Food and Diary Microbiology

Food Microbiology is the science which includes the contamination, spoilage and preservation of food. Food is a highly nutritious, easily metabolized and suitable substrates for the growth of micro-organisms. In other words, activities of microbes in food is called as food microbiology

Food is medicine, coined by Hippocrates- Father of Medicine, followed by siddha medicine – a traditional medicine introduced by Siddhars.

Louis Pasteuer (1857) who called as the Father of Food Microbiology, he said living creatures would arise from non-living matter like food and other things, he also developed Pasteurization technique, the word fermentation was introduced by Pasteur. Microbes responsible for souring and alcohol production from fermented food.

Food is the chief source for spreading various types of diseases.

Growth of microbes in food

Growth of microbes in food that results in three effects 1, they can cause spoilage(e.g., growth of ***Pseudomonas*** in meat that result in bad odor unfit for consumption) 2, they can cause food borne illness or vectors for transmitting human pathogens) (*E.coli* causes gastroenteritis) 3, they can transform a food's properties in a beneficial way – food fermentation (curd, idli),

Preservatives

Preservatives are defined as substances capable of inhibiting, retarding or arresting the growth of microorganisms**.**

Important microorganisms of food includes bacteria, mold and yeast, mold responsible for Soy sauce production, yeast responsible for bread, wine and beer production. Bacteria responsible for curd and other dairy products

Food as a Substrate for Microorganisms

Food Microbiology is the science which includes the activities of microorganisms in food. Food is a highly nutritious, metabolizable and suitable substrates for the growth of micro-organsms.

Foods for human consumption can be divided into eight main groups. **Four of plant origin** e.g., 1, Cereals and cereal products. 2, Sugar and sugar product. 3, Vegetables and vegetables products. 4, Fruits and fruits products. **Foods from animals origin** e.g., 1, Meats and meat products. 2, Poultry and poultry products. 3, Fish and other sea food.4, Milk and milk products.

Mineral food- Sodium chloride is a mineral food, a flavoring materials and a chemical preservatives.

Fortified food – food is added with minerals e.g., iron and calcium are added into flour .

Factors affecting the growth and survival of microorganisms in foods.

There are two factors 1, **Intrinsic factors** (Physico – chemical factors) it includes Nutrients, pH, Redox potential, Water activity, Antimicrobial constituents and Antimicrobial structures. 2, **Extrinsic factors** (Environmental factors) it includes Relative humidity, Temperature and Gaseous atmosphere.

Nutrients - Microbes can use food as a source of nutrients and energy**.**

Foods for energy - Simple sugars (glucose, lactose, sucrose, maltose), esters, peptides , amino acids, fats and organic acids and their salts, complex carbohydrates e.g., cellulose, starch are serve as energy and carbon source for microbes. For degradation of starch the microbes produces amylase enzymes e.g., ***Bacillus sp, Aspergillus sp***, for fats the lipase enzymes degrade fat into fatty acids and glycerol are seve as energy source for microbes. The peptides , amino acids are energy source for proteolytic microbes e.g., ***Pseudomonas spp.***

Food for growth – Proteins and their products peptides and amino acids are serve as a source of nitrogen for growth e.g., ***Pseudomonas sp, Clostridium sp, Penicillium sp***, are proteolytic are able to produce protease enzymes that degrade proteins into peptides and amino acids.

Food for vitamins - Some microbes are unable to synthesize vitamins for their growth but obtained from food, e.g., meat contain rich source of vitamins B, fruits contain vitamin C ascorbic acid are utilized by different microbes. Long storage of food can reduce their vitamin contents , Heat processing of food also lose their vitamins e.g., thiamine, pantothenic acid, folic acid and ascorbic acid are heat labile.

pH

Every microbes require optimum pH for their better growth, based on pH the food can be classified into three types.1, acid food (below 4.5) e.g., fruits, fermented milk, sauerkraut and pickles are spoiled by yeasts and molds (acidophiles – grow well in low pH environments) 2, neutral food (around 7) e.g., milk, meat, vegetables, egg, cereals, pulses are spoiled by bacteria ***E. coli, Salmonella sp, Staphylococcus aureus, Shigella, Clostridium sp, Bacillus sp*** that are neutrophile that are grow well in neutral pH environments.3. alkali food e.g., white (albumen)of egg is spoiled by proteolytic bacteria e.g., ***Pseudomonas spp***, that are alkalophile grow well in alkali environments.

Redox potentials or oxidation – reduction potentials.

Microbes can use oxygen for their growth is called aerobic, microbes that can not use oxygen for growth is called as anaerobic, food in aerobic environment that are spoiled by aerobic e.g., ***E. coli, Salmonella sp, Staphylococcus aureus, Shigella,*** **and** ***Bacillus sp*** , food in anaerobic environment that are spoiled by anaerobic e.g., ***Clostridium sp,***. The oxidation – reduction potentials is usually written as ***Eh.*** The oxidized substrate would have positive *Eh*, reduced substrate would have negative *Eh*,

Water activity - Every Microbes require water for their growth, without water no growth can occur. The water requirement is expressed in terms of available water or water activity $a_{w..}$ Each microbes has maximal optimal, minimal a_w for growth. As the a_w reduced below the optimum level, there is a lengthening of the lag phase of growth or decrease in the rate of growth.

a_w Values	Food
0.98	Fish, meat, milk, vegetables and fruits
0.60 – 0.85	Cereals, flour, nuts, jams and cheese
Below 0.60	Dried eggs, milk, vegetables, honey

Factors that affect a_w requirements of microorganisms includes : 1, adding of solute (e.g., potassium chloride, sodium chloride)will reduce the a_w in food. 2, microbe can tolerate low a_w at their optimum temperature, oxygen level and *pH*.

Bacteria can grow well in medium contains water activity a_w of more than 1.00 level.

a_w Values	microbes
0.97	*Pseudomonas sp*
0.96	*E. coli*
0.95	*Bacillus subtillus*
0.88	*Yeasts*
0.62	*Molds- Mucor, Rhizopus and Botrytis*

Antimicrobial structures - Presence of skin in apples, shell in coconut, husk in paddy and wheat are physical barriers provides an inhospitable (bad) environment for microorganisms.

Antimicrobial constituents - Presence of allicin in garlic and onion, phaseolin in green beans, lactoperoxidase in milk and lysozyme in egg are antimicrobial constituents, These antimicrobial constituents can kill Gram – negative bacteria or inhibit the Gram - positive bacteria .

Extrinsic factors (Environmental factors)

Relative humidity – If food with low availability of water ($a_{w\ Values}$) is stored in an atmosphere of high relative humidity water will transfer from the gas phase to the food. If relative humidity is low in storage room of vegetables and fruits will lose water and become flaccid. If it is too high then condensation may occur and microbial spoilage may be initiated.

Temperature – Microbial growth in food can occur a temperature range from about – 8^0 C to 100^0C. Based on temperature requirement the microbes can be classified into three types 1, **Thermophiles** (microbes can grow best in high temperature environment) can grow best at 35-75^0C. 2, **Mesophiles** (microbes can grow in moderate temperature environment)can grow best in temperature 30-40^0C. 3, **Psychrophiles** (microbes can grow in low temperature environment)can grow best at temperature 12-15^0C.

Gaseous atmosphere - Some microorganisms in food are killed by prolonged exposure to CO^2 that are aerobes, Some microorganisms in food are killed by prolonged exposure to oxygen that are anaerobes, oxygen level in atmosphere is 21%.

Microorganisms important in food microbiology

It is necessary to study about the characteristics of microorganisms, because bacteria and fungi are responsiple for spoilage of foods.

Fungi - are eukaryotic(having nucleus covered by neuclear membrane – true neucleus) heterotrophs (depend on organic matter for their growth), non-photosyntheic (lack of chlorophill pigments), aerobic (require oxygen for their growth), unicellular –single celled e.g., Yeasts, some are multicellular e.g., molds(*Mucor, Rhizopus, Penicillium* and *Aspergillus),* moisture environments for better growth,reproduce by asexually (Budding- Yeasts) – production of asexual spores e.g., conidiospores*(Penicillium* and *Aspergillus*,) sporongiospores(*Mucor, Rhizopus*), arthrospores, chlamydospores, production of sexual spores e.g., zygospores(*Rhizopus*), oospores (aquatic molds oomycetes), ascospores(Yeasts), basidiospores (*Agaricus sp*- Basidiomycetes).

Molds – are multicellular, filamentous fungi, their growth on foods is recognized by fuzzy or cottony appearance, produces coloured spores.

Morphology characters - some are macroscopic, some microscopic nature, it produces branched filaments is called as **hyphae** (singular-**hypha**), mass of or more hyphae is called as **mycelia** (singular **mycelium)**, some hyphae are fertile involved in reproduction, some vegetative nature involved in nutrition transport of molds, the mycelium and hypha are called as **thallus**, under unfavourable or bad or adverse environmental condition the mycelium converted into thick walled structure is called as **sclerotia** (singular **sclerotium**). Hypha having cross walls or septum is called as **septate** *(Penicillium* and *Aspergillus)*, without cross walls is called as **non-septate** or noncoenocytic(*Mucor, Rhizopus*),.

Reproduction

They reproduce by asexually (Budding- Yeasts) – production of asexual spores e.g., conidiospores*(Penicillium* and *Aspergillus*,) sporongiospores(*Mucor, Rhizopus*), arthrospores, chlamydospores(thick walled resistant spores, withstand adverse environmental condition),

production of sexual spores e.g., zygospores(*Rhizopus*), oospores (aquatic molds oomycetes), ascospores covered by ascus – a sac like structure (Yeasts - ascomycetes), basidiospores (*Agaricus sp*- Basidiomycetes).

The structure that bears conidiospores is called as **conidiophore**, sporongiospore – **sporongiophore**, the tip of the **sporongiophore** is called as columella, the tip of the **conidiophore** is called as sterigma (plural sterigmata).

Sexual reproduction – it means production of spores after union of two neuclei from two strains. It occurs in favorable or good environmental condition.

Asexual reproduction - it means production of spores there is no union of neuclei

Fungi imperfecti- Fungi with only asexual cycle e.g., *Aspergillu and Penicillium sp.*

Fungi perfecti – Fungi with both sexual and asexual cycle e.g., Ascomycetes – Yeasts(*Saccharomyces cerevisiae*)

Molds which are involved in the spoilage of food

Mucor, Rhizopus (bread mold because it will spoil the bread), *Absidia, Thamnidium, Aspergillus, Penicillium(*produces blue-green conidia), *Trichothecium*(pink mold), *Geotrichum, Neurospora, Sporotrichum*(white spot on food), *Cephalosporium, Pullularia, Cladosporium* (black spot on food), *Helminthosporium, Alternaria, Fusarium, Endomyces, Trichoderma*, and *Neurospora* (red bread mold)

Molds which are involved in the food industries.

Aspergillus sp is useful in the production of citric acid, gluconic acid and enzymes.

Penicillium roqueforti help in the production of Cambert cheese.

Mucor – production of Oriental foods.

Culture Medium – Potato Dextrose Agar is used for isolation of molds from food.

Lactophenol blue stain used for staining molds.

Yeasts

Yeasts are unicellular and ovoid or spheroid fungi , reproduce by budding or fission, It spoiled various type of food e.g., sauerkraut, fruit juices, meats, wine, beer . It also involved in the commercial production of beer, wine, vinegar, alcohol(ethanol) and bread (yeast –Baker's Yeasts used for leavening of bread).

True Yeasts – which produces sexual spores ascospores e.g., *Saccharomyces cerevisiae*.(Ascomycetes).

False yeasts - Which produces no ascospores, produces asexual spores chlamydospores- fungi imperfecti e.g., *Candida, Rhodotorula* and *Cryptococcus.*

Wild Yeasts – natural, not used in industries.

Top Yeast – are very active fermenters, grow rapidly at 20^0C, clumbing of the cells and rapid evolution of CO_2, sweep the cells to the surface, responsible for production of ale, wines,alcohol,glycerol and vertase. e.g., *Saccharomyces cerevisiae var. ellipsoideus.*

Bottom Yeast - slower growth, absence of clumbing characters, slow production of CO_2, e.g., *S.uvarum,* responsiple for production of beer.

Culture medium

It produces cream colored colonies on fugal agar medium (SDA- Sabouraud's Dextrose Agar)

Osmophilic yeasts- grow at high concentration of sugar and salt e.g., *Saccharomyces rouxii and S. mellis.*

Water activity a_w.

Most Yeasts require more moisture than molds.

Optimum temperature is 25-30^0C.

Lactose fermenters - *S.fragilis* and *S.lactis* are ability to ferment the lactose used in dairy industries –for milk products production.

Yeasts which are involved in the spoilage of food.

Rhodotorula, Trichosporon, Candida, Torulopsis, , S.rouxii , S. mellis, pichia, and Zygo*saccharomyces,*

Bacteria

It is necessary to study about bacteria because they are responsible in spoilage of food.

Bacteria are prokaryotes (presence of nuceoid(DNA- not covered by neuclear membrane) instead of neucleus, reproduce by binary fission (asexual), most of them heterotrophs, some are autotrophs (synthesise food itself), areobic- *Bacillus*, anaerobic – *Clostridium* sp, microscopic,

Shape – coccoid(round shaped- *Micrococci*), bacilli(rod- *Lactobacillus),* cocci in chains(*Streptococci*), cocci in clusters(*Staphylococci*).

Size – 1.5- 3µm.

Motility – presence of flagella in bacteria as their locomotive organs , motile – *E. coli, Pseudomonas sp, Salmonella sp,* Non- motile e.g., *Staphylococci sp,*

Gram's stain reaction – this stain used for staining of bacteria based on their reaction to Gram stain, Gram negative appears in pink color e.g., *E.coli,* Grams positive appear in violet color e.g., *Staphylococci sp.*

Capsulated – Some bacteria their cell wall is covered by capsules (is made up of polysaccharide (*Klepsiella pneumonia*) helps or protect the bacteria from heat or harmful chemicals or phagocytosis e.g., *Klepsiella pneumonia, Bacillus cereus.* Capsules are causes sliminess or ropiness of food (milk).

Endospores - are formed at an intracellular site of bacteria, are very refractile, and are resistant to heat, ultraviolet light and desiccation, Lysis of the vegetative cell releases the free endospores, which may remain dormant for years. Endospores (made up of Ca^{2+} and dipicolinic acid) producing bacteria e.g., *Bacillus sp* (aerobic), *Clostridium sp* (anaerobic).

Toxin- two types of toxin 1, exotoxin (heat labile) e.g., enterotoxin of *E.coli* causes gastroenteritis, 2, endotoxin (heat stable).

Medium for isolation

Nutrient agar used for isolation of bacteria from food.

Mac conkey agar used for isolation of coliform bacteria(*E. coli* and *Enterobacter sp),*, Mannital salt agar used for isolation of *Staphylococcus aureus*

Bacteria which are involved in spoilage of food

E. coli, Staphylococcus aureus, Salmonella, Shigella, Streptococcus faecalis,S.faecium, Aeromonas hydrophila, Vibrio, Bacillus subtilis, B.coagulans,B. polymyxa, Campylobacter jejuni , Clostridium perfringens and C. butyricum, Erwinia, Enterobacter, Flavobacterium, Lactobacillus trichodes, Listeria monocytogenes, Micrococcus varians,.M.luteus, Mycobacterium tuberculosis, Proteus, Pseudomonas, Serratia, Alcaligenes viscolactis, Corynebacterium diptheriae, C.bovis, Halobacterium salinarium, Pediococcus damnosus, Yersinia enterocolitica and Shigella.

Bacteria important in food microbiology

Lactic acid – forming bacteria or Lactics

Lactobacillus, Leuconostoc, Streptococcus and *Pediococcus* are responsble for production of lactic acid. This acid is used for making cheese and sauerkraut.

Homofermenter – microbes ferment sugar (lactose) to lactic acid with small amount of acetic acid and carbon dioxide e.g., *Lactobacillus acidophilus, L.lactis, L.thermophilus, L. bulgaricus* and *L.fermentum.*

Heterofermenter microbes ferment sugar (lactose) to lactic acid with appreciable amount of acetic acid, alcohol and carbon dioxide e.g., *Lactobacillus brevis, L. higardii, L. trichode*.

Acetic acid forming bacteria or Acetics

Acetobacter and *Gluconobacter* can oxidize ethyl alcohol to acetic acid, these bacteria used in the production of vinegar or acetic acid.

Butyric acid forming bacteria or Butyrics

Clostridium sp produces butyric acid.

Propionic acid forming bacteria or Propionics – Propionibacterium spp.

Proteolytic bacteria – bacteria which produce proteinase enzymes (it wiil breakdown or hydrolyze the protein into peptides and aminoacids) , producers *Proteus, Pseudomonas, Clostridium sp, Bacillus sp.*

Lipolytic bacteria- produce lipase it will breakdown the lipid into glycerol and fatty acids, producers *Pseudomonas, Staphylococcus, Micrococcus and Serratia.*

Saccharolytic bacteria – produce amylase enzymes it will breakdown starch into simple sugars(glucose), producers *Clostridium sp, and Bacillus sp.*

Pectinolytic bacteria- produces pectinase it will breakdown pectin (complex carbohydrates) ,producers *Clostridium sp, Bacillus sp, Achromobacter, Aeromonas, Erwinia, Arthrobacter,* and *Flavobacterium*

All above the enzymes are commercially valuable.

Thermophilic bacteria or **Thermophiles** – bacteria can grow 55^0C or above. E.g., *Bacillus stearothermophilus*.

Thermoduric bacteria - bacteria can survive a heat treatment such as pasteurization e.g., *Micrococcus, Bacillus, Streptococcus, Clostridium sp,* and molds such as *penicillium* and *Apergillus sp*

Psychrophilic bacteria or psychrotrophs - bacteria can grow at commercial refrigeration temperatures e.g., *Pseudomonas, Flavobacterium, Lactobacillus, Alcaligenes ,Micrococcus,* and *Yeasts and molds.*

Halophilic bacteria - can grow at high salt environments e.g., *Halobacterium, Halococcus* can grow at15-30% . The *Pseudomonas, Flavobacterium, Acnetobacter, Vibrio* can grow at0.3-3.0% salt. The*Acnetobacter, Vibrio, Morexella,* and *Micrococcus sp* can grow at*3-15%* salt.

Osmophilic bacteria or Saccharophilic bacteria – can grow best at high concentration of sugar environment e.g., *Leuconostoc.*

Pigmented bacteria - produces pigments to protect from sun light and ultraviolet radiation e.g., *Halobacterium* and *Halococcus* -pink,red, orange color pigments. *Flavobacterium* – yellow to orange, *Serratia* – red, *Micrococci* also produce pigments.

Slime or ropiness forming bacteria

Alcaligenes, Enterobacter aerogenes and *Klebsiella oxytoca* are cause ropiness in milk. *Lactobacillus plantarum* cause ropiness in fruits and vegetables , *Bacillus* cause ropiness in bread.

Gas forming bacteria

Leuconostoc sp,Lactobacillus sp Clostridium sp, Bacillus sp, Proteus sp, Enterobacter sp, and *E. coli.*

Coliform and feacal coliform group.

These are Gram negative, non-spore forming, aerobic and facultative anaerobic which ferment lactose with gas formation. E.g., *E.coli,* and *Enterobacter aerogenes*.

Actinomycetes are filamentous bacteria belonging to Streptomyces produces conidiospores

Bacterial agents of Foodborne illness.

Foods are complex mixture of chemicals responsible for maintenance of life, it can also be responsible for ill health. Food provide adequate protein, energy, minerals and vitamins, lacking of any one that giving rise to characteristic deficiency syndromes such as Kwashiorkor (protein deficiency) goiter (iodine deficiency), pellagra (nicotinic acid), beriberi (thiamine) and scurvy (ascorbic acid). Several vitamins are toxic if consumed in excessive amounts and many food plants produce toxic secondary metabolites to discourage their attack by pests.

Potatoes contain the toxic chemicals alkaloid solanine , its concentration is more in aerial parts of the plants and the peel, green potatoes and potatoes sprouts. Cassava contains hydrogen cyanide. The body's detoxification pathways converts cyanide to thiocyante which can interfere with iodine metabolism giving rise to goiter and cretinism.

Poor hygienic practices in the preparation of food and the use of contaminated water are often implicated diarrhea, 70% diarrhoea in young children are food associated.

Microbes and food

I, they can cause spoilage

2, they can cause food borne illness,

3, they can transform a food properties in a beneficial way- food fermentation.

Food borne illness/ food poisoning (food-poisoning outbreaks) – Diseases transmitted from one person to another by faecal-oral route of transmission or Disease is transmitted from one person to another after ingestion of contaminated food or infectious organisms. The pathogens are called as gastrointestinal pathogens. These are produces enterotoxins affect the gastrointestinal tracts that leads to gastroenteritis(dysfunction of the gut) , symptoms includes diarrhoea, vomiting, abdominal pain, and nausea. The food act as vehicle for these pathogens.

F.borne illness or f,b,disease symptoms can be seen later stage, it took prolonged period for developing symptoms (typhoid), but in **f.poisoning** the symptoms are appear in early stage symptoms includes diarrhoea, vomiting , abdominal pain, and nausea.

Sporadic means outbreaks from one country to another.

F.B.Ilness is mostly originated from meat, poultry, egg, milk, fish and shellfish.

The alimentary tract: its function and microfora

The al.tract is not an internal organ of the body but a tube passing through it from the mouth to anus. Its principle functions are the digestion and absorption and the excretion of waste. It offers congenial environment for micro-organisms.

In the mouth, food is mixed with saliva and broken down mechanically to increase the surface area for attack by digestive enzymes. Saliva is an alkaline fluid containing starch degrading enzymes amylase, and the antimicrobial factors lysozymes, immunoglobulin IgA, lactoferrin and lactoperoxidase. It provides lubrication to assist chewing and swallowing, rinsing teeth and mouth to remove debris. On average, an adult secretes and swallows about 1.5 liter of saliva each day.

Dental plaque

It is an organic film in which bacteria are embedded in a matrix derived from salivary gland glycoproteins and microbial polysaccharides. ***Fusobacterium*** and ***Streptococci*** are common components. Plaque are protective environment for bacteria , causes dental caries.

Mouth – oesophgous – stomach- Duodenum- jejunum – ileum –colon and appendix – anus.

In stomach , food is blended with gastric juice, an acidic fluid HCL, normally acid –tolerant vegetative cells and spores survive, lactobacilli are frequently found in stomach. The enzymes

proteases such as pepsin and lipase are present, which can partially digest the stomach contents. The gastric mucosa secretes protein responsible for efficient absorption of Vitamin B_{12}.

The partially digested food+ gastric juice = chime, periodically released into the small intestine. In this muscular tube is 6 metres long most of the digestion and absorption of food occur. Its internal lining is finger like projection is known as villi – absorption.

In duodenum , large scale digestion occur, the chyme is mixed with digestive juice (pancreatic juice supplies a battery of digestive enzymes) from pancreas and bile from gallbladder which neutralize the chyme's acidity, bile salts responsible for absorption of fat soluble vitamins, duodenum is relatively short section, only 2% of its overall length.

The food is now move from duodenum into jejunum – ileum by peristalsis (waves of muscles contraction). During this passage, nutrients such as amino acids, sugars, fats, vitamins, minerals, and water are absorbed by passive diffusion into capillaries in the villi.

The microbial load is increases from duodenum towards, 10^4, 10^5, 10^6, despite the presence of antimicrobial factors lysozymes, immunoglobulin IgA, lactoferrin and lactoperoxidase and bile.

Obligate anaerobes ***Bacteroides*** and ***Bifidobacterium*** make up 99% of the flora of the large intestine, ***E.coli(***produce variety of vitamins***)***, ***Lactobacillus, Clostridium***, and ***Fusobacterium*** plus ***staphylococci,yeast, pseudomonads,*** at lower levels.

