SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC. MICROBIOLOGY

UNIT – I – FOOD & DIARY MICROBIOLOGY – SMB2202

Components of Food

Food is essential for the survival of living organisms. It provides us with energy to carry out daily activities and develop, grow and repair our body parts in case of any damage. Therefore understanding the importance of consuming the right kind of food becomes a necessity.

Furthermore, most of the food items available today are adulterated in many possible ways. The food that we consume comes from numerable sources and the variety is huge. You can think of the last meal you had, and list down sources from which your food was obtained. This helps us understand how food varies in its sources and components.

What Do Different Food Items Contain?

Each dish we eat is prepared using more than one ingredient containing different nutrients along with dietary fibres and water. Some nutrients in our food are fats, minerals, vitamins, proteins and carbohydrates.

- Carbohydrates are present in the form of starch and sugars in our food. The presence of carbs in our food can be tested using a dilute iodine solution, an indication of the presence of starch is shown by black-blue colouration.
- Presence of protein in food is tested using a solution of copper sulphate and caustic soda. If the solution turns violet, proteins are present.
- Presence of fat in food is indicated when food wrapped in paper turns oily after releasing its moisture content.

Also Read: Balanced diet

What Do Various Nutrients Do For Our Body?

- Nutrients play a vital role in determining the health of our body. These nutrients are used up during the process of nutrition by each cell in our body to carry out their basic functionality.
- Fats provide more energy to our body as compared to carbohydrates.
- Proteins are bodybuilding foods.
- Vitamins help our body to fight against diseases and also helps to keep our eyes, gums, bones in a healthy shape. There are different kinds of vitamins such as Vitamin A, B complex, C, D. E, K etc. Vitamin C protects our body against diseases.

Sources of different Vitamins	
Vitamin A	Carrot, Mango, Papaya
Vitamin C	Tomato, Guava, Lemon, Orange
Vitamin B	Rice, wheat

Vitamin D	Liver, fish, milk, butter

- Roughage, also known as dietary fibres, is obtained from plants such as fruits, whole grains etc. They do not provide nutrients but add bulk to our food and are essential to our body as they aid in digestion.
- Water helps absorb nutrients from food and aids in getting rid of wastes in the form of sweat and urine

Components of Food (Tabular Representation)

Food Component	**Functions**
Carbohydrates	These are digested and broken down into glucose and provide energy to the body
Fats	Store energy, protects and insulates the important organs
Proteins	Help in metabolism, act as enzymes, and hormones
Vitamins	These help in maintaining healthy bones, boost the immune system, heal wounds, repair and damage of cells and converting food into energy
Iodine	Formation of thyroid hormone
Calcium	Helps in the proper functioning of the nervous system and maintain healthy bones
Phosphorus	Helps to maintain acid-base balance in the body
Sodium	Controls the blood pressure
Iron	Facilitates the formation of haemoglobin
Fibres	They help in food absorption and prevents constipation
Water	They help in absorbing nutrients from the food and release waste from the body in the form of urine and sweat.

Balanced Diet

A diet, which contains the right amount of nutrients, roughage and water is called a balanced diet. Eating a balanced diet can never lead us to obesity. Consuming the right food is as important as having knowledge of the nutrient content of your food.

Deficiency Diseases

Deficiency diseases occur in our body due to lack of nutrient supply over a long period of time. Listed below are a few deficiencies caused as a result of lack of nutrients.

Name of Nutrient	Disorder caused	Symptoms
Vitamin C	Scurvy	Takes a long time to heal, bleeding gums
Iron	Anaemia	Weakness
Calcium	Tooth decay and bone	Tooth infections, Weak bones

Growth of Microorganisms in Food: Intrinsic & Extrinsic Factors

The interaction between microorganisms and other living things in the earth is natural, constant and which plays a significant role in maintaining the ecological balance and stability of biogeochemical cycling. As microorganisms are associated with living things in nature they play a significant role for survival of plants and animals.

Majority of food materials are obtained from plants and animals and it is rich in different type of microorganisms. Some microorganisms serve as food for human and animal, e.g. mushrooms, some present in food are helpful and some others are harmful to our health. Microorganisms use food as the substrate for their growth and colonization.

Depending on the type of microorganisms the growth of many organisms in food can result in improving overall quality of food and in some cases they can deteriorate the quality also. Growth of harmful microorganisms especially bacteria and fungi in food constitutes food spoilage and sometimes cause several diseases on consumption of such food.

The major reason for food spoilage is due to increase in number of microorganisms, utilization of nutrients, causing enzymatic changes resulting in bad flavors due to breakdown of some food materials or synthesis of new compounds. Food becomes unfit for human consumption because of such microbial activities. Microorganisms can oxidize reduced carbon; nitrogen and sulfur compounds present in dead plants and animals and can contribute the minerals to the biogeochemical cycling.

Food acts as good medium for transmission of many diseases and infections. If the food is contaminated by pathogenic microorganisms or their spores, they can grow and increase their population and can produce various types of toxins which may leads to several diseases.

Sometime microorganisms may not grow in food but food can act as transmission route of many diseases. Therefore, food act as good medium for spread of diseases. Several food borne diseases are the result of microorganism present in food or their growth in them.

Growth of microorganisms in food is dependent on various parameters. The factors influencing the growth of microorganisms are physical, chemical and biological in nature. The factors can be generally classified as intrinsic and extrinsic factors.

The intrinsic and extrinsic factors affecting the growth of microorganisms in food are explained below:

Intrinsic Parameters in Food:

The parameters present in substrates in which the microorganisms are growing, that are internal parts of the substrate are called as intrinsic parameters.

The most important types of internal factors in food are:

1. Hydrogen Ion Concentration (pH):

All the microorganisms have a minimal, maximal and optimal pH for their growth, survival and activity of their enzymes. Growth of microorganisms is affected by the pH of growth environments in food (growth medium) resulting large number of enzymes responsible for metabolism and growth. Influence of pH of food not only has effect on growth of microorganisms but also on processing conditions. Food having acidic contents promotes growth of acid loving microorganisms such as yeasts, moulds and some acidophilic bacteria.

Mould can grow over a wider range of acidic pH than bacteria and yeast. Most of the fermentative yeasts can grow at pH of about 4.0 to 4.5, as in fruit juices and acid food such as sauerkraut and pickles. A food with an acid pH would tend to be more microbiologically stable than neutral or alkaline food. Because of this restrictive pH the food such as fruits, soft drinks, fermented milks, sauerkraut and pickles are stable against bacterial spoilage.

Most of the bacteria, except acid fermenters are favored alkaline or neutral pH. Most of the bacteria preferred a pH range between 7.0-7.5 but some proteolytic bacteria can grow on food substrate with high pH. The buffer content in the food is important to maintain the stability against microbial spoilage.

Buffers permit an acid (or alkali) fermentation to go on longer with a greater yield of products and organisms. Vegetable juices have low buffering capacity permitting a decrease in pH with the production of only small amount of acid by the lactic acid bacteria during the early stage of sauerkraut and pickle fermentation. This helps to inhibit the growth of pectin hydrolyzing and proteolytic competing bacteria in food.

Food acidification by fermentation in home food preparations is the oldest practice man has been doing. It is due to production of organic acids in food by growth and fermentation of microorganisms such as lactic and acetic acid bacteria. The inhibitory properties of many of the

organic acids such as citric acid, lactic acid, benzoic acid, propionic acid, sorbic acids, etc. can be used as effective acidulants or chemical preservatives against food spoilage bacteria.

2. Water Activity or Moisture Content (a_w):

Water is an excellent solvent for all life processes in every living organism for biocatalytic activity. The amount of water required varies for different organisms. Water requirement of microorganisms is expressed as available water or water activity a_w. Water activity is the vapor pressure of the solution (of solutes in water in most food) divided by the vapor pressure of the solvent (usually water).

In other words it is defined by the ratio of the water vapor pressure of food substrate to the vapor pressure p of pure water at the same temperature – $a_w = p/p_o$, where P is the vapor pressure of the solution and Po is the vapor pressure of the solvent (usually water). The a_w content is very well related to relative humidity (RH) in the following way: RH = 100 x a_w.

Pure water has an a_w of 1.00, a 22% NaCl solution (w/v) has an a_w of 0.86, and a saturated solution of NaCl has an a_w of 0.75. The water activity (a_w) of most fresh foods is above 0.99. In general, bacteria require more water activity than moulds and yeasts. Gram-negative bacteria have higher water requirements than gram-positive bacteria.

Most of the food spoilage bacteria do not grow below a_w 0.91, while spoilage moulds can grow even at a_w 0.80. The aerobic food poisoning bacterium Staphylococcus aureus is found to grow at a_w as low as 0.86 while anaerobic Clostridium botulinum does not grow below a_K 0.94. Moulds differ considerably in optimal a_w for vegetative growth and spore germination.

