hepatitis A outbreaks are caused mostly by asymptomatic food handling carriers.

Parasites

Many parasites, such as helminths, have more than one host involved in a complicated lifecycle. For these parasites, the primary path of transmission to humans is the food route. The pattern tends to be the eating of undercooked pork or beef, or the consumption of raw salads washed in polluted water.

Solium of Taenia and T. Saginata: also called tapeworms for pigs and beef. Their cysts are swallowed, present in the muscle of the species, and the adult worm grows in the gut. The ova will grow into larvae and, as a result, can enter other tissues, such as the brain, forming cysticercosis and significant neurological disorders.

Trichinella spiralis: It is present in pork that is undercooked. Tissues may be attacked by the larvae to develop a febrile disease.

Giardia lambila: This infection may be transmitted through food, water or transferred by interpersonal communication. It induces acute or subacute diarrhea, with malabsorption, stomach pain and bloating, and oily stools.

Entamoeba histolytica: Transmission is primarily transmitted through food or drink. Because they are extremely immune to chemical disinfectants, including chlorination, the cysts pose a serious issue. Typically, the virus is asymptomatic, but may occur either as a moderate chronic diarrhea or as a fulminant dysentery.

Food Spoilage

It is the alteration in food's texture, consistency, taste and scent, and is caused by bacteria, moulds andyeasts.

Bacteria: Examples of action of bacteria involved in food spoilage:

1. Lactic acid formation: Lactobacillus, Leuconostoc
2. Lipolysis: Pseudomonas, Alcaligenes, Serratia, Micrococcus
3. Pigment formation: Flavobacterium, Serratia, Micrococcus
4. Gas formation: Leuconostoc, Lactobacillus, Proteus
5. Slime or rope formation: Enterobacter, Streptococcus

Moulds: Some strains produce mycotoxins under certain conditions

1. Aspergillus produces aflatoxin, ochrtoxin, citrinin and patulin
2. Fusarium
3. Cladosporium
4. Alternaria

Mycotoxins can penetrate into the parts of food that are not visibly mouldy as well. It is therefore necessary to throw away all of the food if any part of it is mouldy. They are also notoriously difficult to destroy as they are stable to both heat and chemicals.

- Hepatotoxins: aflatoxins, sporidesmins, luteoskyrin
- Nephrotoxins: ochratoxin, citrinin
- GIT toxins: trichocetens
- Neuro- and myotoxins: tremorgens, citreoviridin
- Dermatotoxins: verukarins, psoralen, sporidesmins, trichocetes
- Respiratory tract toxins: patulin

Foods most at risk for moulds:

1. Grains and grain products - many mycotoxin types
2. Peanuts, nuts and pulses - aflatoxin
3. Fruits and vegetables (raw and preserved) - patulin
4. Milk and milk products - aflatoxin

It is important to note that if any contaminated fodder is fed to animals, this is metabolized and the toxic derivatives can be found in animal products consumed by humans, e.g. milk and meat.

Chapter -4 Role of Microorganisms in Food Industry

In household Food processing

The members of the family produce household food for their own consumption. Some microorganisms, such as bacteria and fungi, play a variety of roles in household food production.

Lactobacillus, for example, the bacteria involved in the formation of milk and yogurt curds, is produced by Lactobacillus bulgaricus.

Saccharomyces cerevisiae is a type of yeast used in the household and food processing industries to produce bread.

In order to prepare certain popular beverages like Toddy, microorganisms are often used.

Besides these, some bacteria prepare the most popular foods such as dosa and idly from fermented rice.

Industrial Production

Food engineering is one of the most sophisticated ways of using microorganisms to improve the consistency and quantities of food. The method of planning and upgrading the production process of food products includes food engineering. New foods and high quality biological products can be prepared using microorganisms by food engineering. Even, microorganisms are used in industries to sustain food and its consistency.

Microorganisms play a vital role in the processing of a variety of foodstuffs in commercial food production.

1. Antibiotics against pathogens and diseases are essential components of human welfare. These are produced in factories that use bacteria. Penicillin, for instance, is one of the essential antibiotics and is produced by the bacteria Penicillium notatum.

2. Saccharomyces cerevisiae carries out the processing and storage of drinks such as bourbon, brandy, cider, and rum.
3. In the industrial development of enzymes, microorganisms are also involved. Example: Pro
4. One of the essential commercial chemicals that Saccharomyces cerevisiae produces is ethanol.
5. From the fungus, Trichoderma, immunosuppressive agents like Cyclosporin are prepared.
6. Any of the microorganisms in food processing technology are also used for the preservation of packed food.

Significant Microorganisms in Food Production Microorganisms such as molds, yeasts, and bacteria may develop in food and cause spoilage. Bacteria can cause foodborne illnesses as well. Viruses and parasites can cause foodborne illness, such as tapeworms, roundworms, and protozoa, but they are not capable of developing in food and do not cause spoilage.

A list of diseases and infectious agents of importance to public health is as follows. This list is not complete, but includes most foodborne pathogens that impact beef, poultry, and egg products that have been processed.

• **Bacteria**

- *Bacillus cereus* (*B. cereus*)
- *Brucella* species (*Brucella* spp)
- *Campylobacter* spp
- *Clostridium botulinum* (*C. botulinum*)
- *Clostridium perfringens* (*C. perfringens*)
- *Escherichia coli*
- *Listeria monocytogenes* (*L. monocytogenes*)
- *Salmonella* spp
- *Shigella* spp
- *Staphylococcus aureus* (*S. aureus*)
- *Yersinia enterocolitica* (*Y. enterocolitica*)

Viruses

- Hepatitis A and D
- Norovirus
- Rotaviruses

Chapter -5 Microorganisms in food production

Yeasts, bacteria, moulds, or a mixture of these are the most widely used microorganisms. The fermentation process, resulting in the production of organic acids, alcohols, and esters, is a clear example of the use of microorganisms in food production. They help in:

1. Preserve the food
2. generate distinctive new food products

Yeast in food production

Leavened bread and bakery products: Saccharomyces cervisiae ferments CO2-producing sugars, the gas that gives bakery products their porous shape. By forming alchols, aldehydes, esters etc., it also adds to the flavor.

- Beer
- Wine
- Vinegar
- Pickles

Bacteria in food production

- Fermented milk products: *Lactobacillus, Lactococcus, Bifidobacterium*
- A variety of foods, including Indian dosa, rabri: Leuconostoc mesenteroides fermentation, S. Fecalis
- Probiotics: live dietary additives found in yoghurt and other products of fermented milk. Lactobacillus acidophillus and Bifidobacterium bifidum are included. To have some meaningful impact, a minimum of 108 bacteria per 1 ml must get to the colon alive. The microbial spectrum in the gut is strengthened by these bacteria and thus leads to the following effects:

    1. Influences immunity and thereby eliminates or mildens diarrheal diseases
    2. Lowering the risk of bowel cancer

3. Diminish the synthesis of cholesterol

4. It creates acids that reduce the pH of the intestine, thus increasing the absorption of minerals such as calcium and phosphorus.

Mould in food production

- Cheese: Penicillium roqueforti and Penicillium camemberti (note that at 25 ° C this produces mycotoxin, so the processing of cheese must take place at 15 ° C)
- Dry salami: making use of moulds of Penicillium and Scopulariopis.
- Soy sauce: Aspergillus spp, especially A. Oryzae are interested in this manufacturing. A subsequent lactic fermentation is often carried out in which lactic bacteria produce lactic acid.
- Sake: developed using a mixture of yeast and Aspergillus oryzae mould.

Chapter-6 Factors Affecting Growth of Microorganisms

The food processor eliminates microorganisms' possible issues in many ways:

Remove or kill them by trimming, cleaning, boiling, choosing, applying additives, or promoting competition from species that form acid or alcohol.

Minimizing pollution from buildings, persons, the environment, and unprocessed food.

Minimizing microbial growth on facilities, by washing and sanitizing, and by changing storage temperature, pH, and other environmental variables in the substance itself.

While the presentchapter discusses each factor influencing development independently, these factors exist concurrently in nature. Their inhibitory effects are cumulative where more than one situation is very detrimental to microbial development.

Temperature

The most powerful method of regulating microbial growth is temperature. Microorganisms are loosely categorized as follows, based on their tolerance to wide temperature ranges:

1. Psychrophies only develop at the temperature of refrigeration.

2. At refrigeration temperatures, psychrotrophs grow well, but best at room temperature.

3. At or above human body temperature, mesophiles grow best, but grow well at room temperature.

4. Thermophiles only thrive at temperatures that are almost as hot as the human hand can tolerate, and normally not at or below body temperature at all.

To be more detailed on these limits of growth temperature is to step into the controversy that has continued from the beginning of microbiology, and in temperature ranges there are many species that overlap them. However, for food microbiology, these assumptions are relevant: to be more precise about these growth temperature limits is to step into the debate that has persisted since the beginning of microbiology, because there are several organisms that overlap these temperature ranges. However, these conclusions are relevant for food microbiology:

1. In foods below freezing, some psychrotrophic microorganisms develop very slowly, but typically not below 19 ° F. There are a few growth reports, typically of molds, at 14°F, but there are no credible growth reports below that temperature. This suggests that microbial growth is not allowed by the normal storage temperature for frozen foods, O°F. Few microorganisms withstand freezing, however (Michener and Elliott, 1964).Most psychrotrophs have difficulty growing above 90°F.

2. It is difficult for most psychrotrophs to grow above 90°F.

3. Many species with foodborne pathogens are mesophiles. In the awareness that foods kept above or below the limits in Figure 1 and properly rotated will stay healthy, the food processor will feel comfortable. Storing perishable foods below 40°F or over 140°F is a safe rule of thumb.