Salmonella

Introduction - human pathogen, typhoid bacillus was first observed by German bacteriologist **Elberth and Koch** in 1880. The paratyphoid bacilli were first isolated by **Achard and Bensaud** in 1896 and by Gwyn 1898. Salmonellosis is a most important foodborne illness worldwide. In Europe 50 per one lakh inhabitants, 120 per one lakh in Hungary and Finland, in USA 19 per one lakh.

The **Kauffman- White** serotype scheme – useful for differentiating species within the genus. On the basis of their somatic (o) and flagellar(H) antigens and by capsular (Vi) antigens, the scheme contained around 2200 serotypes.

Human pathogens – ***S.typhi, S.paratyphi*** A and B. ***S.typhimurium, S. cholera-suis*** are animal pathogens.

The Organisms and its characteristics

Salmonellas are members of enterobacteriaceae, Gram-negative, non-sporeforming rods, size 1-3 µm. Facultatively anaerobic, catalase +, Oxidase -, motile with peritrichous flagella, optimum 37^0C, 5-47^0C.Heat sensitive can be destroyed by pasteurization temperature.Survive well in dried foods, optimum pH is 7. Inhabitants of the gastrointestinal tract of rodents,wild animals,birds, reptiles and insects, they can be disseminated via faeces to soil, water, foods, and feeds to other animals including humans.***S.pullorum***, ***S.gallinarum*** in poultry and ***S.cholera-suis*** in pigs. Animal to animal transmission and vertical transmission are common in animals(parents infecting offspring)

Pathogenesis and Clinical features

Salmonellas are responsible for number of different clinical syndromes , grouped into two types – enteritis and systemic disease.

Enteritis – gastrointestinal infections , by salmonellas originated from animals and humans. ***S.enteritidis, S.typhyimurium*** and ***S.virchow-*** enterotoxin, heat- labile which stimulate fluid secretion.confirmed by ileal loop test using ***S. typhimurium.***

Incubation period 6 to 48 hours, symptoms – mild fever, nausea, vomiting, abdominal pain and diarrhoea.

Systemic – typhoid fever(enteric fever) – caused by typhoid and paratyphoid bacilli – ***S.typhi***, and ***S.paratyphi*** A, B and C. Invasive species.

I.period – 10 to 20 days. Invasive Salmonella penetrate the intestinal epithelium and are then carried by the lymphatics to the mesenteric lymph nodes. After multiplication in the macrophages, they are released into the blood stream and are then disseminated around the body. They are removed by macrophages but continue to multiply within them. This eventually kills the macrophages which then release large numbers of bacteria into the blood stream causing a septicaemia. Symptoms – fever, headache, abdominal tenderness and constipation and appearance of rose red spot on the body.

In second stage – this bacteria multiplies in gall bladder, the infected bile reinfects the small intestine causing inflammation and ulceration.

Antibiotics like chloramphenicol, ampicillin and amoxicillin are used for treating typhoid.

Isolation and identification

Modified Selinite – cystine broth which contains cystine to stimulate growth of Salmonellas and dulcitol and trimethylamine oxide (TMAO) ferment dulcitol and reduce TMAO to trimethylamine.

Muller-Kaufffman tetrathionate broth, containing tetrathionate, brilliant green, and bile,

Rappaport- Vassiliadis (RV) broth, which contains malachite green, magnesium chloride are selective factors.

Deoxycholate and or brilliant green or Bile salt agar

Agglutination test using polyvalent 0 antisera.

ELISA and Gene probe kits for detection of Salmonellas are also available.

Association with foods

Zoonotic since the intestinal contents of infected animals is a major source, ingested with food or water , transmission by faecal-oral route.

Undercooked Meat, milk, poultry and eggs are primary vehicle, Direct person to person spread by the faecal – oral route is also possible that occur in hospitals, old people's home and nurseries. Transfer of ***Salmonella*** between animals is seen in during transport, at markets. Vertical transmission can be seen in poultry. Eggs are most frequent vehicle , found in yolk of egg. Raw milk contains Salmonella. Since birds, rodents, insects , infected food handlers or infected foods can all contaminate foods directly or indirectly. Salad vegetables from infected is possible for outbreaks of salmonellosis.

ESCHERICHIA COLI

Introduction - this bacterium was first isolated from children faeces by German bacteriologist **Thodor Escherich in 1885**, is an universal inhabitant of the gut of humans and other warm blooded animals, Generally a harmless commensal, it can be an opportunistic pathogen causing Gram –negative sepsis, UTI, pneumonia in immunocompromised patients and meningitis in neonates. Strains of E. coli (EPEC), (EIEC) and (ETEC) were first recognized as a cause of gastroenteritis by workers in England investigating summer diarrhea in infants in 1940.

The Organisms and its characteristics.

E.coli is a member of Enterobacteriaceae, catalase+, oxidase-, lactose fermentative, short, Gram-negative, non-sporing, In the IMViC tests, most strains of ***E.coli*** are indole+ and methyl red + and VP and Citrate are negative. Optimum temperature is 37^0C, (7-50^0C) Three antigens are present lipopolysaccharide somatic o , flagellar H, and polysaccharide capsular K antigens are proposed by Kauffman in 1940.

Pathogenesis and Clinical features.

Based on distinct , plasmid encoded virulence properties, four diarrhoeagenic ***E.coli*** species are available.

ETEC –Enterotoxigenic E.coli. I. period is 12 to 36 hours. It produces two types of toxin one is heat stable(ST) , can withstand heating at 100^0C for 15 minutes, and heat labile toxin(LT) which are inactivated at 60^0 C. The LT is similar to cholera toxin, it consists of five B subunit(M,11.5kDa) which are responsible for binding of the toxin to the epithelial cells and A subunit (M,25kDa) which is translocated into the epithelial cells where it activates adenylate cyclase. The subsequent increase in c AMP levels then inhibits Na+, Cl^- and water absorption by the villus cells and stimulates their loss from intestinal crypt cells thus leading tp profuse watery diarrhoea.

Enteroinvasive E.coli (EIEC) - Classical symptoms of EIEC is similar to bacillary dysentery by ***Shigella***. EIEC invades and multiplies within the epithelial cells of the colon causing ulceration and inflammation. Clinical features are fever, severe abdominal pains, malaise and often watery diarrhoea – stools containing blood , mucus, and leucocytes. The plasmid is ~ 140 MDa.

Enteropathogenic E.coli (EPEC) – I. period is 12-36 h. symptoms malaise,vomiting, and diarrhea with stools containing mucus but rarely blood.

Enterohaemorrhagic E.coli. – Other name is Verotoxin producing ***E.coli*** (VTEC). I.period is 3-8 days. Clinical features includes haemorrhagic colitis(bloody diarrhoea, stomach cramps, watery diarrhoea), haemolytic uraemic syndrome(acute renal failure,haemolytic anaemia(reduction in number of red blood cells), thrombocytopaenia (reduction in number of platelets) and thrombotic thrombocytopaenic purpura – similar to second one is)

EHEC can produce cytotoxin and kill Vero (African Green Monkey Kidney)Cells.

Isolation and Identification.

The selective and differential medium –MacConkey Agar was devised by Mac Conkey in 1905. It contains Bile salts , Crystal violet act as inhibitors of Gram positive and fastidious Gram negative bacteria, lactose is fermentable carbohydrate with a pH indicator neutral red , Strong acid producer like ***E.coli, Klepsiella*** and ***Enterobacter*** produce red colonies, non-lactose fermentotrs such as ***Salmonella, proteus.***

Eosine / Methylene blue agar is a selective and differential medium in North America. The aniline dye eosine and methylene blue are selective agents, lactose fermenting produces green black colonies with a metallic sheen.

Suspect colonies from selective and differential media can be confirmed by further biochemical testing.

Association with foods- Faecal contaminated water and food and contaminated food handler are responsible for outbreaks of ***E.coli***.

Shigella

Introduction- It cause of bacillary dysentery by the Japanese microbiologist Kiyoshi Shiga in 1898. It consists of four species and regarded as human pathogens – ***S. dysentriae, S. flexneri, S.boydii*** and ***S.sonnei***. The ***S. dysentriae*** is responsible for severe bacillary dysentery in tropical countries. These are biochemically inactive,

The organism and characteristics

These are members of enterobacteriaceae, non-motile, non- sporeforming, Gram-negative, catalase+, oxidase -, facultative anaerobes, produce acid not gas from glucose, fragile organisms which do not survive well outside their natural habitat, mesophiles, gut of humans and other primates, temp- 10-45^{0}C, $_P$H 6-8

Pathogenesis and Clinical features.

I.period is 7 h-7 days, Infectious dose is 10-100 organisms, Symptoms – abdominal pain, vomitting, fever accompanying diarrhoea bloody stool with mucus and pus. ***S. sonnei*** causes watery diarrhea**Isolation and Identification** Gram negative broth and selenite broth are used. PCR is used for detection of virulence plasmid, DNA/DNA hybridization is also used.

Aeromonas hydrophila

Introduction

A. hydrophila is a foodborne pathogen, grow at low temperature, it can cause extra – intestinal infections in immunosupperesed/ immunocompromised individuals, the first report of **gastroenteritis** due to ***Aeromonas*** came from Jamaica in 1958.

The organism and Characteristics

Gram –negative rods, catalase +, oxidase +, ferment glucose, motile by a single polar flagellum,

Optimum temp-28^{0}C, more in sewage, resistant to chlorine, found in potable water.

Pathogenesis and Clinical features.

Aeromonas causes gastroenteritis in children under five years old. Symptoms –watery diarrhea,no vomiting, it is due to production of β- hemolytic, cytotoxic enterotoxin,52kDa, like cholera toxin, stimulate accumulation of high levels of Camp within epithelial cells.

Isolation and Characteristics

Starch ampicillin agar, blood ampicillin agar, alkaline peptone water are used. Bile salt and ampicillin are used as a selective agents.

Association with foods.

Aeromonas hydrophila is isolated from wide range of fresh foods like fish, meat, poultry, raw milk, and salad vegetable as well as water.

Brucella

The genus ***Brucella*** is discovered by Sir David Bruce in 1887. It is a causative agent of undulant fever. (Brucellosis, Mediterranean fever, Malta fever),zoonotic disease, four species that are human pathogens is associated with a particular host, ***B.abortus*** (Cattle), ***B.melitensis*** (Sheep and goats), ***B. suis***(pigs), ***B.canis*** (dogs). Brucellosis is an occupational disease of farmers, slaughterhouse workers, veterinarians, herdsman while handling infected animals.

The organism and Characteristics

Brucella are Gram negative, catalase +,oxidase +, short oval rods, non-motile, occur in single or pair, optimum temperature is 37^0C,

Pathogenesis and Clinical features.

Brucellosis is a protracted and debilitating illness characterized by an I.period from one to six weeks, a chronic, relapsing fever with sweats, headache, constipation, anorexia, pains in the limbs and back and weight loss.

It is a facultative parasite and can live intracellularly or in extracellular body fluids

Pathogenesis and Clinical features.

It is a fastidious organisms, no culture media available**, ring test** is used , Stained antigen is mixed with the test milk, if antibodies to Brucella are present then they will cause the antigen to clump and rise with the milkfat on standing to form an intense blue- violet ring at the top of milk.

Association with foods.

Inadequately cooked meat from an infected animal, raw milk are principle food vehicles.

STAPHYLOCOCCUS AUREUS

Introduction

The staphylococci were first described by Scottish surgeon, Sir Alexander Ogston in 1882. The staphyle means bunch of grapes, coccus means grain, causes pyogenic (pus forming) infection in humans.

Vaughan and Sternberg who described that staphylococci were responsible for food poisoning. Barber in 1914 demonstrated that staphylococci were able to cause food poisoning by consuming milk from a cow with staphylococcal mastitis. The food poisoning was caused by a filterable enterotoxin.

Genus staphylococcus contains 27 species and 7 subspecies, all produce enterotoxin,

The organism and Characteristics

Staphylococci are Gram- positive cocci, cells about 1µm in diameter, cell division occur in more than one plane so that cells form bunches of grapes.

Staphylococci are catalase +,oxidase -, facultative anerobes, ferment glucose, mesophile 37^0C, pH 6-7, can withstand up to 20% NaCl, aw of 0.86, the principle habitat of Staphylococci is the skin, skin glands and mucous membrane of warm blooded animals, ***Staph.hyicus*** (pigs), ***Staph.gallinarum*** (chickens), ***Staph.aureus*** most frequently on the skin of higher primates,

Pathogenesis and Clinical features

Food poisoning by ***Staph.aureus***, I.period 2- 4h.Nausea, vomiting, stomach cramps, ***Staph.aureus*** produces seven exotoxin A(chromosomal),B(chro/plasmid),C1(chro/plas),,C2(not known),C3,(not known)D(plasmid) and E(chromosomal). The toxins are small (M,27.5 -30Da), they are resistant to gut protease and heat stable.

Isolation and Identification

A selective agar Baired-Parker agar, devised by Baired-Parker in 1960**.** ***Staph.aureus*** produces shiny, jet-black colonies with clear zone around it due to lysis of egg-yolk protein lipovitellenin, confirmed by coagulase (clumping factor or protein A) test, and nuclease test using toludine blue/DNA agar.

ELISA for detection of enterotoxin.

Association with foods – raw milk contains enterotoxin that are resistant to heat and then used for preparation milk products like chocolate milk and dried milk- outbreaks occurs when consumed it.

Vibrio

Introduction

Robert Koch who firmly eatablished the causal link between *Vibrio* cholera and cholera in 1885.

***Vibrio* species associated with human diseases**.

Species	**Diseases**
V.cholera, O1(agglutinable)	Cholera,wound infection.
V.cholera, non-O1(non-aggl)	Diarrhoea, gastroenteritis, wound infection,
V.parahemolyticus	Diarrhoea, gastroenteritis, otitis media
V.mimicus	Diarrhoea, gastroenteritis, wound infection
V.hollisae	Diarrhoea,
V.vulnificus	Wound infection, primary and sec.septicaemia
V.alginolyticus	Wound infection, otitis media.

The Organism and their Characteristics

Vibrio are Gram-negative pleomorphic, short rods, motile – polar flagella, tolerate 8% NaCl, a_w 0.93., 37^0C, natural habitat is marine and esturine environment, most environmental isolates of both *V.cholerae*, and *V.parahemolyticus*(99%) are non- pathogenic. The majority of the ***V.cholerae*** are non-01 serotypes. Generation time is 9-11 minute.

Pathogenesis and Clinical features.

V.parahemolyticus causes food poisoning, produces hemolysin and are designated Kanagawa-positive (Ka+) while 99% of environmental isolate are Ka-, the haemolysin can lyse human and rabbit blood cells but not horse blood, this phenomenon is known as Kanagawa reaction. The haemolysin has also been shown to have enterotoxin, cytotoxin, and cardiotoxic activity.

V. vulnificus causes primary septicaemia, fatality rate is (50%), severe in patients with liver disease, diabetes or alcoholism.

Vibrio cholera - cholera toxin (MW 84000) consists of five B subunits and a single A subunit. The B subunit bind to specific ganglioside receptors on the enterocyte surface. This creates a hydrophilic channel in the cell membrane through which the A unit can pass. Once inside the cell A unit enzymatically to transfer an ADP to a protein regulating the acivity of the enzyme adenylate cyclase. As a result the enzyme is locked inti its activity state leading to accumulation of cyclic adenosine monophosphate (Camp) which inhibits absorbtion of Na+, and Cl- ions while stimulating the secretion of Cl-, HCO3-, and Na+ ions. To maintain an osmotic balance the transfer of electrolytes is accompanied by a massive outflow of water into the intestinal lumen and result in a profuse watery diarrhoea.

Isolation and identification

Enrichment media – alkaline peptone water (pH 8-9), tellurite / bile salt broth(pH 9-9.2), TCBS-thiosulfate citrate bile salt sucrose agar- ***V. cholerae*** produce yellow colonies due to fermentation of sucrose, ***V.par***-produces green coloured colonies – biochemical test.

Association with foods- cholera is primarily waterborne infection, improperly washed fruits and vegetables, undercooked seafood are possible source for outbreaks of cholera.

CLOSTRIDIUM BOTULINUM

Introduction

C. botulinum is causes botulism – is a form of bacterial food poisoning, botulus means sausage (made from pig's stomach) because of 13 people fell and 6 later died after eating this sausage in 1793. ***C. botulinum*** was discovered by **Van Ermengem** in 1923, this bacteria produces heat – labile toxin , obligate anaerobes, spore-forming bacillus.

The Organism and their Characteristics

Gram-positive, spore-former, peritrichous flagella, slightly curved rods,2-10 µm long, form central or subterminal oval spores, eight serotypes are present each produces one kind of toxin, although there are exceptions, (A,B,C_1,C_2,D,E,F, and G)., Physiologically the bacteria have four groups, Most cases of botulism in humans are due to types A, B, or E. Although it is rarely found in the alimentary tract of birds and mammals, is essentially a soil saprophytes, aquatic mud provide a moist, anaerobic, nutrient rich environments in which clostridia can flourish, pH 8.5,

Pathogenesis and Clinical features.

I.period - 8hr-8days, after consumption of toxin containing food, symptoms – vomiting, constipation, urine retention, double vision, dysphagia, dry mouth, dysphonia.

Isolation and identification

Enrichment broth is cooked meat broth for seven days, horse blood agar or egg yolk agar incubated anaerobically for three days , smooth colonies 2-3 mm in diameter with irregular edge and showing lipolytic activity on egg –yolk agar.

Association with foods- The food has been contaminated with spores or vegetative cells of ***Cl.botulinum,*** Soil contamination is a major of ***Cl.botulinum*** in foods, Salting and smoking is insufficient to eliminate ***Cl.botulinum,*** Fish can be contaminated ***Cl.botulinum*** particularly type ***E.***

CLOSTRIDIUM PERFRINGENS

Introduction

Cl. perfringens, formerly ***welchii***, causes wound infection, gas gangrene, it was first described by American bacteriologist Welch in 1892.. In 1945 firmly established. ***Cl. perfringens*** as a cause of food poisoning, five types A-E based on the production of four major exotoxins, α,β,€ and 1, and eight minor ones. ***Cl. perfringens*** type A is responsible for food poisoning and gas gangrene produce only the α toxin which has lecithinase activity it will hydrolyse the lecithin and other phospholipids attacking cell membranes it produces local tissue disruption.

Cl. perfringens type C which produces α and β toxins causes enteritis, β toxins damages the intestinal mucosa causing necrosis , symptoms – abdominal pain and bloody diarrhoea

The Organism and their Characteristics

Gram positive, rod shaped, anaerobic , sub terminal spores, 3.9 µm, non-motile, catalase negative, occasionally grow in the presence of oxygen, optimum temperature is 43-47^{0}C, pH 6-7.5, aw is o.95 –o.97, 6% salt, it can be isolated from water, sediments, dust, raw and processed food and found in human gastrointestinal tract.

Pathogenesis and Clinical features.

Cl. perfringens is responsible for food poisoning symptoms nausea, abdominal pain, diarrhea, incubation period is 8-24 hours after consumption of food containing ***Cl. perfringens***

Isolation and identification

TCS medium tryptose/sulfide/cycloserine incubated for 24 hours at 37^{0}C, produce white colonies in pour plates, ability to reduce nitrate to nitrite, lactose +, gelatin liquefaction +, confirm test Nagler reaction to find the α toxin on lactose egg yolk agar , to test the enterotoxin ileal loop test is done.

Association with foods-

Food contain spores of ***Cl. perfringens*** is responsible for outbreak of ***Cl. Perfringens.***

YERSINIA ENTEROLYTICA

Introduction

It causes gastroentrities, the genus Yersinia is named after the French bacteriologist Alexander Yersin in 1894**.**

The Organism and their Characteristics

Y. enterolytica is a member of Enterobacteriaceae, 1-2 µm, Gram negative rod, facultative anaerobic, catalase +, oxidase negative, optimum 29^0C, non-motile at 37^0C, motile at less than 30^0C with peritrichous flagella, pH 7- 8 ,isolated from soil, fresh water and intestinal tract of many animals. Many environmental isolates are non pathogens.

Pathogenesis and Clinical features.

Foodborne illness caused by ***Y. enterolytica*** is common in children under seven years old. I.period is 1-11 days, symptoms are abdominal pain and diarrhea accompanied by a mild fever, vomiting is rare. A heat stable enterotoxin is (9000-9700 Da) produced by ***Y. enterolytica*** its role for pathogenesis.

Isolation and identification

The medium contains deoxycholate and crystal violet as selective agents and mannitol as fermentable carbon source, incubation at 28^0C for 24hours, typical colonies are dark red centre surrounded by a transparent border

Association with foods

Food contain ***Y. enterolytica*** of is responsible for outbreak of ***Y. enterolytica*** , pigs are primary *chronic carriers of* ***Y. enterolytica***.

BACILLUS CEREUS AND OTHER BACILLUS SPECIES

Introduction

Food poisoning caused by ***Bacillus cereus*** was made in 1906, this bacteria responsible for two distinct types of foodborne illness 1, diarhoeal syndrome, 2, emetic syndrome.

The Organism and their Characteristics

Gram positive, aerobic, sporefoming rods, size is 3-5µm in chains.temperature is 8-55^0C, optimum is 28-35^0C,widely distributed in the environment, isolated from soil, water, vegetation.

Pathogenesis and Clinical features.

I.period is 8-16 hours after consumption of the food contains ***B.cereus***, symptoms- abdominal pain, watery diarrhea nausea and vomiting, Emetic syndromes lasting for 6-24 hours, symptoms includes diarrhoea and nausea and vomiting, two distinct toxins the diarrhoeal illness toxin

molecular mass is 50 k Da, it is sensitive to proteolytic enzymes such as trypsin and pepsin, the emetic toxin m.mass is 5kDa, heat resistant resistant to proteolytic enzymes and low pH.

Isolation and identification

Polymyxin/pyruvate/egg yolk/mannitol / bromothymol blue agar is used for isolation, it can not ferment mannitol and produce blue colonies with zone of egg-yolk precipitation caused by lecithinase activity,

Association with foods

Contaminated meat products, soups, vegetables and starchy products such as rice.

CAMPYLOBACTER

Introduction

Campylobacter coli , C.laridis, C. concisus, **and** ***C.hyointestinalis*** are responsible for diarrhoeal illness. The ***C. pylori*** now reclassified as ***Helicobacter pylori*** causes stomach and duodenal ulcers.

The Organism and their Characteristics

They are non sporeforming, oxidase +, Gram negative rods, cells may be 5-8 µm in length 0.5µm in diameter, slender, curved, or spiral shape dating motility with polar or amphitrichous flagella, macroaerophile require 5-10% of oxygen and3-5% of carbon dioxide, grow at 37^0C,, these are commensals of rodents, dogs, cats, dairy cattle, sheep, pigs, poultry, wild birds.

Pathogenesis and Clinical features.

I.period is 3-5 days, symptoms- malaise, fever, abdominal pain, and diarrhoea**.**

Isolation and identification

The incubation atmosphere of 5-6% oxygen, 10% carbon dioxide and media containing oxygen scavenging compounds such as blood,pyruvate, ferrous salt, charcoal and metabisulfite are commonly used.

Association with foods

As a common inhabitant of the gastrointestinal tract of warm-blooded animals.

Hepatitis – A and E.

These are + Sense single stranded RNA virus, foodborne viruses, shows fecal- oral route of transmission, causes hepatitis – inflammation of liver, if it is untreated it causes jaundice

dissemination of bilirubin throughout the body via blood, the body and color becomes yellow, bilirubin is a product of degraded R.B.C , degraded by liver,

Liver responsible for detoxification of toxic chemicals from blood, prevent the microbial infection by macrophages (Kupffer cells), store the vitamins, helps in digestion of food by secreting enzymes, average weight of liver is one and half kilograms.

Pathogenesis- enter the body via infected food, multiflies in the gut epithelial cells – incubation period is 3 to 8 weeks, symptoms tiredness, and stomach discomfort, fever, decreased appetite, diarrhoea, light colored stools. If it is untreated the pathogen goes to liver via blood and causes hepatitis due to multiplication of viruses in liver cells then causes jaundice lead to death.