The lowest a_w value for foodborne bacteria is 0.75 for halophiles ("salt-loving"), whereas xerophilic ("dry-loving") moulds and osmophilic (preferring high osmotic pressures) yeasts have been reported to grow at a_w values of 0.65 and 0.61. The lowest water activity values permitting growth of spoilage microorganisms is given in the Table 3.1.

Table 3.1: Lowest a_w values for different types of microorganisms spoiling food

Group of Microorganism	Minimal (a_w) value
Bacteria	0.91
Yeasts	0.88
Moulds	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.65
Osmophilic yeasts	0.60

The effect of lowering a_w below optimum is to increase the length of the lag phase of growth and to decrease the growth rate and size of final population of microorganisms. This is due to adverse

influences of lowered water on all metabolic activities in microorganisms since all chemical reactions in cells require an aqueous environment.

The a_w is influenced by other environmental parameters such as pH, Eh (redox potential) and growth temperature required for microorganisms. The other factors which influence the water activity are the kinds of solute employed to reduce water activity, the nutritive significance of culture medium, temperature, supply of oxygen, hydrogen ion concentration and presence of inhibitors.

3. Redox Potential (Eh):

The reducing and oxidizing power of the food will influence the type of organism and chemical changes produced in the food. The concentration of oxygen in food, chemical composition and type of microorganisms associated contribute to the oxidation-reduction (O-R) potential of food and affect growth of microorganisms in them. The O-R potential of a food may be defined as the ease with which the substrate loses or gains electrons.

The Redox potential of food is determined by characters such as:

(a) Oxygen tension of atmosphere above the food,

(b) Access of atmosphere to the food,

(c) Resistance of food to the changes occurring and

(d) O-R state of materials present in food.

On the basis of the ability of microorganism to utilize oxygen, organisms are classified as aerobic, anaerobic and facultative anaerobes. Aerobes require free oxygen and anaerobes don't prefer oxygen as it is toxic to them, hence, it is grow in the absence of molecular oxygen. Facultative may grow both aerobic and anaerobic conditions.

Generally fungi- mould and yeasts are aerobic. But bacteria are variables of these aspects. Some are aerobic, some are anaerobics and others are facultative anaerobes. If oxidation potential is high then aerobes will grow better than anaerobes, but if conditions become more reduced then anaerobes will be the predominant organisms.

The O-R potential is written as Eh and measured and expressed as millivolts (mV). If the substrate is highly oxidized would have a positive Eh and substrate is reduced is a negative Eh. Aerobic microorganisms such as bacilli, cocci, micrococci, pseudomonas, acinetobacters require and grow at positive O-R potential and anaerobe such as Clostridia and bacteriodes require negative O-R potential for their growth.

Most of the fresh plant and animal food have low redox potential because of reducing substances present in them. Fresh vegetables and fruits contain reducing substances such as ascorbic acid, reducing sugars and animal tissues have sulfhydryl (-SH) and other reducing group compounds considered as antioxidants.

Fresh vegetables, fruits and meat promote growth of aerobic microorganisms in the surface regions because of positive redox potential. However, the anaerobic microorganisms grow in inner parts of vegetables, fruits and meat because of negative redox potential. Most of processed plant and animal food gain positive redox potential therefore promote growth of aerobic organisms.

4. Composition of Nutrients:

Nutrients are one of the most important compounds for the growth and functioning of microorganism. Nutritional quality of food depends on the chemical composition, nutritive value or nutrients, their proportion and growth promoting ability to the microorganisms.

The most important factors which have to be considered are the energy substances in food, nitrogen substances, growth promoting substances, accessory food substances or vitamins, minerals, and water content which all are very essential for growth or energy production of organisms.

The most energy sources of organisms are carbohydrates. Complex carbohydrates such as cellulose, hemicelluloses, starch, pectin, etc. can be utilized by various types of microorganisms. At the same time other carbon compounds such as esters, alcohols, peptides, amino acids, organic acid and their salt are also serving as energy sources for many organisms.

Bacteria are identified and classified based on their ability or inability to utilize various sugars and alcohols. Most organisms can hydrolyses complex carbohydrates and can use glucose as energy source. Some organisms have the ability to hydrolyze pectin by producing the enzyme pectinase.

Some microorganism can hydrolyze triglycerides and other types of fats by microbial lipase and produces glycerol and smaller fatty acid. In this step triglycerides are hydrolyzed in to diglycerides then monoglycerides and glycerols under alkaline condition by microbial lipase. The glycerol and fatty acids are excellent sources of carbon and energy sources of many aerobic organisms.

Hydrolytic products of proteins and peptides serves as sources of nitrogen for many proteolytic bacteria such as Pseudomonas sps. The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids. A large number of other nitrogenous compounds may serve this function for various types of organisms. Some microbes are able to utilize nucleotides and free amino acids, whereas others are able to utilize peptides and proteins.

In general, simple compounds such as amino acids will be utilized by almost all organisms before any attack is made on the more complex compounds such as high-molecular-weight proteins. Protein rich food promotes more growth of bacteria than moulds and yeasts.

Some microorganisms require vitamins and other growth factors for their growth and that has to be supplied with the growth medium. Such microorganisms are called fastidious organisms.

Food contains different vitamins, minerals and other growth factors and their composition and content may vary.

Fresh plant and animal food contain vitamin B complex and fruits are low, but fruits are high in ascorbic acid. Processing of food often reduces the vitamin content. Thiamine, pantothenic acid, folic acid and ascorbic acid are heat-labile and drying cause's loss in vitamins such as thiamine and ascorbic acid.

Storage of food for long may also result in decrease in vitamins and other growth factors. Some microorganisms produce vitamins and other growth factors which support growth of others organisms present in food. Each kind of microorganisms has a range of food requirements.

Water is another very important component for food nutrients. The water requirement of organisms will depend on the type of organisms. Generally moulds have the lowest requirement, followed by gram-negative bacteria, yeasts, and gram-positive bacteria.

5. Inhibitory Substances:

Inhibitory substances are present in the food as its own origin, or added purposely by preventing or inhibiting the growth of organisms. The stability of certain foods against attack by microorganisms is due to the presence of certain naturally occurring substances that possess and express antimicrobial activity.

Some plant species are known to contain essential oils that possess antimicrobial activity. Eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage and carvacrol (isothymol) and thymol in oregano are some of the best studied examples. Milk contains several antimicrobial substances, including lactoferrin, conglutinin and the lactoperoxidase system.

Milk casein and some fatty acids have been shown to be antimicrobial property against some organisms. Lactoferrin is an iron-binding glycoprotein that is inhibitory to a number of foodborne bacteria and its use as a microbial blocking agent on beef carcasses. Eggs contain lysozyme; ovotransferrin and conalbumin have shown some antimicrobial properties.

6. Biological Structures:

The natural covering of some foods provides excellent protection against the entry and subsequent damage by spoilage organisms. The inner part of healthy tissues of living plants and animals are sterile and contains less microbial count. The protective covering of food such as the skin of eggs, the skin on poultry, rind on fruits and vegetables, shell on nuts and artificial coating helps to protect its inner structures from microbial contamination and spoilage.

The physical protection of the food my help for preservation and determination of kind, rate and course of spoilage. Layers of fat over meat may protect that part of the flesh, or scales may protect the outer part of fish. In the case of nuts such as pecans and walnuts, the shell or covering is sufficient to prevent the entry of all organisms.

Once cracked nut meats are subject to spoilage by moulds. The outer shell and membranes of eggs prevent the entry of all microorganisms when stored under the proper conditions of humidity and temperature. Fruits and vegetables with damaged covering undergo spoilage much faster than those not damaged.

Extrinsic Parameters:

The extrinsic parameters are substrate independent and in this case the storage environment that affect both the food and their microorganisms.

The main extrinsic parameters influence the foods are:

1. Relative Humidity (RH):

The relative humidity of the storage environment is important extrinsic parameter both from the standpoint of a_w within foods and the growth of microorganisms at the surfaces. When foods with low a_w contents are placed in high RH environments, the foods takes up more moisture until equilibrium has been established.

Similarly foods with a high a_w lose moisture when placed in an environment of low RH. There is a relationship between RH and temperature that should be borne in mind in selecting proper storage environments for foods. Generally, if the temperature high then the RH low and vice versa.

2. Atmospheric Gases:

Like O_2, Carbon dioxide (CO_2) is also most important atmospheric gas that is used to control microorganisms in foods. Modified atmosphere packaged (MAP) foods are make use of this types of gases. Ozone (O_3) is the other atmospheric gas that has high antimicrobial properties; it has strong oxidizing property hence it should not use for fat rich food as it will undergo auto-oxidation. It has been noticed that ozone extend the shelf life of many foods and it has shown to be effective against a variety of microorganisms.

