UNIT - I
FOOD AS A SUBSTRATE FOR MICRO ORGANISMS

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The nutritional requirements of various microorganisms may differ appreciably but all of them require carbon, nitrogen, and phosphorus sources, as well as other minerals and frequently vitamins. Sources of nitrogen (ammonia, ammonium salts, and nitrates) and of phosphorus are well known.

These as well as sources for other minerals such as calcium, magnesium, potassium and trace elements are infrequently mentioned in the technical literature because of the overriding importance of the carbon source for the technical and economic feasibility of the biomass process. In some instances, vitamins such as biotin, thiamine, or others have to be supplied to the growth medium.

Hydrogen ion concentration (pH)

The pH, or hydrogen ion concentration, [H+], of natural environments varies from about 0.5 in the most acidic soils to about 10.5 in the most alkaline lakes. Since the pH is measured on a logarithmic scale, the [H+] of natural environments varies over a billion-fold and some microorganisms are living at the extremes, as well as every point between the extremes. The range of pH over which an organism Grows is defined by three cardinal points: The minimum pH, below which the organism cannot grow, the maximum pH, above which the organism cannot grow, and the optimum pH, at which the organism grows the best. Microorganisms which grow at an optimum pH well below neutrality (7.0) are called acidophiles. Those which grow best at neutral pH are called neutrophiles and those that grow best under alkaline conditions are called alkalophiles.

In general, bacteria grow faster in the pH range of 6.0- 8.0, yeasts 4.5-6.5 and filamentous fungi 3.5-6.8, with the exception of lactobacilli and acetic acid bacteria with optima between pH 5.0 and 6.0 (Table 4.6). The approximate pH ranges of some common food commodities are shown in Table 4.7.

Table 4.6 Approximate pH ranges of different microbial groups

Microbe	Minimum	Optimum	Maximum
Most Bacteria	4.5	6.5 – 7.5	9.0
Yeasts	1.5 – 3.5	4.0 – 6.5	4.0 – 6.5
Molds	1.5 – 3.5	4.5 – 6.8	8.0 – 11.0

Table 4.7 Approximate pH ranges of some common food commodities

Product	pH
Citrus fruits	2.0-5.0
Soft drinks	2.5-4.0
Apples	2.9-3.3
Bananas	4.5-4.7
Beer	3.5-4.5
Meat	5.6-6.2
Vegetables	4.0-6.5
Fish (most spp)	6.6-6.8
Milk	6.5-6.8
Wheat flour	6.2-6.8
Egg white	8.5-9.5
Fermented shark	10.5-11.5

Water Activity (aW)

Water is often the major constituent in foods. Even relatively dry'foods like bread and cheese usually contain more than 35% water. The state of water in a food can be most usefully described in terms of water activity. Water activity of a food is the ratio between the vapour pressure of the food, when in a completely undisturbed balance with the surrounding air, and the vapour pressure of pure water under identical conditions. Water activity, in practice, is measured as Equilibrium Relative Humidity (ERH) and is given by the formula: Water Activity (aW) = ERH / 100

Water activity is an important property that can be used to predict food safety, stability and quality. The various applications of water activity include maintaining the chemical stability of foods, minimizing non enzymatic browning reactions and spontaneous autocatalytic lipid oxidation reactions, prolonging the desired activity of enzymes and vitamins in foods, optimizing the physical properties of foods such as texture. Water activity scale extends from 0 (bone dry) to 1.00 (pure water). But most foods have a water activity in the range of 0.2 for very dry foods to 0.99 for moist fresh foods. Based on regulations, if a food has a water activity value of 0.85 or below, it is generally considered as non-hazardous. This is because below a water activity of 0.91, most bacteria including the pathogens such as *Clostridium botulinum* cannot grow. But an exception is Staphylococcus aureus which can be inhibited by water activity value of 0.91 under anaerobic conditions but under aerobic conditions, it requires a minimum water activity value of 0.86. Most molds and yeasts can grow at a minimum water activity value of 0.80. Thus, a dry food like bread is generally spoiled by molds and not bacteria. In general, the water activity requirement of microorganisms decreases in the following order:

Bacteria > Yeast > Mold. Below 0.60, no microbiological growth is possible. Thus the dried foods like milk powder, cookies, biscuits etc are more shelf stable and safe as compared to moist or semi-moist foods. Factors that affect water activity requirements of microorganisms include the following- kind of solute added, nutritive value of culture medium, temperature, oxygen supply, pH, inhibitors, etc. Each microorganism has a minimal water activity for growth as shown in Table 4.4

Table 4.4 Minimum water activity values of spoilage microorganisms

Microbial group	Minimum a_w	Examples
Most bacteria	0.91	*Salmonella* spp. *Clostridium botulinum*
Most yeasts	0.88	*Torulopsis* spp.
Most molds	0.80	*Aspergillus flavus*
Halophilic bacteria	0.75	*Wallemia sebi*
Xerophilic molds	0.65	*Aspergillus echinulats*
Osmophilic yeasts	0.60	*Saccharomyces bisporus*

Water activity of some foods and susceptibility to spoilage by microorganisms is shown in Table 4.5. Water acts as an essential solvent that is needed for most biochemical reactions by the microorganisms. Water activity of the foods can be reduced by several methods: by the addition of solutes or hydrophilic colloids, cooking, drying and dehydration: (e.g., egg powder, pasta), or by concentration (e.g. condensed milk) which restrict microbial growth so as to make the food microbiologically stable and safe.

Table 4.5: Water activity of some foods and susceptibility to spoilage by microorganisms

Water activity	Microorganisms grow at this a_w and above	Food examples
1.00 – 0.95	*Pseudomonas, E. coli, Proteus, Shigella, Klebsiella, Bacillus, Clostridium perfringens* & some yeasts	Highly perishable fresh foods & canned fruits, vegetables, meat, fish, milk, eggs; foods containing up to 40% (w/w) sucrose or 7% NaCl.
0.95 – 0.91	*Salmonella, Vibrio parahemolyticus, Clostridium botulinum, Serratia, Lactobacillus, Pediococcus,* some molds and yeasts	Some cheeses (cheddar, Swiss), cured meats; some fruit juice concentrates; bread; high moisture prunes; foods containing 55% (w/w) sucrose or 12% NaCl
0.91 – 0.87	Many yeasts like *Candida, Torulopsis, Hansenula; Micrococcus*	Fermented sausages; sponge cakes; dry cheese; margarine; foods containing 65%(w/w) sucrose (saturated) or 15% NaCl
0.87 – 0.80	Most molds, *Staphylococcus aureus*, most *Saccharomyces spp, Debaromyces*	Most fruit juice concentrates, sweetened condensed milk, flour, rice, pulses containing 15-17% moisture, salami
0.80 – 0.75	Most halophilic bacteria, Mycotoxigenic *Aspergilli*	Jam, Marmalade, Soy sauce
0.75 - 0.65	Xerophilic molds, *Saccharomyces bisporus*	Rolled oats containing 10% moisture; Fudge; marshmallows; Jelly; Some dried fruits; Nuts, Peanut Butter
0.65 - 0.60	Osmophilic yeasts, few molds	Dried fruits containing 15-20% moisture; Honey
0.50	No microbial proliferation	Pasta containing 12% moisture; spices containing 10% moisture

Oxidation-Reduction potential

Microorganisms display varying degrees of similarity to Oxidation-Reduction potential of their growth medium. The O/R potential is the measure of tendency of a revisable system to give or receive electrons. When an element or compound looses electrons, it is said to be oxidized, while a substrate that gains electrons becomes reduced.

Thus a substance that readily gives up electrons is a good reducing agent, while one that readily gains electrons is a good oxidizing agent. When electrons are transferred from one compound to another, a potential difference is created between the two compounds and is expressed in as milk volts (mV). If a substance is more highly oxidized, the more positive will be its electrical potential and vice versa. The O/ R potential of a system is expressed as Eh. Aerobic microorganisms require positive Eh values for growth while anaerobic microorganisms require negative Eh values (reduced).

The redox potential we measure in a food is the result of several factors: redox couples present, ratio of oxidant to reductant, pH, poising capacity, availability of oxygen and microbial activity. Some redox couples typically encountered in food material and their standard redox potential (Eh) values are shown in Table 4.8. With the exception of oxygen, most of the couples present in foods, e.g, glutathione, cysteine, ascorbic acid and reducing sugars tend to establish reducing conditions. pH of the food has a bearing on the redox potential and for every unit decrease in the pH the Eh increases by 58 mV (Table 4.8).

Table 4.8 Some important redox couples and their standard redox potential (Eh) values

Couple	E_0 (mV)
½ O_2/H_2O	+820
Fe^{3+}/Fe^{2+}	+760
Dehydroascorbic acid / ascorbic acid	+80
Methylene blue ox/ red	+11
Pyruvate/ lactate	-190
$NAD^{+}/NADH$	-320

As redox conditions change there will be some resistance to change in food 's Eh, and is known as poising and is similar to buffering of the medium. Poising is maximum when the two redox couples are present in equal amounts. Oxygen is the most powerful of redox couple present in food system and if the food is stored in the presence of air, high positive potential will result. Thus, increasing the exposure to oxygen in air by mincing, cutting, chopping, grinding of food will increase the Eh as evident by high positive Eh of minced meat as compared to raw meat (Table 4.9).

Finally, microbial growth in the food reduces the Eh due to oxygen depletion. The decrease in Eh due to microbial activity forms the basis of some tests used frequently in raw milk such as platform MBRT test.

Table 4.9 Redox potential of some foods

Food	Eh(mV)	pH
Raw meat	-200	5.7
Minced meat	+225	5.9
Whole wheat	-350	6.0
Ground grain (Barley)	+225	7.0
Fruit juices	+300 to +	3.0-5.5
Grape	+410	3.8
Lemon	+380	2.2

Nutrient content

Like all other living beings, microorganisms need water, a source of carbon, an energy source, a source of nitrogen, minerals, vitamins and growth factors in order to grow and function normally. Since foods are rich source of these compounds, they can be used by microorganisms also. It is because of these reasons that various food products like malt extracts, peptone, tryptone, tomato juice, sugar and starch are incorporated in microbial media. The inability to utilize a major component of the food material will limit its growth and put it at a competitive disadvantage compared to those that can. In general, molds have the lowest requirement, followed by yeasts, Gram-negative bacteria, and Gram-positive bacteria. Many food microorganisms have the ability to utilize sugars, alcohols, and amino acids as sources of energy. Few others are able to utilize complex carbohydrates such as starches and cellulose as sources of energy. Some microorganisms can also use fats as the source of energy, but their number is quite less. The primary nitrogen sources utilized by heterotrophic microorganisms is amino acids. Also, other nitrogenous compounds which can serve this function are proteins, peptides and nucleotides. In general, simple compounds such as amino acids are utilized first by a majority of microorganism.

MICROORGANISMS IMPORTANT IN FOOD MICROBIOLOGY

Bacteria

Acinetobacter

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

Bacillus cereus

B.cereus is a thick long rod shaped gram positive, catalase positive aerobic spore former and the organism is important in food borne illness. It is a normal inhabitant of soil and is isolated from a variety of foods. It is quite often a cause of diarrheal illness due to the consumption of desserts, meat, dishes, dairy products, rice, pasta etc that are cooked and kept at room temperature as it is thermoduric. Some of the B. cereus strains are psychrotrophic as they grow at refrigeration temperature. B. cereus is spread from soil and grass to cows' udders and into the raw milk. It is also capable of establishing in cans. It is also capable of producing proteolytic and amyloltic enzymes and also phoslipase C (lecithinase). The production of these enzymes by these organisms can lead to the spoilage of foods. The diarrheal illness is caused by an enterotoxin produced during the vegetative growth of B. cereus in small intestine. The bacterium has a maximum growth temperature around 48°C to 50°C and pH range 4.9 to 9.3. Like other spores of mesophilic Bacillus species, spores of B. cereus are also resistant to heat and survive pasteurization temperature.

Bacillus subtilis

Bacillus subtilis known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium commonly found in soil. A member of the genus Bacillus, B. subtilis is thin short rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. B. subtilis produces the proteolytic enzyme subtilisin. B. subtilis spores can survive the extreme heat during cooking. B. subtilis is responsible for causing ropiness a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides in spoiled bread dough. A strain of *B. subtilis* formerly known as *Bacillus nattois* used in the commercial production of the Japanese food natto, as well as the similar Korean food cheonggukjang. It is used to produce amylase and also used to produce hyaluronic acid, which is useful in the joint-care sector in healthcare.

Corynebacterium

Corynebacterium is a genus of gram-positive rod-shaped bacteria. They are widely distributed in nature and are mostly innocuous. Some are useful in industrial settings such as *C. glutamicum*. Others can cause human disease. *C. diphtheriae*, for example, is the pathogen responsible for diphtheria. Some species are known for their pathogenic effects in humans and other animals. Perhaps the most notable one is *C. diphtheriae*, which acquires the capacity to produce diphtheria toxin only after interacting with a bacteriophage. Diphtheria toxin is a single, 60,000 molecular weight protein composed of two peptide chains, fragment A and fragment B, held together by a disulfide bond.

Clostridium perfringens

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

Clostridium botulinum

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

Campylobacter

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

The organism is commonly present in raw milk, poultry products, fresh meats, pork sausages and ground beef. The infective dose of *C.jejuni* may be <1,000 organisms.

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

Enterococcus is a genus of lactic acid bacteria. Enterococci are gram positive cocci that often occur in pairs (diplococci) or short chains and are difficult to distinguish from streptococci on physical characters mentioned above. The two species are commensal organisms in the intestine of humans. The Enterococci are facultative anaerobic organisms non spore forming that grows optimally at 35°C. However, they tolerate wide range of environmental conditions (10-45°C) pH (4.5 to 10.5) quite high NaCl concentration (6.5%) and can survive heating at 60°C for 30 min. Catalase-negative, oxidase negative-bacteria of the genes Enterococcus are ubiquitous organisms that often occur in large numbers on vegetables, plant materials and foods especially those of animal origin such as meat and dairy products. Enterococci also constitute a large preparation of autochthonous bacteria associated with the mammalian gastro-intestinal tract. The resistance of enterococci to pasteurization temperatures and their adaptability to different substrates and growth conditions in food products manufactured from raw materials and in heat treated food products is of great significance. Enterococci may constitute an important part of the microflora of fermented cheese and meats.

***Escherichia coli*:**

E. coli strains are associated with food borne gastroenteritis. These are Gram-negative asprogeneous rods that ferment lactose and produce dark colonies with a metallic sheen on Endo agar. The organism grows well on a large number of media and in many foods. They grow over a wide range of temperature (4 to 46 °C) and pH (4.4 to 9.0). However, they grow very slowly in foods held at refrigerator temp. (5 °C). They belong to the family Enterobacteriaceae. The organism is also an indicator of fecal pollution. The organism is also capable of producing acid and gas and off-flavours in foods. E. coli strains involved in foodborne illness can be placed into five groups: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC) and facultatively enteropathogenic (FEEC). The organism also grows in the presence of bile salts. The primary habitat of E.coli is the intestinal tract of most warm blooded animals. E.coli 0157: H7 strains are unusually tolerant of acidic environments.

Lactococcus

L.lactis subsp. *lactis*
L.lactis subsp. *cremoris*
L.lactis subsp.*lactis biovar diaectylactis*

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

Lactobacillus and Streptococcus.

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

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

Lactobacillus (*L. bulgaricus*, *L. helveticus*. *L. plantarum*, *L. acidophilus*, *L. casei, L. lactis*, *L. fermentum*):

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

Leuconostoc

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

Listeria monocytogenes

Listeria monocytogenes in foods has attracted worldwide attention due to the serious illness it causes in human beings. The Listeria are Gram positive non spore forming, nonacid-fast rods. The organism is catalase positive and produces lactic acid from glucose and other fermentable sugars. The organism grows well in brain heart infusion (BHI), trypticase soy, and tryptose broths. However, the medium should be fortified with B. vitamins and the amino acids. It is a mesophilic organism with optimal growth temperature 37°C but it can grow at refrigerator temperature also. Strains grows over the temperature range of 1°C to 45°C and pH range 4.1 to 9.6.4 35 *Listeria monocytogenes* is widely distributed in nature and can be isolated from decaying vegetation, soil, animal feces, sewage, silage and water. The organism has been found in raw milk, pork, raw poultry, ground beef and vegetables. The HTST treatment of pasteurization is good enough to destroy the organism in milk. The most significant virulence factor associated with *L. monocytogenes* is listeriolysin O. The virulent strains produce β-hemolysis on blood agar and acid from rhamnose. L. monocytogenes grows well in moderate salt concentrations (6.5%). *L. monocytogenes* is unique among foodborne pathogens while other pathogens excrete toxins or multiply in the blood stream, L. monocytogenes enters the host 's cells and grows inside the cell. In humans it crosses the intestinal barrier after entering by the oral route. Ready to Eat (RTE) foods that are preserved by refrigeration pose a special challenge with regard to L. monocytogenes infection

Micrococcus

Micrococcus occurs in a wide range of environments, including water, dust, and soil. Micrococci are Gram positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and typically appear in tetrads. Micrococcus has a substantial cell wall, which may comprise as much as 50% of the cell mass. Some species of Micrococcus, such as *M. luteus M. roseus* (red) produce yellow or pink colonies when grown on mannitol salt agar. Micrococcus is generally thought to be a saprophytic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune systems, such as HIV patients.

Proteus

Since it belongs to the family of Enterobacteriaceae, general characters have been applied on this genus: It is oxidase negative, but catalase and nitrate reductase positive. Three species P. vulgaris, *P. mirabilis*, and *P. penneri* are opportunistic human pathogens. Proteus includes pathogens responsible for many human urinary tract infections. *P. mirabilis* causes wound and urinary tract infections. Most strains of *P. mirabilis* are sensitive to ampicillin and cephalosporins. *P. vulgaris* is not sensitive to these antibiotics.

However, this organism is isolated less often in the laboratory and usually only targets immune suppressed individuals. *P. vulgaris* occurs naturally in the intestines of humans and a wide variety of animals; also, manure, soil and polluted waters. P. mirabilis, once attached to urinary tract, infects the kidney more commonly than E. coli. P. mirabilis are often found as free-living organisms in soil and water.