Diagnosis – blood test.

Prevention – Hepatitis A Vaccines are available, administration of Hepatitis A immunoglobulins

Treatment – Ribavirin,

Mycotoxin

A **mycotoxin** is a toxic secondary metabolite produced by organisms of the fungus and is capable of causing disease and death in both humans and animals. The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops. One mold species may produce many different mycotoxins, and several species may produce the same mycotoxin.

Production

Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity and temperature are sufficient. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high. The reason for the production of mycotoxins is not yet known; they are not necessary for the growth or the development of the fungi. Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to better the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and these substances vary greatly in their toxicity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms.

Major groups

Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*. The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B_1, B_2, G_1, and G_2. Aflatoxin B_1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species. Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios, and maize.

Ochratoxin is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochratoxin A. *Aspergillus ochraceus* is found as a contaminant of a wide range of commodities including beverages such as beer and wine. *Aspergillus carbonarius* is the main species found on vine fruit, which releases its toxin during the juice making process. OTA has been labeled as a carcinogen and a nephrotoxin, and has been linked to tumors in the human urinary tract, although research in humans is limited by confounding factors.

Citrinin is a toxin that was first isolated from *Penicillium citrinum*, but has been identified in over a dozen species of *Penicillium* and several species of *Aspergillus*. Some of these species are used to produce human foodstuffs such as cheese (*Penicillium camemberti*), sake, miso, and soy sauce (*Aspergillus oryzae*). Citrinin is associated with yellowed rice disease in Japan and acts as a nephrotoxin in all animal species tested.[13] Although it is associated with many human foods (wheat, rice, corn, barley, oats, rye, and food colored with Monascus pigment) its full significance for human health is unknown. Citrinin can also act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys.

Ergot Alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps*, which are common pathogens of various grass species. The ingestion of ergot sclerotia from infected cereals, commonly in the form of bread produced from contaminated flour, cause ergotism, the human disease historically known as St. Anthony's Fire. There are two forms of ergotism: gangrenous, affecting blood supply to extremities, and convulsive, affecting the central nervous system. Modern methods of grain cleaning have

significantly reduced ergotism as a human disease, however it is still an important veterinary problem. Ergot alkaloids have been used pharmaceutically.

Patulin is a toxin produced by the *P. expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species. *P. expansum* is especially associated with a range of moldy fruits and vegetables, in particular rotting apples . It is destroyed by the fermentation process and so is not found in apple beverages, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals.

Fusarium toxins are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and maize. They include a range of mycotoxins, such as: the **fumonisins**, which affect the nervous systems of horses and may cause cancer in rodents; the **trichothecenes**, which are most strongly associated with chronic and fatal toxic effects in animals and humans; and **zearalenone**, which is not correlated to any fatal toxic effects in animals or humans. Some of the other major types of *Fusarium* toxins include: beauvercin and enniatins, butenolide, equisetin, and fusarins.

Mycotoxin exposure is almost always accidental whereas with mushrooms improper identification and ingestion causing mushroom poisoning is commonly the case. Ingestion of misidentified mushrooms containing mycotoxins may result in hallucinations. The cyclopeptide-producing *Amanita phalloides* is well known for its toxic potential and is responsible for approximately 90% of all mushroom fatalities. The other primary mycotoxin groups found in mushrooms include: orellanine, monomethylhydrazine, disulfiram-like, hallucinogenic indoles, muscarinic, isoxazole, and gastrointestinal (GI)-specific irritants.

. Some of the mycotoxins in the indoor environment are produced by *Alternaria*, *Aspergillus* (multiple forms), *Penicillium*, and *Stachybotrys*. *Stachybotrys chartarum* contains a higher number of mycotoxins than other molds grown in the indoor environment and has been associated with allergies and respiratory inflammation.

FOOD SANITATION AND HYGIENE

Food hygiene constitutes a basic necessity of Good Manufacturing/Agricultural Practices and the development of Hazard Analysis Critical Control Point (**HACCP**), as well being as a component of all **GFSI**-benchmarked **food safety standards**. Government, industry and consumers all play a role in safe sanitation and food hygiene practices.

Studies have shown that an appreciable percentage of **foodborne illness** cases can be attributed to poor sanitation and food hygiene, including poor personal hygiene and contamination of equipment and/or environments. Examples of **food recalls** related to sanitation issues include the contamination and subsequent recall of deli meats in Canada in 2008, when cells of Listeria monocytogenes were transferred to the product after surviving in equipment niches, where they were protected from sanitation procedures. The company was very public about the changes made to the sanitation program since then, including regular testing to monitor the success of the strategy in reducing environmental contamination.

Due to the risk, the **Codex Alimentarius Commission** provides an international code of practice concerning food hygiene: **http://www.fao.org/docrep/005/Y1579E/y1579e02.htm**. Within it, the following general prerogatives of the guidelines are laid out:

- Identify the essential principles of food hygiene applicable throughout the food chain (including primary production through to the final consumer), to achieve the goal of ensuring that food is safe and suitable for human consumption
- Recommend a **HACCP-based approach** as a means to enhance **food safety**
- Indicate how to implement those principles
- Provide guidance for specific codes which may be needed for – sectors of the food chain; processes; or commodities; to amplify the hygiene requirements specific to those areas

When designing a food hygiene and sanitation program, a total supply chain approach is crucial. The major areas to cover are:

- Equipment
- Environment
- Air
- Water

A key thing to note about these areas is that they function not as a static entity, but as a constantly evolving system. This is why good food hygiene programs need to be responsive to the dynamics of the plant environment and emerging risks – the same proactive approach used when developing HACCP.

According to the **Codex Alimentarius Commission**, food hygiene should cover all of these elements throughout the supply chain (all GFSI-benchmarked standards have similar requirements for housekeeping and food hygiene, with details laid out in their respective guidance documents):

- Primary Production (environmental hygiene, hygienic production, handling storage & transport, cleaning, maintenance and personnel hygiene)
- Establishment – design and facilities (location, premises and rooms, equipment, facilities)
- Control of operation (food hazards, hygiene control systems, incoming materials, packaging, water, management & supervision, documentation & records, recall procedures)
- Establishment – maintenance and sanitation (maintenance & cleaning, cleaning programmes, pest control systems, waste management, monitoring effectiveness)

- Establishment – personal hygiene (health status, illness and injuries, personal cleanliness, personal behaviour, visitors)
- Transportation (general, requirements, use & maintenance)
- Product information and consumer awareness (lot identification, product information, food labelling, consumer education)
- Training (awareness & responsibilities, training programmes, instruction & supervision, refresher training

Cleaning and food hygiene procedures for the building, plant and equipment should be validated using visual, analytical or microbiological methods – and records should be maintained. For instance, swab samples can be taken from various places on equipment, floors, walls or drains, to test for the presence of contamination. Then, after applying a sanitation step, samples can be taken again and compared with the original results to ensure that the step is effective at reducing harmful microbes to safe levels. For certain high-risk materials (e.g. allergens, ruminant protein or ready-to-eat products), validation of procedures is mandated, with individual governments designating acceptable methods for cleaning of high-risk materials.

A comprehensive food hygiene and sanitation program leaves nothing to chance. Responsibility should be designated for each parameter:

- Frequency of cleaning
- Method (chemicals used, concentrations, materials – including colour-coded/segregated tools to prevent cross contamination of high-risk materials)
- Verification records to ensure that procedures are being carried out consistently and effectively.
- Acceptable limits for CCPs must also be scientifically-established and maintained with regular monitoring
- Training and communication throughout the organization, with clear leadership from management on food hygiene and sanitation

As with other areas of food safety, sanitation and food hygiene should be proactive. End-product testing is important, but a positive result in the end-product doesn't tell you where the contamination originated. The overall food hygiene system, when applied at each point in the supply chain, is about managing risks before they result in a case of food contamination. Using common sense and **food science** based approaches, a well-designed food hygiene program can provide for proactive responses and risk-mitigation from farm to fork

Non-bacterial agents of food-borne illness

Food may also act as vehicles for other disease causing agents such as helminthes, nematodes, protozoa and viruses as well as toxic metabolites of fungi and algae. These are animal parasites which can be transmitted to humans via food and water. These complex animals do not multiply in food and they cannot be detected and enumerated by cultural methods. Their presence is normally detected by direct microscopic examination, concentration and staining procedure.

Platyhelminths(flatworms)

Liver flukes(Trematoda) and Tapeworms(Cestode)

Liver flukes (*Fasciola hepatica*) -

Definitive host or usual host is sheep, accidental host is humans.

Humans infected by eating undercooked watercress contains encysted metacercariae, it excyst in duodenum, pass through intestinal wall, peritoneal cavity to liver, in liver it will grow into adult and laid their egg. The eggs are pass to intestine and excreted into faeces. The eggs are hatched into motile miracidium larvae and infect water snail (Limnaea truncatula). In snail it develops into non-motile redia and motile cercaria, this cercaria binds to leaves of watercress and encysted into metacercaria.

Clinical features

Hepatomegaly, jaundice, epigastic pain, anorexia, vomiting, eosinophilia is marked.

Distribution- found in all sheep-rearing countries. About one million people are infected worldwide.

Lab-diagnosis - ova are found in faeces.

Taenia saginata (beef tapeworm)

Humans (Natural or definitive host) infected by eating undercooked beef, this beef contain larva ***cysticercus bovis*** and develop into adult worm having size is 8-10 meter. The head is scolex and body is segmented is called as strobila. The eggs are excreted through the faeces (size is 30-40µm) , infect the cattle during grazing, in stomach of cattle it will liberated into larvaor embryo and settle in the muscles .

Cli-features – mild irritation of intestinal mucosa. Occasionally there is alimentary upset.

Lab- dia- ova similar to ***T.sag*** and scolex found in faeces.

Distri- poor hygienic maintain countries,

Taenia solium (Pork tapeworm)

Humans (Natural or definitive host) infected by eating undercooked pork, this beef contain larva ***cysticercosis*** and develop into adult worm having size is 8-10 meter. The head is scolex and body is segmented is called as strobila. The eggs are excreted through the faeces (size is 30-40µm) , infect the pig during grazing, in stomach of pig it will liberated into larva or embryo and settle in the muscles.

Cli-features – mild irritation of intestinal mucosa. Occasionally there is alimentary upset.

Lab- dia- ova and scolex found in faeces.

Distri- poor hygienic maintain countries,

Nematodes –

Round worms (***Ascaris lumbricoides***)

1, Round worms (***Ascaris lumbricoides***) infected by eating contaminated food/water contains egg are ingested by humans, hatches into larvae, migrated and penetrate the mucosa, enter lymphatics, migrate to the right heart and lungs, ascend the respiratory tree and descend the oesophagous to mature in the intestine, ----life span is 10-12 months, male is smaller than female, adult female laid their eggs excreted through the faeces and may contaminate the water or con.water is used for preparation of food, the cycle will be repeated, four types eggs-corticated is normal form, decorticated, embryonated, unfertilized,

Cli- features

Larvae cause pneumonities with eosinophilia, adult worm can cause obstruction of small intestine, bile ducts and trachea, appendicitis, pancreatitis and peritonitis

Lab-diagnosis

Ova are found in faeces.

Distribution

1.47 billion infected worldwide

2, Whip worm (***Trichuris trichura***) similar to A.lum.

Cli- features - rectal prolapsed, rectal bleeding, anaemia, stunted growth.

3,Thread or pin worm (***Enterobius vermicularis***) similar to A.lum.

Cli-features – perianal itching, in female it cause pruritis vulvae or vaginities.

Lab-diag- adhesive tape or a cotton swab moistened with saline.

Protozoa

Giardia lamblia/intestinalis

It exists in two forms trphozoites and cysts, transmitted from one person to person by poor hygiene, responsible for number of outbreaks of diarrhoeal diseases/ foodborne outbreaks, trphozoites are flagellated, which are characterized eight number of flagella and two nuclei, invasive, humans infected by ingestion of food contain cysts of ***Giardia***, ,cysts are excreted through the faeces, resistant to chlorination, symptoms – diarrhoea, abdominal cramps and nausea.

Lab diagnosis – cyst in faeces.

Entamoeba histolytica - causes **amoebiasis**

It exists in two forms **trophozoite** responsible for pathogenesis and **cyst** –resistant stage, highly infectious, endoparasite, survive in larger intestine(colon) of humans,

Cli- features – diarrhoea, abdominal pain, fever and vomiting, endemic in many poor communities in all parts of the world.

Cryptosporidium parvum

It is a sporozoid protozoa, Life cycle of this parasites need any one of host like man or cattle or sheep.

Sarcocystis

It requires two obligatory host –sexual reproduction of this parasites take place carnivores such as cats, dogs or man(Definitive host). Asexual reproduction take place in cattle, sheep and pigs(Intermediate host).

Toxoplasma gondi

cat is a definitie host. Sheep, pig, poultry are intermediate host, humans infected by eating undercooked meat of sheep or poultry or pig. Humans as dead end host in humans this parasite transmitted to fetus through the placenta.

FOOD SANITATION

It is concerned with aseptic practices in the preparation , processing and packaging of food. It is also concerned with sanitation of food processing plant and premises and the health of the

employees, food products may involved in quality control and supply of water that should be free from microbial contamination and prevention of contamination at all stages.

General aspects of sanitation – making inspection, consulting with personnel responsible for sanitation, training personnel in sanitation. If water contains appreaciable number of *Pseudomonas* and *Alcaligenes* might be unsatisfactory. The slimy growth of iron bacteria in water supplies that lead to trouble in food plant. 5 to 7 ppm chlorine in water is used for washing food processing plant.

Antibiotics like chlortetracycline and oxytetracycline is used for dipping dressed chicken.

Treatment of wastes - adequate treatment is need for both solid and liquid waste discharged from food proccesing plant. The solid and concentrated waste should be kept and treat separate from liquid waste may be used for food , feed and fertilizers.

It is recommended that sewage of human origin be kept separate from other plant waters.

Chemical treatment - The liquid waste is treated with flocculant materials like aluminium and iron salts or ferrous sulphate plus lime that cause flocculation as a result the settled materials contains suspended colloidal materials and bacteria.

Disposal of liquid waste –1, Dilution it means liquid waste is allowed into a large body of water. 2, Irrigation it means cultivation of crop plants using treated waste water.3, Lagooning it means oxidation of pond. 4, Trickling filte.5,Activated sludge.6, Anaerobic tank.

GMP – The code does covers regulation that would be interest to food sanitation including plant and equipment and utensils, sanitary facilities, sanitary facilities, control sanitary operation, process and controls.

HACCP – hazard analysis: critical control points.

HA it means identification of ingredients and products which might be consumed by special population, such as infants and elders. CC , it means identification of critical control point, this involves the identification and control over those processing parameters whose loss of control would result in an unacceptable risk to consumers.

Biological spoilage of canned food.

1 Based on spoilage of canned food by microorganisms, it has two types 1, spoilage by thermophilic bacteria 2, by mesophilic bacteria

2, Based on the kinds of changes produced in the food it has four types 1, putrefaction, 2, acid production, 3, gas formation, 4, blackening.

3, Based on spoilage in commercially canned food,, it has four types 1, flat sour spoilage 2, TA spoilage 3, Putrefaction 4, Hydrogen swell

Anaerobic food spoilage occur in canned food due to spore forming ***Clostridium*** sp If can and foos are not sterilized (heat treatment) properly.

Aerobic food spoilage (by spore forming ***Bacillus*** sp and **fungi**) is occur in canned food if this can got damage or cracks or leak.

1, **Flat sour spoilage** , the can of food remain flat during souring or development of lactic acid in the food by flat sour bacteria e.g. *Bacillus coagulans*

2, **TA spoilage -** by thermophilic anaerobes e.g *Clostridium thermosaccharolyticum*(spore forming), it produces acid and gases in foods. The gas is a mixture of carbon dioxide and hydrogen swells the can if it is held long.

3, **Sulfide spoilage** – by *Desulfotomaculum nigrificans*, produces Hydrogen sulfide in the canned peas or corn

Spoilage by mesophilic *Clostridium* species.

C.butyricum and C. pasteurianum, C.putrifaciens, C. botulinum, C. sporogenes. These are proteolytic and putrefactive produces H2S, mercaptans, ammonia, indole and skatole.

Spoilage by mesophilic *Bacillus* species

B. mesentricus, B. subtilus and B. polymyxa

Spoilage by non -spore forming bacteria

Lactobacillus, Micrococcus, Streptococcus faecalis, Leuconostoc species.

Contamination of foods or Primary sources of microorganisms in foods

Food is a solid or liquid mass contains various nutrients used as a human food, it also excellent culture medium for microorganisms. If food is not preserved properly

The food will get microbes from different natural sources that are given below ;

1, **From green plants and fruits.**

The natural surface flora of plants varies with the plant but usually includes *Pseudomonas sp Alcaligenes, Flavobacteruium sp, Bacillus sp, Micrococcus, Coliforms, Lactobacillus brevis, L.plantarum , Leuconostoc mesentroides* and many number of molds and yeasts, The number of microbes depend on the plant and its environment.

From animals

Surface flora(Skin) of animals e.g., β - haemolytic *Streptococci, Staphylococcus aureus,* flora of respiratory tract, flora of gastrointestinal tract e.g., *Salmonella*,

Many animal pathogens transmitted to human being via foods e.g., *Brucella, Mycobacterium tuberculosis, Coxiella, Listeria, Campylobacter, β - haemolytic Streptococci, Salmonella,* Enteropathogenic *E.coli*, Parasites and viruses.

From sewage

The untreated sewage may transmits the human pathogens(enteropathogens e.g., Coliform bacteria, *Salmonella, Shigella* sp to foods.

From soil

Alcaligenes, Flavobacteruium sp, Bacillus sp, Micrococcus, Coliforms, Leuconostoc mesentroides, Chromobacterium, Acetobacter and higher bacteria such as actinomycetes, and the iron bacteria.

From water

Bacillus sp, Micrococcus, Coliforms, Leuconostoc mesentroides, Chromobacterium, Proteus sp, Acetobacter, Presnce of *Coliform* bacteria in food indicates fecal contamination.

From Air

Air contains many number of respiratory pathogens, spores of fungi (*Penicillium* sp , *Aspergillus, Mucor* sp, *Rhizopus* sp sp) and bacteria(*Bacillus sp, Clostridium sp*), some bacteria are pigmented to protect from sunlight *e.g., Pseudomonas sp.*

General principles of food Preservation and Preservatives.

Preservatives are defined as substances capable of inhibiting, retarding or arresting the growth of microorganisms

Most kinds of food are readily decomposed by microorganisms unless special methods are used for their preservation.

The chief methods of food preservation are as follows;

1, Asepsis or keeping out microorganisms

1, Removal of microorganisms

3, Maintenance of anaerobic conditions

4, Use of high temperature

5, Use of low temperature

6, Drying

7, Use chemical preservatives

8, Irradiation.

The following principles are involved

1, Prevention or delay of microbial decomposition

2, , Prevention or delay of self decomposition of the food

Growth curve of microbial culture.

If microbes are added into the food and conditions are favourable, the microbes will begin to multiply and will pass through a successive stages or phases and the results are plotted and gives a growth curve. This curve is divided into four phases 1, **lag phase** (cells become enlarged and initiate multiplication) , 2, **log phase** (rate of multiplication is rapid), 3, **stationary phase** (bacterial numbers remain constant), and 4, decline or **death phase**(death rate is more than new cells formation).

Asepsis – means keeping out microorganisms as a preservative factor. I nature the inner tissues of healthy plants and animals are free from microorganisms, and if any microorganisms are present they will initiate spoilage. If there is a protective covering about the food, the microbial decomposition is delayed and prevented. Examples , shells of nuts, the skins of fruits and vegetables, the husks of rice , corn, the shells of egg, the skin, membranes or fat on meat or fish.

Packaging of food is a widely used application of asepsis.

Removal of microorganisms

Removal of microorganisms is not very much effective in food preservation, it can be accomplished by means of filteration, centrifugation, washing or trimming. Filteration is only effective method compared to others. Washing is used for removal of microbes from vegetables and fruits, Trimming is also used for removal of spoiled part of vegetables and fruits.

Maintenance of anaerobic conditions

Replacement of air by carbon dioxide or by an inert gas such as nitrogen, it prevents the growth of aerobes.

Use of high temperature for preservation of food

Heat or high temperature will kill the microbes and their spores by means of denaturation of their proteins and inactivation of their enzymes required for metabolisms.

Factors affecting heat resistance (Thermal Death Time).

Cells and Spores of microorganisms differ widely in their resistance to high temperature.

Temperature and time relationships

If high temperature is applied , killing time will be decreased, if low temperature , the killing time will be increased.

Concentration of microbial cells and their spores

If their numbers is more, the heat treatment time is increased.

Culture medium

The presence of accessory growth factors in culture medium it will increase the resistance power of microbial cells.

Temperature of incubation

Resistance power of microbes increases as the incubation temperature is raised towards the optimum.

Phases of Growth or age

Bacterial cells are highly sensitive to heat at log phase than lag phase.

Desiccation – Dried spores are highly resistance to heat than moist spores.

Hydrogen – ion concentration (pH)

Microbes are resistance to heat at neutral pH than acid and alkali pH.

Thermal death time

The heat resistance of microbes is expressed in terms is **Thermal death time** or absolute thermal death time. It means time it takes at a certain temperature to kill the microbes under specified condition.

For e.g., *E. coli* can be killed at 57^0C for 20-30 minutes. *Lactobacillus* at 71^0C for 30 minute

Heat resistance of Yeasts and their spores

Both yeasts and their spores are killed by 62.8^0C for 30 minutes.

Heat resistance of Molds and their spores

Both molds and their spores are killed by $6O^0$C for 10 minutes. Sclerotia can survive at 90-100^0C. It will spoil the canned fruits.

Heat resistance of Bacteria and their spores

Most bacterial cells are killed by temperature at 75^0C for 30 minutes and their spores 100^0C for several minutes.

Heat resistance of enzymes

Most food and microbial enzymes are destroyed at 79.4 C. Bovine phosphatase is present in milk and used as a monitor in the pasteurization of milk, if milk is not properly pasteurized the enzymes will be viable detected by chemical test, absence of this enzymes indicate good sterilization of milk, presence of this enzymes indicate bad heat treatment.

Heat penetration

Heat penetration from an external source to the centre of the can may take place by **conduction**, where heat passes from molecule to molecules by **convection** , where the heat is transferred by movement of liquids or gases or by a combination of conduction and convection. Conduction is slow in foods, rapid in metals.

When both conduction and convection are involved in the heating of foods, they function simultaneously. When solid particles of foods are suspended in a liquid, the particles heat by conduction and the liquid heat by convection. Glass has a slower rate of heat penetration than a metal can. The larger a can is the longer it will take to reach s given temperature at the centre. Rotation or agitation of the container of food during heat processing will hasten heat penetration if the food is fluid, but it also cause undesirable physical changes in some foods,

. **Application of heat treatment**

Pasteurzation- a heat treatment of milk , that kills microorganisms in milk (especially *Mycobacterium tuberculosis* causes tuberculosis and *Listeria monocytogens* causes listeriosis and *Coxiella burnetti* causes Q fever are transmitted by milk and other pathogens). It has three types-

Heat treatment of milk

Low temperature holding(LTH)	62.8^0C for 30 minutes
High temperature short time(HTST)	71.7^0 C for 15 seconds
Ultra high temperature	135^0 C for 1 second

LTH is not kill Q fever pathogen if their number is more. Alkalin phosphatase is an enzyme present in milk, which is inactivated in pasteurization, if enzymes are active after pasteurization the pasteurization is not done properly.

If pre- pasteurized milk contains psychrotrophs such as Pseudomonads , it will produces lipases and proteinases that will active in post-pasteurized milk cause rancidity (undesirable chemical reaction in milk that result in unfit for human consumption) and casein degradation. Thermodurics are survive in mild pasteurization , active in post-pasteurization if it is not preserved properly.