4. The psychrotrophs develop more quickly in the temperature range where both mesophilic and psychrotrophic species live (from 41 ° F. to around 90 ° F), causing spoilage and often frequently interfering with the development of foodborne disease species (Elliott and Michener, 1965).

The rate of growth grows exponentially within the growth spectrum as the temperature is increased. Conversely, as the temperature is reduced, microbial growth rates decline quickly and, thus, food spoilage happens even more slowly. Near the freezing point, this impact is extremely marked. Note that a decline would more than double the s from about 41 ° F to about 32 ° F(time before spoilage).

Water Activity

Water activity (aw) is a concept that describes microorganisms' supply of water. It is only roughly related to the moisture percentage. Pure water has 1.00 aw, and a 100 percent equilibrium relative humidity (ERH) would provide the atmosphere above the water in a closed bottle. If we apply an ounce of rock in such a bottle to a quart of water, the ERH and aw will not alter. But if we apply an ounce of salt, it's going to reduce the ERH to around 98% and the aw to 0.98. Rocks do not dissolve in water, but salt does, decreasing the percentage of water that will penetrate the environment. Similarly, there is a decrease in the amount of water accessible to microorganisms found in the solution. Yet in the container with rocks, the percent moisture is the same as in the container with salt, namely, 98 percent.

Water behavior is defined by the GMP regulations for low-acid canned foods as the vapor pressure of the food component separated by the vapor pressure of pure water under equal pressure and temperature conditions. The regulations define low-acid foods as foods, other than beverages, with a finished equilibrium pH value greater than 4.6 and a water activity greater than 0.85.

Table 1. The water activity (a_w) limits for growth of principal foodborne disease organisms.*

Microorganism	Minimal a_w for growth	Reference
Salmonella	0.945	Christian & Scott, 1953
Clostridium botulinum	0.95	Scott, 1957
Clostridium perfringens	0.93	Kang, et al., 1969
Staphylococcus aureus	0.86**	Scott, 1962
Vibrio parahaemolyticus	0.94	Beuchat, 1974

* These limits are the lowest stated, with optimal conditions for all other growth. The minimum aw would be higher if other parameters are less than ideal.

** Troller and Stinson (1975) have shown that the minimum aw in their experiments for toxin output is greater than 0.93 for growth.

In a food or other medium where the aw is less than 0.94, most bacteria struggle to expand. Bacteria need a higher aw than yeasts, requiring a higher aw than molds in exchange. Thus, bacteria, then yeasts, and finally molds are inhibited by any condition that reduces the aw first (Elliott and Michener, 1965). But there are limitations for each species that are interrelated with other growth factors. The aw limits for the development of key foodborne disease species kept under otherwise ideal conditions are presented in Table 2.

On fish immersed in saturated salt solution where the aw is around 0.75, some molds and bacteria can emerge. Any molds with AW 0.62-0.655 can develop in foods (Elliott and Michener, 1965). Development is very sluggish at these lower limits. The aw is around 0.10 for entirely dried foods, such as crackers or sugar, and these items are microbiologically stable solely because of this element. Combinations of variables such as low aw, low pH, pasteurization, organic contaminants, and impervious packaging rely on the consistency of intermediate moisture foods (aw 0.75-0.90), such as dried fruits, preserves, and soft moist pet foods.

pH

pH is a term used to describe the acidity or alkalinity of a solution. At pH 7, there is an equal amount of acid (hydrogen ion: H^+) and alkali (hydroxyl ion: OH^-), so the solution is "neutral". pH values below 7 are acidic, while those above 7 are alkaline. pH expresses the H^+ concentration logarithmically, that is, in multiples of 10. For example, at pH 5 there are 10 times as many H^+ as at pH 6; at pH 3 there are 100 times as many H^+ as at pH 5, and so on.

pH has a profound effect on the growth of microorganisms. Most bacteria grow best at about pH 7 and grow poorly or not at all below pH 4. Yeasts and molds, therefore, predominate in low pH foods where bacteria cannot compete. The lactic acid bacteria are exceptions; they can grow in high acid foods and actually produce acid to give us sour milk, pickles, fermented meats, and similar products. Some strains, called Leuconostoc contribute off-flavors to orange juice. The pH values of certain foods are given in Table 2.

Table 2. Mean pH Values of Selected Foods (Lopez, 1987)

pH Value	Selected Foods
2.3	Lemon juice (2.3), Cranberry sauce (2.3)
3.0	Rhubarb (3.1) Applesauce (3.4), Cherries, RSP (3.4) Berries (3.0 – 3.9), Sauerkraut (3.5)Peaches (3.7), Orange juice (3.7) Apricots (3.8)
4.0	Cabbage, red (4.2), Pears (4.2) Tomatoes (4.3)
4.6	Ravioli (4.6) Pimientos (4.7)
5.0	Spaghetti in tomato sauce (4.9) Figs (5.0)Onions (5.2) Carroes (5.2) Green Beans (5.3), Beans with pork (5.3)Asparagus (5.5), Potatoes (5.5)
6.0	Lima beans (5.9), Tuna (5.9), Tamales (5.9) Codfish (6.0), Sardines (6.0), Beef (6.0) Pork (6.1), Evaporated milk (6.1) Frankfurters (6.2), Chicken (6.2) Corn (6.3) Salmon (6.4)
7.0	Crabmeat (6.8), Milk (6.8) Ripe olives (6.9) Hominy (7.0)

The lowest pH limits for growth of foodborne disease organisms are shown in Table 3. Many of the investigators who reported these values also determined that adverse factors, such as low temperature or low water activity, increased the minimal pH for growth. But the processor can be sure that these minimal values will prevent growth of these pathogens under any and all circumstances.

Table 3. The minimal pH minimal for growth of principal foodborne disease organisms*

Microorganism	Growth reported at but not below	Reference
Staphylococcus aureus	pH 4.5	
Salmonella	4.0	Chung and Goepfer, 1970
Clostridium botulinum		
Types A and B	4.8	National Canners Assn., 1971a
Type E	5.0	National Canners Assn., 1971a
Clostridium perfringens	5.0	
Vibrio parahaemolyticus	4.8	Beuchat, 1973
Bacillus cereus	4.9	Kim and Goepfer, 1971

*Note: These limits are the lowest recorded, with all other growth conditions optimal. If other conditions are less than optimal, the pH limit will be higher.

Population

A high initial bacterial load increases the likelihood that spoilage will occur under marginal circumstances (Chung and Goepfert, 1970) (see Figures 4 and 5) (see Figures 4 and 5). This fact is of major importance to the processor of refrigerated foods, the shelf-life of which is enhanced by good sanitation. A high level of spores also increases the possibility that a few will survive to spoil heat processed products.

Oxygen

Oxygen is essential for growth of some microorganisms; these are called aerobes. Others cannot 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 cannot 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 subsurface growth is not.

Chapter 7 The “Indicator” Organisms

The “indicator” organisms are so called because their presence in large numbers in food signifies one of three contamination possibilities: disease bacteria or filth; spoilage or low quality; or preparation under insanitary conditions.

Aerobic Plate Count

The aerobic plate count (APC) measures only that fraction of the bacterial flora that is able to grow to visible colonies under the arbitrary test conditions provided in the time period allowed. It does not measure the total bacterial population in a food sample, but is the best estimate. Altering conditions, such as composition of the agar medium or temperature of incubation, changes the spectrum of organisms that will grow. It is necessary to adhere rigidly to the standardized test conditions that have encouraged some to call the APC a “standard plate count.”

Depending on the circumstances, a high APC may indicate that a food has been grossly mishandled or that it contains a poor quality ingredient. Interpretation depends on knowing what the normal APC is for this food. An abnormal APC indicates that something is out of control. The microbiologist can frequently suggest that cause, thereby aiding the sanitarian. Some of the problems that investigation of a high APC might reveal include:

- Failure of sorting, trimming, washing, and destroying operations to remove or destroy bacteria from raw ingredients adequately.
- Inadequate heat processing.
- Insanitary equipment, particularly near the end of the process.
- The food has reached or is approaching the end of its refrigerated shelf-life.
- The food has been stored at or above room temperature for too long.
- The food is at least partly decomposed.

Coliform Bacteria

The coliform bacteria in human and animal waste are non-spore-forming rods that exist in vast quantities. They are commonly found in raw animal products, such as beef, milk, and eggs, and are often found naturally in soil, water, and plant surfaces. They are heat sensitive and, during blanching or pasteurizing, die easily. Significant numbers of coliforms indicate an inappropriate degree of post-heating contamination during a heat phase or indicate adequate time-temperature abuse of the food to permit development. In order to identify the cause of infection or temperature mishandling, elevated coliform quantities require investigations.

The appearance in the diet of Escherichia colia, a member of the coliform community, typically suggests overt or indirect fecal infection of humans or livestock. While this may be valid in a general context, a quantitative relationship between the E numbers must not be believed. Coli and the degree of stool infection. E. Coli grows well outside the body of the animal and thrives in Uncle

Food Poisoning

Human infections induced by foodborne microorganisms are commonly referred to as food poisoning. The widespread use of a single grouping is largely due to the resemblance of symptoms of multiple food-related diseases (see Table 5). Foodborne disease may be classified into two main groups, food infection and food overdose, aside from illness related to food allergy or food reaction. When foods infected by pathogenic, invasive, food poisoning bacteria are ingested, food contamination occurs. In the human body, these bacteria then proliferate and ultimately cause disease. The intake of preformed toxic compounds that develop during the development of certain bacterial forms in foods is accompanied by food intoxication.