Pseudomonas fluorescens

Pseudomonas fluorescens is a common Gram-negative, rod-shaped, motile bacterium. The organism is psychrotrophic in nature and grows at refrigeration temperature (7°C). It has an extremely versatile metabolism and can be found in the soil and in water. It is an obligate aerobe, but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. Optimal temperature for growth of Pseudomonas fluorescens is 25-30 °C. It tests positive for the oxidase. *Pseudomonas fluorescensis* also a non-saccharolytic organism. Heat-stable lipases and proteases are produced by Pseudomonas fluorescens and other similar pseudomonads. These enzymes cause milk to spoil, by causing bitterness, casein breakdown, and ropiness due to the production of slime and coagulation of proteins.

Pseudomanas aeruginosa

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

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

Salmonella spp. have been reported to be a leading cause of foodborne illnesses in humans. Foodborne salmonellosis scores over all other foodborne bacterial illnesses in humans. Enteric fever is a serious human disease associated with typhoid and paratyphoid strains. Salmonella belong to the family Enterobacteriaceae. The optimum growth temperature is 37-45 °C. The organism can also grow at about 7°C in foods. It ferments carbohydrates with its production of acid and gas. Salmonella are oxidase negative, catalase positive and grow on citrate as a sole carbon source and produce H2S. Some Salmonella strains can grow at higher temperatures (54 °C) while others exhibit psychrotrophic properties. The organism has the ability to grow at pH values ranging from 4.5 to 9.5, with an optimum pH growth at 6.5 to 7.5. spp. is facultatively anaerobic, small Gram-negative, non spore forming, rod-shaped (2-4 mm) bacteria belonging to the family Milk, meat and poultry are principle vehicles of human foodborne salmonellosis. Ingestion of only a few salmonella cells can be infectious. Low levels of salmonellae in finished food products may, therefore, be of serious public health consequence.

Serratia

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

Staphylococcus aureus

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

Shigella

Bacillary dysentery, or shigellosis, is caused by Shigella species. Shigella is a member of the family Enterobacteriaceae. The growth temperature varies from 10 to 48 °C. Shigella does not usually survive well in low pH foods. Shigella is sensitive to ionizing radiations. species are non-motile, oxidase negative produce acid only from sugars; do not grow on citrate as sole carbon source, do not grow on KCN agar, and unlike Salmonellae do not produce H Shigellosis is a cannot use this word for disease instead use a vulnerable important disease in developed and developing countries. Disease is caused by ingestion of contaminated foods, and in some instances, it subsequently leads to rapid dissemination through contaminated feces from infected individuals. The infective dose may be as low as 100 cells. Contamination of foods usually does not occur at the processing plant but rather through an infected food handler. Humans are the natural reservoir of Shigella. The organism is spread through the fecal-oral route.

Vibrio

Vibrio cholerae and *V. parahaemolyticus* are the two important species of the genus Vibrio. Vibrio cholerae O1 causes cholera, one of the few foods borne illnesses with epidemic and pandemic potential. *Vibrio cholerae* are Gram-negative straight or curved rods and belong to the family Vibrionaceae. Important distinctions within the species are made on the basis of productions of cholera enterotoxin (CT) and serogroup. *Vibrio cholerae* is part of the normal free-living bacterial flora in estuarine areas. Amongst the many different enrichment broths described for the isolation of vibrios alkaline peptone water is the most commonly used. Though *V. parahaemolyticus* can grow in the presence of 1-8% NaCl, the best growth occurs in the salt concentration 2 to 4%.

Yersinia

Yersinia enterocolitica and *Yersinia pestis* are the two important human pathogens while *Y.enterocolitica* causes food borne gastroenteritis, *Y. pestis* is an agent of human plague. Y. enterocolitica also known as newly emerging human pathogen is a heterogeneous species that is divisible into a large number of subgroups. *Y. enterocolitica* is unusual because it can grow at temperatures below 4 °C. The generations time at the 28- 30 °C (Optimum growth temperature) is almost 34 min. It also survives in frozen foods. It grows better in processed foods such as pasteurized milk, vacuum packed meat, boiled eggs, boiled fish, and cottage cheese. Both the species can grow over a pH range of 4 to 10 (optimum pH is 7.6) and tolerate alkaline environment well. They can motile at a temperature < 30 °C. However, both these organisms are susceptible to pasteurization, ionizing and ultraviolet (UV) irradiation. The organism can also tolerate upto 5% NaCl. Infections with Yersinia species are due to transmittance of the organism from animals to humans. The organism is frequently present in pork, lamb, poultry and dairy products.

Yeasts

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

Candida:

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

Debaromyces:

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

Kluyveromyces:

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

Rhodotorula

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

Saccharomyces

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

Torulaspora

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

Molds

Molds are filamentous fungi that grow in the form of tangled mass that spreads rapidly and may cover several inches of area in a very short period. It is also referred to as mycelial growth. Mycelium is composed of branches of filaments referred to as hyphae. The molds of great importance in foods multiply by ascospores or conidia. The ascospores of some of the mold genera are notable for their extreme degrees of heat resistance.

Alternaria

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

Aspergillus

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

Geotrichum

The yeast like fungi, Geotrichum are also referred to as dairy mold

Mucor and Rhizopus

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

GENERAL PRINCIPLES OF FOOD PRESERVATION

All food preservation methods are based upon the general principle of preventing or retarding the causes of spoilage caused by microbial decomposition, enzymatic and non-enzymatic reaction, chemical or oxidative reactions and damage from mechanical causes, insects and rodents etc. Food preservation operates according to three principles, namely:

Prevention or delay of microbial decomposition brought out by

1.Keeping out microorganisms or asepsis

2.Removal of microorganisms e.g. washing, filtration etc.

3.Hindering the growth and activity of microorganisms by controlling the conditions required for the growth and activity of microorganisms by use of low temperature, drying, maintenance of anaerobic conditions or chemicals.

4.Killing microorganisms by heat or irradiation

Prevention or delay of self-decomposition of foods by

1. Destroying or inactivating food enzymes e.g. blanching, low temperature storage, chemical preservation, drying etc

2.Preventing or delay of chemical reactions e.g. prevention of oxidation with the use of antioxidants as oxygen speeds up decomposition of food and antioxidants deprives food from oxygen.

3.Prevention of damage because of external factors such as insects, rodents, dust, odour, fumes, and mechanical, fire, heat or water damage

Eg. Use of boxes, cartons, and shock absorbing materials, sealed tight, vacuum-packaging etc

Asepsis

Food is a living system and in its natural form it has its own protective mechanisms. When the food is removed from the field or protective skin or peel, it begins to deteriorate. Asepsis is a process of keeping microorganisms out of food. An aseptic environment can be created by Proper packaging of the product, which separates the internal environment from the surroundings.

Maintenance of general cleanliness and sanitary conditions while processing and handling the product from raw material to finished stage can help in preventing the entry of microorganisms into the product.

Removal of microorganisms

It is well known fact that microbes are add some connectivity word like present seen everywhere. The dust and dirt adhering to the raw material contain microorganisms and by applying various pre-treatment/ cooking methods, number of microorganisms can be reduced considerably.

Such steps include:

- Washing
- Trimming ingredients
- Discarding dirt
- Filtering
- Centrifugation
- Sedimentation

Maintenance of anaerobic conditions/ packaging

Packaging food in a vacuum environment, usually in an air-tight bag or bottle results in anaerobic environment. As bacteria need oxygen for survival, the vacuum environment in the package slows down the spoilage by them.

Drying

Drying is one of the oldest and the simplest method of preserving food. It refers to removal of water from the food. Dried foods are preserved because the available moisture level is so low that the microorganisms cannot grow, and the enzyme activity is also controlled. Drying can be accomplished by a number of methods viz. sun drying, mechanical/ artificial drying and freeze drying etc. Dried foods are compact and light weight; do not require refrigeration and last much longer than the fresh foods. Dried foods should be stored in airtight containers to prevent moisture from rehydrating them and allowing microbial growth.

Sun drying

Sun-drying takes heat from sun rays, but it is a slow process involving risk of contamination and spoilage. The limitation for sun drying is availability of climate with a hot sun and a dry atmosphere.

Mechanical/ artificial drying

Dehydration process usually implies the use of controlled conditions of heating, with the forced circulation of air or artificial drying (mechanical drier) in contrast to sun drying. Using mechanical driers, fruits, fruit leathers, banana chips, tea, coffee, milk, soups, fish, meat, eggs and vegetables can all be dried year-round.

Freeze drying

Freeze-drying is a form of dehydration in which the product is first frozen and then water is removed under vacuum as vapour by sublimation. The principle behind freeze drying is that under certain conditions of low vapour pressure, water in the form ice evaporates as water vapour directly without turning into liquid phase. The advantage is that the food structure and nutritional properties are better conserved, but the equipment and its maintenance is costly.

Smoking

Smoking has been used as a method of food preservation from time immemorial. In this method, foods are exposed to smoke by burning some special kinds of wood, which has two main purposes, adding desired flavouring and preserving. Smoke contains chemicals like formaldehyde, which is bactericidal. And also, the dehydration occurring due to smoking is responsible for its preservative action. The smoke is obtained by burning wood like oak, maple, walnut and mahogany under low breeze/ wind. Most meat is smoked after curing to aid their preservation. Examples of smoke preserved foods are smoked beef, ham, bacon, fish and meat.

Food concentration

Relatively few liquid foods are preserved by concentration, involving preservative action of reduction in water activity (aw) and development of osmotic pressure, which retard the microbial growth and enzymatic reactions. Concentration of food is usually done for many reasons: reduction in volume and weight; reduction in packaging, storage and transport costs; better microbial stability; and convenience. Examples of food preserved by concentration are tomato paste, fruit juice concentrate, soup and condensed milk. The rate of heating should be controlled to prevent localized burning of the product, particularly when it has become thickened towards the end of boiling.

Use of high sugar or salt content

Sugaring

A strong sugar solution (about 68 per cent or more) draws water from the microbial cells and thus, inhibits the growth of microbes. Examples of food preserved by high sugar concentrations are fruits in heavy sugar syrup (preserve or murraba), jams, jellies, marmalades, candies and sweetened condensed milk.

Pickling

Pickles are the relishing accompaniments in the Indian meals. Microorganisms do not grow well in acidic solutions. And this is the basis of preserving fruits and vegetables by pickling. Pickling uses the salt combined with the acid, such as acetic acid (vinegar). Some of the fruits and vegetables, which are generally pickled, are raw mangoes, limes, Indian gooseberry, ginger, turmeric and green chillies.

Salting or curing

Salting is being done in case of meat and fish preservation since ancient times. Curing preserves the food by drawing moisture from the meat through osmosis and makes it unavailable for microbial growth and enzyme action. Meat is generally cured with salt or sugar, or a combination of the two. Nitrates and nitrites are also often used to cure meat, which contribute the characteristic pink colour to meat, as well as inhibition of *Clostridium botulinum*. Dry salting is used in India for the preparation of preserved tamarind, raw mango, Indian gooseberry, fish and meat etc.

Use of organic acids

Organic acids are used in food preservation because acid conditions inhibit growth of many spoilage microorganisms. Bacteria are generally pH sensitive. Organic acids penetrate the bacteria cell wall and disrupt its normal physiology and thus preserve the food. Acetic acid, lactic acid, citric acid and malic acid are widely used for preservation in food products.

UNIT – II
CONTAMINATION OF FOOD

CONTAMINATION OF FOOD

The process of food contamination takes several steps to get food from the farm or fishery to the dining table. We call these steps the food production chain. Contamination can occur at any point along the chain during production, processing, distribution, or preparation.

Production

Production means growing the plants we harvest or raising the animals we use for food. Most food comes from domesticated animals and plants, and their production occurs on farms or ranches. Some foods are caught or harvested from the wild, such as some fish, mushrooms, and game.

Examples of Contamination in Production

If a hen's reproductive organs are infected, the yolk of an egg can be contaminated in the hen before it is even laid.

If the fields are sprayed with contaminated water for irrigation, fruits and vegetables can be contaminated before harvest.

Fish in some tropical reefs may acquire a toxin from the smaller sea creatures they eat.

Processing

Processing means changing plants or animals into what we recognize and buy as food. Processing involves different steps for different kinds of foods. For produce, processing can be as simple as washing and sorting, or it can involve trimming, slicing, or shredding. Milk is usually processed by pasteurizing it; sometimes it is made into cheese. Nuts may be roasted, chopped, or ground (such as with peanut butter). For animals, the first step of processing is slaughter. Meat and poultry may then be cut into pieces or ground. They may also be smoked, cooked, or frozen and may be combined with other ingredients to make a sausage.

Examples of Contamination in Processing

If contaminated water or ice is used to wash, pack, or chill fruits or vegetables, the contamination can spread to those items.

During the slaughter process, germs on an animal's hide that came from the intestines can get into the final meat product.

If germs contaminate surfaces used for food processing, such as a processing line or storage bins, germs can spread to foods that touch those surfaces.

Distribution

Distribution means getting food from the farm or processing plant to the consumer or a food service facility like a restaurant, cafeteria, or hospital kitchen. This step might involve transporting foods just once, such as trucking produce from a farm to the local farmers' market. Or it might involve many stages. For instance, frozen hamburger patties might be trucked from a meat processing plant to a large supplier, stored for a few days in the supplier's warehouse, trucked again to a local distribution facility for a restaurant chain, and finally delivered to an individual restaurant.

Examples of Contamination in Distribution

If refrigerated food is left on a loading dock for long time in warm weather, it could reach temperatures that allow bacteria to grow.

Fresh produce can be contaminated if it is loaded into a truck that was not cleaned after transporting animals or animal products.

Preparation

Preparation means getting the food ready to eat. This step may occur in the kitchen of a restaurant, home, or institution. It may involve following a complex recipe with many ingredients, simply heating and serving a food on a plate, or just opening a package and eating the food.

Examples of Contamination in Preparation

If a food worker stays on the job while sick and does not wash his or her hands carefully after using the toilet, the food worker can spread germs by touching food.

If a cook uses a cutting board or knife to cut raw chicken and then uses the same knife or cutting board without washing it to slice tomatoes for a salad, the tomatoes can be contaminated by germs from the chicken.

Contamination can occur in a refrigerator if meat juices get on items that will be eaten raw.

Mishandling at Multiple Points

Sometimes, by the time a food causes illness, it has been mishandled in several ways along the food production chain. Once contamination occurs, further mishandling, such as undercooking the food or leaving it out on the counter at an unsafe temperature, can make a foodborne illness more likely. Many germs grow quickly in food held at room temperature; a tiny number can grow to a large number in just a few hours. Reheating or boiling food after it has been left at room temperature for a long time does not always make it safe because some germs produce toxins that are not destroyed by heat.

Different Types of Food Contamination

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

1. Biological contamination
2. Chemical contamination
3. Physical contamination
4. Cross-contamination

Read on to find out more about the different types of food contamination and their effect on your health.

Biological Contamination

Biological contamination is one of the common causes of food poisoning as well as spoilage. Contamination of food items by other living organisms is known as biological food contamination. During biological contamination, the harmful bacteria spread on foods that you consume. Even a single bacterium can multiply very quickly when they find ideal growth conditions. Not just bacteria, but also their process of multiplying can be quite harmful to humans.

The common places where you can find bacteria are:

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

The best way to avoid food contamination is by washing the food items with KENT vegetable and fruit cleaner and wash the kitchen cloths on a regular basis.

Physical Contamination

When harmful objects contaminate the food, it leads to physical contamination. At times, food items can have both physical and biological contamination. Physical contaminants such as rats, hair, pests, glass or metals, which can contaminate food and make it unhealthy. Some of the safety tips that you can follow when handling food items to prevent food contamination are:

Hair-Tie your hair when handling food

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

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

Cross-Contamination

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

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

Disposing Waste- Make sure you store and seal garbage correctly to prevent cross-contamination. Clean and sanitize the waste bins to prevent infestation risk.

CLASSIFICATION OF FOODS BY EASE OF SPOILAGE

Healthy tissues inside foods and foods in their natural form resist infection and they do not harbour microorganisms. On the other hand, spoilage of highly perishable foods is natural. The onset of food spoilage is rather indefinite. It is a gradual process occurring because of poor sanitation, enzymatic or chemical reactions, improper temperature controls, microbial growth or physical abuse starting from the time food is harvested, slaughtered or manufactured till it is consumed. Physical changes, such as bruising or puncturing of tissue and water loss and chemical changes, such as those caused by enzymes; or the effects of microbial growth can make food unappealing.

Foods undergo undesirable changes in the physical and chemical characteristics of food ultimately leading to spoilage of food. In general, food spoilage is a state in which food is deprived of its good or effective qualities. Spoilage of food refers to the undesirable alterations in foods or the food undergoes some physiological, chemical and biological changes, which renders it inedible or hazardous to eat. In extreme cases, the food becomes totally unpalatable and unfit for human consumption. Hence, it is essential to process or preserve foods after it is harvested or slaughtered to combat the problem of food spoilage.

Undesirable Changes in Food due to Spoilage

Food deterioration is manifested by the reduction in aroma, flavour, textural and nutritional values of foods. Different types of undesirable changes which occur due to spoilage in food are listed as follows:

Change in colour:

The fruits like bananas and apples turn black after storing for a long period of time and reduce the acceptability of food.

Change in smell:

Rancid smell of spoiled oils and fats, bitter smell of curd or sour smell of starchy food.
Change in consistency: Splitting of milk, curdling of milk, stickiness and undesirable viscosity in spoiled cooked dal and curries and spoiled cooked vegetables.

Change in texture:

Some vegetables like potato, brinjal and carrot undergo too much softening leading to rotting.

Change due to mechanical damage:

Mechanical damages such as eggs with broken shells, mechanical spoilage of fruits and vegetables during transportation also constitute food spoilage.

Factors Affecting food Spoilage

The types of spoilage of a particular food item depend to a great extent on the following:

The composition of food:

The composition of food influences its susceptibility to spoilage. For example- presence of proteins and carbohydrates especially sugars are preferred by microorganisms for energy source. Very few utilize fat for energy production.

Structure of the food item:

Whole healthy tissues of food from inside are sterile or low in microbial content. Skin, rind or shell on food works as its protective covering from spoilage microorganisms.

Types of microorganisms involved:

The types of microorganisms present in food depend on its composition of food.