Heating at about 100^0C

Blanching – it means immersion of fresh vegetables in a boiling water at 100^0C before freezing or drying, because it will inactivate plant enzymes otherwise it will cause toughness, change in colour, mustiness, loss in flavour, softening and loss in nutritive value, reduction of microbes 99%, enhancement of green colour of vegetables e.g., peas, spinach, better to package, displacement of air entrapped ti the tissue.

Baking – it means preparation of bread, cake other bakery products, the internal temperature is 100^0C, bacterial spores can survive at 97^0C may cause ropiness.

Simmering - it means gentle boiling of food about 100 C.

Roasting – it means roasting of meat at 85 C ,

Frying - foods are get heat at 100 C,

Warming up – means food are get at 100 C,

Heating above 100 C

UHT- means milk is treated at 150 C by use of steam injection or steam infusion followed by rapid cooling(flash evaporation) is called ultra high temperature.

Canning – it means preservation of foods in sealed containers, heat is applied as a principle factor in the prevention of spoilage of canned food. A French man **Nicolas Appert** (1795-1810)Who has been called as father of Canning who conducted an experiment on heating of foods in a sealed containers and to publish a direction for preservation by canning - who referred this method is appertization. **Tin cans** are commonly used, **Enamel** (Zinc oxide) is coated inside the cans to prevents the discoloration of food , foods which are involved in canning is milk,

meats, wine, beer, soups, and some fruit juices. Glass containers also used in canning of food, aluminium containers are not withstand mechanical stress.

Salt plus sugar (**brine solution**) solution is added to canned vegetables and sugar syrups may be added to fruits. Before canning the foods are subjected for heat treatment. **Pressure cooker** is used as modern methods of canning of low acid foods. Co**ld pack** – it means the food is not heated before canning, **Hot pack** means heating of food before canning.

Preservation of food by use of low temperature

The low temperature will retard the chemical reaction and action of food enzymes and to slow down or stop the growth and activity of microorganisms in food. The low temperature is used as a preservative agent.

Low temperature growth of microorganisms are given below; *Cladosporium* and *Sporotrichum* are able to grow on food at -6 C, *Penicillium* and *Monilia* at -4C, Bacteria at -5C on meats , Yeast at -5C on meats, molds at -7C,

Cold storage or Chilling - storage of foods above freezing 0 C, temporary preservation of foods e.g., eggs, dairy products, meats, seafood, vegetables and fruits. The **relative humidity** becomes low that results in loss of moisture and softening of vegetables, if relative humidity is too high that result in initiation of microbial spoilage on food. Ventilation is maintain to maintain uniform relative humidity throughout the room, and also remove the odors and prevents the stale odors and flavors.

Frozen storage - storage of foods below – 18 C, Meats and sea foods are selected for freezing because it will minimize enzymatic and microbial changes. Most foods are packaged before freezing.

Common, or Cellar storage - storage of foods below 15 C,

Irradiation – It is better for exposure of meat to ultraviolet irradiation followed by cooling storage.

Sharp breezing - freezing in air with natural air circulation or electric fans. The temperature is -23.3 C, for 3- 72 hours.

Slow freezing – food is frozen in a short time

Quick freezing - freezing time is 30 minutes

Liquid nitrogen - foods which are frozen by means of liquid nitrogen e.g., fruits and vegetables, fish, shrimp, and mushrooms.

Dehyrdofreezing – fruits and vegetables are subjected for removal of half their moisture before freezing.

Changes during freezing-

The low temperature will retard the chemical reaction and action of food enzymes and to slow down or stop the growth and activity of microorganisms in food, there is an expression in volume of the frozen food and ice crystals form and grow in size, the ice crystals may crush or rupture tissue cells or even microorganisms. It will denature the protein.

The red myoglobin of meat changes into brown meta-myoglobin the fats of meat and meat become oxidized and hydrolysed, the unfrozen concentrated solution of sugars, salts may ooze(release) from packages of fruits and vegetables, this solution may viscous is called as **metacryotic liquid**. Fluctuation in the storage temperature that result in formation of ice crystals and physical damages to the food. When ice crystals evaporates from an area of surface a defect is called as **freezer burn**, this can be seen in fruits , vegetables, meat and fish.

Changes during thawing – thawing refers to sudden changes of temperature from freezing or chilling to normal room temperature, it is due to sudden power failure or fluctuation. During thawing a pink or reddish liquid that comes from meat is called as **drip or bleeding**, if liquid oozing from fruits or vegetables is called as **leakage**. The psychrophiles become activated and initiate spoilage of food.

Lethal effects - Rapid cooling or freezing can kills microorganisms because cooling can denature the proteins and enzymes is called as **cold shock.**

Response of microorganisms to freezing - On the basis of sensitivity to freezing the microbes classified into three types 1, **sensitive or susceptible** e.g., vegetative cells of yeasts, molds and gram – negative bacteria. 2, **moderately resistant** e.g., Gram positive bacteria Staphylococcus aureus and Streptococcus sp, 3, **insensitive or resistant e**.g., spores of bacilli and Clostridia.

Bacteria in log phase become highly sensitive to freezing,

freezing temperature is - 4 to -10 C.

Storage death – gradual reduction of microbes in freezing.

If food contains sugar, salt or proteins, colloides, and fat may protect the microbes from freezing.

Food with high moisture and low pH may kill microbes rapidly.

Alternate freezing and thawing may kill microbes rapidly.

Preservation of food by Drying

Most foods contain enough water to permit the action by their own enzymes and by microorganisms, so that to preserve them by dryness the removal of moisture is necessary, preservation of foods by drying has been practiced for many centuries e.g., grains such as paddy, wheat, pulses etc.

Drying is accomplished by the removal of water, that will reduces the amount of available moisture i.e., the $\mathbf{a_w}$,

Sun dried - exposure of food to the sun rays without any artificial method.

Dehydrated or desiccated food- food is dried by artificially under controlled relative humidity and air flow.

Condensed food or evaporated food - moisture removed from liquid food.

Solar drying

Solar drying or hot sun is used for drying of fruits such as pears, peaches and fishes rice and other grains.

Evaporator or Kiln

It refers to the passage of heated air with controlled relative humidity over the food to be dried. The simplest drier is Evaporator or Kiln. The liquid food is evaporated by passage over a heated drum.

Freeze drying- it refers sublimation of water from a frozen food such as e.g., meats, poultry, seafood, fruits and vegetables.

Treatments of foods before drying – 1, Sorting of food items based on their size, shape and weight, 2, washing e.g., vegetables and fruits. 3, Peeling of vegetables and fruits.4, Subdivision into halves, slices, shreds or cubes by hand, machine 5, alkali dipping - 0.1 to 1.5% sodium carbonates for fruits such as raisins, grapes ,6, Blanching or scalding of vegetables and some fruits 7, Sulfuring - exposure of some fruits and vegetables to sulfur dioxide gas , sulfuring helps the preservation of Vitamin C and Vitamin A and repel insects.

Procedure after drying – 1, **Sweating** means storage of food in bins or boxes e.g., Almonds., **Packaging**- Most foods are packaged soon after drying for protection against moisture, contamination with microbes and infestation with insects e.g., fruits and nuts.

Microbiology dried foods – moderate dried food contains spores of fungi and bacteria are present.

Intermediate moisture products- Numerous commercially prepared foods contain 20 to 40% moisture and have non-refrigerated shelf stability is called as intermediate moisture products

e.g., meat products ham, some dried fishes, honey, jams, jellies, dried fruits etc, these can be eaten without preparation or rehydration.

Preservation of food by food additives or chemical preservatives of food

Food additives are substance or mixture of substances other than basic food stuff, food additives are specifically added to prevent deterioration or decomposition of a food is called as chemical preservatives. The deterioration of food is caused by microorganisms or by food enzymes or by chemical reaction. Preservatives are defined as substances capable of inhibiting, retarding or arresting the growth of microorganisms

The ideal antimicrobial or food preservatives.

It should be non-toxic to human beings or animals, should be economical, should not affect on flavour, taste or aroma of the original food.

Different group of food additives

Groups	**Examples**
Preservatives	Chemical preservatives
Colors	Natural or synthetic
Flavors	Natural extracts
Sweetners	Saccharin, polyols
Emulsifiers and stabilisers	Lecithin
Antioxidants	Vitamin –E,Butylated hydroxytoluene(BHT), Butylated hydoxyanisole(BHA)
Flour improvers	Vitamin C, Cyseine, chlorine.
Anticaking agent	Calcium silicate
Leavening agent	Baking soda, ammonium carbonate
Chelating agent	Polycarboxylic acid
Curing agent	Sodium nitrite, polyphosphates
Nutrient supplements	Thamine, nicotinic acid, iron , calcium
Processing aids	Antifoaming agents, acids, gelling agents
PRESERVATIVES	
Sodium chloride	Antimicrobial, flavouring, preservative agent and used as brine
Sugar	Food is added with sugar and salt will reduce availability of water that result in no growth of bacteria and fungi and used as a preservative.
Sulfur dioxide	Antimicrobial agent in wine
Nitrate and nitrite	Colour stabilizer in cured meat and antimicrobial agent

Sorbic acid	Inhibit the growth of molds and yeasts in cheese and bakery products, friut juices, wines and pickles.
Acetic acid 4% (vinegar)	Antifungal, preservative in pickled vegetables.
Probionic acid	Anitfungal and antibacterial agent in bakery products.
Benzoic acid	Anitfungal and antibacterial agent in fruit juices, carbonated beverages,pickle and sauerkraut.
Parabens	Anitfungal and antibacterial agent in food
Epoxides	Anitfungal and antibacterial agent in spices
Antibiotics	Oxytetracycline and chlortetracycline are used as a antimicrobial agent in fish and poultry.
Diethyl pyrocarbonates	Antimicrobial agent in in fruit juices, carbonated beverages - wine.

Contamination , Spoilage and preservation of milk and milk products

Milk is an animal origin food, highly perishable food if it is not preserved properly, because it contains rich quantity of proteins (casein), sugar (lactose), calcium, vitamin A, and minerals , excellent culture medium for microbes.

Contamination of milk

Milk contains relatively few bacteria when it leaves the healthy cow, during the normal milking operation the milk is subject to contamination from the animal, manure, soil, water, and un cleaned dairy utensils, the contaminating bacteria includes *Streptococci*, coliform bacteria, bacilli, enterococci, micrococci and brevibacteria etc.

Other possible source of contaminations are the hands and arms of the milker or dairy workers and flies. If proper precaution are taken the contamination rate will be decreased.

Other source of contamination includes tanker truck, transfer pipes, sampling utensils, pipelines, vats, tanks, pumps, valves, separators, clarifiers, homogenizers, coolers, strainers, stirrers, filters and clarifiers may serve as possible sources of bacteria.

Spoilage of milk

Milk is an excellent culture medium for different kinds of microorganisms, because it contains rich in microbial foods, neutral pH. It contains inhibitory substances for bacteria includes lactoperoxidase and agglutinins are present in freshly drawn milk but ineffective.

If milk is sours it indicate acid production , if it is curdles it indicate coagulation of milk proteins.

If raw milk at 37 C *Streptococcus lactis* becomes dominated and cause souring, at higher temperature 37 -50 C *Streptococcus thermophilus* and *S.faecalis* , *L. bulgaricus* are dominated and produces more quantity of lactic acids. Butyric acid may be produced by *Clostridium* sp.

Gas production

The chief gas producers in milk are Coliform bacteria *Clostridium* sp and *Bacillus* sp. The gases are carbon dioxide, and hydrogen.

Proteolysis

The hydrolysis or decomposition of milk proteins (casein) by micro-organisms is known as proteolysis. It is accompanied by the production of bitter flavor, it is favored by storage of milk at low temperature. It has three types 1, **acid proteolysis** in which acid production is followed by proteolysis e.g., *Micrcocci*.2, **proteolysis** is followed by little production of acid or alkalinity 3,**sweet curdling** is caused by rennin enzymes 4**, slow proteolysis** is mediated by intracellular enzymes of bacteria after their autolysis 5**, residual proteolysis** mediated by heat stable proteinase enzymes released by autolysed *Pseudomonas fluorescens*.

Examples of proteolytic bacteria – *Micrococcus, Pseudomonas sp, Flavobacterium, Proteus sp , Alcaligenes and Serratia* are non- spore forming bacteria, *Bacillus and Clostridium* sp

Pseudomonas sp, Flavobacterium, Alcaligenes and *Bacillus* are grow well at low temperature.

Ropiness or sliminess – it occur in milk and cream caused by two factors 1, non-bacterial 2, bacterial ropiness.

1, **non-bacterial** is due to mastitis (inflammation of udder), thin films of casein(lactalbumin), thickness of cream

2, **bacterial ropiness** is due to the presence of slimy capsular material from the cells, surface ropiness is caused by *Alcaligenes viscolactis, Micrococci, Streptococcus lactis, Lactobacillus bulgaricus, L. casei*

Changes in milk fat

Milk fat is hydrolysed or decomposed by lipase produced by microbes into fatty acids and glycerol, lipase producers are *Pseudomonas sp Alcaligenes, Bacillus, Micrococci, Clostridium* sp, and many number of molds and yeasts, *Pseudomonas fragi* and *Staphylococcus aureus* produces heat stable lipase it will survive in pasteurization.

Alkali production - *Pseudomonas fluorescens* and *Alcaligenes viscolactis* can hydrolyse the protein that result in the production of ammonia, These bacteria are alkali formers.

Flavor changes

Acid flavor or sour flavor - acid flavor in milk is due to growth of *Streptococcus lactis* .

Bitter flavors - due to proteolysis and lipolysis by coliform bacteria.

Burnt or Caramel flavors – in milk is due to the growth of *Streptococcus lactis* var. *maltigenes.*

Soapiness flavor - by *Pseudomonas sapolactica*

Fruity flavor - by *Pseudomonas fragi*

Fishiness – by *Aeromonas hydrophila*

Potato flavor – by *Pseudomonas mucidolens*

Turnip flavor – by *E. coli*.

Color changes

Blue milk – by *Pseudomonas syncyanea and Geotrichum (*Actinomycetes)

Yellow milk - by *Pseudomonas synxantha* and *Flavobacteruium sp*

Red milk- by *Serratia marcescens* and *Brevibacterium erythrogenes*.

Brown milk- by *Pseudomonas putrefaciens* , and *P. fluorescens*

Thermodurics – it means bacteria survive in pasteurization.

Spoilage of condensed milk – condensed milk are sweetened milk, evaporated milk (unsweetened), frozen milk and dry milk. These are spoiled by molds when the surface is exposed to air.

Bulk condensed milk - is sterilized by temperature less than pasteurization and spoiled by thermophiles and thermoduric bacteria

Frozen desserts - it includes ice cream, ice milk, frozen custards, sherbets, these are prepared by using various ingredients includes coloring materials, flavors, fruits, nuts, sweetening agents, egg and egg products and stabilizers. Any of these ingredients may contribute the contamination of frozen desserts that result in spoilage of frozen desserts.

Butter - is commonly kept at about -17.8 C, Where no microbial growth can take place, however, a little defects occur in long stored butter.

Flavor defects - undesirable flavors may come from the cream, which may receive from the feed of the cow , absorb them from the atmosphere or develop them during microbial grow

Barny flavor by *Enterobacter sp*

Malty flavor by *Streptococcus lactis var. maltigenes*

Yeasty flavor by yeasts

Musty flavor by molds and actinomycetes

Unclean flavor by coliform bacteria

Flat flavor by *Pseudomonas sp*

Fishiness by *Aeromonas* sp

Easterlike flavor by *P.fragi*

Surface taint by *P.putrefaciens*

Color defects in butter

Discoloration is mainly caused by molds , yeasts and bacteria, orange or yellow coloration by *Geotrichum* sp. *Penicillium* sp - green color, *Fusarium* – bright reddish-pink color, Yeasts – pink color, , *Cladosporium* sp– black spot *Pseudomoas nigrifaciens* – reddish brown .

Preservation of milk

Asepsis - Packaging helps to keep microorganisms from bottled milk, fermented milk, packaged butter, canned milk, and packaged cheese.

Removal of microorganisms- Centrifugal procedure used for removal of microbes from milk is called as bactofugation, it is no value in commercial basis.

Heat treatment of milk

Low temperature holding(LTH)	62.8^0C for 30 minutes
High temperature short time(HTST)	71.7^0 C for 15 seconds
Ultra high temperature	135^0 C for 1 second

LTH is not kill Q fever pathogen if their number is more. Alkalin phosphatase is an enzyme present in milk, which is inactivated in pasteurization, if enzymes are active after pasteurization the pasteurization is not done properly.

If pre- pasteurized milk contains **psychrotrophs** such as Pseudomonads , it will produces lipases and proteinases that will active in post-pasteurized milk cause rancidity (undesirable chemial reaction in milk that result in unfit for human consumption) and casein degradation.

Themodurics are survive in mild pasteuriztion , active in post-pasteuriztation if it is not preserved properly.

Use of low temperature

Refrigerated storage or chilling - it means storage of milk in refrigerator at below 7 C. This is recommended in storage in plant, retail market, during delivery in home.

Freezing – it means storage of milk products (butter, frozen milk) at -17 to -18.

Drying- Various milk products are made by removing different percentage of water from the whole or skim milk in order to prevent the microbial growth.

Condensed products – Evaporated milk is made by removing about 60% of the water from whole milk, the high concentration of sugar will inhibit the growth of some bacteria.

Dry products – are prepared in the dry form are skim milk, cream, whey, buttermilk, malted milk, ice cream mix. These are prepared by the roller process.

Use of preservatives

Sorbic or propionic acid and their salts used for preventing mold growth and preservation of cheese, yogurt. Sugar is added into the sweet condensed milk because sugar will reduce the availability of water. Sodium chloride is used for preservation of cheese. Hydrogen peroxide is used for treatment of milk excess peroxide is removed by adding catalase enzymes.

Quality check up

Bacteriology test

1,MBRT – methylene blue reductase test

Preparation of methylene blue dye – take 1 gm of methylene blue in 25 ml of sterile water , it is prepared dye,

Procedure – take 10 ml milk sample is added with 1ml of prepared dye and incubate at 37 C **for 6 hours,**

Observation – colour changes from blue to white.

MBRT- time	**Classification of milk sample**
0- 30 minutes	Very poor quality
31-120 minutes	Poor quality
121 – 360 minutes	Fair quality
361 – 480 minutes	Good quality

2,Pour plate method or Serial dilution agar plating technique

Procedure

1 ml milk sample is serially diluted in 9 ml of sterile peptone water, 1: 10, 1:100, 1:1000, 1:10000, etc.(10^{-1}, 10^{-2}, 10^{-3}, 10^{-4})

Take 1 ml of diluted sample from each tube and mix with warm agar in a petri dish and incubate at 37 C for 24 – 48 hours in an inverted position.

Number of cells / ml = Number of colonies

Amount plated × dilution

For example if 60 colonies were counted on 10^{-4} dilution

$$\frac{60 \text{ colonies}}{1\text{ml} \times 10^{-4}}$$

Result 6,00,000 = 6×10^{4} bacteria per ml or gm of sample

Contamination , spoilage and preservation of fish and other seafood

Fishes are animal origin and important food for humans because of fishes are rich source of proteins, carbohydrates, minerals , vitamins (B- complex) and amino-acids.

Sources- based on sources of fishes, they can be classified into two types 1. Fresh water fish 2, Sea fish.

Contamination

Freshwater fishes are contaminated by bacterial sp e.g., *Pseudomonas sp, Flavobacterium, Corynebacterium, Sarcina, Serratia, Vibrio, Bacillus, Lactobacillus, Brevibacterium, Alcaligenes* and *Streptococcus.*

Sea fish or Marine fishes – are contaminated by *Pseudomonas sp, Flavobacterium, Corynebacterium, Sarcina, Serratia, Vibrio, Bacillus, Alcaligenes, Micrococcus, Acinetobacter* and *Morexella.* Contaminating microorganisms are responsible for spoilage of fishes.

The numbers of microbes on skin and gills of fishes are influenced by various factors as well as method of catching. If fishes are catching from the bottom of the environment the microbes load will high. Dirty water environment also increase the microbial load on fishes.

Spoilage of sea fishes

Fishes are highly perishable (spoilage) if it is not preserved properly,

Factors influencing kind and rate of spoilage

1, The kind of fish – flat and small, fatty fishes are spoiled more rapidly than large and round fishes.

2, The condition of fishes – fish that are exhausted as a result of struggling, lack of oxygen, excessive handling spoil more rapidly than others.

3,The kind of contamination – if bacteria from mud or exterior slime or handlers or intestinal contents may enter into the vascular system through the gills and thus invade the flesh of fish.

4, **Temperature** – chilling or low temperature that will delay the bacterial spoilage of fishes.

Bacteria Causing Spoilage

The *Pseudomonas sp, Flavobacterium, Corynebacterium, Sarcina, Serratia, Vibrio, Bacillus, Alcaligenes, Micrococcus, Acinetobacter , Morexella E.coli, Proteus* and *Clostridium sp.*

Discoloration of fish flesh may occur during spoilage – yellow to greenish color of fish flesh is due to *Pseudomonas sp,* Yellow by *Micrococcus,* red or pink color of fish flesh by *Sarcina, Micrococcus* and *Bacillus.*

Autolysis - Self decomposition of fishes by their own enzymes that result in increasing of bacterial numbers, from the decomposed fishes the bacteria make tri-methylamine, ammonia, amines, lower fatty acids and aldehydes, hydrogen, sulfides, mercaptens and indole which products are indicative of putrefaction.

Putrefaction - (anaerobic decomposition of proteins by bacteria).

Souring – carbohydrate of fishes are fermented by Coliform bacteria, *Streptococci* and *Lactobacilli* that result in production of acids and sour odor.

Evidence of Spoilage – the bright color of fish become fade, dirty, yellow or brown. The slime on the skin of fish become increases at the gills, the eyes gradually sink and shrink, softening of flesh of the fish, the gill turn a light pink to brown color, presence of tri-methylamine, ammonia, amines, lower fatty acids and aldehydes, hydrogen, sulfides, mercaptens and indole which indicate spoilage. The chemical test are used for testing these compounds.

Microbial spoilage of fresh water fish and other sea foods are similar to sea fish spoilage.

Preservation

Eviscertation - it means removal of digestive tract of fish in order to prevent the action of intestinal enzymes involved in autolysis, before preservation of fish the eviscertation should be done.

Rigor mortis - it means a chemical changes in the muscles of fish after death that result in the stiffening of muscles, rigor mortis can retards postmortem autolysis, preserve the fish in good condition for certain hours.

Physical methods

Use of low temperature

Chilling- preservation of fish by chilling temperature(less than $5^{O}C$) is temporary. This method is used when outside temperatures are warm and distances of transportation are great. In this method the fishes are packaged with crushed ice or by mechanical refrigeration it will stop the autolysis and prevent the microbial growth.

Freezing - fishes are preserved with a layer of ice or storage at $-^{0}C$, or at freezer, during storage the fats of frozen fish are subject to hydrolysis and oxidation, fatty deteriorate more rapidly than lean ones. Other sea foods preserved by freezing include clams, oysters, spiny lobster, cooked crab and lobster meat.

Fish carry a flora of psychrotrophic bacteria, most of which survive freezing and are ready to grow on thawing, e.g., *Pseudomonas sp, Acinetobacter, Morexella, Alcaligenes Flavobacterium sp, and Spores of Clostridium botulinum.*

Use of irradiation

Ultraviolet rays has been tried but not used as a commercial basis.

Drying

The salt- drying of fish is a traditional method of drying, oxidation of fish oil is not retarded and may cause deterioration. This method is not allowed in United States.