The incubation period is called the period of time between the ingestion of infected foods and the occurrence of illness. Depending on the causative species or the harmful substance, the incubation time can be anything from less than one hour to more than three days.

Table 5. Characteristics of the important bacterial food intoxications and foodborne infections. (NAS-NRC, 1975)*

Disease	Etiologic Agent	Incubation Period	Symptons
Botulism	*Clostridium botulinum* A.B.E.F toxin	Usually 1 to 2 days; range 12 hours to more than 1 week	Difficulty in swalling, double vision, difficulty in speech. Occasionally nausea, vomiting, and diarrhea in early stages. Constipation and subnormal temperature. Respiration becomes difficult, often followed by death from paralysis of muscles of respiration.
Staphylococcal food poisoning	Staphyloccal enterotoxin	1 to 6 hours; average 3 hours	Nausea, vomiting, abdominal cramps, diarrhea, and acute prostration. Temperature subnormal during acute attack, may be elevated later. Rapid recovery- usually within 1 day.
Salmonellosis	Specific infection by *Salmonella* spp.	Average about 18 hours; range 7 to 72 hours	Abdominal pains, diarrhea, chills, fever, frequent vomiting, prostration. Duration of illness: 1 day to 1 week.
Shigellosis (bacillary dysentery)	Shigella sonnei, s. flexneri, s. dysenteriae, s. boydii	Usually 24 to 48 hours; range 7 to 48 hours	Abdominal cramps, fever, chills, diarrhea, watery stool (frequently containing blood, mucus, or pus), spasm, headache, nausea, dehydration, prostration. Duration: a few days.
Enteropathogenic *Escherichia coli* infection	*Escherichia coli* serotypes associated with infant and adult infections	Usually 10 to 12 hours; range 5 to 48 hours	Headache, malaise, fever, chills, diarrhea, vomiting, abdominal pain. Duration: a few days.
Clostridium perfringens food poisoning	*Clostridium perfringens*	Usually 10 to 12 hours; range 8 to 22 hours	Abdominal cramps and diarrhea, nausea, and malaise, vomiting very rare. Meat and poultry products usually involved. Rapid Recovery.

Bacillus cereus food poisoning	*Bacillus cereus*	Usually about 12 hours; range about 8 to 16 hours	Similar to *Clostridium perfringens* poisoning
Vibrio Parahaemolyticus food poisoning	*Vibrio Parahaemolyticus*	Usually 12 to 14 hours; range 2 to 48 hours	Abdominal pain, server watery diarrhea, usually nausea and vomiting, mild fever, chills and headache. Duration: 2 to 5 days.

Chapter 8 Microbial Nutrition and Growth

Growth Requirements

Microbiologists use the term growth to describe a rise in the population of a microbe rather than an increase in size. Microbial growth is based on the synthesis of nutrients which results in the formation of a distinct colony, a single parent cell assembly of cells. A nutrient is any chemical needed for microbial communities to grow. The most important of these are compounds containing carbon, oxygen, nitrogen, and/or hydrogen.

Nutrients: Chemical and Energy Requirements

To perform metabolism, all cells need three things: a source of carbon, a source of electricity, and a source of electrons or hydrogen atoms.

Sources of Carbon, Energy, and Electrons

Organisms may be classified into one of four categories depending on their carbon source and their use as an energy source of either chemicals or light:

In order to make their own food, photoautotrophs use carbon dioxide as a carbon source and light energy from the atmosphere.

Chcmoautotrophs use carbon dioxide but catabolize organic molecules for energy as a source of carbon.

Photosynthetic species that obtain energy from light and acquire nutrients by organic compound catabolism are photoheterotrophs.

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• For both energy and biomass, chemoheterotrophs use organic compounds.

In addition, organotrophs acquire electrons from organic sources, whereas Iithotrophs acquire electrons from inorganic carbon sources

Oxygen Requirements

As the final electron acceptor of the electron transport chain, mandatory aerobes use oxygen, while mandatory anaerobes are unable to tolerate oxygen and use an electron acceptor other than oxygen. Toxic sources of oxygen induce a sequence of vigorous oxidation and are strongly reactive. Four oxygen forms are toxic:

Singlet oxygen (l02) is molecular oxygen with electrons that have been accelerated, usually during aerobic metabolism, to a higher energy state. Phototropic microorganisms also possess pigments called carotenoids, which, through eliminating the surplus energy of singlet oxygen, avoid toxicity.

• Supcroxide radicals (0:;.-) are formed by anaerobes in the presence of oxygen during the incomplete decrease in oxygen during electron transfer in aerobes and during metabolism. Superoxide dismutase detoxifies them.

• Peroxide anion (022-) is a hydrogen peroxide part formed during superoxide dismutase-catalyzed reactions. Peroxide anion is deroxified by the enzymes catalase and peroxidase.

The consequence of ionizing radiation and the incomplete removal of hydrogen peroxide is hydroxyl radicals (OH ').Hydroxyl radicals are the most reactive of the four toxic sources of oxygen, but the threat of hydroxyl radicals is practically reduced in aerobic cells because hydrogen peroxide does not accumulate in aerobic cells.

Neither strict aerobes nor anaerobes are not all organisms. Facultative anaerobes can sustain life by fermentation or anaerobic respiration, but, in the absence of oxygen, their metabolic efficiency is always decreased. Aerotolerant anaerobes prefer anaerobic environments, but since they have a sort of enzymes that detoxify the toxic sources of oxygen, they can withstand oxygen.

Microaerophiles have low levels of oxygen. Capnophiles thrive best with high carbon dioxide concentrations in comparison to reduced oxygen levels.

Requirements for Nitrogen

Nitrogen is a growth-limiting nutrient for many microorganisms that extract nitrogen from organic and inorganic nutrients. Although nitrogen occupies about 79 percent of the atmosphere, only few animals can use nitrogen gas. A few bacteria convert nitrogen gas to ammonia through a process called nitrogen fixation, which is important for life on Earth.

Other Chemical Requirements

Very small quantities of trace elements, such as selenium, zinc, etc., are required in addition to the key elements present in microbes.Many species still need limited quantities of such organic chemicals that can not be synthesized by them. These are called factors for growth. For instance, for certain microorganisms, vitamins are growth factors.

Physical Requirements

Organisms have physical requirements for growth in addition to chemical nutrients, including precise temperature, pH, osmolarity, and pressure factors.

Temperature

While microbes live within the limits imposed by a minimum temperature for growth and a maximum temperature for growth, the metabolic processes of an organism achieve the highest growth rate at the optimum temperature for growth.

In terms of their temperature requirements, microbes are classified as (from coldest to warmest):

- Temperatures below 20°C are expected by psychrophiles.
- At temperatures ranging between about 20 ° C and 40 ° C, mesophiles grow best.
- Temperatures above 45°C are required for thermophiles.
- Temperatures above 80°C are necessary for hyperthermophiles.

pH

Organisms are sensitive to changes in acidity because hydrogen ions and hydroxyl ions interfere with hydrogen bonding within the molecules of proteins and nucleic acids; 54 Study Guide for Microbiology as a result, organisms have ranges of acidity that they prefer and can tolerate. Most bacteria and protozoa are called neutrophiles because they grow best in a narrow range around a neutral pH, between 6.5 and 7.5. By contrast, other bacteria and many fungi are acidophiles, and grow best in acidic environments where pH can range as low as 0.0. In contrast, alkalinophiles live in alkaline soils and water up to pH 11.5.

Physical Effects of Water

In certain metabolic reactions, microorganisms require water to degrade enzymes and nutrients and to serve as a reactant. Cells are limited to some environments by osmotic pressure. Although some microbes' cell walls shield them from osmotic shock, osmosis can cause other cells to die from either swelling or bursting or shriveling (crenation). Obligatory halophiles, such as those present in salt water, need high osmotic pressure. Facultative halophiles do not need salty conditions, but can accommodate them.

In proportion to its depth, water exerts pressure and the pressure in deep ocean basins and trenches is immense. Organisms existing under intense pressure are referred to as barophiles. In order to preserve their three-dimensional functional forms, their membranes and enzymes rely on pressure, and they can usually not live at sea level.

Ecological Associations

Relationships are called antagonistic in which one organism damages or even destroys another. Members of an association interact in synergistic relationships in such a manner that each gains advantages that outweigh those that would arise if each resided independently. Organisms exist in near dietary or physical association within symbiotic partnerships, being interdependent.

Biofilms are an example of dynamic interactions between multiple entities, often different species, that bind to surfaces together and exhibit metabolic and structural characteristics distinct from those displayed alone by each of the microorganisms. They also develop as a result of quorum sensing, a mechanism in which bacteria use signal and receptor molecules to respond to the density of surrounding bacteria.

Chapter 9- Fermented food and their health benefits

A number of health benefits are associated with fermentation. In fact, fermented foods are often more nutritious than their unfermented form.

Improves Digestive Health

During fermentation, the probiotics produced may help restore the balance of pleasant bacteria in your gut and can ease some digestive problems.

Probiotics may alleviate painful symptoms of irritable bowel syndrome (IBS), a chronic digestive disorder, research indicates.

One 6-week study of 274 IBS adults showed that drinking 4.4 ounces (125 grams) of fermented yogurt-like milk daily strengthened symptoms of IBS, including bloating and diarrhea frequency.

Moreover, the incidence of diarrhea, bloating, gas and constipation can also be decreased by fermented foods.