Conditions of storage of the food:

Conditions of storage of food affect the growth of microorganisms. Even if the proper storage of food is done, the food loses its freshness and nutritive value if it is stored for too long.

Classification of Foods by ease of Spoilage

Foods are classified into three groups based on ease of their spoilage.

Relatively stable or non-perishable foods:

Foods that do not spoil unless handled carefully. E.g. grains, flour, sugar, pulses etc.

Protectable or semi-perishable foods:

Foods those remain unspoiled for a fairly long period if properly handled and stored. E.g. potatoes, apples, onions etc.

Perishable foods:

Foods that spoil readily unless special preservative methods are used. E.g. milk, eggs, meat, fish, poultry, most fruits and vegetables.

Spoilage due to Enzymatic Activity

Enzymes are complex chemical substances, which are present in all living organisms and tissues, which control essential metabolic processes. Different biochemical reactions in foods and plants tissues are catalysed by enzymes. Enzymatic spoilage is the greatest cause of food deterioration. They are responsible for certain undesirable or desirable changes in fruits, vegetables and other foods.

Examples involving endogenous enzymes include:

The post-harvest senescence and spoilage of fruit and vegetables; oxidation of phenols in plant tissues to orthoquinones by phenolases, peroxidases and polyphenol oxidases (PPO). These orthoquinones rapidly polymerize to form brown pigments known as melanin leading to enzymic browning; sugar – starch conversion in plant tissues by amylases; post-harvest demethylation of pectic substances in plant tissues (leading to softening of plant tissues during ripening, and firming of plant tissues during processing).

If enzymatic reactions are uncontrolled, the off-odours, and off-colours may develop in foods.

Spoilage Due to Insects, Pests and Rodents

The main categories of foods subject to insects and pest attack are fruits, vegetables, grains and their processed products. Warm humid environment promotes insect growth, although most insects will not breed if the temperature exceeds above 35°C or falls below 10°C. Many insects cannot reproduce satisfactorily unless the moisture content of their food is greater than 11 per cent.

The presence of insects and pests and their excreta in foods may render consumable loss in the nutritional quality, production of off-flavours and acceleration of decay processes due to creation of higher temperatures and moisture levels and release of enzymes. The products of insect and pests' activities such as webbing, clumped-together food particles and holes can also reduce the food values.

Rats and mice carry disease-producing microorganisms on their feet and/or in their feces and urine and contaminate the food by their presence.

Chemical changes caused by Microorganisms

Chemical reactions take place in the presence of atmospheric oxygen and sunlight. Two major chemical changes, which occur during the processing and storage of fruits and vegetables, are lipid oxidation and non-enzymatic browning which deteriorate sensory quality, colour and flavour. Lipid oxidation is influenced by light, oxygen, high temperature and the presence of iron and copper, and water activity. Control of these factors can significantly reduce the extent of lipid oxidation or rancidity in foods.

Non-enzymatic browning is one of the major causes of deterioration which takes place during frying, cooking, storage of dried and concentrated foods through Maillard, caramelization and ascorbic acid oxidation. Maillard reaction occurs due to reactions between reducing sugars and amino acids in the presence of heat and results in formation of black brown insoluble pigments. Caramelization of sugars occurs in presence of high heat and low moisture content in the food. Oxidation of fatty acids to other chemicals like aldehydes, ketones, alcohols and esters also results in off-flavours.

Spoilage Due to Physical Factors

Physical factors such as temperature, moisture and pressure can also cause food spoilage. Physico-chemical reactions are caused by freezing burning, drying and bruising of fruits and vegetables during storage, handling and transportation, which result in food deteriorations.

UNIT - III
PRESERVATION OF FOOD

PRESERVATION - USE OF HIGH TEMPERATURES

The process of heating was used centuries ago before its action was understood. Food is heated up or cooked. Heat is used to inactivate organisms or enzymes of spoilage significance in the foods. Microorganisms are killed by heat because the application of heat coagulates the food proteins and inactivates the microbial enzymes and thus results in death of microorganisms. The examples include all forms of cooked food, pasteurization, milk sterilized by UHT (ultra high temperature), canning etc. One of the most important modern applications of the heat preservation is the pasteurization of milk.

Heat treatment of food may be given in different ways:

Pasteurization (temperature below 100°C)

Pasteurization is a heat treatment involving temperatures below 100°C that kills a part but not all the microorganisms present in food. Milk, for example, is usually heated to 63°C for 30 min or 71°C for 15 seconds or in UHT 138°C for 2-4 seconds. Examples include milk, wine, beer, fruit juices and aerated waters which are routinely pasteurized. The mode of heating can be steam, hot water, dry heat or electric currents. The products are cooled promptly after the heat treatment. Pasteurization is usually supplemented by other methods to prolong shelf-life.

Pasteurization is a mild heat treatment for relatively brief duration to kill part of the microorganisms and to eliminate human pathogens present in food. It is used specially when the aim is to kill pathogenic microorganisms and where the spoilage organisms are not very heat-resistant, and the product cannot stand high-temperatures or frozen.

The main purpose of pasteurization in low acid foods is destruction of pathogenic microorganisms whereas in acid foods it aims at killing spoilage microorganisms along with enzyme inactivation. For example pasteurization is used to kill pathogenic microorganisms *Brucella abortis*, *Mycobacterium tuberculosis* and *Coxiella burnetti* in case of milk (63°C for 30 minutes; 71.5°C for 15 seconds) and spoilage microorganisms in beers (lactic acid bacteria and yeasts at 65°C–68°C for 20 minutes in bottle), fruit juices (yeast and fungi along with pectinesterase and polygalacturonase inactivation at 65°C for 30 minutes; 77°C for 1 minute; 88°C for 15 seconds) etc. In addition to destroying some microorganisms, pasteurization also inactivates some enzymes. Pasteurization does not change the colour and flavour to any significant level.

Since pasteurization does not kill all the microorganisms, this process is usually combined with another preservation method like refrigeration freezing etc. Typical other preservation methods used in combination with pasteurization include refrigeration as in the case of milk; chemical additives- pickles, fruit juices; fermentation (additives)- sauerkraut, cheeses; and packaging (anaerobic conditions)- beers, fruit juices.

The index microorganism for pasteurization is Mycobacterium tuberculosis. If this microorganism is killed by pasteurization it is assumed that all other pathogens are also destroyed.

Methods of pasteurization

Three methods of pasteurization are used viz. low temperature long time (LTLT), high temperature short time (HTST) and ultra high temperature (UHT) method.

Low temperature long time (LTLT) method:

In LTLT pasteurization, the pasteurization time is in the order of minutes and related to the temperature used; two typical temperature/time combinations are used: 63 to 65°C over 30 minutes or 75°C over 8 to 10 minutes. The minimal heat treatment for market milk is 62.8°C for 30 minutes in (LTLT) holding method and for grape juice is 76.7°C for 30 minutes.

High temperature short time (HTST) method:

Rapid pasteurization involves temperatures of about 85° to 90°C or more and time in the order of seconds. HTST method involves temperature of 71.7°C for about 15 seconds in case of milk pasteurization and grape wines are generally pasteurized for one minute at 81 to 85°C.

Ultra high temperature (UHT) method:

Rapid, high or flash pasteurization involves temperatures of 85-90°C or more and time in order of seconds. These are also known as ultra high temperature (UHT) treatments. Typical temperature/time combinations may be 88°C for 1 minute; 100°C for 12 seconds; 121°C for 2 seconds. This treatment will destroy all but the most heat resistant spores resulting in commercially sterile product.

Bacterial destruction is very nearly equivalent in all temperature-time combinations; however, the 121°C for 2 seconds treatment gives the best quality products in respect of flavour and vitamin retention. Very short holding times to the tune of seconds, however, require special equipment which is more difficult to design and generally is more expensive than the LTLT/ HTST type of processing equipment.

Boiling (temperature at 100°C):

Cooking of rice, vegetables, meat, fish etc. at home is usually done by boiling the food with water and involves a temperature around 100°C.

Canning (temperature above 100°C):

Canning is the process in which the foods are heated in hermetically sealed (airtight) jars or cans to a temperature that destroys microorganisms and inactivates enzymes that could be a health hazard or cause the food to spoil. The vacuum seal formed after heating and cooling in the process ensures that no microorganism can get into the product. The degree of heat and the length of time of heating vary with the type of food and the kinds of microorganisms that are likely to occur in it. High-acid foods such as fruits and tomatoes can be processed or "canned" in boiling water, while low-acid vegetables and meats must be processed in a pressure canner at 121°C (15 psi pressure). Tin-coated steel cans are most commonly used followed by glass containers. Nowadays, containers made of aluminum and plastics in the form of pouches or rigid containers are also increasingly used. Examples of food preserved by canning are- all kinds of tinned foods, such as soup, meat, beans, cereal grains, legumes, nuts, and other various dried food products such as fruit, coffee, milk, soups, fish, meat and vegetables.

Canning is the process of applying heat to food that’s sealed hermetically in a jar to destroy any microorganism that can cause food spoilage.

The food preservation process of canning originated in 1809 when French confectioner Nicolas Appert succeeded in preserving meats in glass bottles that had been kept in boiling water for varying periods of time. In the honour of inventor, canning is also known as "appertization". Canning demonstrates that food can be preserved for quite a longer duration of time when heated and stored in anaerobic condition. Today, the method of canning is one of the most widely used methods for food preservation.
In canning the food is placed in containers, heated, and then sealed, usually under vacuum. It is used for products such as fruit juices, syrups, and sauces. Canning process is advantageous in retaining the stable vitamins and colour and flavour of food items.

Unlike pasteurization, canning of foods normally involves exposure for longer periods of time to higher temperatures created by steam under pressure in order to kill endospore-forming microorganisms. Steam under pressure (e.g. a pressure cooker) is the most effective method since it kills all vegetative cells and spores. The heat treatment generally exceeds 100°C temperature and the food are heated long enough to inactivate the most heat-resistant pathogens (disease-causing organisms) and spoilage organisms. Heating to such high temperatures is achieved by steam injection, which is followed by rapid cooling. Factors affecting the length of time the food must be heated include the kind and number of microorganism's present, acidity of foods, presence of preservatives (salt, sugar). The only dangerous spore forming bacterium which survives the treatment is *Clostridium botulinum*. The whole process of canning includes preparation of food, filling, exhausting, sealing, thermal processing i.e. autoclaving and cooling.

Sterilization

Sterilization refers to complete destruction of microorganisms. It requires heat treatment of 121°C for 15 minutes which destroys all spores. But it has severe effect on heat sensitive nutrients and proteins through maillard reaction. The temperature and time required to sterilize the food varies with the type of food. Such high temperatures can be created by steam under pressure in steam pressure boilers/ sterilizers. Temperature at sea level is 100°C at atmospheric pressure but with 15psi temperature of 121.5°C can be achieved.

Commercial sterilization:

Commercial sterility is achieved when all pathogenic and toxin forming microorganisms have been destroyed along with the spoilage microorganisms. Usually target organism is a heat resistant microorganism, most often a spore or schlerotia forming organism rather than a vegetative one (e.g. spore forming anaerobic bacteria – Clostridium botulinum). Such foods may contain viable spores, but these are not able to grow under normal conditions. If packaged aseptically, these products can be marketed without refrigeration. These products generally have a shelf life of 2 years or more.

Blanching

Blanching is used for variety of purposes. It is defined as a mild heat treatment applied to tissue (usually plant) primarily to inactivate enzymes prior to freezing, drying or canning. It is also known as scalding.

Functions of blanching: Inactivate most enzymes Some cleaning action Removes substances in some products Activates some enzymes (if controlled) Removes undesirable odours/ flavours

Softens fibrous material and decreases volume Expels air and respiratory gasses Preheating of product prior to canning

Reduces number of microorganisms:

Major function of blanching is inactivation of enzymes for frozen or dehydrated foods as enzymes can cause rapid changes in colour, flavour and nutritive value of such food products. Moreover freezing and dehydration processes involve temperatures which are insufficient to inactivate enzymes. Blanching as a pretreatment before drying has many advantages like it helps in cleaning the material and reducing the amount of microorganisms present on the surface; it preserves the natural colour in the dried products; for example, the carotenoid (orange and yellow) pigments dissolve in small intracellular oil drops during blanching and in this way they are protected from oxidative breakdown during drying; and it shortens the soaking and/ or cooking time during reconstitution. Blanching does not allow effective autoclaving and stops the activity of autolytic enzymes.

For canned products, blanching removes gases, shrinks the food to correct fill weight in can and offers preheating, which are very important to provide vacuum in can and proper sterilization. Sometimes canning process may allow sufficient time for enzymatic activity and under blanching may increase the enzymatic activity.

Fruits are not blanched. As a thumb rule, all those vegetables which cannot be eaten raw are blanched. E.g. potatoes, greens green beans, carrots, okra, turnip and cabbage should always be blanched. On the other hand, blanching is not needed for onions, leeks, tomatoes and sweet peppers.

Using sodium bicarbonate with blanching water preserves the green colour of vegetables by preventing the conversion of chlorophyll into pheophytin, unattractive brownish-green colour compound.

Methods of blanching:

Blanching is a delicate processing step. It requires careful monitoring of time, temperature and the other conditions. Effective blanching time necessary to inactivate enzymes is dependent on various factors viz. type of food, method or type of heating, product size and temperature of heating medium etc. There are mainly two typical methods of blanching based on type of heating medium viz. hot water blanching and steam blanching use hot water and steam as heating medium, respectively. The former process involves temperatures below 100°C whereas the latter is carried out at temperatures above 100°C. A third type of blanching system exists which is a combination of hot water and steam blanching.

Blanching of green leafy vegetables especially spinach at boiling point causes loss of green colour but at lower temperature (77°C), it retains the natural green colour, even when heated at higher temperature (121°C) later during sterilization. At lower temperature, the enzyme chlorophyllase remains active for little time and converts chlorophyll to a phyllin, which retains green colour.

Hot water blanching:

In this method, the cleaned food is subjected to hot water (85 to 100°C) until the enzymes are inactivated. Pot blanchers are used at home scale. Generally hot water blanching is done because of low capital costs and better energy efficiency. Disadvantages associated with hot water blanching include loss of water-soluble constituents, risk of contamination and higher cost of water and disposal of effluent.

Steam blanching:

In case of steam blanching, the food product is directly exposed to steam, which avoids the loss of food soluble solids (flavours, vitamins, acids, sugars etc.) to blanching medium as well as solves the problem of disposing blanching medium after processing. Steam blanching is advantageous as it results in less loss of water-soluble constituents, less volume of waste, easy to clean and sterilize. But it has some disadvantages such as higher capital costs, uneven blanching, and low efficiency.

Central Institute of Agricultural Engineering (CIAE), Bhopal has developed the batch type steam blancher, (Figure 2) with 100 kg/ h capacity. It has been evaluated for blanching of cabbage, cauliflower, pea and okra. Results revealed satisfactory performance on account of colour retention in the dried product. On commercial level, tunnel steam blanchers with product conveyers are used.

Blanchers with hot water and steam system:

This type involves three step process viz. product on conveyer belts is exposed to steam consecutively followed by contact between food and hot water and finally immersion in hot water.

Efficacy of blanching:

There are various types of enzymes such as lipoxygenase, polyphenolase, polygalacturonase and chlorophyllase, which cause loss of quality and therefore, must be inactivated. Normally, two heat resistant plant enzymes such as catalase and peroxidase, are used to evaluate blanching efficacy, as appropriate time and temperature is required to inactivate them.

PRESERVATION BY USING LOW TEMPERATURE

Use of low temperature is the easiest, most convenient and least time-consuming method of preserving foods. Refrigeration/ freezing do not sterilize foods or destroy the microorganisms that cause spoilage. It simply slows the growth of microorganisms and the chemical and enzymatic changes that affect quality or cause spoilage.

Low temperature preservation is less effective as compared to thermal techniques of food preservation because microorganisms are more likely to be able to survive cold temperatures than hot temperatures. One of the problems related to use of freezing as a method of food preservation is that microorganisms are only deactivated and not killed, which may again become active while thawing. Similarly, enzyme action is slowed but not stopped during freezing and these enzymes are responsible for colour and flavour changes and loss of nutrients during refrigerated storage.

The metabolism of a living tissue is a function of the temperature of the environment. Low temperature is applied to retard chemical and enzymatic reactions in food. In addition, reducing temperature retards or stops growth and activity of microorganisms in the food. Lower the temperature; the slower will be the rate of above natural activities. Cooling thus slows down or stops the spoilage of foods. Freezing and refrigeration are among the oldest methods of preservation. Mechanical ammonia refrigeration systems invented during 1875 allowed development of commercial refrigerated warehousing and freezing.

Low temperatures retard chemical reaction and action of food enzymes and slow down or stop the growth and activity of microorganisms in foods. As a rule of thumb, for every 10°C temperature change, the rate of reaction changes by a factor of 2 to 3. Pathogen growth is halted below -4°C and spoilage microorganisms don't grow below -10°C.

Low temperatures employed can be:

Cellar storage temperature (15°C)

In cellar storage structures, temperatures are reduced slightly below room temperature (about 15°C). These are underground rooms, which are well insulated to outside temperatures and have proper ventilation. These are meant for short term storage of root crops, potatoes, apples etc. during the winter months as the temperature is not low enough to prevent the action of many spoilage organisms or the plant enzymes. However, deterioration is slowed down considerably. Humidity should be carefully maintained in the storage cell as high moisture promotes fungal growth whereas shriveling and drying may occur in dry conditions.

It is usually used for the storage of surplus foods like root crops, potatoes, onions, apples, etc. for limited periods.

Refrigeration/ chilling temperature (0 to 5°C)

Refrigerated storage of food is generally practiced both at home or industry level. In chilling, the temperature of a food is reduced generally to between -1°C and 7°C and thus subsequent storage at refrigerated temperature extends the shelf life of both the fresh and processed foods. It just increases shelf life and has limited application in preserving foods. It is not the sole method for preserving food therefore it is used as an adjunct process to extend the storage life of mildly processed (e.g. pasteurized, fermented and irradiated) and low-acid foods.

Chilling and refrigerated storage retards the growth of bacteria, particularly the thermophiles and mesophiles. Psychrophillic spoilage bacteria however can spoil food during low temperatures storage, but some psychrophillic pathogens, such as *Listeria monocytogenes*, *Yersinia enterocolitica* etc. found in refrigerated food need attention. Temperatures of 5 to 6°C or less retard the growth of most food poisoning microorganisms except Clostridium botulinum type E.