Use of Preservatives

The salting (4 to 5%) of fishes with sodium chloride is effective method of preservation. The salt should be checked for chemical and bacteriological qualities, chemical test used for finding impurities such as calcium and magnesium salt may hinder the penetration power of the sodium chloride, bacteriological quality test used for finding halophilic or salt tolerant bacteria e.g., *Pseudomonas sp, Acinetobacter, Morexella, Alcaligenes, Flavobacterium sp, and Spores of Clostridium botulinum.*

Benzoic acid , benzoates, Sodium and Potassium nitrites, nitrates, Sorbic acid, Boric acid are used as preservatives for fishes.

Antibiotics

Chloretetracycline and Oxy tetracycline are used as preservatives for fishes.

$C0_2$ - Storage

Fishes are stored in atmosphere containing increased level of CO_2 (Carbon dioxide) that will increases the storage period of fishes.

Pickling of fishes - it means salting with sodium chloride or acidification with vinegar, wine, it will increases the keeping time of fishes.

Thawing – it means increasing of temperature from chilling or freezing to normal temperature(30 – 40 C) , it will allow the bacterial growth predominantly and fungi also. It is due to power failure in refrigerator or freezer.

Germicidal ice – ice contain germicide that will kill fish spoilage microbes, it can be prepared by required quantity of water is mixed with appropriate level of germicide, allowed it to freezing temperature for few hours , now the germicide ice is formed , Canadian government allowed 7ppm of tetracycline for germicidal ice preparation. The germicidal ice is used for preservation of fishes.

Rancidity – means an undesirable chemical changes in the food that result in unfit food for human consumption.

Antioxidants - ethyl gallate, ascorbic acid are antioxidant it will prevents oxidative changes of fats and oil of fish.

Contamination , Spoilage and Preservation of meat and meat products

Meat and meat products are animal origin food, neutral pH food, Protein, mineral, vitamin B complex rich food,

meat includes mutton (Sheep meat) ,chicken(Chicken meat), beef (ox meat), Pork(pig meat). The **meat products includes** bacon, sausage, ham and hamburger.

Contamination - Meat getting contaminants during bleeding, skinning, cutting, handling and processing.

Contaminating bacteria - *Acinetobacter, Moraxella, Pseudomonas, Aeromonas, Alcaligenes* and *Micrococcus.*

Contaminating fungi - Molds - *Cladosporium, Geotrichum, Sporotrichum, Mucor* and *Thamnidium.*

Yeasts – *Candida, Torulopsis* and *Rhodotorula*.

Spoilage

If meats are not preserved properly it will undergoes various changes: 1, **Autolysis** - it refers to hydrolysis of protein and fats by their enzymes that result in souring and sour odor 2, **microbial spoilage** that result in rancidity.

Spoilage under aerobic condition

1, **Surface slime** - caused by species of *Acinetobacter, Moraxella, Pseudomonas, Aeromonas, Alcaligenes* and *Micrococcus, Leuconostoc, Bacillus* and *Lactobacillus.*

2, **Changes in colour of meat pigments**- The red color of meat changes into green, brown due to production of peroxide or hydrogen sulphide by bacteria species of *Leuconostoc, and Lactobacillus*

3, **Changes in fats** – Hydrolysis of fats may be caused by *Pseudomonas, Achromobacter sp* and *yeasts* that result in rancidity of fat take place.

4, **Phosphorescence** - Phosphorescent in the surface of meat is caused by *Photobacterium* spp.

5, **Various surface colours** meat due to pigmented bacteria- red spot by *Serratia marcescens*. Blue spot by *Pseudomonas syncyanae.* Yellow spot by *Micrococcus, and Flavobacterium.* Green spot by *Chromobacterium lividum.*

6, **Off odours and off taste** – it means bad odour and bad taste caused by bacteria and fungi and Yeasts.

By molds - 1, **Stickiness** – the surface of meat become sticky when touch. 2, **Whiskers** – it means molds growth on meat or fuzzy growth.3, **Black spot** by *Cladosporium herbarum*. 4, **White spot** by *Sporotrichum carnis.* 5 **Green batches** by *Penicillium expansum* and *P.oxalicum*. 6, **Off odours and off tastes** and hydrolysis of fats by *Thamnidium* sp .

Spoilage under anaerobic condition -

1, **Souring** – fermentation of meat glycogen into formic, acetic, butyric, propionic acids by *Clostridium sp,*

2, **Putrefaction**- it means anaerobic decomposition of proteins with production of hydrogen sulphide, mercaptens , indole, skatole, ammonia and amines by *Clostridium* sp and some facultative anaerobes *Pseudomonas, Alcaligenes* and *Proteus.*

3, **Taint or off odour or off taste** by *Clostridium sp,*

Preservation –

Asepsis- Hot water used for removal of microbes from surface of meat, covering of meat by films it will keep out bacteria,

Use of heat - most meat and its products are low acid food that are good culture medium for microbes, the heat penetration is more in meat soup than tightly packed meat and meat paste.. Chemicals added to meats such as spices , salt ot nitrates and nitrites also affect the heat processing. Nitrates will kill bacterial spores in the presence of heat.

Commercially **canned meats are two types** – 1, Shelf- stable canned meats , this can be heated up to 98 C, these are not keep refrigerated. 2, Non-shelf-stable, this can be heated up to 65 C, these are keep refrigerated after heat treatment.

Use of low temperature

chilling - storage temperatures is vary from 1-2.2 C, it will preserve the meat for 1to 2 weeks. Storage time can be lengthened by storage of meats in an atmosphere added carbon dioxide or ozone, both will inhibit the growth of microorganisms and increase the storage periods up to 60 days. The carbon dioxide can reduce myoglobin into metmyoglobin(brown color) so that meat become loss its original red color.

Freezing – storage temperature is -12 to -28.9 C, freezing is used to preserve meats during shipment over long distances and in house for several weeks. Freezing temperature will kill microbes. The meats are **frozen meats**.

Changes during thawing of meats – psychrotrophic bacteria become dominated *Acinetobacter, Moraxella, Pseudomonas, Aeromonas, Alcaligenes* and *Micrococcus, Leuconostoc, Streptococcus and Lactobacillus.*

Use of irradiation - ultraviolet rays used for sterilization of microbes which are present in the surface of meat in cold storage and microbes in air of cold storage room. Storage period can be lengthened in conjugation processes like UV sterilization of meat followed by cold storage.

Preservation by Drying – Sun drying of meat will reduce the microbial load , Usually sun drying of meat is combined with salting and smoking.

Curing – it means any preservation process that applied to meats and fishes, the products are cured meats or cured fishes.

Use of Preservatives – **Ozone** and **carbon dioxde** are used in chilling storage of meats. Sodium chloride or common salt (15 to 24%) is used as a preservatve agent and flavoring agent or mineral food. **Sodium nitrate** and **sodium nitrite** are a color fixative by changing muscle myoglobin into oxymyoglobin which are bright red in color in meat and inhibit the growth of anaerobes. The sugar and salts are added to preserve the meat it will reduce the $\mathbf{a_w}$ of meat that result in no growth of microbes.

Brine solution – it means salt solution , sometimes added with vinegar or acetic acid are used for curing or preserve the meats.

Microbiology of brine – halotolerant or halophiles(microbes that tolerate or grow high concentration of salt) such as micrococci and lactobacilli are common in brine.

Smoking – it means burning of wood that generate smoke it will kill microbes in meat is called as smoking.

Antibiotics – oxytetracycline and chlortetracycline are used as a preservative of meats. Usually, animals are injected with these antibiotics before one week of slaughtering,

Notes - for Contamination , Spoilage and Preservation of meat products includes bacon, sausage, ham and hamburger are similar to Contamination , Spoilage and Preservation of meats.

Bacon – is a meat product prepared from cured pork by using large quantities of salt prepared and preserved by by smoking.

Hamburger - is a sandwich consists of one or more patties of ground meat (beef) placed inside a sliced bread.

Ham - is a pork is preserved by salting and smoking or wet curing .

Sausage – is a cylindrical meat products made from ground meat(pork or beef) along with salt, spices and flavouring.

Contamination and spoilage and preservation of vegetables and fruits

It has been estimated that one fourth of all produce harvested is spoiled before consumption. Spoilage of fresh vegetables and fruits occurs during storage and transport.

As soon as fruits and vegetables are gathrered into boxes or baskets or trucks they are subject to contamination with spoilage organisms from each other and from containers.

Contaminating microorganisms are **pectinolytic**, of vegetables - Bacteria - ***Pseudomonas*** *sp, Alcaligenes,* ***Erwinia, Xanthomonas*** *, Micrococci, Bacillus, Lactic acid bacteria.*

Molds- *Fusarium, Alternaria, Aureobacidium, Penicillium, Sclerotina, Botrytis, Rhizopus.*

Fruits - Bacteria - *Pseudomonas sp, Alcaligenes, Erwinia, Xanthomonas , Micrococci, Bacillus, Lactic acid bacteria*

Molds- *Fusarium, Alternaria, Aureobacidium, Penicillium, Sclerotina, Botrytis, Rhizopus, Cladosporium, Phoma, Trichoderma, Phtyophthora infestans.*

Spoilage of vegetables and fruits

General types of microbial spoilage in vegetables :

Microorganisms	Vegetables	Symptoms
Bacteria		
Corynebacterium sepedonicum	Potato	Ring rot
Pseudomonas solanacearum	Potato	Soft rot
Erwinia carotovora	Potato	Soft rot
Streptomyces scabies	Potato	scab
Xanthomonas campestris	Cauliflower, Cabbage	black rot
Fungi		
Botrytis cinera, B.allii	Onions	neck rot
Trichothecium roseum	Tomato	pink rot
Fusarium coeruleum	Potato	dry rot
Aspergillus alliaceus	Onions, Garlic	Black rot

Entry of pathogens- through the wounds, cracks, insect damage, injuries or lesions caused by plant pathogens.

Pectins are methyl ester of α- 1, 4 – poly- D- galacturonic acid,

Pectinase are enzymes destroy the pectin , pectin is a important component of plant cell wall. The enzymes includes pectin esterase, pectin hydrolase, pectic acid hydrolase,trans eliminase.

Animal and birds faeces spreads *Salmonella sp, Shigella sp, E.coli*, *Enterobacter* sp to vegetables. Soil spreads anaerobic spore forming *Clostridium botulinum*. Sewage sludge spreads *Aeromonas hydrophila and Yersinia enterocolitica, Listeria monocytogens*.

Listeria monocytogens can grows on shredded cabbage and salad vegetables at 5 ^{o}C.

1, **Bacterial soft rot** - by *Erwinia, Pseudomonas sp, Bacillus,* and *Clostridium sp.*

2, **Gray mold rot** - by *Botrytis sp.*

3, **Rhizopus soft rot** - *Rhizopus sp.*

4, **Anthracnose** - by *Colletotrichum* sp , spotting of leaves and fruits.

5, **Alternaria rot** – by *Alternaria sp*

6, **Blue mold rot** – by *Penicillium sp*

7, **Downy mildew** – by *Phytophthora* sp, and *Bremia* sp.

8, **Watery soft rot** – by *Sclerotinia* sp.

9, **Black mold rot** - by *Aspergillus* sp

10, **Black rot** - by *Physalospora sp*

11, **Pink mold rot** - by *Trichothecium* sp

12, **Fusarium rots** – by *Fusarium sp*

13, **Green mold rot** – by *Cladosporium,Trichoderma sp*

14, **Brown rot** – by *Sclerotinia* sp.

15, **Sliminess or souring** – by Saprophytic bacteria.

Preservation – chilling, sun drying for onions and garlic,

Vegetables products – soy sauce, tomato sauce, potato chips, tempeh.

Spoilage of fruits

Fruit juices may be extracted or squeezed directly from fruits and vegetables, the juices may be concentrated by evaporation or freezing and may be preserved by canning, freezing, or drying. Most fruit juices are acid enough and have sufficient sugar that favour the yeasts. The deficiency of B-vitamin discourages some bacteria. But usually wild yeast produces moderate amount of alcohol and considerable amount of volatile acid.if temperature above 30 C, *Lactobacillus brevis* and *Leuconostoc mesentroides* will dominate and produces lactic acid if fruit juices is not preserved properly.

Vegetable juices contain sugars and less amount of acid than fruit juices. The presence of sugar and acid favours the growth of Yeast and acid-sugar tolerant bacteria(*Lactobacillus, Leuconostoc sp.)*

Contamination and spoilage and preservation of poultry notes are similar to meats .

Dressed and eviscerated chicken may be canned, preserved by either chilling – preserved chicken for short periods 2 days at 10 C , 6 days at 4.4 C, 14 days at 0 C. and freezing - preserved at -17.8C.

Contamination, spoilage and preservation of eggs

Most freshly laid eggs are sterile, atleast inside, but shells soon become contaminated by fecal matter of hen, by the cage or nest, by wash water, *Salmonella* spp may be on shell is common.

Spoilage

Changes caused by microrganisms , the egg get the contaminant the following ways 1, contaminate the shells by bacteria or fungi 2, penetrate the pores of the shell to the shell membrane3, grow through the shell membrane to reach the white4, grow in the white or yolk , where they can grow readily and spoilage or rots of the egg , the rots are different typ[es , 1, Green rot by Pseudomonas fluorescens. 2, Colorless rot by *Pseudomonas* sp, *Acinetobacter*, *Alcaligenes*, certain coliform bacteria 3, Black rots by *Proteus* sp, *Pseudomonas* sp, *Aeromonas* sp, 4, Pink rots by *Pseudomonas* sp, 5, Red rots by *Serratia* sp.

1,**Pin spot molding** - it means initial growth of fungus in egg,2, Superficial fungal spoilag- it means over growth of fungus both inside and outside of eggs . 3, Fungal rotting – it means final stage of fungal growth in eggs , Spoilage fungus includes *Penicillium sp, Cladosporium sp, Sporotrichum, Mucor sp, Thamnidium sp, Botrytis sp and Alternaria sp*.

Off flavors e.g., Mustiness by *Achromobacter sp, Pseudomonas* sp, *Streptomyces* sp

Hay odor by Enterobacter cloacae, Fishy odor by *Escherichia coli*.

Preservation of eggs

Asepsis - Great care is taken to reduce the contamination of the outside of the shell by hen feces and nests.

Removal of microoganisms.

The use of 1 to 3% acetic acid was effective in removing flora but result in reduction of shell thickness and egg quality.

Use of heat

Dipping of eggs into hot water will reduce microbial load. Heat treatments emblyoed in United States for the elmination of *Salmonella* sp. Pasteurization is required for most of the egg products.

Use of low temperature

Candling – Before preservation the egg is subjected for candling. it refers to the egg is held and rotated in front of light to examine it for defects such as cracks, rots, molds, blood, developing embryo, sided yolk, weak white.

Chilling – preservation ofeggs at less than 5 C

Freezing - The eggs first are sorted and selected by candling and then are washed mechanically with 500ppm chlorine and then broken on automatic machines. The spoiled eggs should be eliminated. All equpment that touches the egg shell or their contents must be cleaned and sanitized daily. The contents are filtered to remove the pieces of shell. And frozen at -20 C.

Drying - spray dryer is used for drying of egg, where the liquid is sprayed into a current of dry heated air. The whole –egg or yolk products can be pasteurized before drying.

A variety of organisms found in dried eggs, including micrococci, streptococci, coliform bacteria, salmonella , spore forming bacteria and molds.

Use of preservatives – Sealing of shells with a dimethylourea has been found effective in inhibiting the mold growth. Some of the substaces are used to keep the shell dry and reduce the penetration of oxygen into the eggs and passage of carbon dioxide and moisture out.

Fumigation with gaseous ethylene oxide effective against the bacterial growth. The atmosphere air is replaced with ozone and carbon dioxde that improve the keeping quality of eggs.

Saurkraut

Saurkraut is a fermented cabbage products or acidic or sour cabbage, In North Korea fermented vegetables is called as kimchi.

Nutritive value of saurkraut

Excellent source of vitamin K, Vitamin C.

Taste – sour or clean acid flavour

Flow chart for saurkraut production

Fresh cabbage ------ trimming ------- washing--------- shredding(2-5mm thick)---------- salting(2-3%)-------filling into vats ---------- cover and seal---------- fermentation--------pasteurization ---------- packaged.

Trimming means removal of spoiled part of cabbage, **washing** will remove undesirable organisms such as chromogens, coliform bacteria, yeasts amd molds, **shredding** means cutting of cabbage into small pieces, **salting** means adding of salt , **vats** means fermentor or device or vessal, **fermentation** means biochemical reactions carried out by anaerobes such as (lactic acid bacteria) *Leuconostoc mesentroides, Pediococcus sp* and *Lactobacillus plantarum*. **Pasteurization** means heat treatment of fermented product.

Salt will extract moisture or water by osmosis (flow of water from its higher concentration to its lower concentration) from shredded cabbage it form the brine in which fermentation will take place.The salt also inhibit the natural micrflora of cabbage such as *Pseudomonas spp* otherwise it will spoilage fermentaion processes and helps to select the lactic acid bacteria. It helps maintain crisp texture of the cabbage by withdrawing water and inhibit the protease enzymes activities and salt also contribute to the flavour of the product.

Ingredients - cabbage, salt.

Fermenting microbes – *Leuconostoc mesentroides* and *Lactobacillus plantarum*. The fermentation processes is initiated by *Leuconostoc mesentroides* (Gram positive cocci) , it ferment the carbohydrates hexoses and pentoses to lactic acid and acetic acids lower the pH 3.5. As no starter culture are added to

the system, this is termed as **wild fermentation.** The *L.mesentroides* can produces diacetyl compound it is an flavoring agent, adding aroma and flavour to the product.

Duration of fermentation – 4- 8 weeks.

Temperature – 21-24C.

Products – 1-2.3% lactic acid, 1-4% volatile non-volatile acid, vitamin K, and Vitamin C.

Saurkraut can be eat immediately, if not eaten, it is to be refrigerated or preserved by canning.

It is an anaerobic fermentation , if oxygen enter it will initiate acid loving molds and yeasts and spoil the saurkaut production. Spoilage of Saurkraut – due to high acidity, the finished product is spoiled by molds. Pink kraut by *Torula glutinis*, Slimy kraut by *Lactobacillus spp*.

Pickles

Pickles are the fermented products of vegetables (fresh cucumber), fermenting microbes is *Lactobacillus plantarum* and *Leuconostoc mesentroides* , **preparation method for pickle is similar to saurkraut**. For production the cucumer is sliced or ground are placed in wooden brine tanks at 5% Salt or NaCl.

During fermentation the carbohydrates are converted into lactic acid which softens the tissues and sours the cucumber. After fermentation the vinegar or spices are added to sour pickle, sugar is also added to sweet pickles.

Most pickles are pasteurized or further acidified to increase their shelflife. Mango or tomato is also used instead of cucumber for pickle preparation.

Major ingredients

Cucumber, salt , spice,and dill(a annual herb their leaves, seeds are used as a spice, flavouring -taste and aroma- smell of pickle) etc.

fermenting microbes- *Lactobacillius plantarum, L. Brevis. Pediococcus sp* and *Leuconostoc mesentroides*..

Bread

Bread is a stable food which is prepared by baking, it is made by using (ingredients) wheat flour, water,salt and a leavening agent the yeast *Saccharomyces cerevisiae*, which converts the fermentable sugars present in dough to carbon dioxide, it causes dough to expand or rise forms pockets or bubbles, when the dough is baked the pokets remain giving the bread product a soft and spongy texture.

Nutritive value of bread per 100 gm.

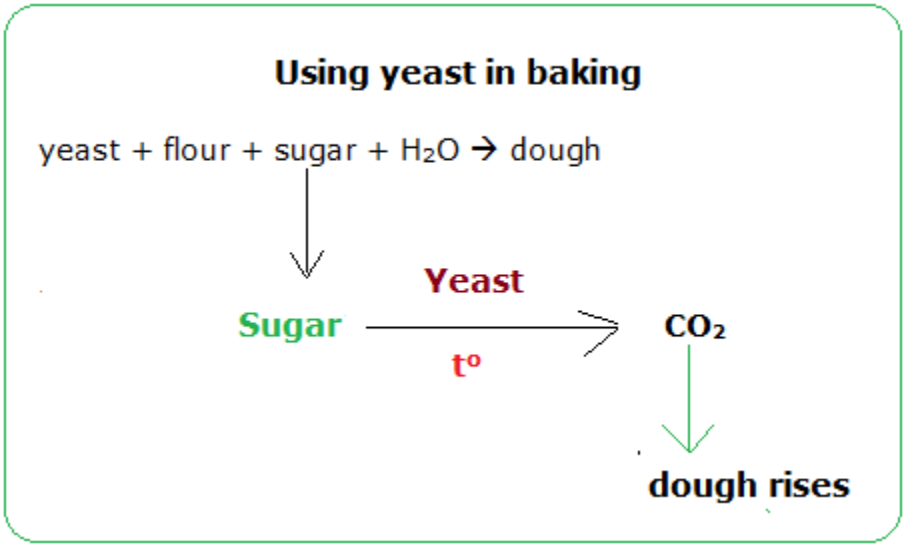
Carbohydrates 46gm, dietry fibre 7gm, fat 4gm, protein 10gm, vitamins (thiamine, riboflavin, niacin), sodium 0.5gm.

Maida – is a wheat flour, bleaching agents benzoyl peroxide, nitrogen dioxide, chlorine dioxide, , calcium peroxide----- maida ---- for parota and bakery products

Ingredients - for bread making

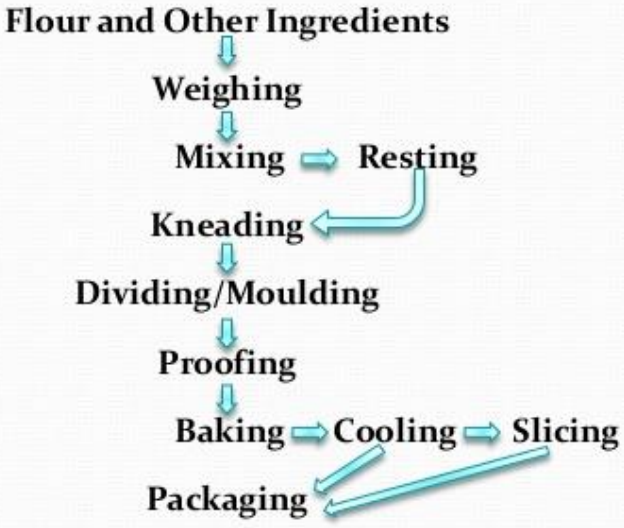
Wheat flour, Salt, water and ***Saccharomyces cerevisiae*** (Baker's Yeast), other ingredients include milk or milk solids, oxidants, surfactants, enzymes and additives for fungus protectans.