3. Temperature:

Microorganisms can grow over a wide range of temperatures. The lowest temperature at which a microorganism has been reported to grow is -34°C; the highest is somewhere in excess of 100°C.But some spore producing bacteria such as Bacillus stearothermophilus, Clostridium tetani and Clostridium perfringens can grow above 100°C.

Based on the temperature range microorganisms are classified as three groups –

i. Psychrophiles (Psychrotrophs), those organisms are grown between the temperature ranges of 2°C to 20-30°C.

ii. Mesophiles, the organism preferably grow at the temperature between 20°C and 45°C and

iii. Thermophiles, the organisms grow better in range of 55°C-65°C.

The most important psychrotrophs include Alcaligenes, Shewanella, Brochothrix, Corynebacterium, Flavobacterium, Lactobacillus, Micrococcus, Pectobacterium, Pseudomonas,

Psychrobacter, Enterococcus and others. The psychrotrophs found most commonly on foods are those that belong to the genera Pseudomonas and Enterococcus.

These organisms grow well at refrigerator temperature and cause spoilage at 5-7°C of meats, fish, poultry, eggs, and other foods normally held at this temperature. Mesophilic species and strains are known bacteria among all genera and may be found on food held at refrigerator temperatures. Most important thermophilic bacteria in food belong to the genera Bacillus, Paenibacillus, Clostridium, Geobacillus, Alicyclobacillus and Thermoanaerobacter.

Like bacteria fungi are also able to grow over wide ranges of temperature. Many moulds are able to grow at refrigerator temperatures, especially some strains of Aspergillus, Cladosporium, and Thamnidium, which may be found growing on eggs, sides of beef and fruits. Yeasts prefer psychrotrophic and mesophilic temperature ranges but generally not within the thermophilic range.

4. Other Microbial Flora:

Microorganisms present in the food can undergo various types of negative interactions. These kinds of interaction cause inhibition of some microorganisms as they are undergoing competitions and antibiosis. Some organisms especially moulds can produce various types of secondary metabolites such as antibiotics that are toxic to many bacteria. Some foodborne organisms produce substances that are either inhibitory or lethal to others; these include bacteriocins, hydrogen peroxide and organic acids.

Natural flora and source of contamination of foods in general

The foods of plants and animal origin carry several microorganisms associated with their natural habitat. Plants carry typical micro-flora on their surface and also get contaminated from outside sources. Animals carry microorganisms on their surface and intestine, and also contain contaminants from surrounding environment. Through their excretions and secretions animals release microorganisms in to surrounding environment. Besides, both plants and animals carry pathogenic microorganisms capable of causing human illness. The food associated microorganisms are influenced by the availability of specific nutritional requirements and the environmental parameters. The primary sources of entry of microorganisms in to foods are from the soil, water, air, during handling, processing transportation and storage of foods.

Soil

Soil being the rich source of several kinds of microorganisms immediately contaminates the plants and edible plant parts, and the surface of animals with the soil associated microorganisms. As the soil particles are carried in to aquatic environment through wind, rain and other means contamination of water takes place with several soil micro-flora. Therefore, it is not uncommon to find several microorganisms both in soil and water environment. These soil derived

microorganisms form part of the the microbial flora involved in spoilage of foods of plant and animal source. Thus, there is a need to reduce the load of soil microorganisms in foods which can be achieved by removing the soil by washing the surface of foods with good quality water, and by avoiding contact with soil/ dust.

Water

Natural waters not only contain several microorganisms native to the aquatic environment but also from soil, raw/treated sewage and pollutants entering the water body. The microbial numbers and types vary in different water bodies depending on the nutrient status. Thus, all kinds of microorganisms found in water are likely to be associated with the aquatic organisms as surface flora. Use of such water for food processing will add microorganisms from water to food. Sewage waters containing human pathogenic microorganisms contaminate foods when such waters are used without proper treatment. The water used in food processing should meet agreeable chemical and bacteriological characteristics.

Air

Air contains several microorganisms which may get deposited on the food being processed and handled. Though the air does not contain natural flora of microorganisms, whatever microorganisms encountered are those associated with the suspended solid material and water droplets. The sources of microorganisms to air are from dust, dry soil, and water spray from natural surface waters, droplets of moisture from coughing, sneezing and talking by food handlers, from sporulating moulds growing on walls, ceilings, floor, foods and food ingredients. Thus, it is likely that the microorganisms persisting in air get deposited on the food being processed and contribute for microbial load and subsequent spoilage of food.
The number of microorganisms present in air depends on factors such as extent of movement of air, sunshine, humidity, location and amount of suspended dust in air. Quiet air allows settling of microorganisms but the moving air brings in microorganisms and keeps them suspended. Thus, the number of microorganisms in air is increased by air currents caused by movement of people, by ventilation and by breeze. The rain or snow removes microorganisms from the air.

Microorganisms of Plants

Plant surfaces have natural microflora called phylloplane flora (on leaf) and rhizoplane flora (on roots) and this can include fungi like *Cladosporium, Penicillium, Aspergill*us etc. yeast of genera *Sporobolomyces* and *Bullera* and some bacteria like *Erwinia, Pseudomonas, Xanthomonas, Lactobacilli, Streptococci, Leuconostoc* etc. *Erwinia* can cause blackleg disease of potato; and soft rot of potatoes during storage. Fungi like *Botrytis cineria* may infect strawberry. Cereal grains can be infected by *Cladosporium, Alternaria, Chaetomium, Helminthosporium, Penicillium, Aspergill*us etc.

Microorganisms of Animals

Intestinal tract of animals has many microorganisms and if shed into waterbodies can cause spoilage of food when used for washing foods. Their skin also contains many microorganisms that can cause food contamination. *Staphylococcus* and *Corynebacterium* are important in this regard.

Utensils

The utensils used to store the harvested fruits and vegetables are contaminated with surface organisms present on them. These may lead to contamination of other products put into them later on.

Food handlers

Microorganisms on hands and outer garments of handlers can contribute towards microflora of the concerned food. Nasal cavity and mouth are also important sources of microorganisms.

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MICROBIAL SPOILAGE OF VEGETABLES

Introduction

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

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

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

Types of Spoilage in Vegetables

The microbial spoilage of vegetables is predominately of following types Spoilage due to pathogens. The plant pathogens which infect stem, leaves, roots, flowers and other parts or the fruit itself.

Spoilage due to saprophytes

Vegetables have general microflora inhabiting them. These organisms under certain conditions can grow on these vegetables and spoil them. The list of these organisms is given in Table -1.

Table - 1 Normal microflora of vegetables

There are certain secondary invaders which may enter the healthy food or grow after growth of pathogens. It is well known that plant diseases are mostly caused by fungi. Thus most of the spoilage causing pathogens in vegetables is fungi.

Fungi have specific characteristics when spoiling food as it leads to mushy areas which may be water soaked. The fungi produce characteristic spores which may be pigmented. The pigmentation helps in identification of the type of spoilage by fungi. The bacterial diseases too cause spoilage of vegetables but to a lesser extent.

Table - 2 The major types of spoilages by pathogens in vegetables

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

Bacterial Soft Rot Caused by *Erwinia carotovora* and *Pseudomonas* such as *P. marginalis*. *Bacillus* and *Clostridium* spp. are also implicated.

Breaks down pectin, giving rise to a soft, mushy consistency, sometimes a bad odour and water soaked appearance. Vegetables affected onions, garlic, beans, carrot, beets, lettuce, spinach, potatoes, cabbage, cauliflower, radishes, tomatoes, cucumbers, watermelons.

Soft rot in tomato caused by *Erwinia carotovora.* Blue mould rot in tomato caused by *Penicillium* spp. *Penicillium, Cladosporium, Rhizopus, Aspergillus* spp. are responsible for various defects in vegetables. Gray mold rot – caused by *Botrytis cinera* in vegetables. Favoured by high humidity and warm temperature

Table -3 Examples of fungal spoilage of vegetables

Some common Fungal Fruit and Vegetable Spoilage Conditions, Etiologic Agents, and Typical Products Affected

Common Fungal Fruit/ Vegetable Spoilage Conditions	Etiologic Agents	Typical Products Affected
Black rot cabbage	*Aspergillus niger, Alternaria*	Onions,
Black rot	*Ceratocystis fimbriata*	Sweet potatoes
Blue mold rot	*Penicillium digitatum*	Citrus fruits
Dry rot	*Fusarium* spp.	Potatoes
Gray mold rot	*Botrytis cinerea*	Grapes, many others
Green mold rot	*Penicillium digitatum*	Citrus fruits
Rhizopus soft rot	*Rhizopus stolonifer*	Sweet potatoes, tomatoes
"Smut" (black mold rot)	*Aspergillus niger*	Peaches, apricots
Sour rot	*Geotrichum candidum*	Tomatoes, citrus fruits

MICROBIAL SPOILAGE OF FRUITS AND FRUIT JUICES

Introduction

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

Microorganisms Associated with Spoilage in Fruits and Juices

The microorganisms associated with fruits depend on the structure of fruit. The fruits contain different organic acids in varying amounts. **The types of acids which are predominately found are citric acid, malic acid and tartaric acid. The low pH of fruits restricts the proliferation of various types of organisms.** The pH and type of acids found in different fruits is given in Table -1.