Foods kept at this temperature slow down the microbial activities and chemical changes resulting in spoilage. Mechanical refrigerator or cold storage is used for this purpose. Examples of this include meats, poultry, eggs, fish, fresh milk and milk products, fruits, vegetables, etc. which can be preserved for 2-7 days by refrigeration.

Freezing (-18 to -40°C)

Freezing is the removal of heat from the packaged or whole foods resulting in the temperatures between slightly below the freezing point of food to -18°C. Frozen foods last many months without spoiling however, some quality loss may occur.

Some microorganisms grow even at sub-freezing temperatures as long as water is available. Conversion of water to ice increases the concentration of dissolved solutes in unfrozen water and leads to low water activity. Freezing prevents the growth of microorganisms due to reduced water activity. The concerted effect of low temperatures, reduced water activity, and pre-treatment of blanching prior to freezing of products yield longer shelf life.

Different types of freezing systems are available for foods. No single freezing system can satisfy all freezing needs, because of the wide variety of food products and process characteristics. The selection criteria of a freezing system will depend on the type of the product, reliable and economic operation, easy cleanability, hygienic design and desired product quality.

Although all commercial freezing processes are operated at atmospheric conditions, there are potential applications of high-pressure assisted freezing and thawing of foods. The pressure–induced freezing point and melting point depression enables the sample to be super cooled to low temperature (e.g. -22°C at 207.5 M pa) resulting in rapid and uniform nucleation and growth of ice crystals on release of pressure.

In freezing, water in food turns into ice and hence, makes the water unavailable for reactions to occur and for microorganisms to grow. Most perishable foods like poultry, meats, fish, ice-creams, peas, vegetables, juice concentrates, etc. can be preserved for several months at this temperature. In vegetables, enzyme action may still produce undesirable effects on flavour and texture during freezing. Heating, like blanching, therefore, must destroy the enzymes before the vegetables are frozen.

Freezing systems based on time taken for freezing

Freezing systems based on time required to freeze foods can be classified into two types i.e. slow and quick freezing. Rate of freezing affects the quality of frozen food.

Slow freezing:

Slow freezing occurs when food is directly placed in freezing rooms called sharp freezers. It is also known as sharp freezing. This method involves freezing by circulation of air by convection i.e. through a specially insulated tunnel, either naturally or with aid of fans. The relatively still air is a poor conductor of heat and that is the reason for long time required to freeze the food. The temperature ranges from -15 to -29°C and freezing may take from 3 to 72 hours. The ice crystals formed are large and found in between cells i.e. extra-cellular spaces because of which the structure of food is disrupted. The structure of food is not maintained, and thawed food cannot regain its original water content. Large ice crystals create quality problems like mushiness in vegetables etc.

Quick freezing:

Vigorous circulation of cold air enables freezing to proceed at a moderately rapid rate. In this process, the temperature is kept between -32°C to -40°C and the food attains the stage of maximum ice crystal formation in 30 minutes or less. Small ice crystals are formed within the cells and therefore, it does not damage the structure of food. On thawing, the structure of original food is maintained.

Slow vs. quick freezing

The difference between sharp and quick freezing has been given in Table 1

Table 1. Difference between sharp and quick freezing

Sharp freezing	Quick freezing
1. Rates of cooling of less than 1°C/min. Ice crystals form in extra-cellular locations	1. Produces both extra-cellular and intracellular (mostly) locations of ice crystals
2. Large ice crystals formation	2. Small but numerous ice crystals
3. Maximum dislocation of water	3. Minimum dislocation of ice crystals
4. Shrinkage (shrunk appearance of cells in frozen state)	4. Frozen appearance similar to the unfrozen state
5. Less than maximum attainable food quality	5. Food quality usually superior to that attained by slow freezing

Freezing Systems Based on Mode of Operation

Batch freezing

This type of freezing is mostly used for small operations. If a variety of products are to be frozen, a batch freezer may be selected over continuous as they are more versatile. Such freezing systems are also likely to be used for products with longer freezing times since with a batch freezer there is better utilization of floor space due to the multi-layer arrangement of loading. It is difficult to choose on an exact line of demarcation, but generally freezing times longer than one hour would usually require a batch mode of operation.

Continuous freezing:

This process of freezing is used in large-scale production lines. Continuous freezers are best used for freezing individual portions, such as small pieces of vegetables. The main advantage in using a continuous freezer for these smaller and/or thinner products is that since they freeze quickly, they will also thaw quickly and the delays that occur with a batch- freezing operation may be overcome. Continuous freezing allows quick handling after freezing and a quick transfer to the cold store.

Batch/ continuous freezing

These kinds of freezers are usually batch type freezers operated with trolleys which are loaded in sequence at fixed-time intervals rather than all at one time as in the truly batch freezer.

Quality loss Due to freezing Temperatures

Freezing is done to preserve food by reducing the product temperature, thereby slowing the quality deterioration processes. But still some changes take place on long storage of frozen foods like oxidation of fat, growth of microorganisms, enzymatic reactions and loss of surface moisture (dehydration) which needs attention.

- Chemical and physical changes of food
- Flavour changes of food
- Textural changes
- Colour changes
- Moisture loss

- Freezer burn
- Nutritional value of frozen foods

PRESERVATION USING DRYING (DEHYDRATION)

The principle behind drying is that sufficient moisture is removed, which is essential for growth of microorganisms and for enzyme activity. Removal of moisture increases the storage life of the product due to reduced water activity. If the moisture content is reduced to 1 to 5 per cent then the product can be stored for more than a year. The processing should be done in such a way that the food value, natural flavour and characteristic cooking quality of the fresh material are retained after drying. A good dried product on reconstitution with water should resemble the original product.

Advantages of Drying

Preservation is the main reason but not the only reason for dehydrating foods. Food may be dehydrated for other reasons also viz. to decrease weight and bulk; to retain size and shape of original food; to produce convenience items. Dehydration/ drying is advantageous for being cheaper than the other methods of preservation with less requirement of equipments. Storage of dried food products does not require special facilities like refrigeration etc. Dried food products are simple to store and pack because of their low volume.

Dehydrated foods, however, are less popular because of some undesirable changes in colour, taste and flavour during storage and distribution. Dehydration techniques have been improved to overcome most of these defects.

Factors in control of drying:

Various factors affecting rate of drying in a fresh produce include the following:

1. Composition of raw materials: Foods containing high amount of sugar or other solutes dry slowly.
2. Size, shape and arrangement of stacking of produce: Greater the surface area greater is the rate of drying.
3. Temperature as well as humidity and velocity of air: Greater is the temperature differential between the product and the drying medium faster the product dries. Lower the humidity of environment the faster the drying will be.
4. Pressure (atmospheric or under vacuum): Lower the atmospheric pressure the lower the temperature required to evaporate water.
5. Heat transfer to surface (conductive, convective and radiative): The fastest method of heat transfer is radiation consecutively followed by convection and conduction.

Types of Drying

Basically, drying can be done by two processes viz. natural drying and mechanical dehydration or artificial drying based on source of energy. Natural drying takes place under the influence of sunlight and wind and is of three types viz. sun, solar and shade drying. In natural drying there is no control over temperature, air flow and humidity whereas in artificial drying, these conditions are well controlled.

Mechanical dehydration or artificial dehydration can be further classified into atmospheric and sub-atmospheric types based on the conditions employed in drying process. On the basis of mode of drying process, drying at atmospheric pressure conditions can be further divided in batch and continuous types. Mechanical drying includes the methods of drying by (1) heated air, (2) direct contact with heated surface e.g. drum drying and (3) application of energy from a radiating microwave or dielectric source.

Commercial dehydrators are generally large in size and various types of dehydrators can be based on circulation of air as

1. Natural
2. Forced draught

In natural draught, the rising of heated air brings about drying of food in the natural draught method. Examples include kiln, tower and cabinet driers. Forced draught employ currents of heated air that move across the food usually in tunnels. An alternative method is to move the food or a conveyor belt or trays through heated air. Examples include tunnel or belt drier. In forced draught drier, the temperature and humidity can be carefully controlled to get a good dehydrated product but are not in general use because of the cost.

Sun drying:

Drying the food product under natural sunny conditions is called as sun drying. No energy is required for the drying process. To practice sun drying of foods, hot days are desirable with minimum temperatures of 35°C with low humidity. Poor quality produce cannot be used for natural drying to achieve good quality dried product. The lower limit of moisture content by this method is approximately 15 per cent. Problems of contamination and intermittent drying are generally encountered with sun drying. It is only possible in areas of low humidity.

Solar drying:

Solar drying uses designed structures to collect and enhance solar radiation. Solar driers generate high air temperature and low humidity which results in faster drying. This drier is faster than sun-drying, and also requires less drying area. But it cannot be used on cloudy days. Generally, three types of solar driers are used, as

1. the absorption or hot box type driers in which the product is directly heated by sun
2. The indirect or convection driers in which the product is exposed to warm air which is heated by means of a solar absorber or heat exchanger
3. Drier, which is combination of first and second type

Shade drying:

This kind of method is used for foods which lose their colour when exposed to direct sunlight for drying. Generally, herbs, green and red chillies, okra and beans etc. are dried under shaded area with good air circulation.

A home scale dehydrator or drier:

It consists of a small galvanized box having dimensions of 90x90x60 cm. The lower portion consists of perforated iron tray. The box is fitted on to a wooden frame which is kept about 2-3 feet above ground. At the top there are two slits which can be closed by shutters. About seven trays can be kept in the drier. The material to be heated is kept on trays and heating source can be a gas stove or any other source. The initial temperature of the dehydrator is usually is 43°C which is gradually increased to 60-66°C in the case of vegetables and 66-71°C for fruits. For a home scale drier 100-200 g of sulphur is required for 25 kg fruit. Time required for drying is generally ½ hour to 2 hours.

Oven drying:

A conventional oven with a thermostatic setting of 60°C is suitable for oven drying of fruits, vegetables, fruit leathers and meats. This is a kind of cabinet drier.

Kiln drier:

Also known as kiln evaporator. It consists of two floors. On the top floor, food to be dried is spread and on the lower floor, the furnace is housed. Heat is conveyed by a ventilator. Generally, it is used for large pieces of food.

Belt-trough drier:

In this drier, belt is in the form of a trough, which is made of metal mesh. Hot air is blown through the mesh and food pieces lying on the trough are dried in the process.

Spray drying:

Spray drier is used to dry purees, low viscosity pastes and liquids, which can be atomized. The material is sprayed in a rapidly moving current of hot air. The dried product drops to the bottom of the drying chamber and is collected. Atomization into minute droplets results in drying in a matter of seconds with common inlet air temperature of about 200°C and properly designed system quickly removes the dried particles from heated zones. This method of dehydration can produce exceptionally high quality with many highly heat sensitive materials including milk and coffee.

Microwave drying:

In this method, microwaves are used to dry the food product.

Freeze drying:

Foods in the pieces and liquids are dried by this method. Fruit juice concentrates are manufactured using freeze drying. The material is frozen on trays and then dried under vacuum. Due to vacuum drying, the material dries directly without passing through the intermediate liquid stage. The principle behind freeze drying is that under certain conditions of low vapour pressure, water can evaporate from ice without the ice melting. Freeze drying is generally used to dry sensitive and high value liquid as well as solid foods such as juices, coffee, strawberries, chicken dice, mushroom slices etc. The dried product is highly hygroscopic and reconstitutes readily. Taste, flavour and reconstitution property of fruit juice concentrates are excellent. Method is costly because of the equipment cost. Freeze drying in combination with air drying is advantageous in reducing cost of drying. For example- vegetables pieces may be air dried to about 50 per cent moisture and then freeze dried down to 2-3 per cent moisture.

PRESERVATION BY USE OF FOOD ADDITIVES

Preservatives are the chemical agents which serve to retard, hinder or mask undesirable changes in food. More precisely, preservatives are substances when added to food to retard, inhibit or arrest the activity of microorganisms such as fermentation, acidification and decomposition of food or of masking any of the evidence of putrefaction but it does not include salt, sugar, vinegar, glycerol, alcohol, spices, essential oils etc.

According to Prevention of Food Adulteration (PFA) Act (1954) and Food Standards and Safety Act (FSSA) of 2006, a 'preservative' means a substance which when added to food, is capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food.

Preservatives may be anti-microbial preservatives, which inhibit the growth of bacteria and fungi, or antioxidants such as oxygen absorbers, which inhibit the oxidation of food constituents. Common anti-microbial preservatives include calcium propionate, sodium nitrate, sodium nitrite and sulfites (sulfur dioxide, sodium bisulphite, potassium hydrogen sulphite, etc.) and ethylenediamine tetra acetic acid (EDTA). Antioxidants include butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT).

Sulphur dioxide (including sulphites) and benzoic acid (including benzoates) are among the principle preservatives used in the food processing industry.

Chemical preservatives are food additives, which are specifically added to prevent the deterioration or decomposition of a food. Chemicals are used to inhibit the factors causing spoilage. These are also used to complement other food preservation techniques.

Preservation of foods by the chemicals is affected by interfering with the cell membrane of the microorganism, their enzyme activity and genetic mechanism; and by acting as antioxidants. In food preservation, the added chemical preservatives may be grouped into two classes.

Class I preservatives:

The first one includes the use of sugar, salt, spices, acetic acid (vinegar) and alcohol, and is referred to as class I preservatives and is considered to be relatively safe to humans.

Class II preservatives:

The second group includes the use of benzoic acid, sulfur dioxide, nitrates and nitrites and a variety of neutralizers, firming agents and bleaching agents and referred to as class II preservatives and is considered to be relatively safe to humans, but within the permissible doses prescribed by the food regulatory bodies of the country because higher concentrations can be a health hazard.

Permitted usage levels of chemical preservatives in foods

Chemical preservative	Concentration (ppm)	Foods
Sorbic acid and its salts (calculated as sorbic acid)	50	Nectars, ready to serve beverages in bottles/pouches selling through dispenser
	100	Fruit juice concentrates with preservatives for conversion in juices, nectars for ready to serve beverages in bottles/ pouches selling through dispensers
	200	Fruit juices (tin, bottles or pouches)
	500	Jams, jellies, marmalades, preserve, crystallized glazed or candied fruits including candied peels fruit bars
Benzoic acid and its salts	120	Ready to serve beverages
	200	Jam, marmalade, preserve canned cherry and fruit jelly
	250	Pickles and chutneys made from fruits or vegetables
	600	Squashes, crushes fruit syrups, cordials, fruit juices and barley water or to be used after dilution; Syrups and sherbets
	750	Tomato and other sauces; Tomato puree and paste
Sulphur dioxide	40	Jam, marmalade, preserve canned cherry and fruit jelly
	150	Crystallized glace or cured fruit (including candied peel)
	350	Squashes, crushes fruit syrups, cordials, fruit juices and barley water or to be used after dilution; Syrups and sherbets; Fruit and fruit pulp
	2000	Dehydrated vegetables
Sodium and / or Potassium nitrite expressed as Sodium nitrite	200	Pickled meat
Lactic acid	No limit	Fermented meat, dairy and vegetable products, sauces and dressings, drinks.
Citric acid	No limit	Fruit juices; jams; other sugar preserves
Acetic acid	No limit	Vegetable pickles; other vegetable sauces, chutney

Benzoic Acid and Related Compounds

Sodium benzoate was the first chemical preservative permitted in foods by the FDA, and it continues to be widely used in large number of foods. Benzoic acid and its related compounds possess antimicrobial activity and their antibacterial action increases in the presence of CO_2 and acid. **For example**- *Bacillus subtilis* cannot survive in benzoic acid solution in the presence of CO_2.

As used in acidic foods, these act essentially as a mould and yeast inhibitor. In fact, benzoic acid is more effective against yeasts than against moulds. It does not stop lactic acid and acetic acid fermentation. Benzoates have greatest activity at low pH especially in food products with pH below 4.5. Optimum functionality occurs between 2.5 and 4.0 pH. Benzoic acid is mostly used in coloured products of tomato, phalsa, jamun, pomegranate, strawberry, coloured grapes etc. as in the long run, it may darken the product.

Sodium benzoate, sodium salt of benzoic acid, is very effective as it is nearly 180 times more soluble in water than benzoic acid. It produces benzoic acid when dissolved in water. It should be used at low levels to avoid possible off-flavours in some products. The maximum level allowable by PFA act is 0.075 per cent. It is used in fruit products, jams, relishes, beverages etc. and is effective against yeasts, some bacteria (food borne pathogens but not spoilage bacteria) and some moulds.

Sorbic acid and related compounds:

Sorbic acid and related compounds have antimicrobial properties. They are available as sorbic acid, potassium sorbate, sodium sorbate or calcium sorbate. Salts of sorbic acid are used in many cases as they are highly soluble in water and produce sorbic acid when dissolved in water. The potassium salt of sorbic acid i.e. potassium sorbate is much more soluble in water than the acid. It does not impart any noticeable flavour at normal usage concentrations. Maximum level allowable by PFA act is 0.3 per cent. It is effective up to pH 6.5 but effectiveness increases as the pH decreases. It has about 74 per cent of the antimicrobial activity of the sorbic acid, thus it is required in higher concentrations than pure sorbic acid. It is effective against yeasts, moulds, and select bacteria, and is widely used at 0.025 to 0.10 per cent levels in cheese, beverages, fermented and acidified vegetables, smoked and salted fish. In wine processing, sorbates are used to prevent refermentation.

Propionic Acid and Related Compounds

Propionic acid is inhibits mould and rope bacteria growth but negligible effect on yeast. Propionic acid and its salts, sodium and calcium propionates, are approved by USFDA as GRAS (Generally Recognized As Safe) substances for food use and also by PFA act in India. Propionates are effective up to maximum limit of 5.5 pH. They are used in preserving cheese, non-alcoholic beverages, jams and jellies. Typical usage level of propionic acid and propionates is 0.2 per cent.

Parabens

Parabens are effective at higher pH values from 3 to 8 and also stable at low and high temperatures, even up to steam sterilization. But they are not as widely used due to high cost and objectionable flavour. They are used in beverages, jams, jellies, preserves, smoked fish and pickles.