Preparation – The wheat flour contains fructose, glucose and wheat protein gluten, now the flour is added with yeasts(2%) and water , salt(1-2%) , mix well, the mixture is dough allowed it for few hours at 25 C, during fermentation the the yeasts ferment the sugar into ethanol and carbon dioxide which inflates the dough and lighter, finer, viscoelastic that retains gas and texture,



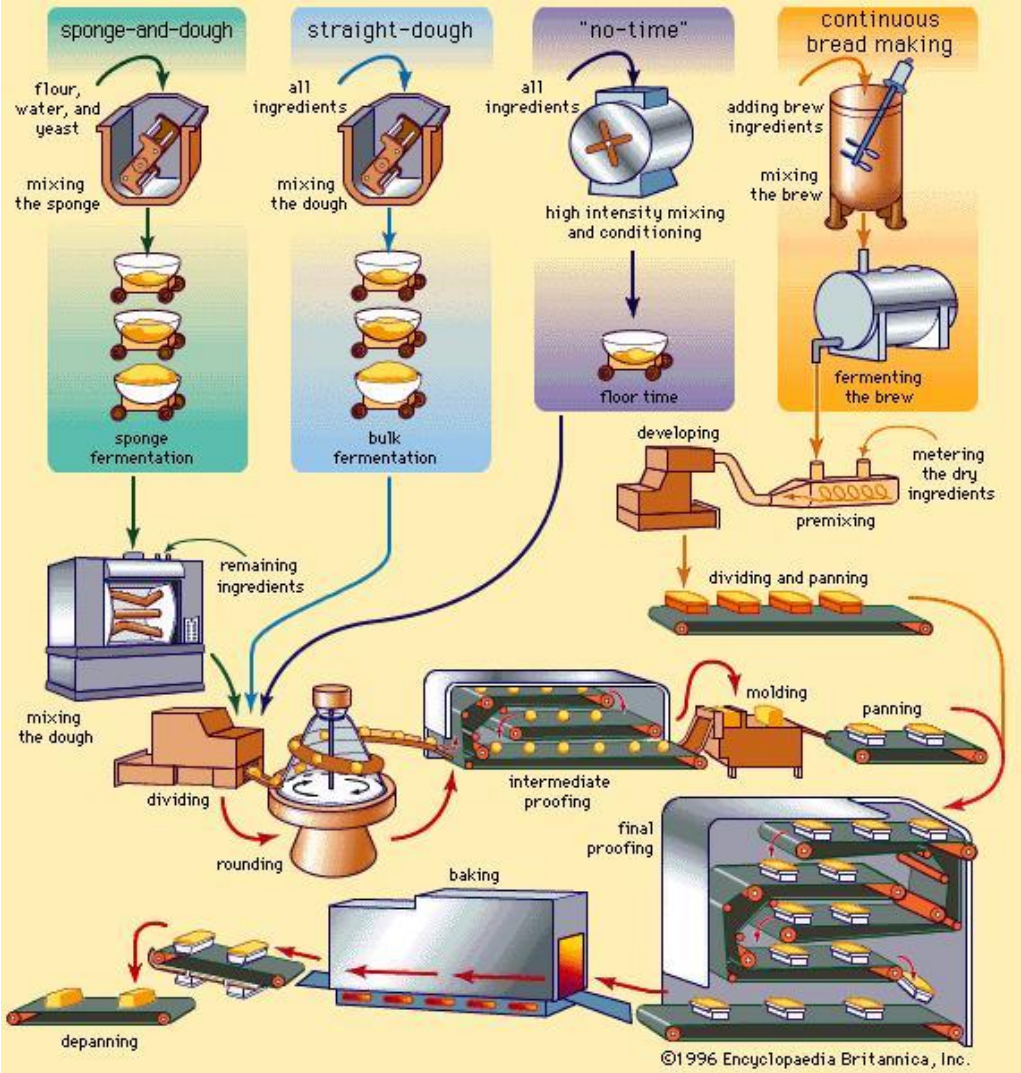
Kneadig means squeezing of dough wth the help of hand, dividing means cutting into small pieces, moulding means dough is made into correct shape.

Bread Production Flow Chart



Golden Penny Products

"A promise of quality"



Bakers yeast producton

Now the dough is divided and rounded and placed in tin and baked at 220 C for 35 minutes in a oven. Remove the loaf or bread from the tin once it is baked and return it to the oven for five minutes for a good crust to all over. Dry yeast means yeasts is preserved by lyophilization.

Bread is cut into pieces and wrapped in plastic film and stored in air tight containers.

Bread is not preserved properly it is spoiled by *Rhizopus sp, Aspergillus sp and Penicillium* sp,

Figure : Shows flow diagram for bread making

Baker's Yeast- it means pure strain of ***Sacchromyces cerevisiae*** is used for bakers yeast making.

Baker's yeast producton

Medium – It contains molasses as the carbon and energy source,

Fermentors - flask culture – small sized aerated fermentor – intermediate fermentor – large size fermentor (40,000 – 200,000 litres capacity)

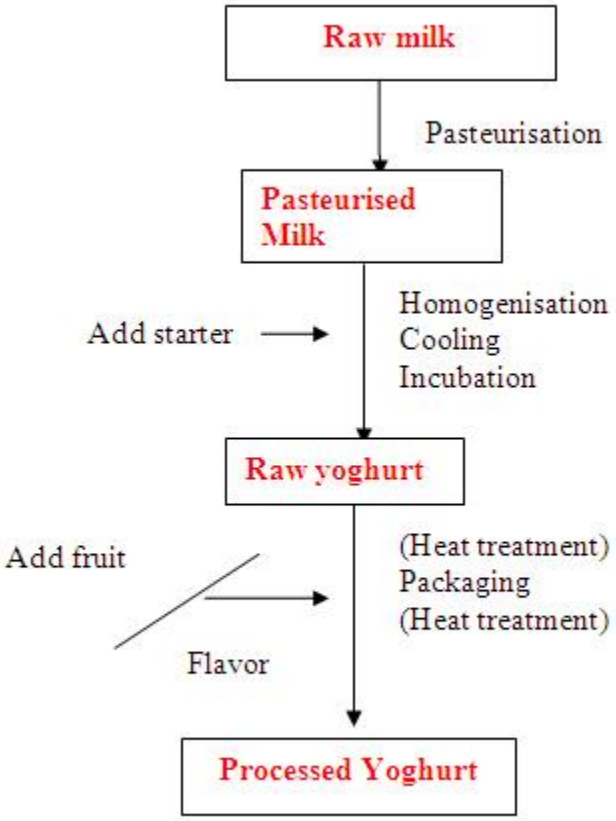
Oxygen supply – culture shoud be aerated with oxygen

Harvesting - at the end of fermentation the yeasts cells are harvested by vaccum filteration,

Bakers yeasts are sold as fresh yeast cake, and marketed as a food supplement.

Yoghurt. Or curd or dahi

It is a thick , sour milk product produced by starter cultures *Streptococcus salivarius* subsp *themophilus* and *Lctobacillus bulgaricus*, the coccus grows faster than the rod, The milk is heated to 82-93C for 30 minutes. This temperature will kill contaminating microbes, After cooling to 45C, the milk is inoculated with mixed culture of starter cultures.the ratio is 1:1., the inoculated milk is incubated at 45C for 3-5 hours,

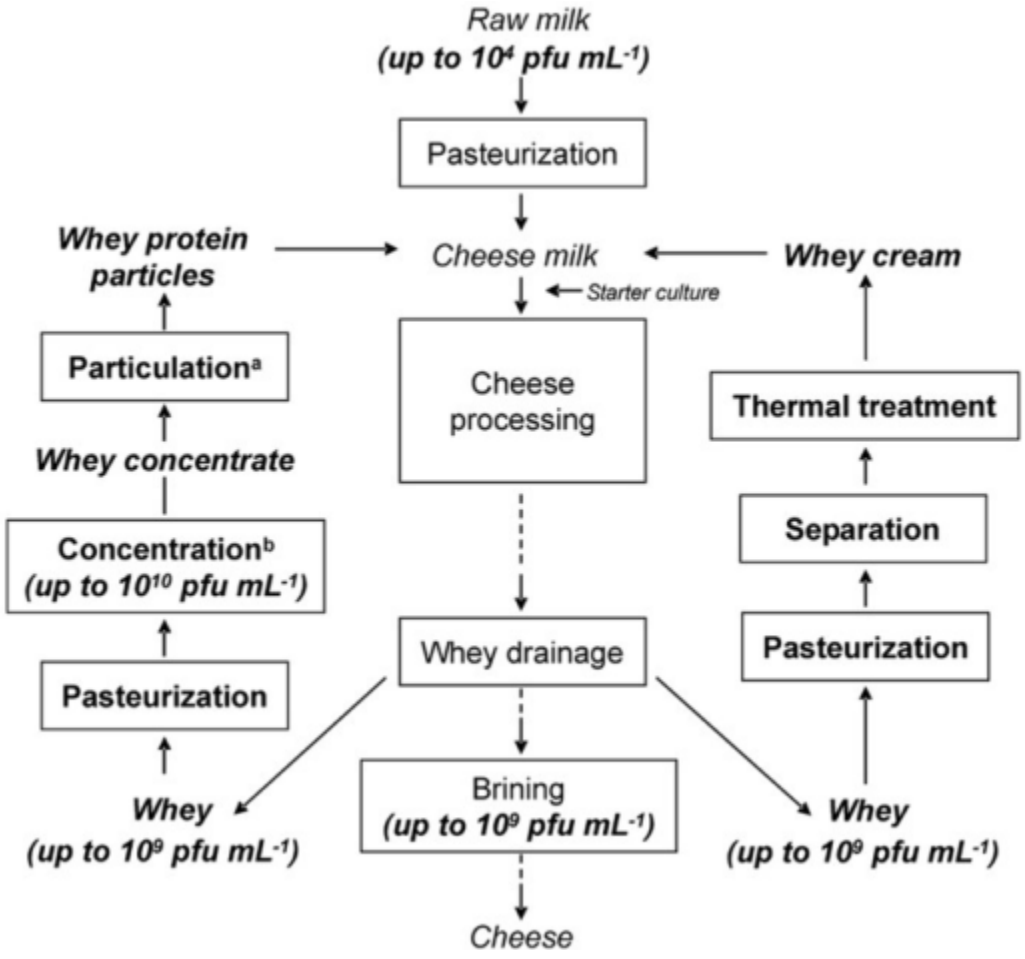


The volume of inoculam is 2-3 spoons of previous batch curd per liter of prepared milk are used. The inoculated milk is poured into the individual pots before fermentation, sometimes mold inhibitors or dye is added to increase the shelf life. The shelf life of yoghurt is usually 3-8 days at below 10^0 C. Freshly prepared curd contains 10^9 bacteria per gram. The shelf life of yoghurt is usually up to 60 days at 5 ^{0}C.

The yoghurt made from skimmed cow or buffalo milk containing probiotic bacteria(bacteria that inhibit the growth of bad bacteria) *Lactobacillus acidophilus* and *Lctobacillus casei* exhibit three important effects on humans., it delays the progression of diabetes, lowers bad cholestrol and raises the good cholestrol level.

Cheese

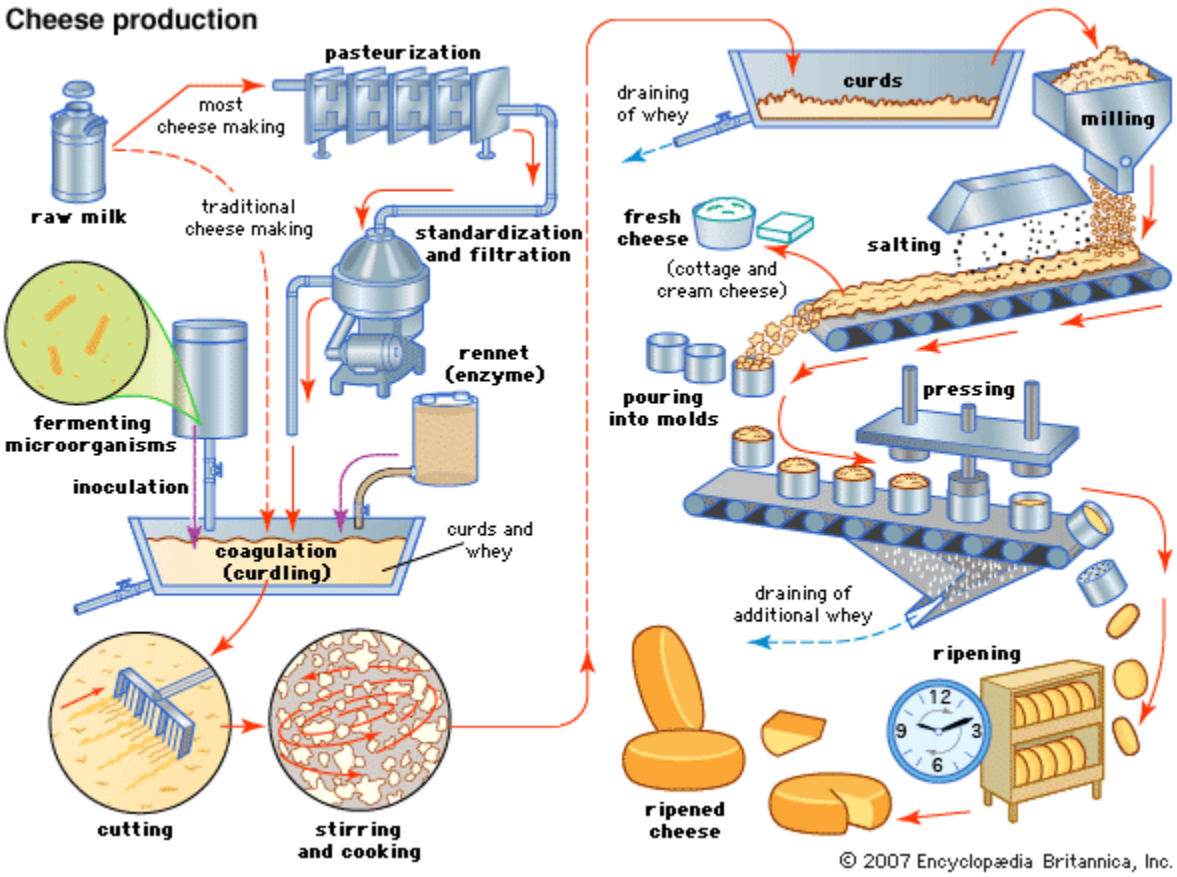
Cheese is a concentrated form of milk protein , it is formed by the combined action of lactic acid bacteria and enzyme rennin or rennet (an enzyme from calves stomach) and the bacterial enzyme.



The bacteria ferment the milk sugar lactose into lactic acid , this acid will coagulate milk protein casein to form a solid portion is called as cheese and liquid portion is called as whey. All cheeses are salted it means adding of salt , salting performs three functions it will remove

the water , prevents the growth of undesirable microbes and contributes to the flavour of the cheese. Two types of cheeses are made , 1, hard cheese (Chedder and Edam) - it is prepared after complete removal of whey and pressed. 2, Soft cheese or Panneer cheese it contains little quantity of whey and are not pressed .

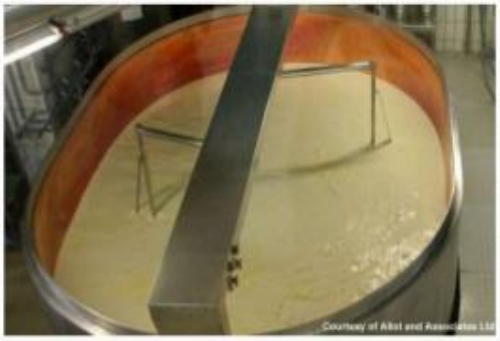
Swiss cheese – it is a hard cheese having high moisture, fat is 43%, prepared from skimmed milk or coagulated with acid and rennet. Milk is first pasteurized and inoculated with starter culture *Streptococcus thermophilus* and *Lactobacillus helveticus* and incubated at 30 C, It is followed by the addition of **rennet** (a protease – an enzyme from calves stomach) and incubated for formation of firm curd, that is cutted into small pieces using copper wires called **cheese harp**. Most cheeses are stored at refrigerated temperature. The milk curd is basically an unripened cheese , it may be called as **cottage cheese**, **pot cheese** or **ricotta cheese**.



CHEESE PRODUCTION

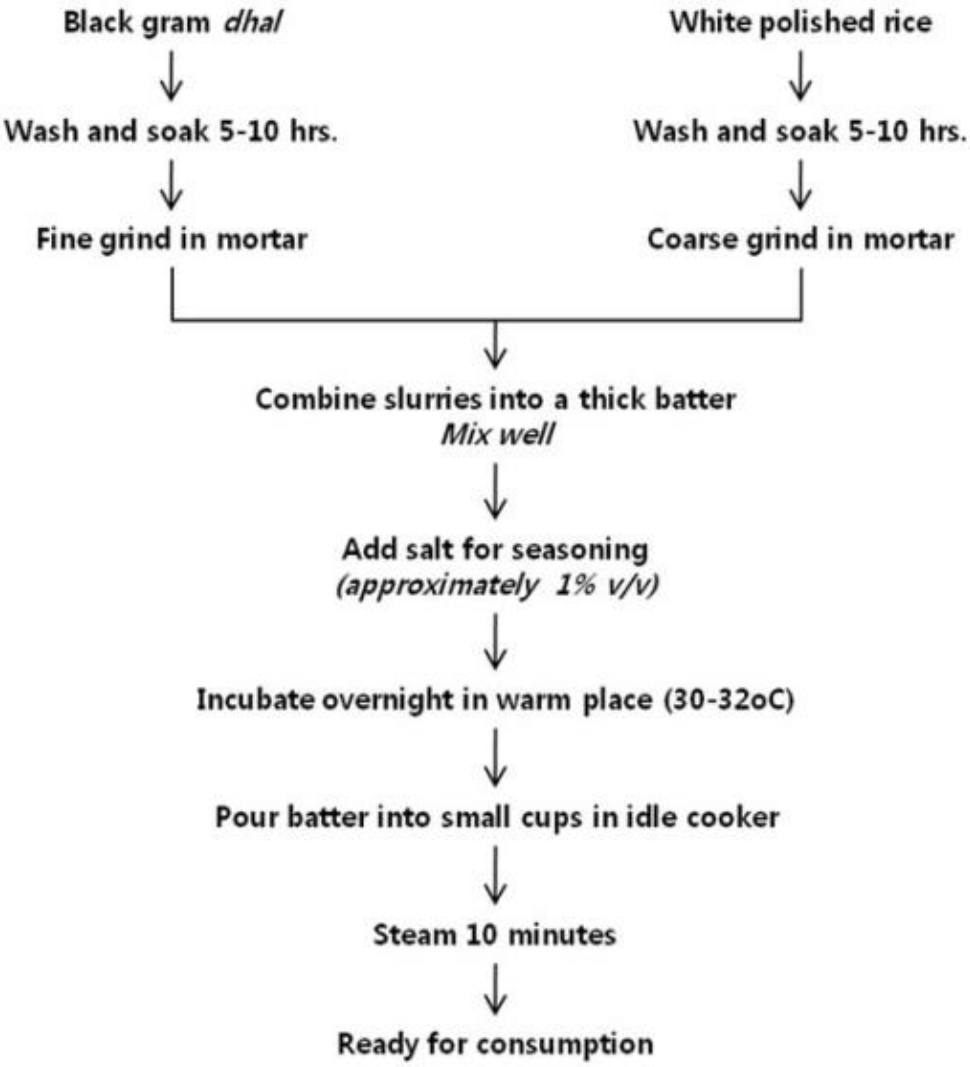
Virtually all cheese is made by coagulating milk protein (casein) in a manner that traps milk solids and milk fat into a curd matrix. This curd matrix is then consolidated to express the liquid fraction, cheese whey. Cheese whey contains those milk solids which are not held in the curd mass, in particular most of the milk sugar (lactose) and a number of soluble proteins.

- **Milk receipt, pre-treatment and standardisation**
- **Pasteurisation**
- **Addition of starter culture**
- **Coagulation**
- **Extraction of whey**
- **Cutting and cooking of curd**
- **Salting**
- **Ripening**
- **Packaging**
- **Distribution**



10

Idli – is a fermented food of India, is made from rice and black gram mungo in equal parts, the ingredients are washed and soaked separately, ground mixed and finally allowed to ferment overnight. *Leuconostoc mesentroides* grows first in the batter, leavening it , and is followed by *Streptococcus faecalis* and *Pediococcus cerevisiae*, When the batter has risen enough, it is cooked by steaming and served hot


Black gram *dhal*
White polished rice
Wash and soak 5-10 hrs.
Wash and soak 5-10 hrs.
Fine grind in mortar
Coarse grind in mortar
Combine slurries into a thick batter
Mix well
Add salt for seasoning
(approximately 1% v/v)
Incubate overnight in warm place (30-32oC)
Pour batter into small cups in idle cooker
Steam 10 minutes
Ready for consumption

Tempeh

Tempeh is a fermented soybean product of Indonesian origin. It consists of cooked, dehulled whole soybeans which have been fermented by **Rhizopus** moulds. It is a moist solid cake with a mild, pleasant taste . It is usually sliced, dipped into a salt solution and deep-fried in oil. The traditional product is highly perishable and is usually consumed the day it is made. In industrial production, it can be preserved by drying or freezing (after blanching to inactivate the mould and its enzymes.)

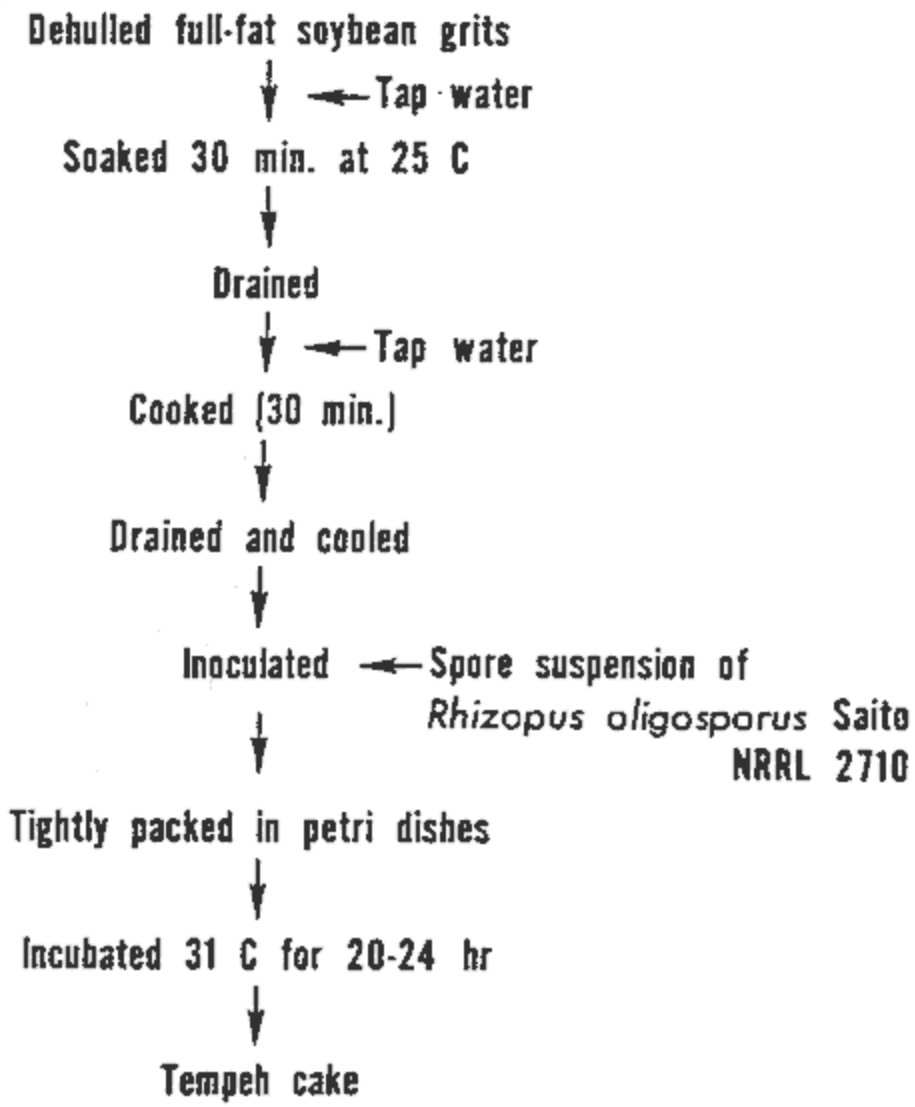
Production methods vary as with most traditional foods. In a typical process, soybeans are soaked in water, dehulled and then cooked in boiling water for one hour. After draining the soybeans are spread out for air-drying of the superficial moisture. Tempeh from the previous day is used as a starter. The prepared soybeans are thoroughly mixed with the starter, wrapped in banana leaves and left to ferment for one to two days. Mould growth is vigorous and the whole mass is soon covered and bound together by Rhizopus mycelium.

Typically, tempeh contains 35% dry matter, half of which is protein. Unlike the previously discussed fermented soy products, tempeh does not contain salt.