Table -1 Type of acid associated with fruits and their pH

Due to the low pH, a large number of microorganisms are restricted to grow on fruits. Fungi are most dominating organisms to grow on fruits because of the ability of yeasts and molds to grow under acidic conditions. A small number of bacteria which are aciduric (ability to resist acidic conditions) also grow. Also the dry conditions prevailing on the skin and surface do not allow the growth of certain microorganisms. Besides, these plants also produce certain antimicrobial components too.

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

Yeasts

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

Molds

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

1. Some of the members are xerophilic, thereby having potential to spoil foods of low water activity such as dried fruits and fruit juice concentrates.
2. Some of the species have heat resistant spores such as ascospores which can survive the commercial pasteurization treatments that are given to most fruit products.
3. Growth of molds on processing equipment such as wooden tanks can result in the generation of off flavors in wines, juices, and other fruit products.
4. Mold infected raw fruit may become soft after processing because pectinases were not inactivated by the thermal treatment.

5. The metabolic products of many molds are toxic to humans. Of these toxins, mycotoxins are important components. Molds are aerobic microorganisms, but many of them are very efficient scavengers of oxygen. Due to this property of molds, processed fruits, including those hermetically sealed in cans or glass, are susceptible to spoilage. In case of limited vegetative growth, evidence of spoilage may be the changes produced by fungal enzymes such as the breakdown of starch or pectins while in case of heavy growth, colonies develop in the headspace or as strands throughout a beverage or similar product. *Penicillium italicum* (blue mold) and *Penicillium digitatum* (green mold) seen in oranges, lemons and citrus fruits.

Bacteria

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

- • Lactic acid bacteria
- • Acetic acid bacteria
- • Spore formers

Lactic acid bacteria

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

Growth of lactic acid bacteria in juices and other fruit products cause the formation of haze, gas, acid, and a number of other changes. Certain hetero fermentative lactobacilli lead to slime in cider. The lactobacilli and leuconostocs that are present in citrus juices generate acetyl methyl carbinol and diacetyl compounds that give the juices undesirable, butter milk like flavor. Some strains, being extremely tolerant to ethanol grow in wines. *Lactobacillus fructivorans* can grow in appetizer and dessert wines containing as much as 20% ethanol. Lactic acid bacteria have the ability to decarboxylate malic acid to lactic acid. This malolactic fermentation is often desirable in high acid wines because the acidity is reduced and desirable flavors are produced.

Acetic acid bacteria

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

oxydans produce microfibrils composed of cellulose, which leads to formation of flocs in different fruit juice beverages.

Spore formers

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

SPOILAGE OF MEAT AND MEAT PRODUCTS

- Spoilage in different food stuffs have been showed by numerous microorganisms.

- Spoilage of milk, fruits and meat occurs quickly as compared to other food products.

- Approximately 25% of the world's food produced post harvest or post slaughter is lost to microbial degradation of food alone.

- Meat is an ideal culture medium for many organisms because it is high in moisture ,rich in nitrogenous content and various degrees of complexity and plentiful of minerals and accessory growth factors.

- The breakdown of fat, protein and carbohydrates of meat impart offodors, offflavor and slime formation.

- Majority of the spoilage microorganisms are contaminants comes from external sources

- Molds (*Cladosporium, Thamnidium, Mucor, Penicilium, Alternaria,*and *Monilia*) can come in contact with the surface of meats and grow in this environment.

- Yeasts present on meat are mostly asporogenous.

- Major bacteria associated meat spoilage are *Pseudomonas, Acinetobacter, Alcaligens, Micrococcus, Sarcina, Leuconostoc, Lactobacillus, Proteus, etc.*

- Spoilage of sea foods (fish and shellfish) caused by various microbes e.g. *Pseudomonas, Moraxella, Shewanella, Flavobacterium, Bacillus, Micrococcus,Clostridium*, etc.

- ***Brochothrix thermosphacta:*** is able to grow under both aerobic and anaerobic conditions for which meat is an ecological niche. The presence of this microbe often seen in irradiated meat and poultry. These compounds, or their derivatives, are responsible for the foul odor of meat.

- ***Carnobacterium:*** is gram-positive genus and *C. divergens*and *C. maltaromaticium*are commonly associated with meat products and seafood. Theses microbes are anaerobic and can even grow at high CO2concentration and high pressure. Due to production of H2O2, *C. divergens*has been shown to result green discoloration of ham.

Microbes Present in Meat and Meat products

- ***Clostridium tetani:*** is a rod-shaped, gram positive, anaerobes, which are recognized as toxin-producing pathogens. *Clostridium*produces large amounts of gas in packaged meat, which usually coupled up with foul odors and causes the package to appear in a blown pack.

- Enterobacteriaceae***:*** gram-negative, straight rods, and sometimes motile species. This family composed of more than 150 bacterial strains that consist mostly of*E. coli*, *Klebsiella pneumonia*, *Klebsiella oxytococa*, and *Enterobacter cloaeces*. *Serratia*, *Enterobacter*, *Proteus*and *Hafnia*often contribute to meat spoilage.

Microbes Present in Meat and Meat products

- ***Leuconostoc :***is the member of lactic acid producing bacteria which produces D-lactate and ethanol. These microbes are responsible for the discoloration, gas production, and buttery smell of spoiled meat.

- ***Pseudomonas:*** predominant bacteria associated with spoiled meat. The most common *Pseudomonas*species found in beef, pork, lamb and poultry meat is *Pseudomonas fragi*.

Microbes Present in Meat and Meat products

General types of Spoilage of meats and meat Products

Spoilage under Aerobic condition

- **Surface slime:**Spoilage of meat due surface slime is produced by species of *Pseudomonas, Acinetobacter, Moraxella, Alcaligens, Micrococcus, Streptococcus, Leuconostoc,* and*Bacillus.*
- **Changes in colour of meat pigments**: The red colour of meat, changed to green, brown, or grey. Species of *Lactobacillus*(mostly heterofermentative) and *Leuconostoc*are reported to cause the greening of sausage.

Spoilage under Aerobic condition

- **Changes in fats:**The oxidation of unsaturated fats in meat takes place chemically. Lipolytic bacteria may cause some lipolysis & caused by lipolytic species of *Pseudomonas*and *Achromobacter*or by yeasts.

- **Phosphorescence**: *Photobacterium*spp., growing on the surface of the meat. Discolorations are caused by bacteria with yellow pigments, usually species of *Micrococcus*or *Flavobacterium*. *Chromobacterium lividum*.

- **Off odours and off tastes**: Taints, or undesirable odours and tastes, which appear in meat as a result of acid producing microbes. *Actinomycetes*may be responsible for a musty or earthy flavour.

Spoilage due to molds in aerobic condition

- **Stickiness**: Budding growth of molds causes the surface of the meat sticky to the touch known as stickiness.

- **Whiskers**: White, fuzzy growth in freezing caused by a number of molds, including *T. chaetocladioides*, or *T. elegans, Mucor mucedo, Rhizopus*, etc.

- **Black spot**: commonly caused by *Cladosporium herbarum*.

- **White spot**: *Sporotrichum carnis*is the most common cause of white spot.

- **Green patches**: *Penicillium*are responsible for the green patches on meat.

- **Decomposition of fats**. Hydrolysis of fats due lipase produce by molds causes the oxidation of fats.

- **Off odours and off tastes**. Musty flavor to meat caused by mold **Spots of surface**. Spoilage caused by yeasts and molds

Spoilage under Anaerobic Conditions

- **Souring**: Souring of meat caused by lactic or succinic produced by microbes. Souring can result from either from action of the meat's own enzymes during aging or ripening, anaerobic production of fatty acids or lactic acid by bacterial action.

- **Putrefaction**. Decomposition of protein is known as putrefaction, which produced by anaerobic microbes with foul-smelling compounds. Generally, *Clostridium*spp., *Pseudomonas*, *Proteus*, *Clostridium*and *Alcaligenes*cause putrefaction in meat and meat products

Preservation and storage of meat

- **Cold storage:** Refrigerated and Freezer storage is another superior method of meat preservation.

- **Vacuum packaging:** vacuum-packaging extends the storage life under refrigerated conditions to approximately 100 days.

- **Canning:** meat is sealed in a container and then heated to destroy all microorganisms capable of food spoilage.