Lactic acid

Lactic acid is formed by microbial fermentation of sugars in preserved food products such as sauerkraut and pickles. The acid produced decreases the pH to levels unfavourable for growth of spoilage organisms such as putrefactive anaerobes and butyric-acid-producing bacteria. It does not control yeasts and mould growth, which can grow at such pH levels. Inclusion of other preservatives such as sorbate and benzoate may be used in that case.

Acetic acid

It is also known as vinegar. Acetic acid is a general preservative inhibiting many species of bacteria, yeasts and to a lesser extent moulds. It is also a product of the lactic-acid fermentation. It is more effective in preservative action than lactic acid at same pH levels. It is mainly used in products such as pickles, sauces and ketchup.

Sulphur dioxide and sulphites

Sulphur dioxide and its derivatives have been widely used in foods as a food preservative. It serves both as an antioxidant and reducing agent and prevents enzymatic and non-enzymatic reactions, leading to microbial stability. The commonly used forms are sulphur dioxide gas and sodium, potassium and calcium salts of sulphite, bisulphite or meta bi-sulphite, which are powders. Various sulphite forms dissolve in water and yield 50 to 68 per cent sulphur dioxide gas. It has bactericidal and bacteriostatic properties and is more effective against bacteria especially gram negative bacteria than moulds and yeasts.

Sulphur dioxide gas (SO2) is one of the oldest known fumigants and a wine preservative. The gaseous form is produced either by burning sulphur or by its release from the compressed liquefied form. It is a colourless, suffocating, pungent-smelling, non-flammable gas and highly soluble in cold water.

Sulphites are effective in producing more SO2 ions at pH values less than 4.0. Meta bi-sulphite are more stable to oxidation than bisulphites and the latter show greater stability than sulphites.

Sulphites inhibit microbial growth by reacting with the energy rich compound like adenosine triphosphate; inhibiting some metabolic pathways; and blocking cellular transport systems. Sulphur dioxide also inhibits browning, both enzymatic and nonenzymatic, reactions in fruits and prevents darkening of colour and alterations of flavour.

Nitrites and Nitrates

Nitrites have been used in meat curing for many centuries. It is used along with a mixture of salt, sugar, spices, and ascorbate for curing meats. Nitrite contributes to the development of the characteristic colour, flavour and texture improvement in addition to preservative effects. Sodium nitrite is quite soluble in water and is more effective below neutral pH (below 7.0). Along with salt, nitrite exhibits stronger antimicrobial action.

Nitrates break down in the body to nitrites and this stops the growth of bacteria, especially Clostridium botulinum, the bacteria that cause botulism poisoning. This is the reason nitrites and nitrates are used mainly among the packaged meats.

Nitrites also stabilize the red colour in cured meat and stop it from turning grey. Nitrates get readily converted into nitrites, which then react with the protein myoglobin to form nitric oxide myoglobin. During cooking, this is converted to nitrosohemochrome, a stable pink pigment, which impart a pink, fresh hue to cured meat. This chemical stabilizes the red colour of the meat and gives an appearance of fresh meat. That is why nitrites are a preferred preservative of meat processors even though its excess use is restricted in many countries.

Nitrite salts should be used with precaution because they can react with certain amines in food at acidic pH to produce nitrosamines, which are known to cause cancer by giving rise to compounds like nitrodimethyl-amine. Addition of sodium ascorbate inhibits nitrosamine formation and reduces the problem of nitrosamines. Nitrites and nitrates are permitted as preservatives in cured meat and meat products including poultry at levels below 200 ppm by USFDA and FSSA in India.

Antibiotics

Antibiotics are antimicrobial substances produced by microorganisms have been allowed for food use only in recent years. But they are not widely used in food preservation due to the risk of ill effects on consumer and possibility of appearance of resistant strains. However, nisin and have been permitted in some foods.

Nisin

Nisin is a polypeptide produced by *Steptrococcus lactis* (now called *Lactococcus lactis*). Its solubility depends on the pH of the medium and it is more soluble in acidic pH. Its antimicrobial action increases as the pH decreases. Nisin has a narrow spectrum affecting only gram-positive bacteria, including lactic acid bacteria, streptococci, bacilli, and clostridia. It does not inhibit gram-negative bacteria, yeasts or moulds. Nisin has been permitted in processed cheese up to 12.5 ppm under FSSA.

Natamycin

Natamycin is produced by the bacterium *Streptomyces natalensis*. The compound has a large lactone ring which is substituted with one or more sugar residues. Natamycin is primarily effective against yeast and moulds and is ineffective against bacteria. It has been permitted for surface treatment of hard cheese under FSSA with maximum level of application not to exceed 2mg/dm3

Ethylenediamine Tetra Acetic Acid (EDTA)

EDTA is a metal-chelating agent which removes the metal cofactors that many enzymes need, thus preventing the food spoilage. But it is not mentioned under FSSA as preservative.

Antioxidant preservatives

Antioxidant preservatives prevent foods from becoming rancid, browning, or developing black spots by suppressing the reaction that occurs when foods combine with oxygen in the presence of light, heat, and some metals. Antioxidants also minimize the damage to some essential amino acids and loss of some vitamins.

Antioxidant preservatives, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), and propyl gallate (PG), stop the chemical breakdown of food that happens in the presence of oxygen by inhibiting the free radicals that help initiate and propagate these reactions.

Sanitation

According to the World Health Organization (W.H.O), “Sanitation refers to the maintenance of hygienic conditions, through services such as garbage collection and waste disposal. Inadequate sanitation is a major cause of disease world-wide and improving sanitation is known to have significant beneficial impact on health both in households and across communities."

Sanitation in Food

There are three main types of hazards or contaminants that can cause unsafe food: Biological, chemical, and physical. Biological includes microorganisms; chemical includes cleaning solvents and pest control; and physical means hair, dirt, or other matter.

In our research, we've come up with five frequently mentioned sanitation tips to prevent food borne illnesses in food service and retail businesses. They are:

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

Proper employee education and training, as well as monitoring and recordkeeping by management of clean and sanitation tasks, also are important.

HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP)

A systematic approach to the identification, evaluation, and control of food safety hazards

Hazard:

A biological, chemical, or physical agent that is reasonably likely to cause illness or injury in the absence of its control.

Hazard Analysis:

The process of collecting and evaluating information on hazards associated with the food under consideration to decide which are significant and must be addressed in the HACCP plan.

Hazard Analysis Critical Control Points (HACCP) is a system which provides the framework for monitoring the total food system, from harvesting to consumption, to reduce the risk of foodborne illness. The system is designed to identify and control potential problems before they occur. In its Model Food Code, the Food and Drug Administration has recommended the HACCP system "because it is a system of preventive controls that is the most effective and efficient way to assure that food products are safe" (1999 FDA Model Food Code). The application of HACCP is based on technical and scientific principles that assure safe food.

HACCP consists of seven steps used to monitor food as it flows through the establishment, whether it be a food processing plant or foodservice operation. The seven steps of the HACCP system address the analysis and control of biological, chemical and physical hazards.

In August of 1997, the National Advisory Committee on Microbiological Criteria for Foods adopted new recommendations on "Hazard Analysis and Critical Control Point Principles and Application Guidelines." These guidelines are designed to facilitate the development and implementation of effective HACCP plans. The new recommendations are reflected in this document. For more information on HACCP principles, in particular formal HACCP, contact your local extension educator. Also, the reference list includes several excellent resources on HACCP.

HACCP terminology

Critical Control Point (CCP)

A procedure/practice (control) in food handling/preparation that will reduce, eliminate or prevent hazards. It is a "kill" step that kills microorganisms or a control step that prevents or slows their growth.

Hazard

Unacceptable contamination, microbial growth, persistence of toxins or survival of microorganisms that are of a concern to food safety

Monitoring

Checking to determine if the criteria established by the critical control point(s) (CCP) have been achieved

Risk

Probability that a condition(s) will lead to a hazard

Severity

Seriousness of the consequences of the results of a hazard

Formal HACCP seven steps:

Conduct a hazardous analysis.

The purpose of a hazardous analysis is to develop a list of hazards which are likely to cause injury or illness if they are not controlled. Points to be considered in this analysis can include: skill level of employees; transport of food; serving elderly, sick, very young children, immune-compromised; volume cooling; thawing of potentially hazardous foods; high degree of food handling and contact; adequacy of preparation and holding equipment available; storage, and method of preparation. The next step is to determine if the factors may influence the likely occurrence and severity of the hazard being controlled. Finally, the hazards associated with each step in the flow of food should be listed along with the measures necessary to control the hazard.

Determine Critical Control Points (CCP's)

A critical control point is any step-in which hazards can be prevented, eliminated or reduced to acceptable levels. CCP's are usually practices/procedures which, when not done correctly, are the leading causes of foodborne illness outbreaks. Examples of critical control points include cooking, cooling, re-heating, holding.

Establish Critical Limits

A critical limit ensures that a biological, chemical or physical hazard is controlled by a CCP. Each CCP should have at least one critical limit. Critical limits must be something that can be monitored by measurement or observation. They must be scientifically and/or regulatory based. Examples include temperature, time, pH, water activity or available chlorine.

Establish Monitoring Procedures

The monitoring system should be easy to use and meet the needs of the food establishment, as well as the regulatory authority. It is important that the job of monitoring be assigned to a specific individual and they be trained on the monitoring technique.

Establish Corrective Actions

Corrective actions may range, for example, from "continue cooking until the established temperature is reached" to "throw out the product," depending on the severity of the situation.

HACCP plans should include the following: who is responsible for implementing the corrective action and what corrective action was taken. They should be established in advance as part of the HACCP plan.

Establish verification procedures

Verification can be accomplished by expert advice and scientific studies and observations of the flow of food, measurements and evaluations. Another means of verification is an onsite review of the established critical limits. Each CCP will have one independent authority. This verification step provides an opportunity to make modifications to the plan if necessary.

Establish record-keeping and documentation procedures

Record-keeping and documentation procedures should be simple to complete and include information that illustrates that the established standards are being met. Employees need to be trained on the record-keeping procedures and why it is a critical part of their job. Examples of records include time/temperature logs, checklists, forms, flowcharts, employee training records, and SOP's.

PERSONAL HYGIENE

Personal hygiene begins at home, with the essential elements for good hygiene being a clean body, clean hair and clean clothing. Hair in food can be a source of both microbiological and physical contamination. Hairnets and beard covers should be worn to assure food product integrity. Long-sleeved smocks should be worn to cover arm hair. Clean uniforms, aprons and other outer garments that are put on after the employee gets to work can help minimize contamination. While working, clothing should be kept reasonably clean and in good repair. Removal of smocks, lab coats or aprons should take place when leaving the work area to go to the employee break room, restroom or exiting the building. Personal items such as meals and snacks should be stored in a locker or break room area that is located away from processing areas or areas where equipment and utensils are washed.

The only jewellery allowed in a food plant is a plain wedding band and/or one small post earring in each ear. No other jewellery is to be worn because it may fall into the product, it can present a safety hazard and it cannot be adequately sanitized against bacterial transmission. It should be removed prior to entering the processing facility.

Employees must wear different colored smocks when going from a raw processing part of the establishment to the cooked processing side. They should also step into a sanitizer footbath between the two processing areas to eliminate the bacteria on their shoes.

No employee who is affected with, has been exposed to, or is a carrier of a communicable disease, the flu or a respiratory problem, or any other potential source of microbiological contamination shall work in any area where there is a reasonable possibility that food or food ingredients can be contaminated. The number one symptom of a foodborne illness is diarrhoea. Other symptoms include fever, dizziness, vomiting, and sore throat with fever or jaundice. Any employee with these symptoms should not be allowed to work around food.

A company policy should be established requiring that employees report any active case of illness to supervisors before beginning work. If an employee has been diagnosed with a foodborne illness, exclude them from the establishment, and contact the local health department. The health department must be notified if the employee has been diagnosed with one of the following foodborne illnesses: Salmonella typhi, Shigella species, shiga toxin-producing E. coli, or hepatitis A virus.

ORIENTAL FERMENTED FOOD

Oriental fermented foods mean the traditional fermented foods made in the orient, including for example Japan, Indonesia, India, Pakistan, Thailand, Philippines, Taiwan, China, Korea, and the encompassing areas.

Piden

Piden or the Chinese preserved egg is made from duck eggs coated with a slurry of soda, burned straw, salt and slaked lime and covered with rice husks.

The eggs then are kept in sealed clay jars for a month or longer. As assortment of bacteria grow in the egg, but coliform bacteria and species of Bacillus apparently are predominant.

Minchin

In this case starch free but gluten rich wheat is used. The moist raw glutens placed in a closed jar and allowed to ferment for 2 to 3 weeks, after which it is salted. A typical specimen was found to contain several species of molds, some of bacteria and a few of yeast, the final product is boiled, baked or fried.

Fermented coffee

Fermentation is the part of coffee processing that enables the removal of the mucilage (sticky flesh layer) from the coffee seed. The majority of the coffee's sugars are found in the sticky mucilage, the process of fermentation is where sugars are transferred from the coffee fruit to the seeds.

Wet fermentation

Traditional washed coffee includes a period of wet fermentation. This means the pulped coffee is soaked in a tank of water. The water allows the sticky mucilage to degrade slowly due to pectinolytic bacteria. Wet fermentation is undertaken in 12-72 hours. Followed by an acid fermentation by lactic acid bacteria such as Leuconostoc species. Fermentation is complete when the coffee has no stickiness or sliminess left. Longer fermentation times can lead to increased acid complexity (eg. Acetic, Citric and Malic). The coffee is then rinsed and put out to dry. This style of processing encourages clean, bright acidity and sweetness.

Dry fermentation

Dry fermentation is a much more aggressive process. Coffee is pulped, then placed into large soaking tanks. No water is added, so the coffee is more exposed to the effects of sun, oxygen and weather conditions. Common dry fermentation periods are 8-24 hours. This fermentation style creates a coffee with more body, similar to a honey processed coffee.

After fermentation of coffee beans to improve the taste and aroma of roasted product

Soy sauce

Of the many Oriental fermented products, soy sauce is the one most widely consumed and the only one that has become well known in the cookery of Western countries. Soy sauce is a dark brown liquid with a salty taste and a distinct pleasant aroma suggestive of meat extracts. It is a seasoning agent used as substitute for salt in preparation of food as well as a table condiment.

Soy sauce is made by fermentation of a combination of soybeans, wheat grain, water and salt.

Cooking Ingredients

Soybeans are steamed. Wheat is roasted and crushed. They are mixed and used for koji (malt) making. Salt is dissolved in water.

Making Koji

Koji mold (*Aspergillus oryzae* or *Aspergillus sojae*) is added to the mixed soybeans and wheat, and then incubated for three days to make koji. The word “koji” means soybeans and wheat with their surface covered with koji mold. It contains various enzymes that greatly contribute to the following moromi fermentation and maturation process.

Making Moromi

Koji is mixed with salt water. This mixture is called “Moromi”. Moromi is then transferred to a fermentation tank.

Fermentation and Maturation

Moromi is slowly fermented and matured for a period of six months. Koji enzymes, yeast and lactic acid bacteria (*Pediococcus soyae*) act in this process and create various tastes and flavors.

Filtration

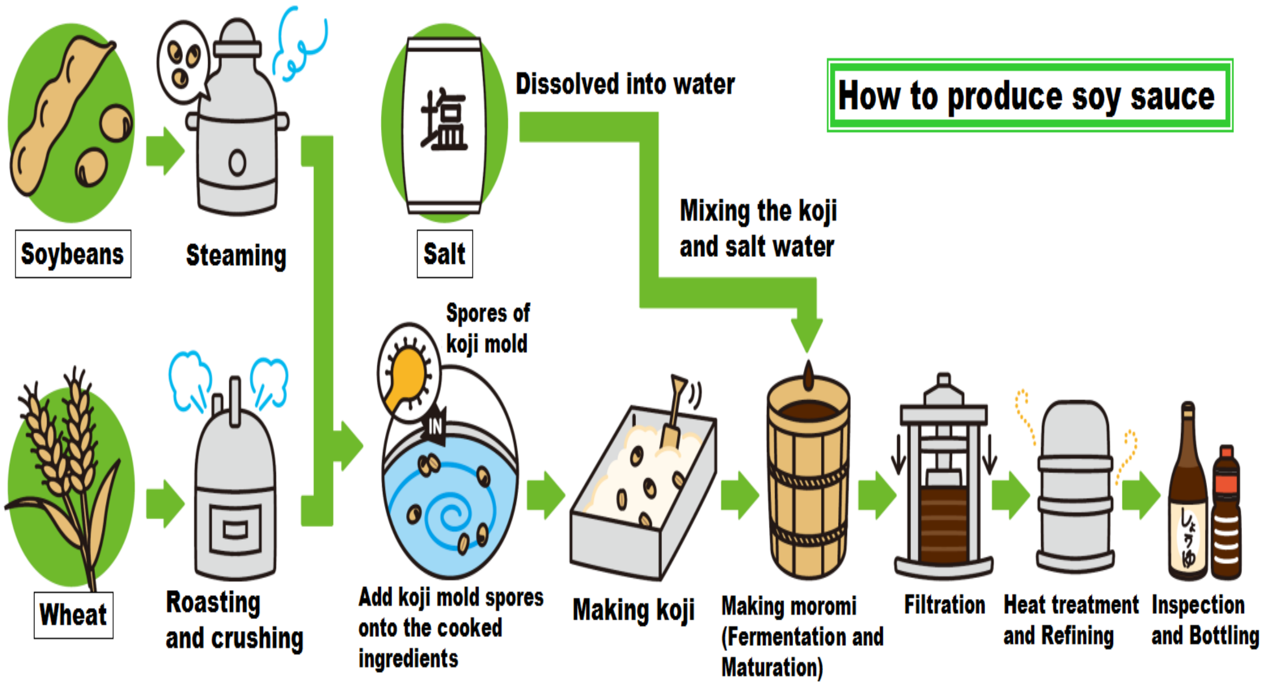
After fermentation and maturation, moromi is wrapped one by one with cloths and they are stacked into many layers. Then they are slowly compressed and the moromi is filtered through the cloths. In this way, raw soy sauce liquid called “Kiage” is obtained from the moromi.

Heat treatment and Refining

Raw soy sauce is heat-treated for sterilization and then refined. The color, aroma and flavor of soy sauce are further enhanced during this process.

Inspection and Bottling

After the inspection, soy sauce is filled into glass or plastic bottles.