Tempeh is still, predominantly a home-made or cottage-industry product in Indonesia and Malaysia. It is practically unknown in Japan. However , it has been introduced to the U.S.A. and to Europe, mainly for the population of Indonesian origin and for the rapidly expanding Indonesian restaurant trade. It is made in modern, sanitary plants, using stainless steel equipment and sometimes, pure cultures of mould.

The traditional banana leave is replaced by suitable plastic materials or foil, perforated to provide the moderate aeration necessary for mould growth without excessive sporulation. Commercial dehulled full-fat grits can be used instead of whole soybeans.

A diagram describing a modern variation of the tempeh production process, developed at the Northern Regional Research Centre, in Peoria, Illinois, is shown in Fig


Dehulled full-fat soybean grits
Tap water
Soaked 30 min. at 25 C
Drained
Tap water
Cooked (30 min.)
Drained and cooled
Inoculated
Spore suspension of
Rhizopus oligosporus Saito
NRRL 2710
Tightly packed in petri dishes
Incubated 31 C for 20-24 hr
Tempeh cake

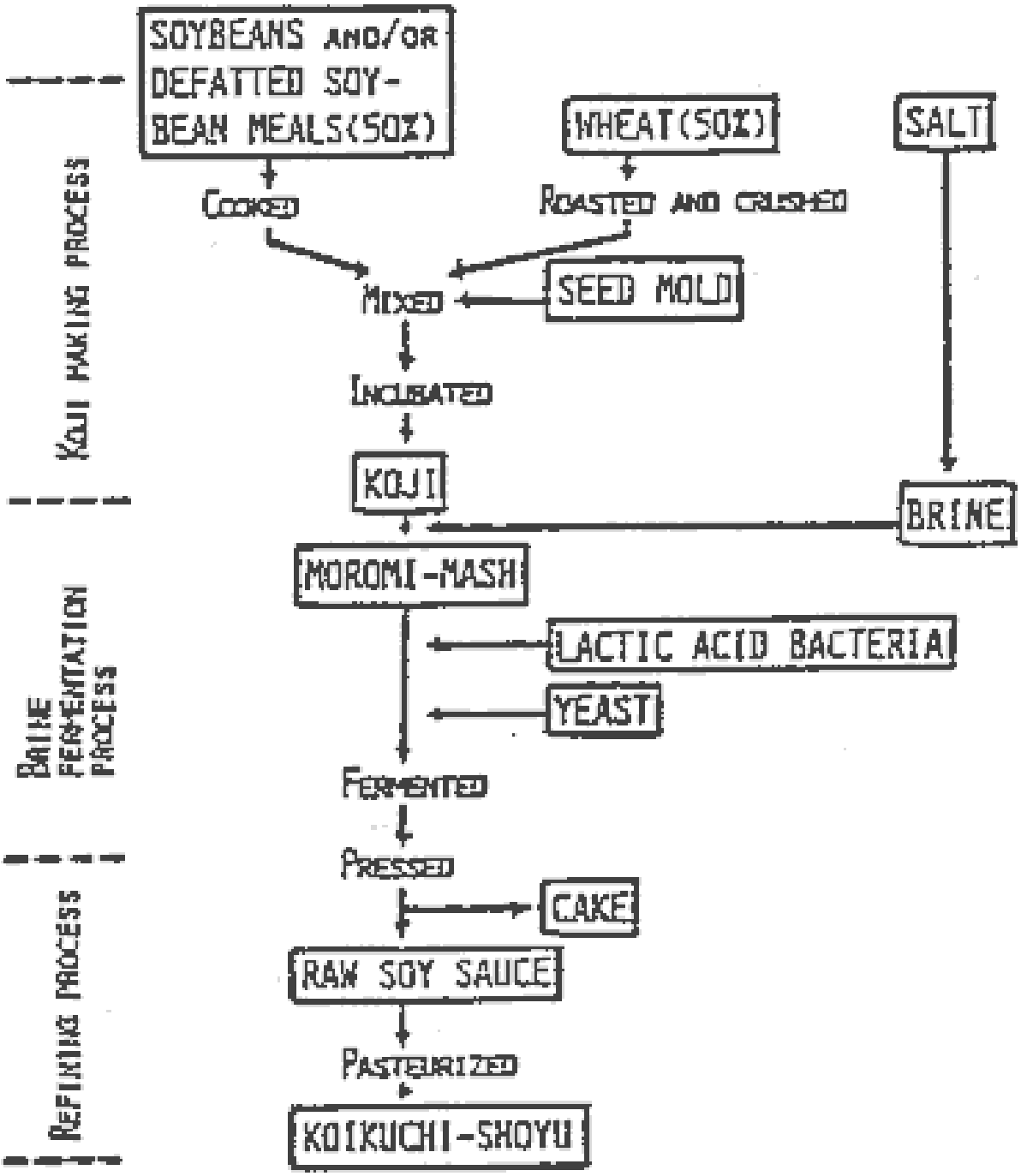
Soy sauce

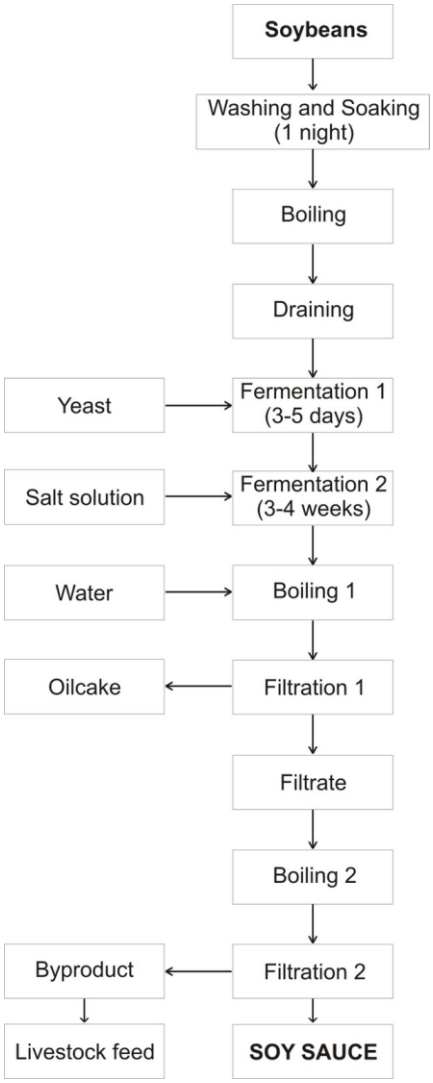
Soy sauce is a dark brown salty liquid with a peculiar aroma and a meaty taste. It is the chief savory seasoning agent in Oriental cookery, but it is becoming increasingly popular in many other regions of the world. Once a home-made staple, it has become an important industrial product. Industrialization has altered the production process, changed the raw materials used, standardized the products and modified somewhat their characteristics.

The main ingredients of soy sauce are soybeans (or defatted soybean meal), wheat, salt and water. The heart of the manufacturing process is a complex fermentation whereby the carbohydrates are fermented to alcohol and lactic acid and the proteins are broken down to peptides and amino acids. Chemical reactions between the original components and the fermentation products create the colour , consistency and aroma of soy sauce. Formulations and processing conditions differ from one country to another and in function of the type of soy sauce to be produced.

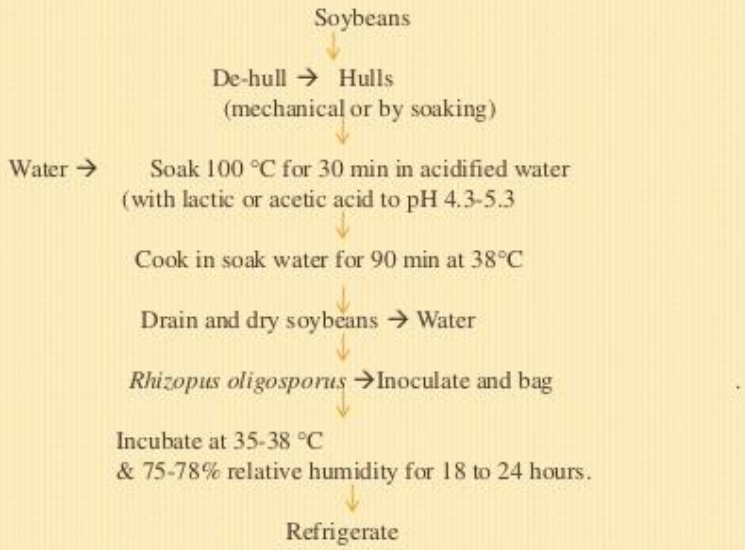
The process of manufacturing soy sauce can be divided into three stages: preparation of the "koji", fermentation in brine and product refining.

Fig.42 describes the basic outline of the manufacturing process of "koikuchi" sauce, a representative type of Japanese soysauce. Soybeans and/or defatted soybean meal are cooked in continuous pressure-cookers and mixed with roasted and coarsely broken wheat. The mass is inoculated with a mould- Aspergillus orizae (or A. sojae) and incubated in shallow vats with perforated bottoms and air is forced through the mass. After three days of incubation under controlled conditions of temperature and moisture, mould growth covers the entire mass which turns greenish as a result of sporulation. This mass is called "**koji"**. Koji is the essential ingredient of most fermented products of the Orient. It is a concentrated source of amylolytic and proteolytic enzymes necessary for the decomposition of the carbohydrates and proteins.


SOYBEANS AND/OR DEFATTED SOY-BEAN MEALS(50%)
WHEAT(50%)
SALT
COOKED
ROASTED AND CRUSHED
MIXED
SEED MOLD
INCUBATED
KOJI
BRINE
MOROMI-MASH
LACTIC ACID BACTERIA
YEAST
FERMENTED
PRESSED
CAKE
RAW SOY SAUCE
PASTEURIZED
KOIKUCHI-SHOYU
KOJI MAKING PROCESS
BRINE FERMENTATION PROCESS
REFINING PROCESS


Soybeans
Washing and Soaking (1 night)
Boiling
Draining
Yeast
Fermentation 1 (3-5 days)
Salt solution
Fermentation 2 (3-4 weeks)
Water
Boiling 1
Oilcake
Filtration 1
Filtrate
Boiling 2
Byproduct
Filtration 2
Livestock feed
SOY SAUCE

FLOW CHART


Soybeans
De-hull → Hulls
(mechanical or by soaking)
Water → Soak 100 °C for 30 min in acidified water
(with lactic or acetic acid to pH 4.3-5.3
Cook in soak water for 90 min at 38°C
Drain and dry soybeans → Water
Rhizopus oligosporus →Inoculate and bag
Incubate at 35-38 °C
& 75-78% relative humidity for 18 to 24 hours.
Refrigerate

Tempeh

Food

Tempeh is a traditional soy product originating from Indonesia. It is made by a natural culturing and controlled fermentation process that binds soybeans into a cake form. Wikipedia

Nutrition Facts

Tempeh Tempeh

Amount Per 100 grams 100 grams

Calories 193

% Daily Value*

Total Fat 11 g	16%
Saturated fat 2.2 g	11%
Polyunsaturated fat 3.8 g	
Monounsaturated fat 3 g	
Cholesterol 0 mg	0%
Sodium 9 mg	0%
Potassium 412 mg	11%
Total Carbohydrate 9 g	3%
Protein 19 g	38%

Vitamin A	0%	Vitamin C	0%
Calcium	11%	Iron	15%
Vitamin D	0%	Vitamin B-6	10%

Vitamin B-12 1% Magnesium 20%

*Per cent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

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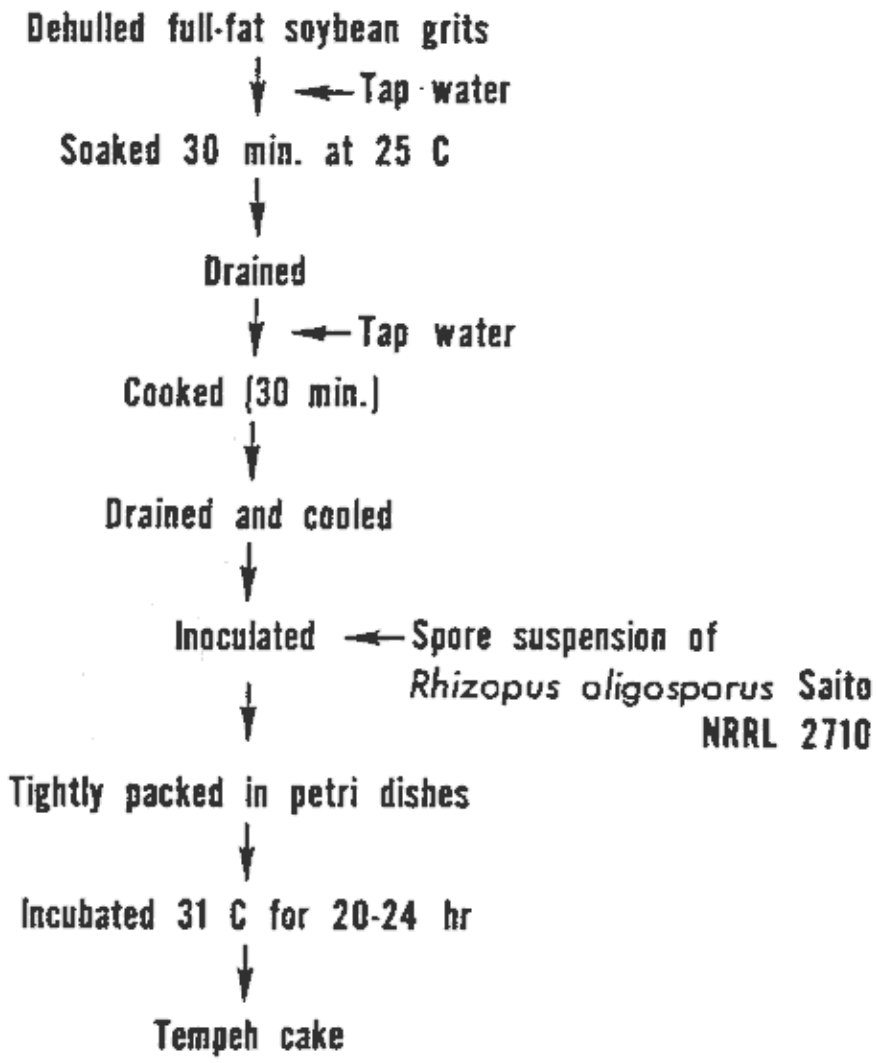
Tofu

Wheat gluten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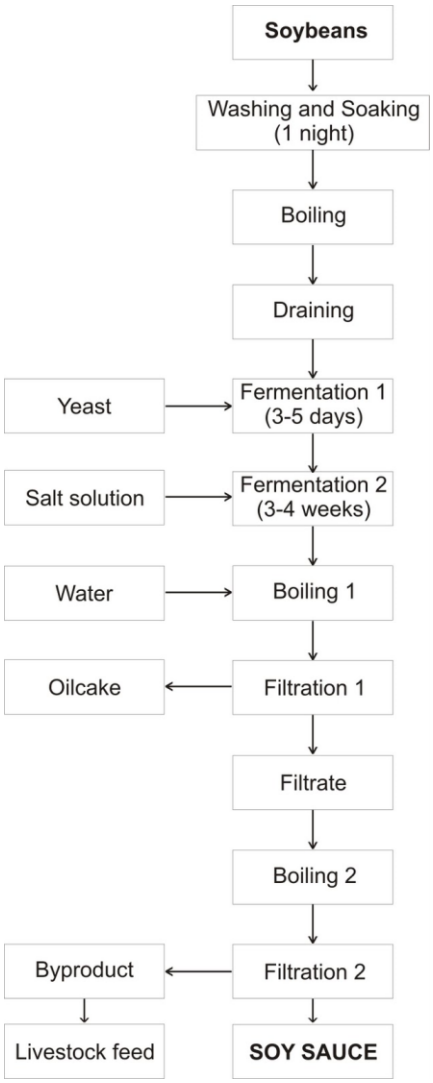
Soybeans
Washing and Soaking (1 night)
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Yeast
Fermentation 1 (3-5 days)
Salt solution
Fermentation 2 (3-4 weeks)
Water
Boiling 1
Oilcake
Filtration 1
Filtrate
Boiling 2
Byproduct
Filtration 2
Livestock feed
SOY SAUCE

Soy Sauce(soy&wheat)

Nutrition Facts

Serving Size 1 ounce (28g)

Amount Per Serving

Calories 15	Calories from Fat 0
	% Daily Value*
Total Fat 0g	0%
Saturated Fat 0g	0%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 1578mg	66%
Total Carbohydrate 2g	1%
Dietary Fiber 0g	1%
Sugars 0g	
Protein 2g	

Vitamin A	0%	• Vitamin C	0%
Calcium	1%	• Iron	3%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

NutritionData.com

5. Japanese soy sauce

- Japanese soy sauce (Japanese Shoyu)

  : Major sources are soybean and wheat.

- Making Japanese soy sauce

  : Koji making (Aerobic growth of fungi to produce hydrolyzing enzymes)

  + Fermentation & Maturation (lactic acid bacteria + yeast)

  : Koji → enzyme sources for hydrolysis of protein and carbohydrates in soybean

  M/O : *Aspergillus oryzae* or *A. sojae*

- Types of Japanese soy sauce

① Koikuchi type : more than 90% of Japanese soy sauce. Dark color

- Source : mixture of the same amount of soybean and wheat
- Hydrolysis : A. oryzae
- Fermentation & maturation : lactic acid and alcohol fermentation
- Sterilization : High temperature condition

② Usukuchi type : less than 10%. 1.2% total nitrogen content. Bright color

Nutrition Facts

Serving Size 226 g

Amount Per Serving	
Calories 773	Calories from Fat 92
	% Daily Value*
Total Fat 10.3g	**16%**
Saturated Fat 1.9g	**9%**
Cholesterol 0mg	**0%**
Sodium 914mg	**38%**
Potassium 290mg	**8%**
Total Carbohydrates 151.6g	**51%**
Dietary Fiber 3.0g	**12%**
Sugars 2.2g	
Protein 14.5g	
Vitamin A 3% •	Vitamin C 5%
Calcium 7% •	Iron 47%
Nutrition Grade B+	

* Based on a 2000 calorie diet

CHAPTER 9
TOFU, TEMPEH, SOYSAUCE AND MISO

9.1 Introduction

The differences in consumption patterns for soy protein foods between the West and the Orient were outlined by D. Fukushima of Kikkoman Foods, Inc. (U.S.A.), who wrote: " There are significant differences in the use of soy proteins for human consumption in the U.S. and in the Orient. In the U.S., the soy proteins are used as ingredients of a wide variety of foods, e.g. hamburgers, sausages, meat loaves, dairy products, breads, pastries and cookies. In the Orient, however, soy proteins have been consumed for thousands of years, not as an ingredient, but as characteristic, unique soy protein foods." (Fukushima, 1981).

A detailed discussion of the multitude of traditional soy foods of the Orient is beyond the scope of the present work. Only four traditional products and their production technology will be described. These four products were selected, not only because of their leading position in the Orient, in terms of consumption levels, but also in view of the growing interest that they have recently created among Western consumers and food manufacturers.

Traditional soy protein foods are usually divided into two groups: fermented and non fermented. Miso, soysauce and tempeh are representatives of the first group. Tofu, together with soymilk discussed in the previous chapter, belong to the second. Nevertheless, fermented varieties of tofu and soymilk also exist.

It should be stressed that each one of the four foods is, in fact, a group in itself, comprising many distinct variations.

Historically, most traditional soy protein foods originated in China and were introduced later to other countries in East and Southeast Asia. At present, however, Japan occupies the leading position in this field, with respect to industrialization, technology development, equipment manufacture and worldwide marketing.

Soy sauce

Soy sauce is a dark brown salty liquid with a peculiar aroma and a meaty taste. It is the chief savory seasoning agent in Oriental cookery, but it is becoming increasingly popular in many other regions of the world. Once a home-made staple, it has become an important industrial product. Industrialization has altered the production process, changed the raw materials used, standardized the products and modified somewhat their characteristics.

The main ingredients of soy sauce are soybeans (or defatted soybean meal), wheat, salt and water. The heart of the manufacturing process is a complex fermentation whereby the carbohydrates are fermented to alcohol and lactic acid and the proteins are broken down to peptides and amino acids. Chemical reactions between the original components and the fermentation products create the colour , consistency and aroma of soy sauce. Formulations and processing conditions differ from one country to another and in function of the type of soy sauce to be produced.

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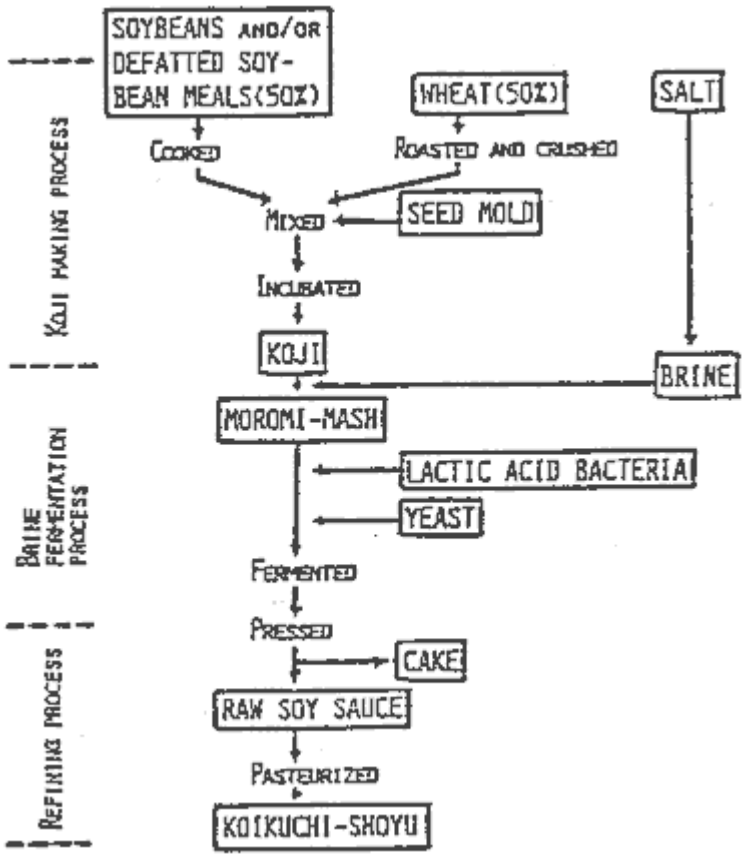

Figure 42: Production of "koikuchi" Soysauce Source:

Fukushima (1981)

The koji is mixed with a brine containing 22 to 25 % salt (weight by volume) and transferred to deep fermentation tanks.Lactic acid bacteria and osmophilic yeast cultures are added and the slurry (called "moromi-mash) is allowed to ferment at controlled temperature and occasional aeration. The high salt concentration effectively inhibits growth of undesirable "wild" microorganisms. The starch is transformed to sugars which are fermented to lactic acid and alcohol. The pH drops from near-neutral to 4.7- 4.8. The moromi is held in the fermentation tanks for 6 to 8 months.

In the refining stage, the fermented mash is pressed to separate the sauce from the solid residue. the sauce is filtered, clarified and heated to 70-80^{o} C. Heating is necessary to pasteurize the sauce and to develop the characteristic colour and aroma. After final clarification the sauce is bottled.

The use of defatted soybean meal instead of whole soybeans is justified, since most of the oil is lost in the residue.

Tamari soy sauce is popular in Southeast Asia. It contains a much lower proportion of wheat and therefore less alcohol in the final product.

In modern soy sauce plants, all the steps of the process , including the making of koji, are mechanized and the process conditions are fully controlled. Material losses are minimized through recovery operations. Thus, 90% of the raw material nitrogen is recovered in the product.

Soy sauce typically contains 28% soluble solids (18% salt and 7% protein).