- **Drying:** process removes moisture from meat products so that microorganisms cannot grow.

- **Fermentation:** process involves the addition of harmless bacteria to meat to inhibit the growth of many pathogenic microorganisms

- **Irradiation:** effective approach to killing meat spoilage microorganisms.

- **Curing and Smoking:** improves the safety and shelf life of meat products as well as color and flavor quality. Smoking & curing decreases moisture on the surface of meat products and hence prevents the microbial growth

Microorganisms responsible for seafood spoilage

- ***Clostridium botulinum:*** *C. botulinum*is widely distributed in soil, aquatic sediments and fish.

- ***Vibrio spp.:*** Most vibrios are facultative anaerobe and are of marine origin and needs sodium for growth.

- ***Aeromonasspp****.:Aeromonas*is ubiquitous in freshwater environments and this organism also found in meat, fish and seafood and other foods.

- ***Plesiomonasspp****.: Plesiomonas*commonly occurs in water, both fresh water and seawater.

- ***Listeriaspp.:*** Most of these environmental strains are probably non-pathogenic.

- ***Salmonellaspp.:*** *Salmonella*are members of the family *Enterobacteriaceae*and most common in the gut of man and animals.

- ***Shigellaspp***.: *Shigella*is also a member of the *Enterobacteriaceae*and its presence in the environment is associated with fecal contamination

- ***Staphylococcus aureus:*** This pathogen can easily transmit from air, water and dust to sea food.

- **Viruses:** Viral disease transmission to human via consumption of seafood Hepatitis, Norwalk virus, Snow Mountain Agent Calicivirus and Astrovirus common in seafood.

Microorganisms responsible for seafood spoilage

Microbial spoilage of processedfish

- **Spoilage of CO2and vacuum packing:** *Photobacterium phosphoreum &* Lactic acid bacteria are another group of bacteria that has the ability to adapt to the anaerobic packing environment.

- **Spoilage of salted foods:** Salt-loving bacteria called halophiles can survive in salt concentrations of up to 10-20% NaCl.

- **Spoilage of Heating/pasteurization food stuffs:** *Clostridiumsp.*and *Bacillus anthracis*are able to spread in the face of pasteurization by producing spores.

- **Spoilage of food containing preservatives:** lactic acid bacteria and yeasts are able to remain active in this environment and become part of the surviving spoilage domain.

Preservation of Seafood

- **Salting:** Wet and dry salting is carried out in sea food to avoid the microbial growth.

- **Smoking:** hot and cold carried out to preserve the seafood. Hot smoking cooks the seafood but it preserves partially. Cold smoking is another preservation process; seafood is not cooked in process.

- **Marinating:** marinating liquid usually consists of an acidic base consisted of lime and mild vinegar. Herbs, spices, onions or soy sauce also be added to impart flavour.

- **Pickling:** immersion of sea food it into an acidic solution, which may also be salted or flavored.

Illness caused due to spoiled seafood and spoiled meat

- **Scombrotoxicfish poisoning:** caused by bacterial spoilage of finfish. The common symptoms include rash, sweating, diarrhea, flushing, headache, and vomiting

- **Botulism:** caused by *Clostridimbotulinum*. Double vision, speech difficulty, inability to swallow are the major symptoms.

- **Campylobacteriosis:** caused by *Campylobacter jejuni*. The symptom includes abdominal cramping, diarrhea, fever, and sometimes bloody stools.

- ***E. coli*O157:H7:** rare illness. Seafood and meat become contaminated during slaughter or when it is ground. Symptoms include abdominal cramps, severe bloody diarrhea and; sometimes the infection causes non-bloody diarrhea or no symptoms.

- **Listeriosis:** caused by *Listeria monocytogenes*, Fever, headache, nausea, and vomiting are the sign of illness.

- **Perfringens food poisoning:** caused by *Clostridium perfingens*Abdominal pain and diarrhea, and sometimes nausea and vomiting are the common symptoms of this illness.

- **Salmonellosis:** Salmonella is most frequently involved in many foods including seafood. The illness caused by salmonella involves abdominal pain, nausea, vomiting and diarrhea.

- **Staphylococcal food poisoning:** Staphylococcal sea food poisoning caused by contaminated food. Abdominal pain, cramps, and prostration Diarrhea, vomiting and nausea are common symptoms.

Illness caused due to spoiled seafood and spoiled meat

Vibrio Infection:found in coastal waters and cause chills and fever.

Amebiasis: *Entamoeba histolytic a*re main common. Polluted seafood spread the infection. Severe crampypain, loss of weight, fatigue, and sometimes anemia are major symptoms.

Giardiasis: Giardiasis in seafood is most frequently associated with contaminated water. Abdominal cramps, anorexia, nausea, and vomiting are the common symptoms.

Calcivirus: Calcivirus cause of acute gastroenteritis. Nausea, vomiting, diarrhea, abdominal pain is symptoms such type of illness.

Hepatitis A virus: Raw shellfish are especially potent carriers of Hepatitis A, as cooking does not always kill the virus.

Spoilage of eggs:

1. Contamination of eggs:

- Freshly laid egg is sterile but the egg shell soon becomes contaminated by fecal matter of hen by nest, by washing water, by handling and by other material in which it is stored.
- If a total number of micro-organisms per shell of hen's egg has been reported to range from 10^2-10^7 with average of 10^5.
- *Salmonella* spp. may be found on shell or inside egg.

2. Non-microbial spoilage of eggs:

- These include loss of moisture and hence loss of weight during long term storage.
- Change in physical state of egg contents also occur during long term storage.
- They include thinning of egg white and breaking of yolk membrane.
- As the yolk membrane weaken and break, yolk becomes flat and homogenously mixed in egg white.

3. Microbial spoilage of eggs:

- In order to cause spoilage of shell of egg, microorganisms must contaminate the shell, penetrate through the pores in shell and inner membrane, reach the eggwhite and yolk and grow there.
- Some microorganisms cannot grow in egg white but can grow rapidly in egg yolk.
- Change in storage temperature facilitates penetration of organism through shell and hence facilitates microbial spoilage.

1. Bacterial spoilage of egg:

- Bacteria are more common spoilage organism than mold.
- Bacteria cause rots in egg.

- When bacteria grow within the egg, they decompose the content and form byproduct.
- This result in characteristic odor, appearance or color from which various microorganisms acquire their name:
- **Green rot:**
  - It is caused by *Pseudomonas fluorescence.*
  - Green egg white shows fluorescence when exposed to UV light.
  - In later stage of spoilage, egg yolk disintegrates and mask green color of egg white.
  - Odor is lacking or fruity or sweetish.
- **Colorless rot:**
  - It may be caused by *Pseudomonas, Acetobacter, Acinatobacter* and *coliform.*
  - In later stage of spoilage, egg yolk disintegrates or at least have incrustations.
- **Black rot:**
  - It is caused by *Proteus* and sometimes *Pseudomonas* and *aeromonas.*
  - Egg yolk blackens and then breakdown to give whole egg content muddy brown color.
  - Odor is putrified due to H_2S.
- **Pink rot:**
  - It is caused by *Pseudomonas* usually at the later stage of green rot.
  - They are similar to colorless rot except that pink coloration occurs in yolk and white.
- **Red rot:**
  - It is caused by *Serrotia marcesceus.*
  - These eggs are distinguished by a rod dissociation of egg white and the surface of the yolk in ammonical i.e. putrified odor.
- **Custard rot:**
  - In this rot, yolk is incrusted with custard like material and occasionally have green to olive pigment.
  - The albumin become thin with orange coloration.
  - This type of spoilage is caused by Citrobacter and *Proteus vulgaris.*

2. Fungal spoilage of egg:

- Fungal spoilage goes through following stages:
- **Pin spot molding:**
  - In this case, small compact colonies of mold appear on the shell and usually just inside the shell.
  - The color of pin spots varies with the type of mold. For example: *Cladosporium* give black spot and *Sporotrichum* give pink spot.
- **Superficial fungal spoilage:**
  - This occurs if eggs are stored in atmosphere of high humidity.
  - In this case, molds grow on shell in the form of whiskers.
- **Fungal rotting:**
  - It is the final stage of spoilage by mold.

- In this case, mycelium of the mold grows through the pores and cracks in the shell.
- Jellying of egg white may occur and colored spots may be produced.
- Hypha of mold grows through the yolk membrane and rupture it, so that yolk mixes with the white.

- Molds causing spoilage of egg include *Penicillium, Sporotrichum, Mucor, Botrytis, Alternaria, Thamnidium* etc.