For these factors, if you often have intestinal disorders, it might be helpful to incorporate fermented foods to your diet.

Boosting Immune System

There is a huge effect on the immune system from the bacteria that reside in your gut.Fermented foods will give your immune system a boost because of their high probiotic content and reduce the chance of illnesses like the common cold.

When you're ill, eating probiotic-rich diets will also help you heal quicker.

In addition, vitamin C, iron, and zinc are rich in many fermented foods, all of which have been shown to lead to a stronger immune system.

Makes Food Easier to Digest

Fermentation helps break down dietary nutrients, making them easier to absorb than their counterparts that are not fermented.

For example, during fermentation, lactose, the natural sugar in milk, is broken down into simpler sugars-glucose and galactose.

As a consequence, fortified milk such as kefir and yogurt is normally consumed well for anyone with lactose intolerance.

In addition, fermentation helps to break down and kill antinutrients that interfere with nutrient absorption, such as phytates and lectins, which are compounds present in seeds, nuts, grains, and legumes.

The intake of fermented beans or legumes such as tempeh thus improves the absorption of valuable nutrients, making them more nutritious.

Other Potential Benefits

Studies have shown that fermented foods may also promote:

- **Mental health**: A few studies have linked the probiotic strains *Lactobacillus helveticus* and *Bifidobacterium longum* to a reduction in symptoms of anxiety and depression. Both probiotics are found in fermented foods.
- **Weight loss**: While more research is needed, some studies have found links between certain probiotic strains — including *Lactobacillus rhamnosus* and *Lactobacillus gasseri* — and weight loss and decreased belly fat.
- **Heart health**: Fermented foods have been associated with a lower risk of heart disease. Probiotics may also modestly reduce blood pressure and help lower total and “bad” LDL cholesterol.

Nutritional Highlights

- Fermented foods are high in probiotic microbes, so you introduce helpful bacteria and enzymes to the general intestinal flora by eating fermented foods, increasing the health of the gastrointestinal microbiota and digestive tract, and strengthening the immune system.

Digestion and absorption

Fermented foods are easier to absorb when more of the carbohydrates and starches of food have been broken down by the process. For example, fermentation breaks down the lactose in milk into simpler carbohydrates, glucose and galactose, which will make it theoretically easier to eat things like yogurt and cheese if you are lactose intolerant.

- **Synthesis and availability of nutrients**

- The supply of vitamins and minerals for our bodies to consume can also be improved by fermentation. In addition, you are supporting their ability to develop B vitamins and synthesize vitamin K by enhancing the beneficial bacteria in your stomach.

Immune functions

- The gut houses a significant proportion of the immune system. You help the mucosa (gut lining) as a natural shield by eating probiotic-rich ingredients, rendering the immune system more resilient. A lack of beneficial bacteria enables the development of diseases that cause microbes that cause inflammation in the intestinal wall. Probiotic diets are especially beneficial if you have just taken a course of antibiotics.

Phytic Acid

- By fermentation, certain natural compounds which interfere with the absorption of nutrients can be extracted. For example, phytic acid, which is present in legumes and seeds, binds minerals such as iron and zinc and, when ingested, decreases their absorption. During fermentation, however, phytic acid may be broken down such that the minerals become available.

Mood and behaviour

Via the hypothalamic-pituitary-adrenal (HPA) axis, the gut and the brain are linked. The intestine, scientifically called the enteric nervous system, is packed with neurons that can trigger our thoughts and emotions. Serotonin, a mood-involved neurotransmitter, is produced in the intestine, and research further suggests that they are also related to a healthier mind as probiotic bacteria add to a healthy gut.

Chapter 10 Bacteriocin of lactic acid bacteria

Introduction

A large number of Gram (+) and Gram (-) bacteria develop protein-structured substances (either proteins or polypeptides) with antimicrobial activities, called bacteriocins, during their development. Although bacteriocins may be labeled as antibiotics, they are not. The key distinction between bacteriocins and antibiotics is that the action of bacteriocins is limited to strains of species similar to the producing species and to strains of the same species in particular. In the other hand, antibiotics have a broader range of action and this does not indicate any preferential effect on closely related strains, even though their activity is limited.In addition, during the primary phase of development, bacteriocins are ribosomally synthesized and generated, while antibiotics are typically secondary metabolites. Bacteriocins typically have a low molecular weight (rarely more than 10 kDa) and can be easily degraded by proteolytic enzymes, especially proteases from the mammalian gastrointestinal tract, making them safe for human consumption. In general, bacteriocins are cationic, amphipathic molecules as they contain excess residues of lysyl and arginyl.They are normally unstructured when incorporated in aqueous solutions, but they form a helical shape when subjected to structure supporting solvents such as triofluroethanol or combined with anionic membranes of phospholipids. Among the bacteria that have Gram positive (+),

Due to the development of bacteriocins, lactic acid bacteria (LAB) have gained special interest nowadays. It is possible to add these compounds as natural preservatives in the food industry. In general, the use of LAB and its metabolic products is regarded as healthy (GRAS, Grade One).

Classification of LAB Bacteriocins

Most LAB bacteriocins are cationic, heat-stable, amphiphilic and membrane-permeabilizing peptides that are small (<10 kDa). They can be classified into three key groups, because of the extensive research realized, their classification has been continuously revised over the last decade. All of these bacteriocins tend to have very little precision of adsorption. Gram positive

(+) bacteria have a cell wall that allows comparatively large molecules to move through. In the initial interaction of anionic bacteriocins formed by Gram positive (+) bacteria, anionic cell surface polymers such as teichoic and lipoteichoic acids, which are part of the cellular wall, are essential.At lower pH values (below 5), LAB bacteriocins have greater antibacterial activity, indicating that their adsorption to the cell surface of Gram-positive (+) bacteria, including the forming cells, is pH based. There can be amino acid sequence homologies not only within the mature peptide, but also in the N-terminal leading region and the related proteins in the secretion and processing of bacteriocin within any class of bacteriocin.

Class I: The Lantibiotics

Class I, the lantibiotics, are a class of peptide substances that contain the characteristic polycyclic thioether amino acids lanthionine or ethyllanthionine, as well as the unsaturated amino acids dehydroalanine and 2- aminoisobutyric acid. They are further devised into two types based on structural similarities. Type A comprises of relatively elongated, screw shaped, positively charged, amphipatic, flexible molecules. Their molecular mass varies between 2 to 4 kDa and they generally act through pore formation, through membrane depolarization, of the cytoplasmic membrane of the sensitive target species, nisin and lacticin 3147 are the major representatives of this group.Type B lantibiotics, are globular in structure and interfere with cellular enzymatic reactions. Their molecular mass, lies between 2 to 3 kDa and either they have no net charge or a net negative charge.

Class I LAB bacteriocins are small (<5kDa) heat stable peptides, which are extensively modified after translation resulting in the formation of characteristic thioether amino acids lanthionine (Lan) and □- methyllanthionine (MeLan). These arise after a two-step process. Firstly gene encoded serine and threonine can be subject to enzymatic dehydration to give rise to dehydroalanine (Dha) and dehydrobutyrine (Dhb)..

Subsequently, the double bond of Dha and Dhb producing both Lan and MeLan is invaded by thiol groups from adjacent cysteins. This condensation between two neighboring residues results in the creation of covalently closed rings that impart both structure and functionality within the previously linear peptide. Members in this category include D-alanine as well. This latter residue of amino acids is derived from dehydroalanine, arising from the serine residue dehydration.

Class II: the Non-Lantibiotics

Also small (<10 kDa), Class II bacteriocins are relatively heat-stable, non-lanthionine-containing membrane active peptides. They are broken into 2 subclasses. An N-terminal consensus sequence Tyr-Gly-Asn-Gly-Val-Xaa-Cys is available in subclass II a, pediocin-like or listeria active bacteriocins subclass. When the corresponding amino acid sequences are aligned, they display a high degree of homology (40 percent -60 percent) and are synthesized with a leader peptide that is removed by proteolytic processing, typically after a double glycine residue, such as pediocin PA-1, sakacin A.Subclass II b refers to twocomponent(twoseparate peptides) bacteriocins by means that require two peptides to work synergistically in order to have anantimicrobial activity. Lactacin F and lactococcin G are members of this group.

1.1.3. Class III: Bacteriocins

This group consists of heat labile proteins which are in general of large molecular weight (>30 kDa). Thisgroup has not been extensively investigated. Bacteriocins representing this group are helveticin I by*Lactobacillus helveticus and* enterolysin produced by *Enterococcus faecium*.

Table Most important bacteriocins produced by *Lactobacilli*

Bacteriocin Bacteriocin Producing Strain

Lactacin F *L. johnsonii spp.*

Lactocin 705 *L. casei spp.*

Lactoccin G *L. lactis spp.*

Lactococcin MN *Lactococcus lactis var cremoris*

Nisin *Lactococcus lactis spp.*

Leucocin H *Leuconostoc spp.*

Plantaricin EF, Plantaricin W Plantaricin JK,

Plantaricin S

L. plantarum spp

2. Biosynthesis and Immunity of LAB Bacteriocins

Ribosomally synthesized and posttranslationally transformed peptides are bacteriocins as previously described. The genes encoding bacteriocin development and immunity are typically arranged in operon clusters and may be found on mobilisable elements such as transpons-associated chromosomes or on plasmid. The bacteriocins are mainly synthesized as biologically inactive prepeptides possessing a plasmid. Other proteins or amino acids encoded by the bacteriocin gene cluster before export are then modified by the prepeptides.For example, there are thioether cross-links termed lanthionines (Lans) or methyllanthionines (MeLans) additionally to amino acids 2.3-didehydroalanine (Dha) and (2).-2-3-didehydrobutyrine (Dhb). These amino acids are introduced by the dehydration of serine and threonineresidues followed by stereo selective intermolecular addition of cysteins onto the unsaturated amino acids.Bacteriocin producing strains have to protect themselves from the action, the toxic effect of their ownbacteriocin.This occurs through the production of specific immunity proteins. The immunity protein coding genes are in close genetic similarity to other structural and processing genes of bacteriocin. The structural gene of bacteriocin and the immune gene may also be put on the same operon. Two forms of immune mechanisms have been identified for LAB bacteriocins. One mechanism depends on the unique Lan I immunity, while the second system depends on a separately designated multicomponent ABC transporter (Lan EFG). Most likely, the Lan I protein is bound to the cytoplasmic membrane outside.It provides immunity to the producer cells by preventing pore formation by the bacteriocinmolecules that have inserted into the membrane, back to the surrounding medium and thus keeping theconcentration of bacteriocin in the membrane under a critical level.