How to produce soy sauce
Soybeans
Steaming
Salt
塩
Dissolved into water
Mixing the koji and salt water
Wheat
Roasting and crushing
Spores of koji mold
IN
Add koji mold spores onto the cooked ingredients
Making koji
Making moromi (Fermentation and Maturation)
Filtration
Heat treatment and Refining
Inspection and Bottling

UNIT - IV
CONTAMINATION, SPOILAGE OF FOODS

Spoilage of food can be defined as any visible or invisible change which can makes food or product derived from food unacceptable for human consumption.

Spoilage of food not only causes health hazard to the consumer but also cause large economic losses. Spoilage not only leads to loss of nutrients from food but also cause change in original flavor and texture. It is estimated that about 25% of total food produced is spoilt due to microbial activities only despite range of preservation methods available. Thus, the spoilage of food is not only a health hazard but also carry lot of economic significance too.

In total, the food spoilage is considered a complex phenomenon whereby a combination of microbial and biochemical activities take place. Due to such activities, various types of metabolites are formed which aid in spoilage. The detection of these metabolites helps in detection of spoilage.

SPOILAGE OF CEREALS AND CEREAL PRODUCTS:

Cereals are important foods which provide bulk of our dietary requirements. They are also source of carbohydrates which are metabolized by body for energy generation. Besides cereals also provide minerals, proteins and vitamins. India produces a large variety of cereals such paddy, wheat, maize, barley millets like, jowar, bajra, ragi. Various types of products are prepared from cereals.

Cereal products can be broadly classified into the following groups:

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

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

Spoilage Factors

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

Physical

Physical losses are caused by spillages, which occur due to use of faulty packaging materials.

Physiological

Physiological losses include respiration and heating in grains, temperature, humidity and oxygen.

Biological

Biological losses occur due to micro-organisms, insects, rodents, etc.

The sources of contamination in cereals are:

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

Cereal Grains and Flours

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

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

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

- Type and number of microorganisms
- Moisture content of more than 12-13%
- Storage temperature
- Physical damage

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

Fig-9.1. Stem rot and head blight of wheat and barley- *Fusarium culmorum* & *Fusarium graminearum*.

The process of flour making such as washing, milling reduces the microbial content. Moisture content of less than 15% does not allow growth of molds. Most molds and bacteria in flours can grow only above 17% moisture, thus moistening of flours is essential for spoilage by microbes.

Spoilage of Bread

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

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

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

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

Some spoilage of bread are shown in Figure

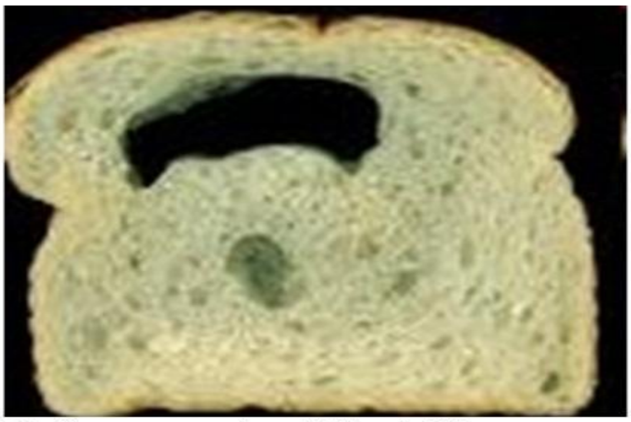


Fig.9.2. Green spored mold-*Penicillium expansum*

- Bread mold- Rhizopus stolonifer

- White cottony mycelium and black spots

Fig. 9.3. Red bread mold- *Neurospora sitophila*

Fig. 9.4. Chalky bread

- Ropiness of home-made breads- Bacillus subtilis (*Bacillus mesentericus*).
- Ropyness due to hydrolysis of flour protein by proteinase of the bacillus and capsulation of bacillus
- Chalky bread—chalk like white spots due to yeast like fungi ----*Endomycopsis fibuligera* and *Trichonospora variable*

CONTAMINATION AND SPOILAGE OF VEGETABLES

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

These sources include:

- Soil
- Water
- Diseased plant
- Harvesting and processing equipments
- Handlers
- Packaging and packing material
- Contact with spoiled vegetables

The conditions in which vegetables are stored and transported after harvesting also contribute to rate of spoilage. Other than microbial, sources, the spoilage of vegetables can also occur due to the activity of native enzymes.

Types of Spoilage in Vegetables

The microbial spoilage of vegetables is predominately of following types

Spoilage due to pathogens

The plant pathogens which infect stem, leaves, roots, flowers and other parts or the fruit itself.

Spoilage due to saprophytes

Vegetables have general microflora inhabiting them. These organisms under certain conditions can grow on these vegetables and spoil them. The list of these organisms is given in Table 8.1. There are certain secondary invaders which may enter the healthy food or grow after growth of pathogens. It is well known that plant diseases are mostly caused by fungi. Thus, most of the spoilage causing pathogens in vegetables are fungi. Fungi have specific characteristics when spoiling food as it leads to mushy areas which may be water soaked. The fungi produce characteristic spores which may be pigmented. The pigmentation helps in identification of the type of spoilage by fungi. The bacterial diseases too cause spoilage of vegetables but to a lesser extent. Figure 8.1 represent bacterial and fungal diseases of tomato.

Table 8.1 Normal microflora of vegetables

Bacteria	Fungi
Alcaligens	Alternaria
Bacillus	Aureobasidium
Erwinia	Botrytis
Micrococci	Fusarium
Pseudomonas	Penicillin
Lactic acid bacteria	Rhizopus
Xanthonomas	

Table 8.2 The major types of spoilages by pathogens in vegetables

Type of Spoilage	Causative organisms	Symptoms
Bacterial soft rot	*Erwinia carotovora* *Pseudomonas marginalis* *Clostridium*	Water soaked appearance, soft-mushy, bad odor
Alternaria rot	*Alternaria tenuis*	Greenish brown to black brown spots
Rhizopus soft rot	*Rhizopus sp*	Cottony mold growth with small black dots
Blue mold rot	*Penicillim digitatum*	Bluish green color
Downy mildew	*Phytophthora, Bremia*	White woolly mass
Black mold rot	*Aspergillus niger*	Brown to black mass, referred as smut
Fusarium rot	*Fusarium*	
Sliminess or souring	*Saprophytic bacteria*	
Stem end rots	*Diplodia* *Alternaria* *Phomopsis*	Involve stem ends
Watery soft rot	*Sclerotenia sclerotinium*	

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

Bacterial Soft Rot

- Caused by Erwinia carotovora and Pseudomonas such as *P. marginalis*. Bacillus and Clostridium spp. are also implicated.
- Breaks down pectin, giving rise to a soft, mushy consistency, sometimes a bad odour and water-soaked appearance.
- Vegetables affected- onions, garlic, beans, carrot, beets, lettuce, spinach, potatoes, cabbage, cauliflower, radishes, tomatoes, cucumbers, watermelons.

Some types of spoilage in vegetables by bacteria are shown in the Figure 8.1 to 8.7

Fig. 8.1 Bacterial and fungal disease of tomato
Soft rot in tomato caused by *Erwinia carotovora* (Left)
Blue mould rot in tomato caused by *Penicillium* spp (right)

Fig. 8.2. Bacterial soft rot in Tomato, Capsicum and Tomato

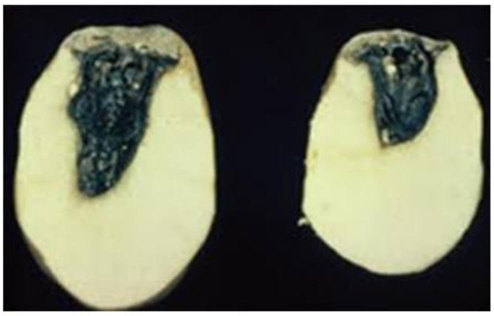


Fig. 8.3. Black leg of potatoes- *Erwinia carotovora* var. *atroseptica*

Fig. 8.4. Black rot of cabbage and cauliflower- *Xanthomonas campestris*

Fig.8.5. Bacterial wilt of beans- *Corynebacterium flaccumfaciens*

Fig. 8.6. Slime of lettuce- *Pseudomonas marginalis*

Fungal spoilage of vegetables

Penicillium, Cladosporium, Rhizopus, Aspergillus spp. are responsible for various defects in vegetables. Some types of spoilage in vegetables by fungi are shown in the Figure 8.8 to 8.13. **Gray mold rot** – caused by *Botrytis cinera* in vegetables. Favoured by high humidity and warm temperature

Fig. 8.9. Black mold rot – *Aspergillus niger*, dark brown to black masses

Fig. 8.10. Pink mold Rot – *Trichothecium roseum*

Fig. 8.11. Fusarium Rot – *Fusarium* spp.

Fig. 8.12. Green Mold Rot – *Cladosporium* and *Trichoderma*

Fig. 8.13. Brown Rot – Sclerotinia spp

Table 8.2 Examples of fungal spoilage of vegetables

Examples of Commodities Most Affected	Genus	Type of Spoilage
Most vegetables especially carrot, lettuce, celery, cabbage	*Botrytis*	Grey mould rot
Most vegetables. Especially carrot, lettuce, legumes, *Brassica* spp.	*Sclerotinia*	Watery soft rot
Legumes, carrot, *Brassica* spp.	*Rhizopus*	Soft rot
Tomato, cucumber, asparagus, potato	*Fusarium*	Dry rots
Tomato, potato, carrot	*Phytophthora*	Brown rots (blight)
Tomato, potato, beetroot Cucumber, legumes	*Phoma* *Pythium*	Dry brown, black rots Cottony leak

CONTAMINATION AND SPOILAGE OF FRUITS:

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

Microorganisms associated with spoilage in fruits and juices:

The microorganisms associated with fruits depend on the structure of fruit. The fruits contain different organic acids in varying amounts. The types of acids which are predominately found are citric acid, malic acid and tartaric acid. The low pH of fruits restricts the proliferation of various types of organisms. The pH and type of acids found in different fruits is given in Table 7.1. Due to the low pH, a large number of microorganisms are restricted to grow on fruits. Fungi are most dominating organisms to grow on fruits because of the ability of yeasts and molds to grow under acidic conditions. A small number of bacteria which are aciduric (ability to resist acidic conditions) also grow. Also, the dry conditions prevailing on the skin and surface do not allow the growth of certain microorganisms. Besides these plants also produce certain antimicrobial components too

Table 7.1 Type of acid associated with fruits and their pH

Fruit	Type of Acid	pH
Apple	Malic, citric, lactic	3.3–4.1
Watermelon	Citric, malic	5.8–6.0
Banana	Citric, malic, tartaric	4.5–5.2
Grape	Tartaric, malic	3.0–4.5
Plum	Malic, quinic	2.8–4.6
Pineapple	Citric, malic	3.2–4.0
Guava	Citric, malic, lactic	3.0–3.2
Lemon	Citric	2.2–2.4
Mango	Citric, tartaric	3.3–3.7
Orange	Citric, malic	3.0–4.0
Papaya	Citric, malic, ketoglutaric	4.5–6.0

Despite the high-water activity of most fruits, the low pH leads to their spoilage being dominated by fungi, both yeasts and molds but especially the latter.

Yeasts

Yeasts are unicellular fungi which normally reproduce by budding. Of the 215 species important in foods, about 32 genera are associated with fruits and fruit products. Only a few species of yeasts are pathogenic for man and other animals. None of the pathogenic species are common contaminants of fruits and fruit products. Fruit that has been damaged by birds, insects, or pathogenic fungi usually contain very high yeast populations. The yeasts are introduced into the exposed tissue, often via insects, and are able to use the sugars and other nutrients to support their growth. Types of yeasts growing in fruits depend upon the nature of the fruit and the strain of yeast. Growth of a strongly fermentative type such as certain strains of *Saccharomyces cerevisiae* may produce sufficient CO2 (90 lb/in. or more) to burst the container. Growth of some species in a clear fruit juice may produce only slight haze and sediment. While carbon dioxide and ethanol are the predominant metabolic products of yeasts, other products such as glycerol, acetaldehyde, pyruvic acid, and a -ketoglutaric acid are also formed. Oxidative yeasts such as species of Brettanomyces produce acetic acid in wines and other fruit products. Although yeasts produce hydrolytic enzymes which degrade pectins, starch, and certain proteins, enzymatic activity is usually much less than that exhibited by other aciduric microorganisms, molds in particular.

Molds

These are filamentous fungi which are important group of microflorae of fruit products due to following reasons:

1. Some of the members are xerophilic, thereby having potential to spoil foods of low water activity such as dried fruits and fruit juice concentrates.

2. Some of the species have heat resistant spores such as ascospores which can survive the commercial pasteurization treatments that are given to most fruit products.
3. Growth of molds on processing equipment such as wooden tanks can result in the generation of offflavors in wines, juices, and other fruit products.
4. Mold-infected raw fruit may become soft after processing because pectinases were not inactivated by the thermal treatment.

The metabolic products of many molds are toxic to humans. Of these toxins, mycotoxins are important components.

Molds are aerobic microorganisms, but many of them are very efficient scavengers of oxygen. Due to this property of molds, processed fruits, including those hermetically sealed in cans or glass, are susceptible to spoilage. In case of limited vegetative growth, evidence of spoilage may be the changes produced by fungal enzymes such as the breakdown of starch or pectins while in case of heavy growth, colonies develop in the headspace or as strands throughout a beverage or similar product. Some types of spoilage by fungi are shown in the Figure 7.1 to 7.10. *Penicillium italicum* (blue mold) and *Penicillium digitatum* (green mold) seen in oranges, lemons and citrus fruits (Figure 7.1)

Fig. 7.1: Green and blue mold by *Pencillium* growth on oranges and lemons

Fig. 7.2. Soft rot of apples- *Penicillium expansum* (blue mold)

Fig. 7.3. Grey mold *Botrytis cinerea*

Fig. 7.5. *Rhizopus* soft *Rot*

- *R. stolonifer* cause soft and mushy food, cottony growth of mold.
- Anthracnose -*Colletotrichum lindemuthianum*, cause spotting of leaves and fruits and seed pods

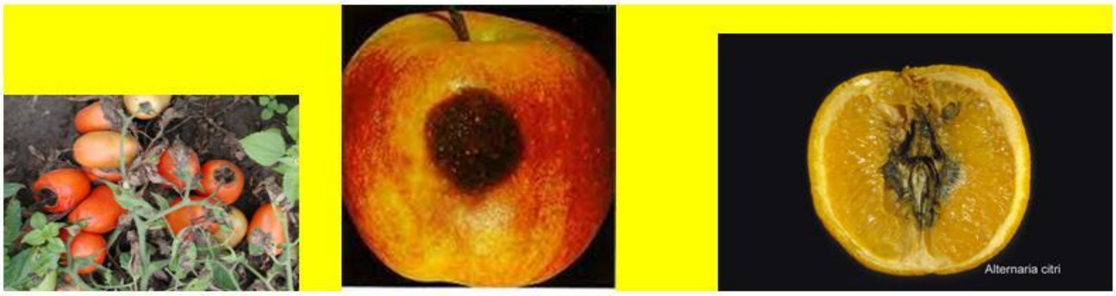


Fig. 7.6. Alternaria rot– *Alternaria tenuis,* Area becomes greenish brown and then black spots

Fig. 7.7. Blue mold rot– *Penicillium digitatum,* bluish green color

Downy mildew:

Fig. 7.8. Downy mildew - *Phytophthora* :wooly masses

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

Watery soft rot

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

Bacteria:

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

- Lactic acid bacteria
- Acetic acid bacteria
- Spore formers

Lactic acid bacteria

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

Growth of lactic acid bacteria in juices and other fruit products cause the formation of haze, gas, acid, and a number of other changes. Certain heterofermentative lactobacilli lead to slime in cider. The lactobacilli and leuconostocs that are present in citrus juices generate acetylmethylcarbinol and diacetyl, compounds that give the juices an undesirable, buttermilk-like flavor. Some strains, being extremely tolerant to ethanol grow in wines. *Lactobacillus fructivorans* can grow in appetizer and dessert wines containing as much as 20% ethanol. Lactic acid bacteria have the ability to decarboxylate malic acid to lactic acid. This malolactic fermentation is often desirable in high-acid wines because the acidity is reduced and desirable flavors are produced. *Oenococcus oenos* is the most acid and alcohol-tolerant species and often is isolated from wines that are undergoing a malo-lactic fermentation.

Acetic acid bacteria

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

Spore formers

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

CONTAMINATION AND SPOILAGE OF MEAT AND MEAT PRODUCTS

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

MICROORGANISMS ASSOCIATED WITH MEAT DURING PROCESSING

Meat spoilages indicate

a) Color changes
b) Textural changes
c) Development of off-flavour or off-odor or slime as a result of microbial growth.

Salmonella is the primary microbial challenge for poultry. The primary microbial to the beef industry is Escherichia coli O157: H7. Listeria, which is an adulterant with zero tolerance, is the major problem for ready to eat meat products. Treatment with organic acids, hot water steam carcass pasteurization and steam carcass vacuuming, trisodium phosphate, acidified sodium chlorite, chlorine dioxide, lactoferrins, peroxyacetic acid, sodium lactate, sodium acetate and sodium diacetate, ozone and radiation have been used as microbial decontaminants during meat processing operations. Carcass washing with hot water of 80°C for 10 seconds can reduce microbial loads by 2 logs. Current regulatory policies and inspection in the meat industry include the HACCP (Hazard Analysis Critical Control Point) food safety system with an objective to provide safe food for consumption and prevent chemical, physical and biological hazards.

Gram-negative bacteria (Aerobes):

Neisseriaceae:

Psychrobacter immobilis, P. phenylpyruvica, Acinetobacter spp., A. twoffii, A. Johnsonii, Pseudomonadaceae: Pseudomonas fluorescens, P. lundensis, P. fragi, P. putida

Gram-positive bacteria:

Brochothrix thermosphacta, *Kurthia zophii*, *Staphylococcus* spp., *Clostridium estertheticum*, *Clostridium frigidicarnis*, *Clostridium casigenes*, *Clostridium algidixylanolyticum* sp. nov.