Contamination of milk:

- Contamination of milk occurs at two levels:
- **On farm:**
  - Freshly drawn milk contains relatively few bacteria however *Micrococcus* and *Streptococcus* are usually found in aseptically drawn fresh milk.
  - During normal milking process, milk is subjected to contamination from udder of animal and adjacent areas.
  - Bacteria found in manure, soil and water contaminate are udder of animal from where they enter into the milk.
  - Other possible source of contamination is hand and finger of milker or other dairy workers.
  - Contamination also occurs from dairy utensils.
- **During transport and at processing plant:**
  - During transport and manufacturing, contamination occur through tanker, transfer pipes, sampling utensils and other equipment.
  - Sometimes, pathogen may contaminate the milk from hand and finger of milk handler.

Microbial Spoilage of milk and mik products:

- Milk is an excellent culture media for growth of many microorganisms.
- Therefore, different types of microorganisms grow in it and cause spoilage.

i. Spoilage of Milk and cream:

- **Souring:**
  - Evidence of souring of milk are sour flavor and then coagulation of milk to form solid like curd.
  - Many lactic acid bacteria, coliform and other bacteria ferment sugar of milk and produce acid.
  - At temperature of 10-37^{o}C, *Streptococcus lactis* is most likely to cause souring with possible growth of *Coliform, Enterococci, Lactobacillus* and *Micrococcus.*
  - At higher temperature, 37-50^{o}C, *Streptococcus thermophilus* and *Streptococcus faecalis* may produce 1% acid and it may be followed by *Lactobacillus* which produces more acid.
  - Little souring occurs in milk held at refrigeration temperature.

- Pasteurization of milk kills more active acid forming bacteria but permit survival of thermoduric lactic acid bacteria such as *Enterococcus, Streptococcus thermophilus, Lactobacillus,* etc.
- Bacteria other than lactic acid bacteria produce acid specially if conditions are unfavorable for lactic acid bacteria.
- For example: *coliform* produce acetic acid, formic acid, ethanol, CO_2, H_2 etc.
- Similarly, *Clostridium* produce butyric acid.

- **Gas production (Strong fermentation of milk):**
  - Sugar fermenting organism produce gas together with acid.
  - Main gas formers, *Coliform, Clostridium, Heterofermentative lactic, Propianics bacillus*, etc.
  - *Coliform, Clostridium,* and *Bacillus* produce both H_2 and CO_2, while others produce only CO_2.
  - Gas production in milk is evidenced by foam at top of liquid milk by gas bubble trapped in curd, by formation of curd.
  - Excessive gas production causes cracking or breakdown of curd causing so called stormy fermentation of milk.
  - *Clostridium perfringens* mainly causes stormy fermentation.
- **Proteolysis:**
  - Proteolysis is facilitated by storage at lower temperature by destruction of lactic acid bacteria or by distribution of already produced acid by mold and yeast.
  - Changed cause by proteolytic organism include:
  - Acid proteolysis in which acid production and proteolysis occur simultaneously.
  - Proteolysis with little acidity or even alkalinity.
  - Sweet curdling which is caused by renin like enzyme of microorganisms.
  - Slow proteolysis by intracellular enzyme of bacteria after their autolysis.
  - Residual proteolytic activity of some heat stable proteinase.
  - Acid proteolysis is caused by *Micrococcus , Streptococcus faecalis var liquefaciens* and some lactose fermenting proteolytic *Bacillus* species.
  - Sweet curdling is caused by *Bacillus cereus.*
- **Ropiness/ sliminess:**
  - Ropiness of milk occur both by bacterial and non-bacterial causes non-bacterial ropiness occurs due to thickness of cream or due to film of cousin or Lactalbumin during cooling.
  - Bacterial ropiness is caused by slimy capsular material of bacteria which usually develop at low storage temperature.
  - Bacteria producing ropiness in milk are *Alcaligenes viscolactis, micrococcus freudenreichii, Enterobacter aerogenes, Klebsiella oxytoca, E. coli.*
- **Change in milk fat:**
  - Various bacteria, yeast and mold hydrolyses fat of milk and cause rancidity.
  - Species of *Proteus, Pseudomonas fragi, Staphylococcus, Bacillus, Micrococcus, Clostridium,* etc. are lipolytic.
  - *Pseudomonas fragi* and *Staphylococcus aureus* produce fairly heat resistant lipase.

- **Alkali production:**
  - *Pseudomonas fluorescence* and *Alcaligene viscolactis* produce alkali.
  - Alkali production is due to formation of ammonia from urea and formation of carbonate from organic acid.
- **Flavor defect:**
  - **Acid flavor:** Acid flavor may be aromatic or sharp. Sharp flavor is caused by production of acetic acid formic acid, butyric acid etc. by *Coliform* and *Clostridium.* It is undesirable. Aromatic flavor is caused by *Streptococcus lactic* and *Leuconostoc* when they grow together. It is desirable.
  - **Caramel or burnt flavor:** It is caused by *Streptococcus lactic var. maltigens.*
  - **Bitter flavor**: It is caused by proteolytic organism.
  - **Other flavor**: They include earthy flavor by *Actinomycetes,* fruity flavor by *Pseudomonas fragi,* soapiness by *Pseudomonas sapolactic* etc.
- **Color defect:** Growth of pigmented bacteria and other organism give undesirable color. Some examples include:
  - Blue milk: It is caused by *Pseudomonas syncyaneum*
  - Yellow milk: caused by *Pseudomonas synxantha* and also by flavobacterium.
  - Red milk: caused by *Serratia marcescencs* and *Micrococcus roseus.*
  - Brown milk: caused by *Pseudomonas putrefaciens* and by enzymatic oxidation of tyrosin by *Pseudomonas fluorescence.*

ii. Spoilage of Butter:

- Many spoilage microorganisms come in butter from cream or milk from which it is prepared.
- **Color defect:**
  - Some color defect of butter is non-microbial. They include pink color caused by sulphur-dioxide refrigerant, surface darkening caused by evaporation of water from surface.
  - Discoloration caused by microorganisms depend on type of organism. For example, *Stemphylium* give black spots, *Penicillium* give green spot, *Alternaria* or *Phoma* give brown spots, *Pseudomonas nigrificans* give reddish brown spot etc.
- **Flavor defect:**
  - Cream and butter have capacity to absorb moisture from surrounding.
  - Butter may gain such flavor from absorption of flavor is developed in butter during microbial growth.
  - Some odors in butter caused by growth of organisms include:
  - Fishiness caused by *Aeromonas hydrophila.*
  - Ester like flavor caused by *Pseudomonas fragi.*
  - Rancid odor caused by lipase producing organism.
  - Yeasty flavor caused by yeast etc.

iii. Spoilage of Cheese:

- Spoilage of cheese occurs either by mechanical damage or by microorganisms.
- Microbial spoilage of cheese occurs during following three stages:
- **Spoilage during manufacturing:**

- During manufacture of most cheese lactic starter culture is added to carry out lactic acid fermentation.
- If these lactic starters are not effective or when contamination is heavy, many contaminating organisms grow in it and bring undesirable changes in cheese. For.eg. if starter culture is not effective, *Clostridium* and *Bacillus* grow and produce holes and other changes.
- Acid proteolytic bacteria may produce bitter flavor.
- *Leuconostoc* may produce holes in cheese.
- Various organism cause proteolysis, gas production, sliminess and off flavor that damage the quality of cheese.
- Cheese with too low acidity because of failure of starter culture or because of addition of cream is often made slimy by *alcaligenes*, *melalcaligenes* and *Pseudomonas fragi.*

- **Spoilage during ripening:**
  - During ripening, spoilage occurs by enzyme released from autolyzed bacteria or by growth of microorganisms during ripening.
  - Main type of spoilage differs with type of cheese.
  - In most of cases, like gas production by *Clostridium, Heterofermentative lactis, Propionibacterium, Yeast* etc. cause eye formation or cracking of cheese.
  - *Clostridium* also produce undesirable flavor by production of butyric acid.
  - Certain lactic streptococci give bitter flavor.
  - Some bacteria and yeast give sweet, fruity and yeasty flavor.
  - In cheese with insufficient acidity, putrefaction is caused by anaerobic *Clostridium.*
  - Microorganisms also caused discoloration on surface of cheese.
  - Bluegreen or black discoloration are produced by reaction of H_2S produced by Microorganisms with metal or metallic salt.
  - Oxidation of tyrosine by bacteria give reddish brown to greyish brown color.
  - *Propionibacterium* grow as yellow, pink or brown colored complex.
- **Spoilage of finished cheese:**
  - Soft cheese is most perishable and hard cheese such as cheddar and swiss cheese are most stable.
  - Most common spoilage organism of finished cheese are molds.
  - They grow on surface or into holes or cracks and cause discoloration.
  - Sometimes off flavor is also produced.
- Some molds causing spoilage of cheese are:
  - *Cladosporium:* It grows on surface and gives black discoloration.
  - Oospora (*Geotrichum)*: *Oospora lactis* (called dairy mold) grow on surface of soft cheese. In this case, curd gradually becomes liquified under the growth. *Oospora crustacea* give red spots.
  - *Penicillium: Penicillium puberulum* and other green spored species grow on surface or into holes and give green coloration.
  - *Monilia: Monilia nigra* grow on surface of hard cheese and give black discs.