Tempeh

Tempeh is a fermented soybean product of Indonesian origin. It consists of cooked, dehulled whole soybeans which have been fermented by **Rhizopus** moulds. It is a moist solid cake with a mild, pleasant taste . It is usually sliced, dipped into a salt solution and deep-fried in oil. The traditional product is highly perishable and is usually consumed the day it is made. In industrial production, it can be preserved by drying or freezing (after blanching to inactivate the mould and its enzymes.)

Production methods vary as with most traditional foods. In a typical process, soybeans are soaked in water, dehulled and then cooked in boiling water for one hour. After draining the soybeans are spread out for air-drying of the superficial moisture. Tempeh from the previous day is used as a starter. The prepared soybeans are thoroughly mixed with the starter, wrapped in banana leaves and left to ferment for one to two days. Mould growth is vigorous and the whole mass is soon covered and bound together by Rhizopus mycelium.

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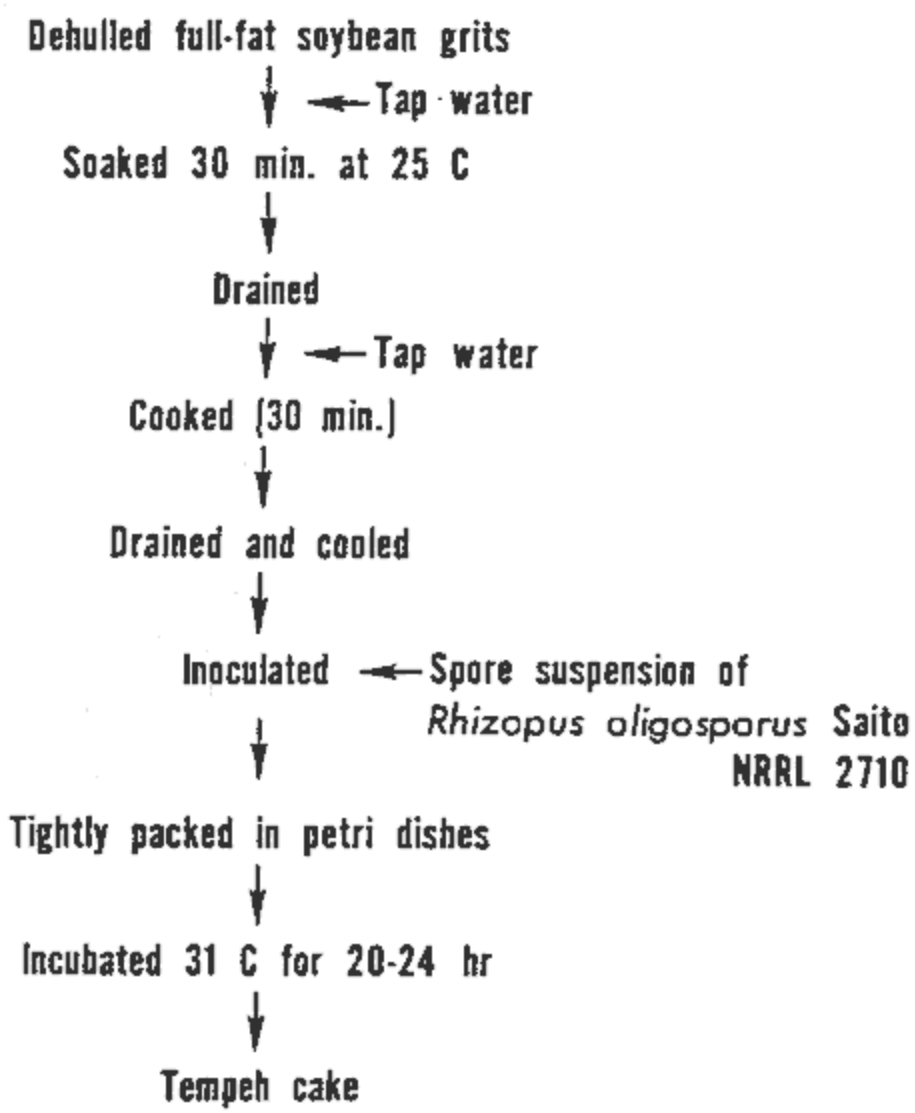

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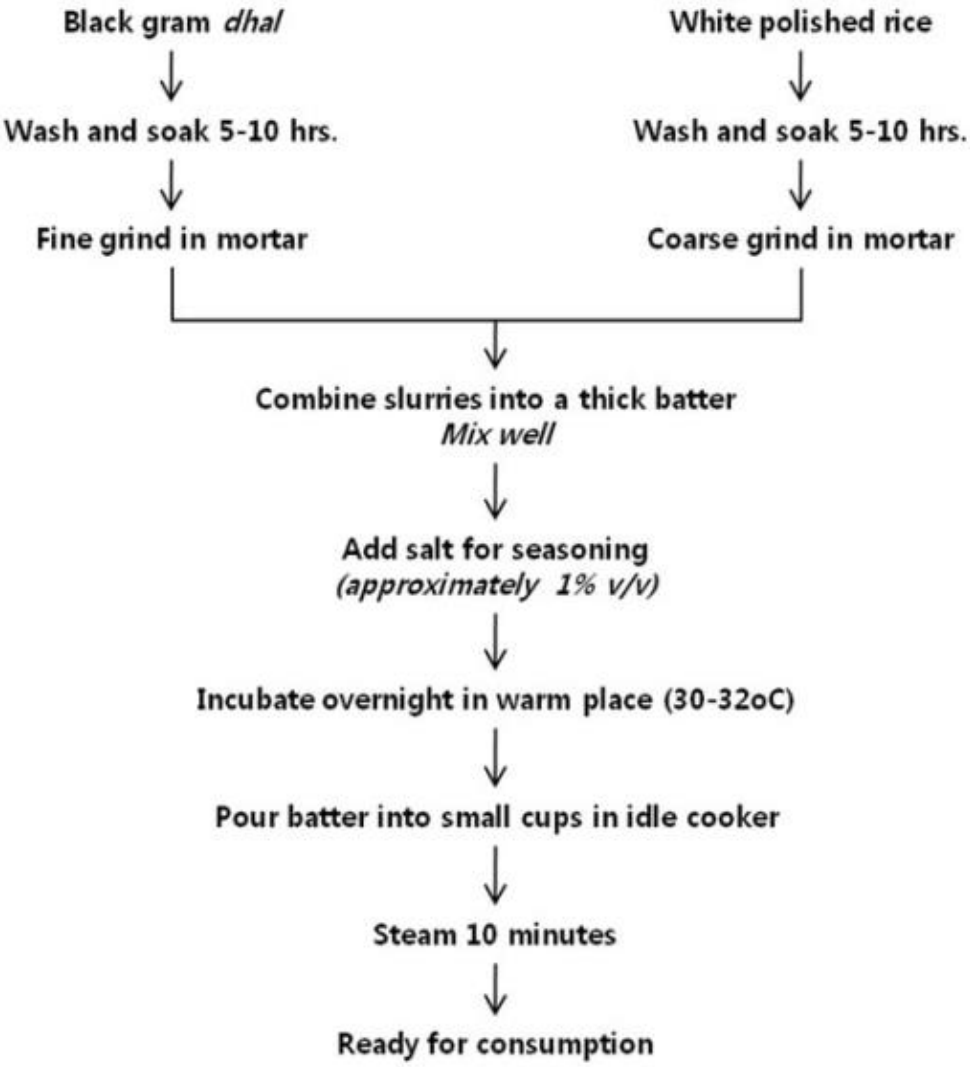
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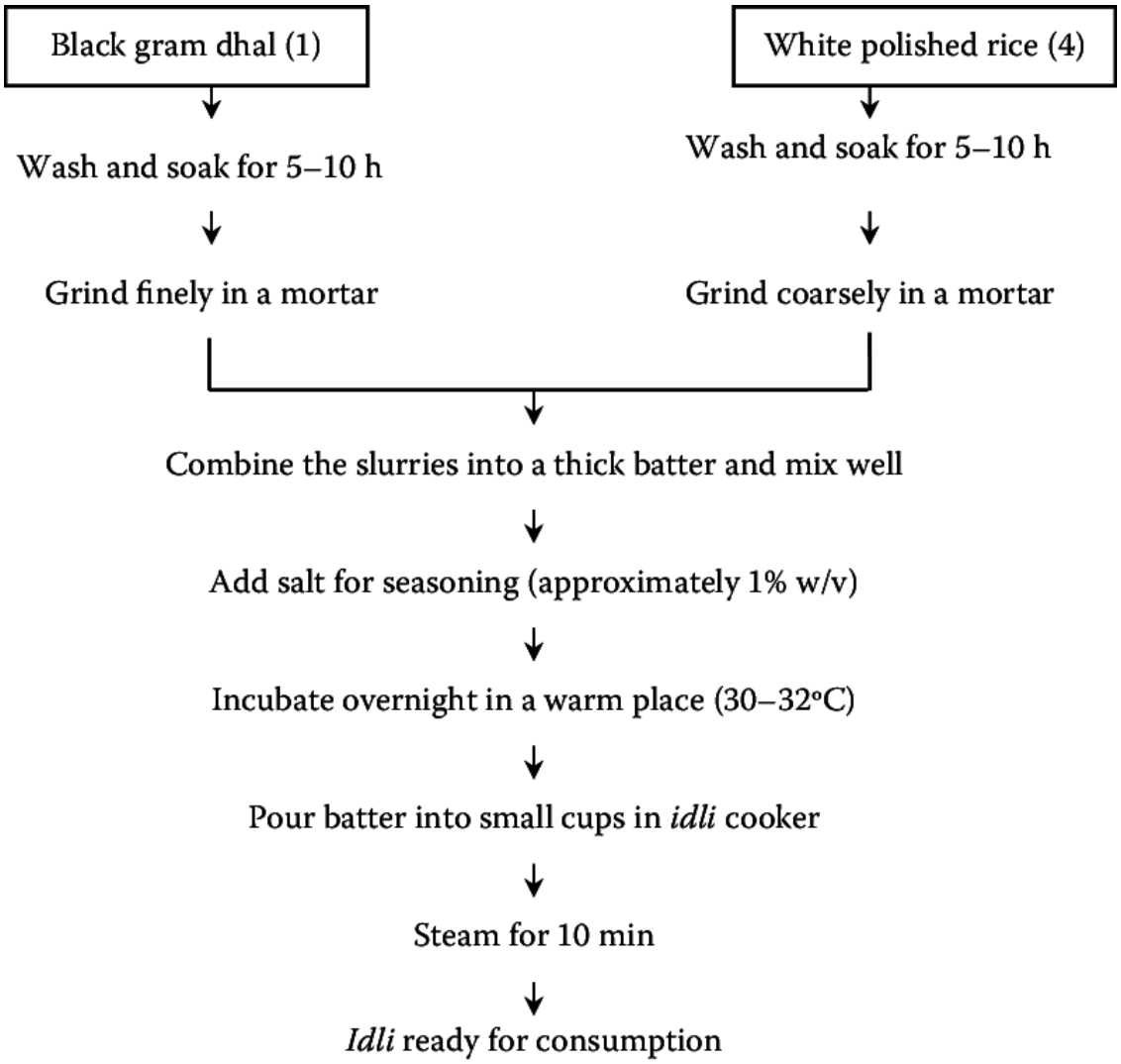
Incubate overnight in warm place (30-32oC)
Pour batter into small cups in idle cooker
Steam 10 minutes
Ready for consumption



Spoilage of Canned Foods (With Diagram) | Microbiology

If a canned food contains viable micro-organisms capable of growing in the product at ambient temperatures, then it will spoil. Organisms may be present as a result of an inadequate heat process, under processing, or of post process contamination through container leakage. Spoilage by a single spore former is often diagnostic of under processing since rarely would such a failure be so severe that vegetative organisms would survive.

A normal sound can will either be under vacuum with slightly concave ends or have flat ends in those cases where the container is brimful. Spoilage often manifests itself through microbial gas production which causes the ends to distend and a number of different terms are used to describe the extent to which this has occurred (Table 4.6).

Table 4.6 *Description of blown cans*

Name	*Description*
Flat	No evidence of swelling.
Hard swell	Both ends of the can are permanently and firmly bulged and do not yield readily to thumb pressure.
Soft swell	Both ends bulged but not tightly; they yield to thumb pressure.
Springer	One end flat, the other bulged. When the bulged end is pressed in then the flat one springs out.
Flipper	A can with a normal appearance which when brought down sharply on a flat surface causes a flat end to flip out. The bulged end can be forced back by very slight pressure.

The spore-forming anaerobes Clostridium can be either predominantly proteolytic or saccharolytic but both activities are normally accompanied by gas production causing the can to swell. Cans may sometimes swell as a result of chemical action. Defects in the protective lacquer on the inside of the can may allow the contents to attack the metal releasing hydrogen. These hydrogen swells can often be distinguished from microbiological spoilage since the appearance of swelling occurs after long periods of storage and the rate at which the can swells is usually very slow.

In cases where microbial growth occurs without gas production, spoilage will only be apparent once the pack has been opened. Bacillus species, with the exceptions of B. macerans and B. polymyxa, usually break down carbohydrates to produce acid but no gas giving a type of spoilage known as a ‘flat sour’, which describes the characteristics of both the can and the food.

The heat process a product receives is determined largely by its acidity: the more acidic a product is, the milder the heat process applied. Although more complex schemes have been described, the essential classification of canned foods is into low acid (pH > 4.5, or 4.6 in the United States) and acid foods (pH < 4.5 or 4.6).

We have already seen how this is applied to assure safety with the requirement that products with a pH > 4.5 must undergo a botulinum cook to ensure 12 decimal reductions of C. botulinum spores. This is not a concern in acid foods and the F_0 applied to products with a pH in the range 4.0-4.5 such as canned tomatoes and sopie canned fruits is generally 0.5-3.0. In higher acidity products such as canned citrus fruits (pH < 3.7) the heat process is equivalent only to a pasteurization.

A product's acidity also determines the type of spoilage that may result from under-processing since it can prevent the growth of some spoilage organisms. At normal ambient temperatures (< 38 °C) only mesophilic species will grow. Typical examples would be C. botulinum, C. sporogenes and B. subtilis in low acid products and C. butyricum and C. pasteurianum in products with a pH below 4.5.

Cans are cooled rapidly after processing to prevent spoilage by thermophiles. Thermophilic spores are more likely to survive the normal heat process but would not normally pose a problem. If however a large assemblage of cans is allowed to cool down naturally after retorting, the process will be slow and the cans will spend some time passing through the thermophilic growth range.

Under these conditions surviving thermophilic spores may be able to germinate and grow, spoiling the product before it cools. This may also occur if cans are stored at abnormally high ambient temperatures (>40 °C) and canned foods destined for very hot climates may receive a more stringent process to reduce thermophilic spoilage.

Thermophilic organisms commonly associated with spoilage of low acid canned foods are the saccharolytic organism C. thermosaccharolyticum, B. stearothermophilus and Desulfotomaculumnigrificans. The last of these causes a type of spoilage known as 'sulfur stinker'. It produces hydrogen sulfide which does not usually distend the can but does give the product an objectionable smell and reacts with iron from the can to cause blackening.

Leakage is the most common cause of microbiological spoilage in canned foods. Cans are the most common containers used for retorted products, although glass jars, rigid plastic containers and soft pouches are also sometimes used. Cans are usually made of two or three parts: the three-

part can consists of a base, body and lid while in two part cans the body and base are made from a single piece of metal.

In a three-part can the body seam is electrically welded but the lid on all cans is held in place by a double seam (Figure 4.7). The correct formation and integrity of this seam are crucial to preventing leakage and monitoring seam integrity is an important aspect of quality control procedures in canning.

During processing cans are subjected to extreme stress, particularly when the hot can is cooled down rapidly from processing temperatures. The negative pressure created in the can under these conditions could lead to micro-organisms on the container's surface or in the cooling water being sucked inside through a small defect in the seam.

The defect in the hot can that allowed leakage to occur may seal up and be undetectable when the can is cool since leaker spoilage can cause cans to blow. Since the micro-organisms enter the can after processing there is no restriction on the type of organism capable of causing leaker spoilage, therefore the presence of a mixed culture or non-sporing organisms is almost certainly a result of can leakage.

To prevent leaker spoilage it is essential that the outside of cans is clean and uncontaminated and that chlorinated water is used to cool them. Failures in this respect have been the cause of a large typhoid outbreak in Aberdeen, Scotland where cans of corned beef made in the Argentine had been cooled with river water contaminated with Salmonella typhi and in an outbreak of botulism associated with canned salmon where the C. botulinum type E spores which were associated with the raw product contaminated the outside of the cans after processing and were sucked into one can during cooling.

There have been occasional reports of pre-process spoilage in canned foods where there was an unacceptable delay between preparing the product and heat processing. During this time spoilage may occur although the organisms responsible will have been killed by the heat process.

Hazard analysis and critical control points

Hazard analysis and critical control points or **HACCP** (/ˈhæsʌp/) is a systematic preventive approach to food safety from biological, chemical, and physical hazards in production processes that can cause the finished product to be unsafe, and designs measurements to reduce these risks to a safe level. In this manner, HACCP attempts to avoid hazards rather than attempting to inspect finished products for the effects of those hazards. The HACCP system can be used at all stages of a food chain, from food production and preparation processes including packaging, distribution, etc. The Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) require mandatory HACCP programs for juice and meat as an effective approach to food safety and protecting public health. Meat HACCP systems are regulated by the USDA, while seafood and juice are regulated by the FDA. All other food companies in the United States that are required to register with the FDA under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, as well as firms outside the US that export food to the US, are transitioning to mandatory hazard analysis and risk-based preventive controls (HARPC) plans. [1]

HACCP is believed to stem from a production process monitoring used during World War II because traditional "end of the pipe" testing on artillery shell's firing mechanisms could not be performed, and a large percentage of the artillery shells made at the time were either duds or misfiring.[2] HACCP itself was conceived in the 1960s when the US National Aeronautics and Space Administration (NASA) asked Pillsbury to design and manufacture the first foods for space flights. Since then, HACCP has been recognized internationally as a logical tool for adapting traditional inspection methods to a modern, science-based, food safety system. Based on risk-assessment, HACCP plans allow both industry and government to allocate their resources efficiently in establishing and auditing safe food production practices. In 1994, the organization of *International HACCP Alliance* was established initially for the US meat and poultry industries to assist them with implementing HACCP and now its membership has been spread over other professional/industrial areas.[3]

Hence, HACCP has been increasingly applied to industries other than food, such as cosmetics and pharmaceuticals. This method, which in effect seeks to plan out unsafe practices based on science, differs from traditional "produce and sort" quality control methods that do nothing to prevent hazards from occurring and must identify them at the end of the process. HACCP is focused only on the health safety issues of a product and not the quality of the product, yet

HACCP principles are the basis of most food quality and safety assurance systems. In the United States, HACCP compliance is regulated by 21 CFR part 120 and 123. Similarly, FAO/WHO published a guideline for all governments to handle the issue in small and less developed food businesses.[4]

History[edit]

In the early 1960s, a collaborated effort between the Pillsbury Company, NASA, and the U.S. Army Laboratories began with the objective to provide safe food for space expeditions. People involved in this collaboration included Herbert Hollander, Mary Klicka, and Hamed El-Bisi of the United States Army Laboratories in Natick, Massachusetts, Dr. Paul A. Lachance of the Manned Spacecraft Center in Houston, Texas, and Howard E. Baumann representing Pillsbury as its lead scientist.[5]

To ensure that the food sent to space was safe, Lachance imposed strict microbial requirements, including pathogen limits (including *E. coli*, *Salmonella*, and *Clostridium botulinum*).[5] Using the traditional end product testing method, it was soon realized that almost all of the food manufactured was being used for testing and very little was left for actual use. Therefore, a new approach was needed.

NASA's own requirements for critical control points (CCP) in engineering management would be used as a guide for food safety. CCP derived from failure mode and effects analysis (FMEA) from NASA via the munitions industry to test weapon and engineering system reliability. Using that information, NASA and Pillsbury required contractors to identify "critical failure areas" and eliminate them from the system, a first in the food industry then. Baumann, a microbiologist by training, was so pleased with Pillsbury's experience in the space program that he advocated for his company to adopt what would become HACCP at Pillsbury.[5]

Soon, Pillsbury was confronted with a food safety issue of its own when glass contamination was found in farina, a cereal commonly used in infant food. Baumann's leadership promoted HACCP in Pillsbury for producing commercial foods, and applied to its own food production. This led to a panel discussion at the 1971 National Conference on Food Protection that included examining CCPs and good manufacturing practices in producing safe foods. Several botulism cases were attributed to under-processed low-acid canned foods in 1970–71. The United States Food and

Drug Administration (FDA) asked Pillsbury to organize and conduct a training program on the inspection of canned foods for FDA inspectors. This 21-day program was first held in September 1972 with 11 days of classroom lecture and 10 days of canning plant evaluations.[5] Canned food regulations (21 CFR 108, 21 CFR 110, 21 CFR 113, and 21 CFR 114)[6] were first published in 1969. Pillsbury's training program to the FDA in 1969, titled "Food Safety through the Hazard Analysis and Critical Control Point System", was the first time that HACCP was used.[5]

HACCP was initially set on three principles, now shown as principles one, two, and four in the section below. Pillsbury quickly adopted two more principles, numbers three and five, to its own company in 1975. It was further supported by the National Academy of Sciences (NAS) that governmental inspections by the FDA go from reviewing plant records to compliance with its HACCP system. A second proposal by the NAS led to the development of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) in 1987. NACMCF was initially responsible for defining HACCP's systems and guidelines for its application and were coordinated with the Codex Committee for Food Hygiene, that led to reports starting in 1992 and further harmonization in 1997. By 1997, the seven HACCP principles listed below became the standard. A year earlier, the American Society for Quality offered their first certifications for HACCP Auditors.[7] (First known as Certified Quality Auditor-HACCP, they were changed to Certified HACCP Auditor (CHA) in 2004.).[8]

HACCP expanded in all realms of the food industry, going into meat, poultry, seafood, dairy, and has spread now from the farm to the fork.[5]

Principles

1. Conduct a hazard analysis

   Plans determine the food safety hazards and identify the preventive measures the plan can apply to control these hazards. A food safety hazard is any biological, chemical, or physical property that may cause a food to be unsafe for human consumption.

2. Identify critical control points

   A critical control point (CCP) is a point, step, or procedure in a food manufacturing process at which control can be applied and, as a result, a food safety hazard can be prevented, eliminated, or reduced to an acceptable level.

3. Establish critical limits for each critical control point

A critical limit is the maximum or minimum value to which a physical, biological, or chemical hazard must be controlled at a critical control point to prevent, eliminate, or reduce that hazard to an acceptable level.

4. Establish critical control point monitoring requirements

   Monitoring activities are necessary to ensure that the process is under control at each critical control point. In the United States, the FSIS requires that each monitoring procedure and its frequency be listed in the HACCP plan.

5. Establish corrective actions

   These are actions to be taken when monitoring indicates a deviation from an established critical limit. The final rule requires a plant's HACCP plan to identify the corrective actions to be taken if a critical limit is not met. Corrective actions are intended to ensure that no product is injurious to health or otherwise adulterated as a result if the deviation enters commerce.

6. Establish procedures for ensuring the HACCP system is working as intended

   Validation ensures that the plants do what they were designed to do; that is, they are successful in ensuring the production of a safe product. Plants will be required to validate their own HACCP plans. FSIS will not approve HACCP plans in advance, but will review them for conformance with the final rule.

   Verification ensures the HACCP plan is adequate, that is, working as intended. Verification procedures may include such activities as review of HACCP plans, CCP records, critical limits and microbial sampling and analysis. FSIS is requiring that the HACCP plan include verification tasks to be performed by plant personnel. Verification tasks would also be performed by FSIS inspectors. Both FSIS and industry will undertake microbial testing as one of several verification activities.

   Verification also includes 'validation' – the process of finding evidence for the accuracy of the HACCP system (e.g. scientific evidence for critical limitations).

7. Establish record keeping procedures.