Spoilage

In contrast to fruits and vegetables, meats are composed mainly of protein and fats rather than carbohydrates. Water content is 71–76% and therefore moisture is not an issue except for spoilage microbes on cured meats. Muscles of healthy animals do not contain any bacteria or fungi but as soon as animals are slaughtered, meat is exposed to contaminants and good sanitation practices are essential to produce high quality meats. The number of spoilage organisms on meat just after slaughter is a critical factor in determining shelf life. The surface of beef carcasses may contain anywhere from 101 to 107 cfu/cm^2, most of which are psychrotrophic bacteria.

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

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

Processed Meat

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

Lactic acid bacteria (LAB) is the group of bacteria primarily associated with spoilage of processed meats. They produce sour off-flavors, gas, slime, and greening, and this spoilage may be more severe than in fresh meat because of the presence of added carbohydrates. Competitive ability of different LAB strains is related to pH and water activity of the meat, cooking and storage temperatures and oxygen and carbon dioxide levels.

Spore formers (Clostridium and Bacillus) are usually not a spoilage problem in processed meats because of the presence of nitrite and other curing salts. However, faulty cooking/cooling procedures, including long cooling periods and temperature abuse, has allowed growth of these organisms in some cases. Spores of these organisms may be introduced with spices or other ingredients. Yeasts cause some spoilage in processed meats but are generally only important when sulfite is used as a preservative or when meats have been irradiated or are stored aerobically in the cold. Slime may be produced along with vinegary or malty off-odors in some sausages.

Fig. 10.1. Spoilage of meat

CONTAMINATION AND SPOILAGE OF MILK AND MILK PRODUCTS:

Milk when secreted into an uninfected animal's udder is sterile and invariably, it becomes contaminated during milking, cooling and/or storage. It is an excellent medium for the growth of bacteria, yeasts and moulds that are the common contaminants of any food material. Their rapid growth, particularly at high ambient temperatures can spoil the milk for liquid consumption and for manufacturing dairy products. This can be avoided to a greater extent by adopting the basic rules of clean milk production.

Spoilage of milk

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

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

Sources of Microbial Contamination of Milk

Microbial contamination of milk can be from the internal and/ or external sources that are described in the following section.

Interior of udder

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

During progress of a milking, bacteria are present in the largest numbers at the beginning and then gradually decrease. This is mainly due to the mechanical dislodging of bacteria, particularly in teat canal, where the numbers are probably highest. Because of this discarding of first few streams of milk helps in lowering the counts of microbes in milk. Milk from different quarters also vary in numbers. Different species of bacteria that are found in milk, as it comes from udder are very limited as given in Table 2.1.

Table 2.1 Presence of different microbial groups in raw milk

Group of microbes	Percent range
Micrococci	30-99
Streptococci	0-50
Asporogenous Gram positive rods	<10
Gram negative rods	<10
Bacillus spores	<10
Other groups of microbes	<10

Though micrococci are slow growing, but if allowed to grow, they cause acid formation and proteolysis. These are mostly non-pathogenic. Streptococci are less frequent than micrococci. Streptococcus agalactiae may be present even in non-clinical mastitis and thus it appears to be a natural inhabitant of udder. Among Gram positive rods, *Corynebacterium bovis* has been found in large numbers. It is non-pathogenic, but if grown causes rancidity. If an animal is infected from mastitis, microbial contamination from within the udder of animal contributes notably to the total numbers of microbes in the bulk milk, when compared with the milk originated from a healthy animal. The influence of mastitis on the total bacterial count of milk depends on the type of the infecting microbe. Most common microbial agents of mastitis in milch animals are given in (Fig 2.3) are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis, Escherichia coli and Corynebacterium pyogenes*.

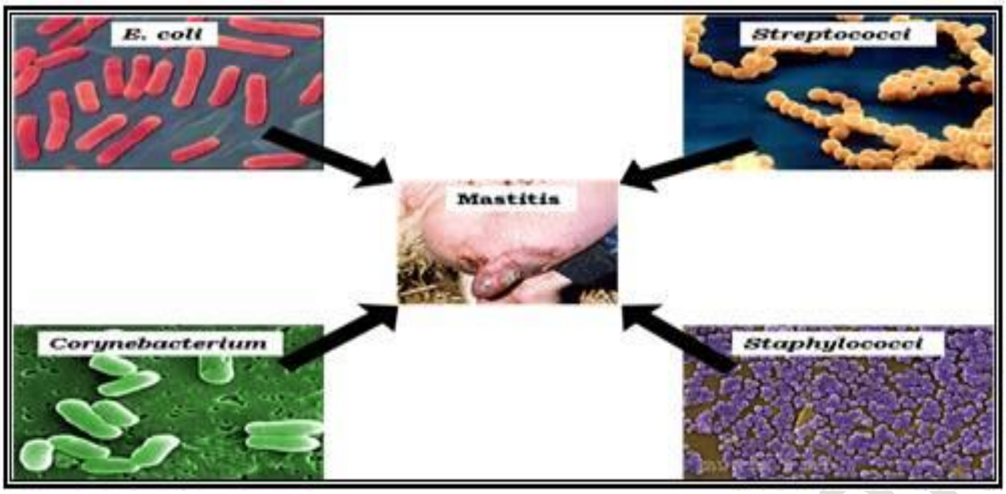


Fig. 2.3 Most common microbial agents of mastitis

Exterior of udder

In addition, to the udder infections, unclean udder and teats of animal also contribute significantly to the total bacterial counts of milk. The microbes that are naturally associated with the skin of the animals as well as those derived from the environment, where the cow is housed and milked are predominant in the milk. The environmental conditions such as soil, manure, mud, feed or bedding; determines what kind of microbes will dominate in milk.

Udder and teat become soiled with dung, mud, bedding material such as saw dust, straw etc. With heavily soiled udder teats the counts may be 1,00,000 cfu/ml. The bedding material in winter has high number of bacteria, mainly psychrotrophs, coliforms and Bacillus spp. Udder microflora is not affected much by simple washing. Economy washing with sodium hypochlorite accompanied by drying, helps in reducing the number of microbes. Different category of microbes that occurs in the exterior of udder are

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

Psychrotrophic and thermoduric bacteria predominate on the teat surfaces. The psychrotrophs that can grow at 7^0C and below are mostly Gram negative rods, and the major ones are Pseudomonas fluorescens, followed by Alcaligenes, Flavobacterium and On the other hand, thermodurics on teat surfaces are often bacterial spores (a dormant and non-reproductive structure; highly resistant to radiations, desiccation, lysozymes, high temperature, starvation and disinfectants) that are typically found in the soil. When these spores enter the bulk milk, they may survive during pasteurization and cause a number of post-pasteurization problems.

Coat of cow

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

Animal shed and surroundings

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

Microbes associated with the bedding materials include:

- Coliforms
- Spore-formers
- Staphylococci
- Streptococci
- Other Gram negative bacteria

Milking staff

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

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

Milking equipment (storage containers and transportation systems)

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

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

Water supplies

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

Airborne contamination

Air contains dust, moisture and bacteria; hence its entry should be minimized in milk. Micrococci, Coryneforms, Bacillus spores, streptococci, and Gram negative rods are the major genera present in air. In general, more air incorporated into milk leads to the faster growth of bacteria.

Colour changes in milk

- Affected by amount and yellowness of butter fat, thinness of milk, content of blood and pus and feed of animal.
- Blue milk - *Pseudomonas syncyanea*, *Streptococcus lactis*, *Actinomycetes* or *Geotrichum* mold.
- Yellow milk - *Pseudomonas synxantha*, Flavobacterium.
- Red milk - *Serratia marcescens, Brevibacterium erythrogenes, Micrococcus roseus*.
- Brown milk - *Pseudomonas putrefaciens, P.fluorescens*.

Spoilage of Butter

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

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

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

Microorganisms of concern in the spoilage of butter are mainly psychrotrophs, which are predominantly Gram-negative, rod shaped microorganisms. The main characteristic of psychrotrophs, which makes them important in the spoilage of butter, is their ability to survive at low temperatures (i.e., 3–7ºC) and the production of enzymes such as lipase and protease which catalyze the hydrolysis of lipids and proteins in the butter, respectively (Ledenbach and Marshall 2009).

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

The major culprit for both is believed to be Pseudomonas spp. *Shewanella putrefaciens* (formerly *Alteromonas putrefaciens*) or Flavobacterium spp. which also play role in development of surface taints in butter. Some species such as *P. putrefaciens* are able to grow on butter surface and produce a putrid odor within relatively short period of time (7–10 days) at refrigeration temperature.

The odor is suggested to be the result of releasing organic acids such as isovaleric acid. Black discoloration and shunk-like odor are also developed in butter by *Pseudomonas nigrificans* and *Pseudomonas mephitica*, respectively.

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

Hydrolytic rancidity in butter can also be the result of activity of Micrococcus spp. (Boor and Fromm 2006) and molds such as Rhizopus, Geotrichum, Penicillium and Cladosporium.

CONTAMINATION, SPOILAGE OF POULTRY

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

The primary causes of poultry products spoilage are as follows:

- Prolonged distribution or storage time
- Inappropriate storage temperature
- High initial bacterial counts
- High post-rigor meat pH

Spoilage factors

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

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

Initial bacterial counts on broiler carcasses may have a direct effect on the shelf-life of fresh product as well. The initial number of bacteria on poultry is generally a function of grow-out procedures, production practices, and plant and processing sanitation. Higher numbers of spoilage bacteria on the chicken immediately after processing, translates to more rapid spoilage.

High post-rigor meat pH is often caused by stress on the birds during grow-out or transportation. This reduces the shelf-life of the meat by up to six days and is due to the fact that spoilage bacteria multiply much more rapidly on meat that is at a pH of 6.2 than on meat that is at a normal post-rigor pH of 5.4-5.6.

Bacteria responsible for spoilage

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

These spoilage bacteria are called psychrotrophic bacteria (psychro=cold; trophic=able to grow) because they are able to multiply under cold conditions. Fresh poultry products held long enough at refrigerator temperatures will spoil as a result of the growth of psychrotrophic bacteria.

In contrast, the bacteria that exist in higher numbers at the time of processing on the skin of chickens and in their intestinal tracts are primarily mesophiles (meso=middle; phile=love). These bacteria do not multiply to an appreciable degree at refrigerator temperatures. Salmonella, E. coli and other bacteria found on chickens are mesophiles. When a company conducts an “Aerobic Plate Count” or “Total Plate Count” on a chicken carcass, it is measuring the mesophiles.

The figure “Mesophilic and psychrotropic bacterial growth during cold storage at 4 C on fresh poultry” shows how these populations of bacteria behave on carcasses during refrigeration.

Origin of spoilage bacteria

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

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

Spoilage species

The bacterial genera most isolated in high numbers on spoiled poultry was *Pseudomonas fluorescens*, putida, or fragi or *Shewanella putrefaciens*. Identification of the genus and species most responsible for spoiling poultry is important because, once identified, it is easier to understand the mechanisms by which they produce spoilage.

High numbers (105 cfu/cm2) of psychrotrophic spoilage bacteria are required on poultry surfaces before off-flavors, off-odors and appearance defects are able to be detected organoleptically. Researchers have reported that higher numbers of bacteria (3.2x107 to 1x109 cfu/cm2) were required to produce slime than were needed for odor to become noticeable.

Causes of spoilage defects

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

Off-odors do not result from breakdown of the protein in skin and muscle, as previously thought, but from the direct microbial utilization of low molecular weight nitrogenous compounds such as amino acids, which are present in skin and muscle.

Concentrations of free amino acids increase as proteolysis occurs throughout the storage period. It has been demonstrated that measurement of these free amino acids, due to the production of aminopeptidases and subsequent breakdown of protein, may be used to rapidly determine the bacteriological quality of beef.

Development of off-odors and slime

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

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

In the final stages of spoilage, the meat may begin to exhibit a pungent ammoniacal odor in addition to the dirty dishrag odor, which may be attributed to the breakdown of protein and the formation of ammonia or ammonia-like compounds. Various authors have reported that degradation of meat by pseudomonads results in the formation of slime.

Effects of cold storage

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

Cellular lipids:

Typically, mesophilic bacteria cease to proliferate below a certain environmental temperature because as temperature decreases so does their cellular absorption of nutrients. Psychrotrophic bacteria species typically exhibit no such temperature-induced difference. This is why the psychrotrophic spoilage bacteria are able to grow rapidly at refrigerator temperatures.

Lipase production:

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

Proteolytic activity:

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

Shelf life: Counting the right bacteria

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

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

CONTAMINATION, SPOILAGE OF EGG AND EGG PRODUCTS

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

The total number of microorganisms per shell of a hen's egg has been reported to range from 102 to 107 with a mean of about 105. The types of microorganisms recovered from the shell are diverse.

Salmonella species may be on the shell or in the egg as laid, build up during processing, and appear in significant numbers in frozen or dried eggs.

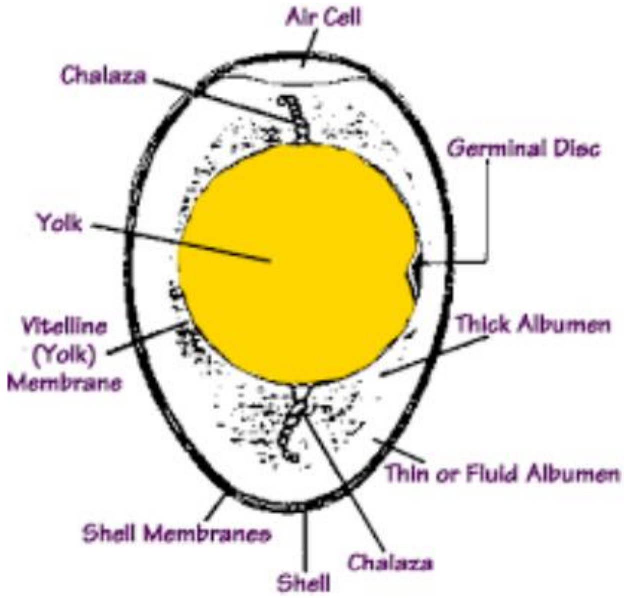
Factors influencing the contamination of eggshells:

The spoilage of eggs is related to eggshell contamination and the ability of some bacteria to penetrate the egg.

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

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

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



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

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

The presence of eggshell cracks or micro-cracks increases the risk of contamination. The manipulation of eggs, especially in the conditioning centres, increases the risk of egg cracking. The external and internal shell membranes represent effective filters composed of anti-bacterial glycoprotein fibres, which may play a role in protection against penetration.

In addition to these physical barriers, egg white, similar to an intracellular fluid, is an important line of defence against invading bacteria because it represents a not favourable environment for microbial development (nutrient-poor, exhibiting an alkaline pH, and high viscosity and heterogeneity), and because it contains several molecules expressing antimicrobial activities, such as lysozyme, ova transferrin, proteinase inhibitors (cystatin, ova mucoid and ovoinhibitor), and vitamin binding proteins (riboflavin binding protein, avidin- and thiamine-binding proteins).

The integrity of these barriers (cuticle, shell, shell membranes, egg white, vitelline membrane) is essential to prevent microbial penetration and proliferation.

Spoilage of Eggs:

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

Defects in the fresh egg

Fresh eggs may exhibit cracks, leaks, loss of bloom or gloss, or stained or dirty spots on the exterior as well as "meat spots" (blood clots), general bloodiness, or translucent spots in the yolk when candled. Changes during storage:¬ The changes that take place in eggs while they are being held or stored may be divided into those due to non-microbial causes and microbial causes.

Changes Not Caused by Microorganisms

1. Untreated eggs lose moisture during storage and hence lose weight. The amount of shrinkage is shown to the candler by the size of the air space or air cell at the blunt end of the egg, a large cell indicating much shrinkage.
2. The change in the physical state of the contents of the egg, as shown by candling or by breaking out the egg.
3. The white of the egg becomes thinner and more watery as the egg ages, and the yolk membrane becomes weaker.
4. When an old egg is broken onto a flat dish, the thinness of the white is more evident, and the weakness of the yolk membrane permits the yolk to flatten out or even break. By contrast, a broken fresh egg shows a thick white and a yolk that stands up strongly in the form of a hemisphere.
5. During storage, the alkalinity of the white of the egg increases from a normal pH of about 7.6 to about 9.5. Any marked growth of the chick embryos in fertilized eggs also serves to condemn the eggs.
6. The poorer the egg, the more the movement is there of yolk and the nearer it approaches the shell when it is twirled during candling.

Changes Caused by Microorganisms

To cause spoilage of an undamaged shell egg, the causal organisms must do the following: (1) contaminate the shell, (2) penetrate the pores of the shell to the shell membranes (usually the shell must be moist for this to occur), (3) grow through the shell membranes to reach the white (or to reach the yolk if it touches the membrane), (4) grow in the egg white, despite the previously mentioned unfavourable conditions there, to reach the yolk, where they can grow readily and complete spoilage of the egg.

In general, more spoilage of eggs is caused by bacteria than by molds. The types of bacterial spoilage, or "rots," of eggs go by different names. Alford (1950) list five groups of rots that are found in eggs. Among the three chief ones are

Green rots:

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

Colourless rots:

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

Black rots:

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

Pink rots:

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

Red rots:

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

Spoilage of Eggs by Fungi:

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

Pin spot molding:

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

Superficial fungal spoilage:

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

Fungal rotting:

The final stage of spoilage by molds is fungal rotting, after the mycelium of the mold has grown through the pores or cracks in the egg. Jellying of the white may result, and coloured rots may be produced, e.g., fungal red rot by Sporotrichum and a black colour by Cladosporium, the cause of black spot of eggs as well as of other foods. The hyphae of the mold may weaken the yolk membrane enough to cause its rupture, after which the growth of the mold is stimulated greatly by the food released from the yolk.

Off-flavours sometimes are developed in eggs:

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

UNIT - V
FOOD POISONING

Foodborne illness, more commonly referred to as food poisoning, is the result of eating contaminated, spoiled, or toxic food. The most common symptoms of food poisoning include nausea, vomiting, and diarrhoea.

Foods contaminated with pathogenic microorganisms usually do not look bad, taste bad, or smell bad. It is impossible to determine whether a food is contaminated with pathogenic microorganisms without microbiological testing. To avoid potential problems in foods, it is very important to control or eliminate these microorganisms in food products. Pathogenic microorganisms can be transmitted to humans by a number of routes.

Diseases which result from pathogenic microorganisms are of two types: infection and intoxication.