Canned foods:

- Heated canned foods are microbiologically more stable than most other foods.
- Better keeping quality of heated canned food is due to:
  - Heat treatment applied during canning kills most of the microorganisms present in original food.
  - Recontamination of food from outside is prevented by sealed can.

Spoilage of heated canned food:

- Spoilage of heated canned foods occurs by two ways:
  - Survival of spores of some thermophilic bacteria during heating of canned foods.
  - Recontamination of microorganisms from outside through leak in container.

Various types of spoilage of heated canned food are:

i. Spoilage of canned food by spore forming thermophilic bacteria:

- Spores of thermophilic bacteria are not killed easily by heat treatment applied during canning.
- Later, spore germinates in food and cause spoilage.
- Three important types of spoilage caused by thermophilic spore formers are:
- **Flat sour spoilage**:
  - This type of spoilage occurs mainly in low acid foods and is caused by species of *Bacillus,* such as *Bacillus coagulase* and *Bacillus stearothermophilus.*
  - Later, spore germinate when canned food is kept hot for sometimes as in case of slow cooling of can.
  - They produce acid without gas in food.
  - Therefore, the can remains flat during spoilage and hence spoilage cannot be detected by examination of can from outside.
- **Thermophile anaerobe spoilage:**
  - A spoilage is caused by *Clostridium thermosaccharolyticum,* which is a thermophilic anaerobe.
  - It produces acid and gas in food. Gas is a mixture of CO_2 and H_2.
  - Due to gas production, can swells and finally burst if it is kept for long time.
  - The spoiled food has usually sour odor or taste.
- **Sulfides or sulfur stinker spoilage:**
  - It is caused by *Desulfotomaculum nigrificans*.
  - Spoilage usually occur in low acid food.
  - Spore of these organisms are usually less heat resistant than those of flat sour and thermophile anaerobes bacteria.

- Therefore, spoilage by this organism indicates inadequate heat treatment during canning.
- The organism is also obligate thermophile. Therefore, spoilage by this organism occur in case of poor cooling or hot storage of can.
- The organism produces H_2S that react with tin of can to form black spots of FeS in food and on inner wall of can.
- H_2S produced give putrid odor which is widened when can is opened.

ii. Spoilage of canned foods by spore forming mesophilic bacteria:

- Spoilage by mesophilic spore formers result from inadequate heat treatment during canning.
- **Spoilage by mesophilic *Clostridium* species:**
  - Several mesophilic *Clostridium* causes spoilage of canned food.
  - Sugar fermenting species such as *Clostridium butyrium* and *C. pasteurianum* produced butyric acid by fermentation of sugar.
  - They also produce hydrogen and carbon-dioxide that sweet the can other species such as *Clostridium botulinum, C. sporogens* and *C. putrifaciens* are proteolytic or putrefactive.
  - They decompose protein to produce H_2S, endole, skatable and mercaptans that give bad odor to the food.
- **Spoilage by mesophilic *Bacillus* species:**
  - Many species of *Bacillus* are aerobic and therefore cannot grow in well evacuated can.
  - They cause spoilage especially in poorly evacuated can.
  - *Bacillus subtilis, B. mesentericus* and *B. polymyxa* cause spoilage of many canned foods such as corn, tomato etc.
  - Since, spores of these organisms are less heat resistant, they usually spoiled food by contaminating through leakage container.

iii. Spoilage of canned foods by non-spore forming bacteria:

- Non spore forming bacteria cause spoilage of canned food if mild heat treatment is applied during canning or, when they enter into the can through leakage during cooling by contaminated water.
- Many thermoduric bacteria such as *Enterococcus, Streptococcus thermophilus, Micrococcus, Lactobacillus* and *Leuconostoc* species can survive pasteurization temperature and spoil the canned food.
- However, in most of the cases, spoilage by non-spore formers indicates leakage of container.
- Type of spoilage depends on type of microorganisms. For example: *Coliforms* and heterofermentative lactic acid bacteria swell the can by production of gas.
- Non gas forming bacteria such as *Pseudomonas, Alcaligenes, Micrococcus, Proteus* etc. also spoiled canned food.

iv. Spoilage of canned foods by yeast and mold:

- When yeast grow in canned food, they cause swelling of can by production of CO_2.

- Spoilage by yeast indicates recontamination through leakage or lack of heat processing plus poor evacuation.
- **Spoilage by mold:**
  - Some species of mold such as *Sclerotia* and *Byssochlamys fulva* are somewhat heat resistant and they survive mild heat treatment.
  - Other molds enter through leakage in can.
  - Mold cause spoilage of high acid and high sugar foods such as jam strain of *Aspergillus penicillium* and *Citromyces* are found commonly in canned food.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC. MICROBIOLOGY

UNIT – III – FOOD & DIARY MICROBIOLOGY – SMB2202

Principle of food preservation:

- Various chemical and physical methods are available for preservation of food. But selection of appropriate method is very important to protect the quality and nutritional value of the food.
- During preservation by these methods, following principle are involved.
- **Prevention or delay of microbial decomposition of food by:**
  - Preventing contamination of food by spoilage organisms (Asepsis).
  - Removing micro-organisms from contaminated food. For.eg. by washing.
  - Slowing down the growth and activity of micro-organisms in food. For.eg. by freezing the food.
  - Killing micro-organisms present in the food. For.eg. by heating the food or by adding some chemicals.
- **Prevention of self-decomposition of food by:**
  - Damaging self-enzyme of food that cause self-decomposition. For.eg. damaging of ripening enzyme of fruits by washing it in hot water.
  - Preventing purely chemical reaction occurring in food that damage the food. For.eg. prevention of oxidative rancidity of lipid by addition of anti-oxidant.
  - Prevention of mechanical damage of food caused by insects, bird, food handling device etc.

Methods for prevention of microbial spoilage of food:

- Various methods of food preservation include:

1. By preventing food contamination (Asepsis):

- Process of preventing contamination of food by spoilage micro-organisms is called asepsis.
- Inner tissue of healthy animal and plants do not contain micro-organisms and if present they usually do not spoil food.
- Most spoilage micro-organisms enter into the food by contamination from external sources like water, air etc.
- Therefore, microbial decomposition of food can be prevented by preventing contamination of food by spoilage organisms.
- Several foods like egg contains natural covering around food that prevent entry of spoilage organism into the food.
- Nowadays, variety of such artificial barrier are made around the food to prevent contamination.
- For.eg. fruits and vegetables are stored for years by coating the wax around them.
- Some other artificial barriers that prevent the contamination includes wrappers, cartoons and cans.
- Safe handling of food and good personal hygiene of food handler are important method to prevent or minimize contamination of food by spoilage organisms.

2. By removing microorganisms from contaminated food:

- It is second level of preservation of microbial decomposition of food.
- If contamination of food by spoilage organism is not prevented, spoilage of food still can be prevented by removing contaminated micro-organisms from the food.
- Some techniques that preserve food by removing micro-organisms include:
- **Filtration:**
  - It is only one successful method for removing micro-organisms from contaminated food.

- Unfortunately, this method is applicable only for clear liquid like clear fruit juice, beverages etc.
- To remove micro-organisms, liquid food is filtered through sterile bacterial filter.

- **Centrifugation/ sedimentation:**
  - Centrifugation removes some but not all micro-organisms from contaminated liquid food.
  - When liquid food is centrifuged suspended particle, debris and some micro-organisms settle to the bottom which is later separated and remove from the food.
  - This technique is commonly used for milk in dairy industries.
- **Washing:**
  - This method is commonly used for vegetable and fruits. Dusts and micro-organisms present on the surface of whole vegetable and fruits are removed by washing with sterile water.
  - One limitation of washing is that it increases water activity of some food and hence facilitates growth of remaining spoilage organisms.
  - Furthermore, if washing water isnot sterile, it adds spoilage organisms in food.
- **Trimming:**
  - Trimming is the process of removing spoiled parts of food like vegetables and fruits by cutting.
  - Trimming prevents spread of micro-organisms from spoiled part into inner healthy tissue.