Most Important Bacteriocins

Plantaricins

L. plantarum has been considered to produce at least 6 distinct bacteriocins. All these peptides were generated primarily as precursors containing a double moiety of glycine. L. Plantarum, via the PlnE and PlnF genes, synthesizes these bacteriocins. The PlnG and PlnH proteins then export and refine these peptides. A separate gene (PlnA) encodes the peptide pheromone for this mechanism and is exported by PlnG and PlnH and detected by the histidine protein kinase PlnB, which eventually phosphorylates two PlnC and PlnD reaction regulators.Plantaricins inhibit a broad range of LAB including their naturalcompetitor *L. plantarum* and other bacteria like *Pediococcus, Carnobacteria, Clostiridia andPropionobacteria*.

Plantaricins JK, EF

As synergetic peptides, these bacteriocins function. They are 30 to 40 residues in length and exhibit no resemblance in sequence to nearly every other plantaricin. These bacteriocins function with strict specificity and, with the exception of JK or EF, any other mixture results in full synergy failure.

Plantaricin S and Plantaricin W

Plantaricin S is a device isolated from L of two peptides. About plantarum spp. Used when green olives are fermented. The structural genes suggest that with a leader containing the double glycine motif, each peptide is initially formed. These peptides have lengths of 26 and 27 amino acids. It is considered that Plantaricin S controls the fermentation mechanism and protects the olives. Plantaricin W, composed of Plwa and Plwb protein molecules, is another two-peptide bacteriocin. These lantibiotic components compose and are considered of 29 and 32 amino acid residues respectively.

Nisin

The most commonly exploited and applied bacteriocin is nisin. It is active against Gram (+) positive bacteria, including microorganisms that are highly pathogenic and spoil food, including S. Aureus, and with L. Monocytogernes. Single. Since 1988, its use in the United States has been approved by the FDA for use in cheese, heat treated soups processed in the chill and pasteurized cheese spreads stored at chill temperature.Nisin belongs to the Class Ilantibiotics, is composed by thirty-four amino acids and has a pentacyclic structure with one lanthionineresidue (Ring A) and four □-methyllanthionine residues (rings B, C, D, E) nisin Z, the natural variant of nisinis different only in that the histidine molecule on place 27 is replaced by asparaginesNisin can, depending on the target strain, be effective at nanomolar concentrations. Nisin is synthesized ribosomally as a precursor peptide that is enzymatically modified later on. This prepeptide is biologically inactive and comprises a c-terminal prepeptide domain, which is separated from the N-terminal leader chain to create the mature antimicrobial compound after a number of posttranslational modification reactions. It is an auto-regulatory two-component mechanism that can be completely triggered by very low sub-toxic concentrations (ng/ml) of nisin.

Nisin is 121^{o}C nheat stable but becomes less heat stable for sustained heating, particularly between pH 5 to 7. Nisin is susceptible to trypsin, elastase, carboxyl peptidase, pepsin, and erepsin but immune to al-chymotrypsin. Nisin is used as a food additive and is manufactured on a commercial basis and is assigned to E234 (ECCU 1983 EEC Commission Directive 8314631EEC). The NICE mechanism is part of the pheromone-dependent quorum sensing systems that have been researched in detail and are widely distributed in Gram positive (+) bacteria.

Mode of Action of Nisin Membrane Insertion & Models of Pore Formation

A high concentration of anionic lipids in the membrane characterizes Gram positive (+) bacteria. Nisin binds quickly to anionic liposomes and this association is strong because nisin has been able to slowly spread to other liposomes. To recognize the regions that are involved in membrane interaction, fragments of nisin were used. Nisin has the potential to communicate with its antimicrobial activity associated with anionic lipids. Interacting with the peptidoglycan precursor lipid II, it forms pores in the lipid membrane.The presence oflipid II enhances the ability of nisin to depolarize the transmembrane electrical potential and disrupting thelipid bilayer organization when it binds to the membrane.Via a sequence of different steps, Nisin forms pores. Nisin tends to orient parallel to the surface of the membrane in equilibrium. Nisin triggers the fluorescent phospholipid transmembrane movement, meaning that the membrane insertion of the nisin Cterminus creates phospholipid intermonolayer touch, creating pores according to the model wedge. As follows, a wedge-like model can be represented. A proton motive force driven by co-insertion of lipids and nisin domains is involved in mediated pore formation. The hinge in the nisin molecule may allow the C-terminal portion to be bent and thus incorporated into the membrane.Multiple inserted nisin moleculesmay give rise to a large local disturbance of the lipid protein pores. Such structures are intrinsically unstabledue to the hydrophobic forces, which are driving the rearrangements of the lipids into their original bilayer organization.

4. Applications of Bacteriocins

Bacteriocins are now commonly used, especially in the field of food preservation. The use of bacteriocins in the food industry has been thoroughly studied, in particular in milk, eggs, vegetables and meat products. Among the LAB bacteriocins, nisin A and its natural counterpart nisin Z have been shown to be highly effective against food poisoning and spoilage by microbial agents. Nisin is still the only bacteriocin officially used in the food industry and its use has been licensed internationally. Numerous techniques of preservation have been used to avoid food poisoning and spoilage, though.These procedures include thermal treatment (pasteurization, sterilization of heating), reduction of pH and water activity (acidification, dehydration) and preservative inclusion (antibiotics, organic compounds such as propionate, sorbate, benzoate, lactate, and acetate). Although these approaches have been shown to be highly effective, there is a rising need for sustainable, microbiologically stable goods with strong health benefits for consumers. Bacteriocins may be added to a purified or crude form or by the use of a substance previously fermented with a strain producing bacteriocin as an ingredient in food processing or by the inclusion of a bacteriocin producing strain. The lack of consistency between the bacteriocin-producing strain and the other cultures needed for fermentation has the downside of introducing a bacteriocin-producing strain. It has been shown, however, that bacteriocin alone in a food is not likely to guarantee full safety; this has been obvious in the case of Gram negative bacteria in particular. The use of bacteriocins must then be paired with other methods capable of destroying the cell membrane so that pathogenic bacteria can be destroyed by bacteriocins. The use of non-thermal therapies such as pulsed electric fields, for example, is useful because it does not affect the functionality of food and dietary functions.This technique may not be financially viable when usedalone, but in lower levels and combined with other treatments such as bacteriocins may be highly effective.

In addition, bacteriocins could be paired with other antimicrobial agents, such as sodium acetate and lactate sodium, resulting in increased bacterial inactivation. Bacteriocins may also be used to boost the consistency and sensory properties of foodstuffs, such as to increase the rate of proteolysis or to avoid gas blowing defects in cheese. Bioactive wrapping, a mechanism which can shield the food from foreign contamination, is another use of bacteriocins. For example, refrigerated food spoilage typically starts with surface microbial growth that enhances the attractive use of bacteriocins used in conjunction with packaging to enhance food safety and self-life.Bioactive packaging can be prepared by directly immobilizing the bacteriocin in the food packaging or by applying a bacteriocin-containing sachet to the packaged food to be released during the food product storage process. The incremental release on the food surface of bacteriocins from a packaging film can have a benefit over dipping and spraying food with bacteriocins, as antimicrobial activity can be lost or reduced due to inactivation of bacteriocins by food components or dilution below active concentration due to migration through foods.One method is to incorporate bacteriocin directly into polymers for example incorporation of nisin into biodegradable protein films. The incorporation of nisin or any other bacteriocin can be achieved through heat press and casting into films made from soy proteins or corn zein. Another method is to coat or adsorb bacteriocins to polymer surfaces; examples include nisin methylcellulose coatings for polyethylene films for the use on poultry meat, adsorption of nisin on polyethylene, ethylene, vinyl acetate, polypropylene, polyamide, polyester acrylics and polyvinyl chloride.

Chapter-11Foodborne Disease caused by Micro-organisms

Escherichia coli

A few of the *E. Coli* strains found in human feces are in themselves pathogenic, causing infection and disease. These are called *EnteropathogenicE. Coli* or EEC. In one extensive study of the feces of food handlers (Hal and Hause, 1966), 6.4% of the workers harbored the EEC organisms as carriers.

Staphylococcus aureus

S. aureus, commonly referred to as "staph," is normally present on the skin, the mucous membranes, and in pimples and boils of human beings and other animals. It is nearly always present in small numbers in raw meats and in foods handled extensively by human hands. The food poisoning strains generally come from human sources. Pasteurizing or cooking destroys the organism, but not its toxin. Foods contaminated by staph organisms can cause food poisoning after the organisms have been destroyed by heat.