- Foodborne infection is caused by the ingestion of food containing live bacteria which grow and establish themselves in the human intestinal tract.
- Foodborne intoxication is caused by ingesting food containing toxins formed by bacteria which resulted from the bacterial growth in the food item. The live microorganism does not have to be consumed.

For a foodborne illness **(poisoning) to occur, the following conditions must be present:**

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

Food poisoning symptoms

If you have food poisoning, chances are it won't go undetected. Symptoms can vary depending on the source of the infection. The length of time it takes for symptoms to appear also depends on the source of the infection, but it can range from as little as 1 hour to as long as 28 days.

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

- abdominal cramps
- diarrhoea
- vomiting
- loss of appetite
- mild fever
- weakness
- nausea
- headaches

Symptoms of potentially life-threatening food poisoning include:

- diarrhoea persisting for more than three days
- a fever higher than 101.5°F
- difficulty seeing or speaking

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

Causes of Food Poisoning

Bacteria and Viruses:

Bacteria and viruses are the most common cause of food poisoning. The symptoms and severity of food poisoning vary, depending on which bacteria or virus has contaminated the food.

Parasites:

Parasites are organisms that derive nourishment and protection from other living organisms known as hosts. In the United States, the most common foodborne parasites are protozoa, roundworms, and tapeworms.

Molds, Toxins, and Contaminants:

Most food poisoning is caused by bacteria, viruses, and parasites rather than toxic substances in the food. But some cases of food poisoning can be linked to either natural toxins or added chemical toxins.

Allergens:

Food allergy is an abnormal response to a food triggered by your body's immune system. Some foods, such as nuts, milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat or soybeans, can cause allergic reactions in people with food allergies.

FOOD BORNE INFECTIONS

A foodborne infection is an inflammation of the stomach and bowels. The infection can happen when you eat or drink something that is contaminated by a bacteria, virus or parasite. Often the inflammation leads to diarrhoea, nausea, vomiting, abdominal pain, abdominal cramps and sometimes fever. A foodborne infection can last between one and three days.

Many foodborne infections occur at people's homes, simply due to poor hygiene. It's as easy as this: preparing food without hand washing after visiting the toilet d food. Cross-contamination is also a risk, for instance if raw meat and lettuce are both chopped on the same cutting board. Even using the same knife to chop both could cause contamination by foodborne pathogens. Eating meat or fish that is not cooked all the way through, or eating raw shellfish, increases the risk of food-borne infections.

Food borne intoxications or food poisoning is caused by ingestion

- Of toxicants found as toxins of certain plants or animals.
- Toxin formed by microbes while they multiply in the foods or after entering the intestines.
- Poisonous substances that may be intentionally or incidentally added to foods during production, processing, transportation or storage.
- Toxicants or toxic substances in food are substances that are found in foods that can produce harmful effects on ingestion by humans and animals.
- Toxicants or toxic substances in food are substances that are found in foods that can produce harmful effects on ingestion by humans and animals.

BACTERIAL INTOXICANTS:

Staphylococcal poisoning

- Most common infection
- Caused by infection with staphylococcus aureus
- Enterotoxins produced are heat stable.
- Toxin causes gastroenteritis. Symptoms appear within 1-6 hours of consumption of contaminated food.
- Symptoms are dose dependent, more the toxin ingested, earlier and severe the symptoms.
- Nausea, vomiting, abdominal pain, diarrhoea, dehydration. If severe and not appropriately treated, it can be fatal.
- Common foods implicated are custard and cream, bakery foods, poultry and ham, fermented meat and dairy products especially 'khoa' in India.
- Following proper hygiene in the preparation and storage is the key to avoid infection

CLOSTRIDIUM - BOTULISM

- Infection by *Clostridium botulinum*
- It produces neurotoxin which is heat sensitive.
- Disease starts within 2 hours to 14 days after ingestion of contaminated food.
- Symptoms are nausea, vomiting, headache, persistent constipation followed by blurred vision.
- Common foods implicated are fermented or smoked marine products, home cured ham and meat products.
- Botulism can be prevented by killing *C.botulinum* spores in the foods.
  - During processing
  - Eliminating recontamination of processed food
  - Destroying the toxin by proper heating of processed food.
  - By proper storage.
  - By discarding the product that has developed signs of spoilage ex. off odour, bulging of cans and gas bubbles on opening the can.

SALMONELLA - SALMONELLOSIS (TYPHOID)

- Incubation period is 6 hours to 3 days.
- Major symptoms are nausea, diarrhoea and fever which may last for several days.
- Foods implicated are egg, meat and milk and their products
- Infection either comes from product of infected animal like meat or milk or from food handler who is a carrier of infection and has not been following strict hygiene.
- Infection can be prevented by strictly adhering to good cooking methods.

Treatment

Symptoms of Salmonella-induced gastroenteritis usually disappear without treatment after 4–7 days.

Treatments may include:

- fluids to prevent dehydration.
- antimotility drugs to reduce cramping and stop diarrhoea
- antibiotics for severe symptoms or if bacteria have entered the bloodstream or are likely to do so

A doctor will not always prescribe antibiotics for Salmonella. Scientists have determined that overuse can lead to antibiotic resistance, and this increases the risk of the infection reappearing.

FUNGAL INTOXICANTS:

Mycotoxins:

Mycotoxins are toxic compounds that are naturally produced by certain types of moulds (fungi). Moulds that can produce mycotoxins grow on numerous foodstuffs such as cereals, dried fruits, nuts and spices. Mould growth can occur either before harvest or after harvest, during storage, on/in the food itself often under warm, damp and humid conditions. Most mycotoxins are chemically stable and survive food processing.

Several hundred different mycotoxins have been identified, but the most commonly observed mycotoxins that present a concern to human health and livestock include aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone and nivalenol/deoxynivalenol. Mycotoxins appear in the food chain as a result of mould infection of crops both before and after harvest. Exposure to mycotoxins can happen either directly by eating infected food or indirectly from animals that are fed contaminated feed, in particular from milk.

The effects of some food-borne mycotoxins are acute with symptoms of severe illness appearing quickly after consumption of food products contaminated with mycotoxins. Other mycotoxins occurring in food have been linked to long-term effects on health, including the induction of cancers and immune deficiency. Of the several hundred mycotoxins identified so far, about a dozen have gained the most attention due to their severe effects on human health and their occurrences in food.

AFLATOXIN

Aflatoxins are amongst the most poisonous mycotoxins and are produced by certain moulds (*Aspergillus flavus* and *Aspergillus parasiticus*) which grow in soil, decaying vegetation, hay, and grains.

Crops that are frequently affected by Aspergillus spp. include cereals (corn, sorghum, wheat and rice), oilseeds (soybean, peanut, sunflower and cotton seeds), spices (chili peppers, black pepper, coriander, turmeric and ginger) and tree nuts (pistachio, almond, walnut, coconut and Brazil nut).

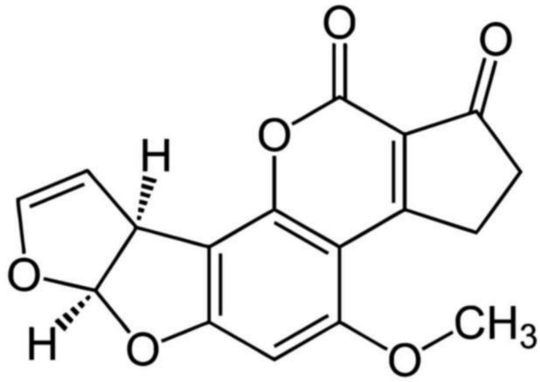
The toxins can also be found in the milk of animals that are fed contaminated feed, in the form of aflatoxin M1. Large doses of aflatoxins can lead to acute poisoning (aflatoxicosis) and can be life threatening, usually through damage to the liver.

Aflatoxins have also been shown to be genotoxic, meaning they can damage DNA and cause cancer in animal species. There is also evidence that they can cause liver cancer in humans.

Aflatoxins are the most important mycotoxins that are produced mainly by *Aspergillus Flavus* and *Aspergillus Parasiticus*, and they are mainly produced by the aspergillus species.

There are four types of aflatoxins named B1, B2, G1, and G2 and can be categorized based on two characteristics: Their fluorescence color under UV light (whether it is blue or green), and their mobility during TLC.

Chemical structure of Aflatoxin



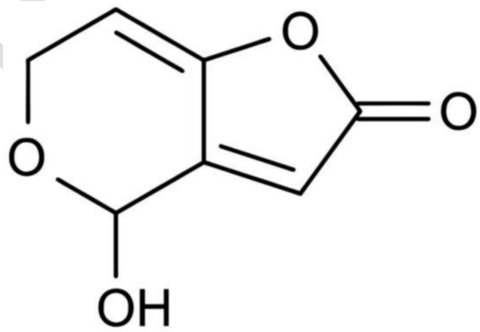
The chemical formula of the aflatoxin is $C_{17}H_{12}O_6$. The biosynthesis of aflatoxins starts with the production of norsolorinic acid which is an anthraquinone precursor joined together by the action of the enzyme II polyketide synthase followed by 15 post-polyketide synthase steps which will yield a series of toxigenic metabolites.

PATULIN:

Patulin is a mycotoxin produced by a variety of moulds, particularly Aspergillus, Penicillium and Byssochlamys.

It found in rotting apples and apple products, patulin can also occur in various mouldy fruits, grains and other foods.

Major human dietary sources of patulin are apples and apple juice made from affected fruit. The acute symptoms in animals include liver, spleen and kidney damage and toxicity to the immune system. For humans, nausea, gastrointestinal disturbances and vomiting have been reported. Patulin is considered to be genotoxic however a carcinogenic potential has not been demonstrated yet. This mycotoxin chemical name is 4-hydroxy-4H-furo[3,2c] pyran-2(6H)-one (64). The chemical formula is $C_7H_6O_4$



Chemical structure of patulin

OCHRATOXIN

Ochratoxin A is produced by several species of *Aspergillus* and *Penicillium* and is a common food-contaminating mycotoxin.

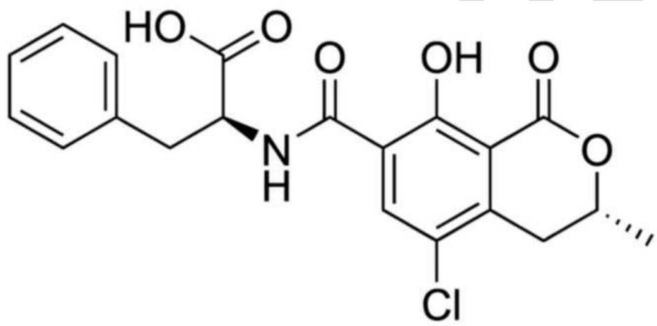
Contamination of food commodities, such as cereals and cereal products, coffee beans, dry vine fruits, wine and grape juice, spices and liquorice, occurs worldwide.

Ochratoxin A is formed during the storage of crops and is known to cause a number of toxic effects in animal species.

The most sensitive and notable effect is kidney damage, but the toxin may also have effects on fetal development and on the immune system.

Contrary to the clear evidence of kidney toxicity and kidney cancer due to ochratoxin A exposure in animals, this association in humans is unclear, however effects on kidney have been demonstrated.

The most important ochratoxin is the Ochratoxin A, which is produced mainly by *Aspergillus ochraceus* and its chemical formula is $C_{20}H_{18}ClNO_6$



Chemical structure of ochratoxin

VIRAL - HEPATITIS

Hepatitis is an inflammation of the liver that results in diffuse hepatic cell death and may lead to areas of liver necrosis. It can be classified as acute or chronic (lasting > 6 months) and may progress to fulminant liver failure, cirrhosis, and, in some cases, hepatocellular carcinoma. Hepatitis may result from infectious (e.g., bacterial, viral, parasitic or fungal) and noninfectious causes (e.g., drugs, metabolic diseases, alcohol, autoimmune diseases). The most common causes in the United States are alcohol abuse and viral infection.

Viral hepatitis is most commonly caused by hepatitis viruses (especially hepatitis A, hepatitis B, and hepatitis C) and herpes viruses (cytomegalovirus, Epstein-Barr virus, varicella-zoster virus, herpes simplex virus).

Common symptoms include fever, nausea, vomiting, fatigue, jaundice, right-upper-quadrant abdominal tenderness, and dark urine and pale stools. Extrahepatic manifestations may occur, particularly with chronic hepatitis. These include amenorrhea, arthritis, skin rash, vasculitis, thyroiditis, gynecomastia, glomerulonephritis, polyarteritis nodosa, and Sjögren's syndrome. Complications of chronic hepatitis include cirrhosis, progressive liver failure, and development of hepatocellular carcinoma.

Hepatitis A (HAV) is a self-limited cause of acute hepatitis and does not result in a carrier state or chronic disease. Transmission occurs via the fecal-oral route and most commonly results from poor hygienic practices and inadequate sanitation.

Hepatitis B (HBV) generally causes a mild or subclinical acute hepatitis but may result in chronic hepatitis or an asymptomatic carrier state. Most symptoms last 1-3 months, although fatigue can last longer. Progression to chronic hepatitis is most common in perinatal infections and young children. Transmission occurs via blood and body fluids (e.g., unprotected sex, intravenous drug use, blood transfusions, tattoos, and body piercing).

Hepatitis C (HCV) is the most common cause of chronic hepatitis in the United States and most common indication for liver transplantation. Acute hepatitis is usually asymptomatic, but many cases do progress to chronic hepatitis.

Hepatitis D (HDV) is dependent on co-infection with the hepatitis B virus. If hepatitis D is acquired at the same time as hepatitis B, complete recovery can be expected. However, hepatitis D occurring as a super infection in a hepatitis B patient can cause a syndrome of accelerated hepatitis, with progression to chronic hepatitis within weeks. Transmission occurs via blood and body fluids.

Hepatitis E (HEV) usually causes a self-limited and mild acute hepatitis. However, the disease may be severe in pregnant women, in whom it may progress to acute onset of liver failure, with mortality as high as 25%. The virus is most commonly spread by fecal-oral route in endemic areas, usually from contaminated water sources. It can also be transmitted by blood transfusions or after organ transplants.

RICKETTSIA

The Rickettsiae are a diverse group of bacteria some of which can be transmitted to humans via the bites of fleas, lice, ticks or mites. Several Rickettsia species present in Australia are capable of causing disease in people.

These species include:

- *Rickettsia australis* - Queensland tick typhus
- *Orientia tsutsugamushi* - scrub typhus
- *Rickettsia honei* - Flinders Island spotted fever
- *Rickettsia typhi* - murine typhus.

Although rickettsial infections are relatively rare, they have been reported along the eastern Australian seaboard, Flinders Island and the east coast of Tasmania, as well as the Fleurieu Peninsula in South Australia and southern coastal Western Australia.

Rickettsia prowazekii (epidemic typhus) is spread by human body lice and can result in outbreaks of disease but is only seen in conflict settings and refugee camps and is not naturally occurring in Australia.

Infections are spread

Rickettsiae are usually injected directly from the saliva of ticks and mites as they feed on humans and, in the case of fleas, by contamination of bite sites by faeces.

Signs and symptoms

There is great variation in the range and severity of symptoms experienced. Commonly a small, hard, black sore (called an eschar) first appears at the bite site where the infection was introduced.

Other typical symptoms may include:

- fever
- headache
- muscle aches
- swollen lymph glands
- cough
- Rash.

Less common severe infections can be associated with confusion and breathing difficulties.

Treatment

Treatment is usually with the tetracycline antibiotic doxycycline which reduces the duration and severity of infection.

TRICHINOSIS

Trichinosis sometimes called trichinellosis is a type of roundworm infection. Roundworm parasites use a host body to live and reproduce. Infection occurs primarily among meat-eating animals (carnivores) such as bears and foxes, or meat- and plant-eating animals (omnivores) such as domestic pigs and wild boar. The infection is acquired by eating roundworm larvae in raw or undercooked meat.

When humans eat undercooked meat containing trichinella larvae, the larvae mature into adult worms in the intestine over several weeks. The adult worms then produce larvae that travel through various tissues, including muscle. Trichinosis is most widespread in rural areas throughout the world. Trichinosis can be treated with medication, though it's not always necessary. It's also easy to prevent.

Symptoms

Abdominal symptoms can occur one to two days after infection. Other symptoms usually start two to eight weeks after infection. The severity of symptoms usually depends on the number of larvae consumed in the infected meat.

Mild cases of trichinosis - those with only a small number of parasites in your body - may cause no recognizable signs or symptoms. Symptoms can develop with moderate or heavy infestation, sometimes progressing as the parasite travels through your body.

Causes

People get trichinosis when they eat undercooked meat — such as pork, bear, walrus or horse — that is infected with the immature form (larvae) of the trichinella roundworm. In nature, animals are infected when they feed on other infected animals. Pigs and horses can become infected with trichinosis when they feed on garbage containing infected meat scraps. Cattle don't eat meat, but some cases have been linked to eating beef that was mixed with infected pork or ground in a grinder previously used for contaminated pork. Due to increased regulation of pork feed and products in the United States, pigs have become a less common source of infection. Wild animals, including bear, continue to be sources of infection.

Prevention

The best defense against trichinosis is proper food preparation. Follow these tips to avoid trichinosis:

Avoid undercooked meat:

Be sure whole cuts of meat other than poultry and wild game are cooked to an internal temperature of 145 F (63 C) throughout, and don't cut or eat the meat for at least three minutes after you've removed it from the heat. Cook ground pork and beef to at least 160 F (71^0 C). They can be eaten immediately after cooking

They can be eaten immediately after cooking:

Using a meat thermometer is the best way to ensure the meat is thoroughly cooked.

Avoid undercooked wild game:

For both whole cuts and ground varieties, cook to an internal temperature of at least 160 F (71 C).

Avoid undercooked poultry:

For whole cuts and ground varieties, cook to a temperature of at least 165 F (74 C). For whole cuts, let the poultry sit for three minutes before cutting or eating.

Have wild-animal meat frozen or irradiated:

Irradiation will kill parasites in wild-animal meat, and deep-freezing for three weeks kills trichinella in some meats. However, trichinella in bear meat does not die by freezing, even over a long period. Neither irradiation nor freezing is necessary if you ensure that the meat is thoroughly cooked.

Know that other processing methods don't kill parasites:

Other methods of meat processing or preserving, such as smoking and pickling, don't kill trichinella parasites in infected meat.