The presence of staph in a cooked food has two levels of significance.

1. Low numbers (not over a few hundred per gram) indicate the degree of contact with human skin or nasal mucous, cross-contamination from raw meat, or survivors of a larger population.
2. High numbers (100,000 or more per gram) indicate that the bacteria were allowed to grow in the food, thereby creating the potential serious hazard of the presence of toxin.

- It's always difficult or impossible to keep foods absolutely free from staph infection. The processor should therefore store the food at temperatures that preclude staph formation. It's only during development that the toxin forms staph. It is tedious to perform an epidemiological examination to ascertain the organism's source, but visual observation of the hands of staff may be beneficial. The well-informed hygienist would also seek time-temperature violations of staph-contaminated foods.
- The National Research Council of the National Academy of Science listed the following measures to regulate the occurrence and degree of staph in foods (NAS-NRC, 1975):

  Reduce direct and indirect exposure of foods, particularly cooked foods, to human contact as much as possible. If handling is necessary, use sanitary rubber or plastic gloves, or sanitize hands. Persons with infected cuts, abrasions, boils, or pimples should never handle cooked foods.

1. Test raw materials and eliminate production lots that contain high levels of S. aureus.
2. Process to destroy the microorganisms.
3. Eliminate cross-contamination from raw to cooked food.
4. Keep cooked foods no longer than 2 to 3 hours between 40°F and 140°F.

Control of staph growth in fermented foods, such as cheese or sausages, requires controlling a number of processing factors(see NAS-NRC, 1975). Low pH, relatively high levels of lactic bacteria, salt, and nitrite help to inhibit toxin formation.

Salmonella

Salmonella infection, or salmonellosis, is almost invariably caused by consuming food or water that has been infected. Contamination originates from humans or animals that host Salmonella species in the intestinal tract. Most adults are able to avoid infection from a few cells, but become sick when millions are consumed. Kids, the elderly, and the infirm are even more susceptible and a few Salmonella cells may affect them. After healing, for a duration ranging from a week to permanency, the survivor can remain a courier.

Domestic animals are vectors of these diseases, such as dogs, pigs, swine, goats, sheep, and cattle. At the moment of killing, carriers exhibit no visible signs of the infection. Salmonella infection of the finished raw meat is unavoidable as long as abattoirs begin collecting Salmonella carriers for slaughter. Slaughtering and dressing procedures, even with seemingly adequate sanitation, may disperse traces of feces from a carrier animal to subsequently slaughtered animals through machinery, water, and hand contact (NAS-NRC, 1969).

Salmonella, as if it were a single entity, is also debated. In fact, within the genus Salmonella, there are over 1,300 serotypes described. All are very heat sensitive, because the body is free of freshly pasteurized or fried foods (USDA, 1966).Cross-contamination of raw foods or animals (via paws, appliances, air, water), recontamination by human carriers or gross undercooking are the major routes of entry into cooked foods. Regulatory authorities are quick to institute seizures, recalls and other legal actions against goods and businesses exporting raw foods tainted with Salmonella.

Fermented sausages that are dry and semi-dry seldom cause foodborne diseases. Recent USDA investigations, however, have demonstrated that Salmonella can withstand the process of fermentation and drying. Salmonella still survives brief salting cycles in normal animal casings, but dies more readily in acidified or alkalized casings.

Salmonella can also develop outside the body of the animal under favorable conditions. For this cause, it has found in a wide range of foods and feeds, in addition to meat and poultry items. Brewer's yeast, coconut meat, cochineal pigment, dried or frozen eggs, pasta, custards, dried animal feed, cottonseed flour, sugar, cocoa, dried milk, fish and shellfish, pastries filled with icing, casings of sausage, and watermelon are some of these. Extensive guidelines for the diagnosis, regulation, and eradication of the Salmonella problem have been made by the NAS-NRC (1969-1975).

Costridium botulinum

C. Botulinum causes an uncommon but sometimes lethal illness called botulism. It is caused by a neurotoxin which is created in the absence of air during development. The preserved spores are harmless, except in the case of infantile botulism. Symptoms of botulism have been produced by children who eat spores, generally from honey. Typically, botulism happens when a food containing the preformed toxin has been consumed, although the parasite infects wounds occasionally, forming the toxin in the victim's muscle. Seven forms of C exist. Botulinum (A to G), four of which are human diseases (A and B associated with meat and plants, E, aquatic climate, and F). Type C has only been reported to cause human disease once.

Fortunately, the toxins have very little resistance to heat, regardless of form, and are inactivated by boiling for 10 minutes. All fresh, but sufficiently cooked foods are therefore healthy (Riemann, 1973). All of C. Spores may be formed by botulinum strains that display differing heat resistance. Types A and B spores are strongly resistant. Class E spores die within a fraction of a minute at 212 °F (Perkins, 1964). Under the professional leadership of the National Food Processors Association (formerly the National Canners Association), the canning industry has set the requisite retorting times and temperatures to ensure the commercial sterility of low-acid canned food (NCA, 1968, 1971b, 1976b).The NFPA also submitted to the FDA the initial petition which eventually developed in the GMP regulations for low-acid canned foods.

Botulinum spores are dispersed uniformly in the soil. In the western states and in New England, type A predominates; in the eastern and southern states, type B. Form E is commonly synonymous worldwide with aquatic or fresh water conditions and is psychrotropic (Riemann, 1973). Form F has been isolated too rarely to decide its pattern of distribution (Eklund et.al., 1967).

C. There will be no botulinum growth below pH 4.8. Botulism is therefore of interest only in foods with low acidity, which are classified as foods with a finished pH equilibrium greater than 4.6. Most outbreaks come from home-made canned vegetables, meats, fish, and over-ripe fruits (USPHS, 1974).

Salt and nitrite are present in dried cured meats. The preservatives safeguard against the production of botulinum spores that may have survived limited handling, sometimes at or below boiling (Halvorson, 1955; Ingram and Hobbs, 1954; Pivnick et. al, 1969).

In the U.S. and Canada, there were 34 outbreaks of type E botulism among fish products prepared in the (Lechowich, 1972). They were mainly smoked or thinly salted items. The FDA isolated types B, E, and F of botulinum from pasteurized blue crab meat. The NAS-NRC (1975) reviewed measures to reduce the risk of smoked fish outbreaks, and the FDA issued legislation to monitor the issue (FDA, 1970).

Clostridium perfringens

C. Perfringens is a spore-forming organism that develops only in the absence of air, analogous to botulinum. In meat or poultry dishes, stews, or gravies stored wet, it grows best. These products fulfill their exacting nutritional requirements and their growth is stimulated by the warm holding temperature, up to 122 ° F. The spores themselves are harmless, but in the intestinal tract of the victim, the vegetative cells that can expand to tremendous quantities in these foods develop spores. The remainder of the vegetative cell dissolves during the sporulation process, releasing the toxin that induces sickness.

The disease-causing vegetative cells are very fragile. By cooking or freezing, they may be lost or reduced to minimal, healthy quantities. The spores are commonly dispersed in nature and present in different foods in limited quantities (Hall and Angelotti, 1965; Strong et. al., 1963). They appear in feces, soils, water, mud, aquatic sediments, raw foods, and even foods that are fried.

C. Poisoning perfringens is a problem that is unique to the food service industry. The issue is only avoided by careful temperature regulation. Holding ready-to-eat moist foods below 40°F or over 140°F is a strong rule of thumb. A serious health threat is time-temperature violence. Epidemiologic investigation of strains to identify the source of spores is a relatively pointless exercise because the spores are everywhere. If serological checks indicate, though, that the same kinds are found in the food and feces of the victim, a certain dish may be incriminated. The biological materials (antisera) for this reason are, sadly, not yet commercially available. The insistence, thus, that vast numbers of C. Perfringens cells are now the most appropriate forensic test.

Bacillus cereus

B. Cereus is a spore-forming organism that spreads and is commonly dispersed in most raw foods in the presence of oxygen. As the spores survive boiling for several minutes, they remain viable in limited numbers in cooked foods. In raw foods, the organism does not compete well with other bacteria, but in moist, cooked dishes kept warm (up to 122 ° F), in a few hours it expands to millions per gram. The food becomes toxic in these circumstances. B. In a large range of cooked foods, such as meats, fish, sauces, puddings, soups, corn, potatoes, and vegetables, cereus grows well. The condition is identical to perfringens, but the disease cause is unclear. There are very mild signs in adults, although small children can become critically ill. The patients heal easily in most cases and do not seek medical treatment. Only big outbreaks are then registered and become part of the historical record.

Close to that of C. Perfringens, by B. Cereus is mainly a food service sector concern. Holding hot foods hot (over 140 ° F) and cold foods cold (under 40 ° F) is the necessary regulation. It is similarly useless to epidemiologically investigate strains to ascertain the origins of the spores.

Vibrio parahaemolyticus

V. parahaemolyticus is a slightly bent, non-spore-forming rod that is closely connected to the organism that causes cholera. It is commonly spread and grows worldwide in the habitats of brackfish, estuarine sediments, raw fish, and shellfish. At temperatures of 41 ° F or above, it competes well with spoiling species. When higher temperatures generate rapid growth, it happens in greater numbers in the summer.

The major source of food poisoning in Japan, where raw fish is frequently eaten, is V. parahaemolyticus. Elsewhere, since the organism dies readily during pasteurization or cooking, the disease occurs less often. Nevertheless, cooked seafoods can be recontaminated from water or raw seafood. The first confirmed outbreaks in the United States occurred in 1971 and 1972 from crabmeat, shrimp, and lobster. In one Japanese outbreak, 22 people died and 250 others became ill.

The human pathogenicity of the organism is determined by the cultivation of salt agar, which includes human blood, in a specific medium. If on this medium, the so-called Kanagawa measure, the organism can expand and kill blood cells, it is labelled "Kanagawa positive" and deemed capable of causing human illness. The Japanese find that Kanagawa is positive for around 1 percent of the strains of V. parahaemolyticus from waters near their shores (Sakazaki et. al., 1968). Twedt et., on the other hand, Al. (1970) indicated that Kanagawa is positive for up to 90% of the strains from U.S. estuarial waters. The value of the Kanagawa examination, however, is not well known.

To reduce the incidence of these outbreaks, the seafood industry should:

- Hold raw seafoods at or below 40°F;
- Keep cooked seafoods carefully apart from raw seafood, sea water, insanitary equipment, and unclean containers; and
- Hold cooked seafood below 40°F or above 140°F

Listeria

Most problems associated with Listeria-induced diseases were linked to cattle or sheep until the 1980s. In Nova Scotia, Massachusetts, California and Texas, this changed with food induced outbreaks. Listeria is now recognised as an important food borne pathogen because of its extensive distribution in the ecosystem, its ability to survive long periods of time under unfavorable conditions, and its ability to thrive at cooling temperatures.

Immunocompromised human beings are particularly vulnerable to virulent Listeria, such as pregnant women or the elderly. The most reliably pathogenic listeriosis-causing bacteria is Listeria monocytogenes. In humans, bacterial consumption can be accompanied by a flu-like condition, or signs can be so slight that they go unnoticed. A carrier state could evolve.

Virulent strains of Listeria can then multiply following the invasion of macrophages, resulting in destruction of these cells and septicemia. The organism has connections to all areas of the body at this time. Death is rare in healthy adults; in the immunocompromised, infant or very young, though, the mortality rate can be about 30 percent.

As described earlier, because it can withstand adverse conditions, Listeria monocytogenes is a particular concern. It can grow in a pH range of 5.0-9.5, in a medium of good growth. The organism has survived the pH 5 of cottage cheese and Cheddar ripening climate. Surviving amounts as high as 30.5 percent over 100 days at 39.2 ° F are salt resistant. But only for five days if it is held at 98.6°F.

The main argument is that the temperature of the coolant does not inhibit the development of Listeria. It is capable of doubling in numbers at 39.2°F every 1.5 days. As the Listeria species can be inactivated by high temperatures, greater than 175 ° F, post-process exposure by environmental sources thus becomes a vital control point for many foods.

Yersinia enterocolitica

While Yersinia enterocolitica in the U.S. is not a common source of human infection, it is frequently implicated in very serious symptoms of the disease. Yersiniosis, a microorganism-induced inflammation, occurs most often in the form of gastroenteritis. Kids are affected more seriously. Many needless appendectomies have resulted in signs of pseudo-appendicitis. Death is unusual and healing normally takes 1-2 days to complete. Arthritis has been recognised as an uncommon but serious outcome of this condition.

Y. Enterocolitica is usually found in meats, but most isolates do not cause illness, with the exception of pork. It is sensitive to heat (122 F., sodium chloride (5%) and acidity (pH 4.6), and will normally be inactivated by environmental conditions that will kill salmonellae.

Campylobacter jejuni

C. Jejuni was isolated from human diarrhoeal stools for the first time in 1971. Since then, as an organism-causing disease, it has steadily achieved recognition in humans.

C. Jejuni in developed countries, enteritis spreads primarily from foods of animal origin to humans. Fecal degradation of food and water and contact with sick people or animals predominate in developing countries, too.

Future studies are required to identify poultry and its components and meats (beef, pork and lamb) as significant reservoirs and vehicles, while milk has been identified most frequently as a vector for Campylobacter worldwide.

*C. jejuni*at ambient and atmospheric temperatures, dies readily and grows poorly in food.

In the regulation of this universal organism, animal science principles will play a significant role. Cross-contamination will be avoided by hygienic slaughter and handling procedures, although the microbial load will reduce due to adequate cooling and aeration. In addition, thorough cooking of meat and poultry products followed by good handling can help safeguard food safety and reduce contamination.

Mycotoxins

Mycotoxins are toxic mold by-products that thrive on food and feed. For years, they have induced serious sickness and death in animals. When 100,000 turkey poults died in England after eating moldy peanut meal from Africa and South America, they first came to the attention of western science in 1960. Aflatoxins, a group of closely associated organic compounds that can cause acute disease and death, were later shown to be the mycotoxins involved. Stimulated by these first findings and antibiotic studies, researchers have identified hundreds of mold strains that contain a wide spectrum of animal-affected mycotoxins. There are over 60 toxins known now. Just a handful of these have been listed as human food toxins. If mycotoxin investigations continue and detection techniques are developed, these figures are expected to rise.

Mycotoxins have, traditionally, been linked with human toxicity and even death. Ergot is among the few mycotoxins that have been identified as affecting humans. A mold forming on cereal grains creates it. The poisoning of Ergot happened in the Rhine Valley in 857 and has since been recorded numerous times. In southern France, the most recent outbreak was in 1951. During World War II, many Russians died from eating moldy grains. Human toxicity from consuming moldy rice was documented by the Japanese; the disease caused serious liver damage, hemorrhaging, and some fatalities (Mirocha, 1969).

While such events are uncommon, there is evidence that low dietary levels of aflatoxin in human beings lead to liver cancer. Extensive laboratory tests have also shown that aflatoxin can produce liver cancer in rodents, mice, monkeys, ducks, ferrets, and rainbow trout, also at very low dietary levels. Southeast Asian and African epidemiological studies have linked a high prevalence of human liver cancer with aflatoxin levels of up to 300 parts per billion (ppb) in 20% of food staples and 3 to 4 ppb in 7% of food staples. 95 per cent of maize and 80 per cent of peanuts contained aflatoxin at an average level of 100 ppb in one geographical region.

While there is no clear evidence that aflatoxins cause cancer of the human liver in the United States, the effects of long-term, low-level intake of a recognized, potentially carcinogenic product on our food supply are of interest to the FDA. In 1965, the FDA established a tolerance standard of informal defect action of 30 ppb on peanuts and peanut products. The level of aflatoxin contamination steadily fell with better harvesting, handling, and sorting methods established by the USDA and industry, and the FDA reduced the level of informal intervention to 20 ppb in 1969. A legislation specifying a 15 ppb tolerance for total aflatoxins in shelled peanuts and peanut products used as human food was proposed by the FDA in the Federal Register of 6 December 1974. The limits today are 0.5 ppb for milk, 20 ppb for dairy, and 100 ppb for food.

On any food not heated in a closed container, molds that form mycotoxins may be present. Therefore, one would conclude that if conditions permit, they are present and ready to produce toxin. However, having a toxigenic mold in a food does not mean that there is a mycotoxin in the food. In comparison, the lack of visible growth of the mold producing aflatoxin does not mean the toxin is absent, since aflatoxins can be produced where there is no visible growth of the mold.

There are many ways to decide if mycotoxins are formed by molds developing in abused food. The food can be stored or inoculated with a toxigenic strain with its naturally contaminating molds, and kept until the molds mature. The food will then be tested for toxin involvement or absence. These studies have shown that molds develop mycotoxins on a wide range of cereal grains and peas, fruit and dried beans, spices, nuts, and cured meats. For optimum growth and toxin production, molds have moisture, temperature, and nutritional requirements, as do bacteria. In most cases, prior to or after harvest, the original mold invasion happens in the fields. During storage, mold growth persists if the moisture content and storage temperatures remain high.

In maize, barley, copra, cassava, spices, dried milk, tree nuts, cottonseed, peanuts, rice, wheat, and grain sorghum, aflatoxin has been discovered worldwide. Maize, figs, grain sorghum, cottonseed, peanuts, and some tree nuts have been found in the U.S.

In order to monitor aflatoxin levels in arid walnuts, the industry focused on electronic and visual sorting processes, as well as blowing and vacuuming. In order to diagnose potential aflatoxin pollution, corn mill operators use high-intensity ultraviolet ('black') light. In certain examples, roasting lowers aflatoxin levels by up to 50%. (Escher et. al., 1973).

The universal solution to the problem is the removal, wherever possible, of conditions that allow mold growth, and thus the prevention of mycotoxin formation. Mold growth and toxin production occur in some cases (corn, peanuts) prior to harvesting. Corn kernels affected by insects and birds are very susceptible; thus, managing these pests can help to mitigate mold problems. For most susceptible foods, when the moisture content is high enough to allow mold formation, the crucial time is immediately following processing, during storage and initial drying.

Chapter 12 -Food preservation from spoilage by common methods

Easy spoilage by bacteria, yeasts, or molds that are not harmful to health is the most common microbiological issue facing the food industry. Chilling delays spoilage; it is completely arrested by proper cooling, drying, canning, and pickling. Until spoiling microorganisms makes them unfit for eating, chilled foods must be shipped to the user. In other methods, the issues of spoilage occur only as they deviate from existing techniques. By taking adequate steps, the occurrence of food spoilage can be significantly minimized and shelf-life increased.

Refrigerated Foods

At a remarkable pace, the use of refrigerated/chilled foods is growing. Any of these items are easy to use and have a picture that is "near to fresh." Prior to chilling, some of these items are partly cooked or processed. This heat decreases the microbial population, but does not make it "commercially sterile." Refrigerated foods have a short shelf-life because of this. That is influenced by client violence and temperature.

For several years, refrigerated food has been available in our shops. In the refrigerated area or deli, products such as milk, butter, yogurt and other dairy products, biscuits and biscuit dough, eggs, salads and processed meat are usually located. 33°F is the optimal storage temperature. Or as near as practicable to freezing. Most refrigerated cases, however, are similar to 45 or even 45 ° F. This fluctuation in temperature limits the shelf life of the goods which can contribute to a concern of interest to public health.

A paper on' Protection Issues for New Generation Refrigerated Foods' was published in the January, 1988 issue of Dairy and Food Sanitation by the Refrigerated Foods and Microbiological Criteria Committee of the National Food Processors Association. From that article, many of the points considered in this section were taken.

Several critical points ought to be considered in terms of planning, handling and delivery. First of all, always assume the presence of pathogenic species in a food product. Secondly, cooling temperatures can delay or discourage most pathogenic microorganisms from replicating, but some will continue to reproduce (psychrotrophs). Yersinia enterocolitica, Listeria monocytogenes, non-proteolytic strains of C, contain psychrotropic pathogens. Any strains of botulinum enterotoxigenic E. The hydrophilia in coli and Aeromonas. Several other species with foodborne diseases that are able to develop at just above 41 ° F include: Vibrio parahemolyticus; Bacillus cereus; Staphylococcus aureus and some *Salmonella* strains. Third, any temperature abuse of foods during storage and delivery can be required by manufacturers; this involves handling at the market level.

The last two points deal with marking for consideration. The declaration "Keep Under Refrigeration" must be conspicuous on the product label and outside of the carton. Furthermore, on these goods, a 'Sale By' or 'Use By' date has to be used. This will assist processors to manage their commodity, but it is not a guarantee against issues. If the stock is not correctly rotated, the commodity that is out of date will also go out. As many therapies as practicable must be integrated into a refrigerated food processor that can help reduce the microbial population and minimize replication. Some of these therapies include: heat, acidification, preservatives, decreased operation of water, and packaging of the changed atmosphere. While the changed atmosphere is included as a possible deterrent, it must be remembered that anaerobic pathogens can currently prefer reduced oxygen atmospheres. The changed environment is really a help for many products to increase product quality rather than protection.

Pasteurized cheese spread is one example of a product which successfully employs the multiple barrier concept. The product uses a combination of reduced water activity (added salt and phosphates) and mild heat treatment to eliminate non-spore forming pathogens and inhibit growth of spore forming pathogenic microorganisms.

Canned Foods

The shelf-life of canned foods results from the destruction of microorganisms capable of growth within the container during normal handling and storage. To attain this optimum situation, canners should:

- Follow the GMP regulations for low-acid foods.

- By maintaining a sanitation program, especially for blanchers and elsewhere where thermophilic spore formers thrive, and by tracking ingredients for spore-forming bacteria, reduce the spore level in the food. As a general rule, in the same or related procedures, food with a high spore level demands greater retort time and/or temperature. For any low-acid and acidified food marketed in the U.S., a procedure approved by a production authority must be submitted with the FDA. Assuming the same retort time and/or temperature, while all other variables are the same, the rate of spoilage in canned food with a high initial spore level would be higher.
- • During the container cooling and post-cooling cycle, adopt proper ventilation and good container handling techniques. It is also necessary to rapidly cool heat treated containers to around l00 °F (38C) because if containers are stacked or cased when heated, thermophilic outgrowth will occur with low spore numbers.
- Maintain strong seams by periodic inspection and checking on cans and secure lids on glass containers.

Table .Effect of level of flat-sour spores on incidence of spoilage of canned vegetables. (Reed and Bohrer, 1961).

Product	**Spores per can before processing (number)**	**Incidence of spoilage (percent)***
Canned peas	2,160	0
	13,000	66
Canned corn	900	16.7
	38,000	100

*After incubation of processed cans at 130°F (54.4°C)

Dry Foods

Dry foods should not degrade until they are sufficiently dry from microbial activity. Before they become solid, most foods require natural or artificial drying. The addition of sugar or salt, as in candied fruit or salted cod, serves the same function as humidity becomes unavailable for microorganisms to use.The appropriate term to express the availability of water to microorganisms is water activity (a_w).

Though dry foods cannot develop microorganisms, those that survive the drying process remain alive for extended periods. Upon rehydration, they rapidly restart their operations. Molds are normally the first to develop due to their broader range of resistance to low aw (Watson and McFarlane, 1948) under unfavorable storage conditions that allow water to access the substance and they thus have less competition from other species.

Fermented and Pickled Foods

Fermented and pickled foods owe their stability to the microbial production by lactic bacteria of organic acids or to the addition to foods of certain acids, especially in the presence of a relatively high salt amount. During the fermentation process or upon storage of the finished product, spoilage may occur. If the bacteriophage attacks the starter culture, if the temperature is unsuitable, or if the amount of fermentable carbohydrate is insufficient, the fermentation may fail.

To prevent spoilage during the fermentation period:

1. Add lactic bacteria as a starter. Keep the starter in pure culture to help eliminate bacteriophage.
2. Add fermentable carbohydrate or organic acid.
3. Maintain the salt level high enough to inhibit spoilage bacteria and to permit the more salt-tolerant lactics to grow.
4. Control the temperature to favor lactics.

To reduce or eliminate spoilage during storage of the pickled or fermented food:

1. Add chemical preservatives, such as benzoates, sorbates, or propionates suitable to the product and acceptable to regulatory authorities.
2. Pasteurize the product, if practicable, to destroy or inhibit spoilage organisms.
3. Store pickles fully covered with brine to inhibit molds and impede yeast development.

Chapter-16 Lethal Effects of Temperature

The most practical and reliable means of killing microorganisms is heat. The reduction of microbial cells steadily happens just above maximum growth temperatures. However, if the temperature is increased, the risk of mortality rises markedly. Pasteurization consists of a temperature of 140°F for 30 minutes, or around 161°F for 16 seconds, and is the destruction of vegetative cells of disease-producing microorganisms. At pasteurization temperatures, yeasts, moulds, and the vegetative cells of spoilage bacteria often perish. A retort capable of working at temperatures above 212°F is necessary to make log-acid foods commercially sterile. For a significant amount of time, often an hour or more depending on the commodity, canners process some canned foods at 240 ° F or 250 ° F and can size. Commercial sterility is the destruction and/or inhibition of public health organisms and non-health-significant organisms that could ruin the commodity. For 15 to 20 minutes, microbiologists sterilize media at 250 ° F (121C). The requirement for high temperatures and enough time to kill a population of bacteria is demonstrated by these examples.

The logarithm of the numbers of survivors is plotted against the period of time test cultures are exposed to a given temperature in thermal degradation tests, also called thermal death time studies. Usually, the outcome is a straight line, though there are several variations. If the temperature is elevated, the slope of this line gets steeper, meaning that less time is taken to kill a population at higher temperatures. A high number of species often takes longer to kill than it does to kill a low population.

The rate of thermal destruction is greater in foods with high a_w than in those with low a_w (Calhoun and Frazier, 1966). Microbial contaminants in dry foods, such as chocolate (Goepfert and Biggie, 1968) or dried bone meal (Riemann, 1968), are hard to destroy with heat. The recommended pasteurization process to destroy Salmonella in liquid egg albumen prior to freezing is 140°F (60C) for 3.5 minutes (USDA, 1969), whereas that for dried egg albumen is 140 (60C) to 158°F (70C) for several days (Banwart and Ayres, 1956). Riemann (1968) was able to kill Salmonella in meat and bone meal more readily at 194°F (90C) after water was added to bring the a_w to 0.90.

Table 4. The effect of the size of the initial spore population on destruction time. (From Reed and Bohrer, 1961)

Microorganism	Spores (number)	Temperature °F (°C)	Destruction time (minutes)
Flat sour #26	45,000	239 (115.9)	62 to 65
	400	239 (115.9)	25 to 28
Clostridium botulinum #90	90,000	221 (105.8)	18 to 20
	900	221 (105.8)	12 to 14

For water activities between 0.2 and 0.4 (dry heat), Clostridium botulinum spores are extremely tolerant to thermal degradation and are much less resistant to heat for water activities beyond this level. For high-temperature-short dry heat sterilization time, this result could be realistic.

The presence or absence of organic matter, oil or fat, pH, strain of species, nature of available nutrients, and age of the culture are other factors that influence the rate of thermal degradation of bacteria. In general, at lower and higher pH values, bacteria are more quickly killed than in more neutral ranges. Careful monitoring of pH is an essential consideration when preparing certain foods.

Chilling to temperatures below the growth level, but above zero, prevents replication but destroys few cells, except for species that are highly susceptible, such as *Clostridium perfringens* vegetative cells. Within a few hours, the freeze kills part of a microbial population and storage tends to be lethal at a much slower pace. The rate of population loss varies with the quality of the food, with orange juice, which is an acid substance, experiencing the most rapid decrease in aerobic plate count ('total count'). During freezing and frozen preservation, bacterial spores die very slowly, if at all. For instance, in general, all *Clostridium perfringens* vegetative cells die, but the spores remain.Staphylococcus aureus and associated organisms live well, but microorganisms, including closely related species, have large differences in susceptibility in most cases (Figure 8). Freezing, in this event, is not a reliable method of killing microorganisms, since certain cells of the original population almost always survive.

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(Only Speed Post is Received at University Campus Address, No. any Courier Facility is available at Campus Address)

Pages	: 66
Book Price	: ₹ 150/-

JPD Women University Press

Jayoti Publication Desk

Year & Month of Publication- 3/9/2021