3. By inhibiting or killing microorganisms:

- Different methods that preserve food either by killing micro-organisms or by slowing down their growth and activity includes:
  - **Preservation of High temperature (Heat)**
  - **Preservation by low temperature**
  - **Preservation by Drying**
  - **Preservation by Radiation (irradiation)**
  - **Chemical agents used in preservation of food**

I. Preservation of food by High temperature (Heat):

- Heat is the most commonly used method of food preservation.
- Heat kills spoilage of micro-organisms by denaturation of cytoplasmic protein and enzyme.
- Two types of heat i.e. dry heat and moist heat are used in preservation of food.
- Moist heat is more effective in killing micro-organisms than dry heat.
- Heat causes denaturation of protein and enzyme by breaking down their bonds.
- Breaking of bond occurs by two mechanisms i.e. hydrolysis and oxidation.
- Hydrolysis require water but oxidation does not.
- Hydrolysis require less energy (heat) for breaking of bond than oxidation.
- Therefore, if moisture is present in food, bonds of protein and enzymes are broken down by hydrolysis even at lower temperature.
- This is the reason why moist heat can kill micro-organisms completely at condition of 121.5^{o}C for 15 min but dry heat requires 160^{o}C for 1-2hrs to kill the same organisms.
- Various factors affect killing of micro-organisms in food by heat.
- Some such factors include:
    - **Time-temperature relationship**
    - **Density of micro-organisms** in food, if density is high, higher temperature is needed.
    - **Types of micro-organisms** present in food, e.g. Spore are difficult to kill than vegetative cells
    - **Growth stage of micro-organisms,** e.g. micro-organisms present in log phase are easily killed but micro-organisms present in late log phase and stationary phase are somewhat heat resistant.
    - Micro-organisms in dry food are difficult to kill than in moist food.
    - If food is acidic or alkaline, micro-organisms present in log phase are easily killed by heat. On the other hand, if food is neutral, high temperature is needed to kill the same organisms.
    - Penetration of heat into inner layer of food also affects killing of micro-organisms.
    - Heat penetration into the food is affected by various factors as given below:
        - Nature of material by which container of food is made, affects heat penetration. E.g. heat penetrates easily into smaller and slim can than through larger and wider can.
        - In case of open heating of food, agitation or stirring of food facilitates heat penetration.

Differences between dry heat and moist heat:

Dry heat	**Moist heat**
It is less effective in killing micro-organisms.	It is more effective in killing micro-organisms
It damages protein and enzyme by oxidation.	It damages protein and enzyme by hydrolysis or coagulation.
It is especially valuable to sterilize the food whose quality is damaged by moisture (e.g. flour) and for lipids foods.	Moisture cannot penetrate into hydrophilic lipid food and moisture damage the quality of powder foods like flour.
Dry heat is applied to the food by spraying moisture free heated air over the food and by hot air oven.	Moist heat can be applied by boiling the food in water or by spraying heated steam over the food.

Conditions for sterilization is 160^{o}C for 1-2 hrs.	Conditions for sterilization is 121.5^{oC} for 15 minutes.

Some technique of food penetration by heat includes:

i. Pasteurization:

- Pasteurization is a selective or gentle heating technique originally developed by Lewis Pasteur for milk and milk products.
- Nowadays, pasteurization is also used for preservation of other beverages like beer, fruit juice etc.
- Pasteurization is a selective heating technique and it kills some but not all micro-organisms present in milk.
- If milk is contaminated by some disease causing micro-organisms, milk cannot be heated at boiling temperature to kill these micro-organisms.
- It is because heating at high temperature brings several undesirable changes in milk. E.g. it kills beneficial lactic acid bacteria present in milk.
- Similarly, casein protein precipitate, lactose caramelizes, flavor of milk changes.
- Such milk becomes unsuitable for production of milk products like icecream, cheese.
- Similarly, beverages like beer and fruit juice cannot be heated at higher temperature because it brings several undesirable changes in flavor, taste etc.
- In pasteurization, milk and beverages are heated at lower temperature that kills spoilage or pathogenic organisms without bringing undesirable changes in the product.
- There are three techniques of pasteurization of milk which differ in their time-temperature relationship.
- Time-temperature relation of pasteurization are selected on the basis of three bacteria which are transmitted through milk (*Mycobacterium bovis, Coxiella buretii* and *Brucella abortus).*
- **Low temperature holding (LTH) or Vat pasteurization:**
  - In this method, milk is heated at 62.8^{o}C for 30 minutes.
- **High temperature short time (HTST) method:**
  - In this method, milk is heated at 71.7^{o}C for 15 seconds.
- **Ultra-pasteurization:**
  - In this method, milk is heated at about 137.8^{o}C for 2 seconds.

ii. Canning:

- Canning is the process of preservation of food by keeping them in sealed container and then heating the container.
- Canning techniques was originally developed by Nicoles Appert.
- There are two types of canned food i.e. heated and unheated canned food.
- During canning at first raw food is washed and then processed.
- Then processed food is placed into a suitable can and the can is then ehated.
- Sealed cans can be heated either by immersing it into boiling water or by spray of super-heated steam or heated dry air.
- **There are two system of canning,**
  - i.e. hot pack system and cold pack system.
- In hot pack system, raw food is preheated before packing into the can and in cold pack system, food is not preheated.
- Canning is commonly used for preservation of many foods like vegetable, meat, fish etc.

- Canned food is microbiologically more stable and remain unspoiled for long time.
- It is because any micro-organism present in raw food are killed during heating the can and sealed container prevents entry of spoilage organism into the food.
- Defective canning is not safe for preservation of food. For.eg. if canned food is not properly heated, some thermophilic organism and endospore of bacteria present in raw food are not killed.
- Such spores then germinate and cause spoilage of the food even if the can is intact.
- Furthermore, can must be completely filled with food.
- If any space or air is present inside the can, this aerobic condition facilitates germination of surviving spores.
- Similarly, can used for packaging the food must be completely sealed.
- If any breaks or leaks are present in can many spoilage organisms enter into the food and spoil the food.
- Furthermore, air enters into the can through leaks that facilitates germination of surviving spores.

iii. Blanching or scalding:

- Blanching is the process of washing vegetables or fruits in warm water before storage.
- Advantages of blanching includes:
    - It inactivates enzyme of food that cause self-decomposition.
    - It reduces number of micro-organisms present on the surface of food.
    - It brings wilting of leafy vegetables and helps in packaging.
    - It enhances fixing of intense green color of fruits and vegetables.

iv. Roasting:

- It is used for meat and meat products.
- During roasting internal temperature of meat reaches (80-85)^{o}C.

v. Baking:

- It is used for bread, cake and other bakery products.
- During baking temperature maximum of 97^{o}C is reached.

vi. Frying:

- It is used for vegetable, meat, fish etc.
- During frying outer surface of food becomes very hot but internal temperature of food never exceed 100^{o}C.

vii. Cooking:

- During cooking temperature around 100^{o}C is reached.

viii. Simmering:

- It is the process of gentle boiling of food with the temperature of about 100^{o}C.

II. Preservation of food by low temperature:

- Low temperature is commonly used physical method of preservation of food.
- Low temperature preserves food by:
  - Slowing down growth and activity of spoilage organism in food.
  - By slowing down the rate of chemical reaction or other enzymatic activity that cause self-decomposition of food.
  - Sometimes low temperature kills spoilage organism in food. E.g. in case of deep freezing.
- Different methods of food preservation by low temperature:

ii. Common or cellar storage:

- Common storage is the technique of preservation of certain foods like potato, onion etc. in home by spreading them in cold room.
- In common storage, storage temperature is slightly lower than that of outside air.
- But this technique food is not stored for long time but it is still used where cold storage and facilities are not available.

ii. Chilling or cold storage:

- In chilling or cold storage, storage temperature is usually between (0-10)^{o}C.
- At this temperature, growth and activity of most bacteria except psychrophiles is significantly slowed down.
- Therefore, by chilling storage, food can be stored for long time than common storage.
- However, if psychrophilic organisms are present in food, they grow in food and cause spoilage.
- Selection of appropriate temperature is very important because quality of some food is damaged at lower temperature storage temperature of some food are (10-12.8)^{o}C for sweet potato, (13.3-16.7)^{o}C for banana etc.
- This method is most commonly used for short term storage of highly perishable foods like meat, milk, fish etc.
- Fish is stored by placing it in cold ice.

iii. Freezing or frozen storage:

- In freezing storage, storage temperature is between (-15 to -29)^{o}C.
- At this temperature, growth of even psychrophilic bacteria is completely inhibited.
- Therefore, food kept in frozen storage remain fresh in long time without undergoing microbial decomposition.
- Furthermore, self-decomposition of food occurs very rarely or not at all at this temperature.
- Sometimes, freezing also kills spoilage organisms present in food.
- Therefore, frozen storage is very effective method of food preservation among these three techniques.
- Killing mechanism of freezing is due to formation of ice-crystal and dehydration of microbial cell.

- When food is frozen, surrounding water freezes to form ice-crystals.
- Then cytoplasmic water from microbial cell continuously diffuses out and is added in surrounding ice-crystal.
- The remaining cytoplasm in microbial cell gradually becomes more and more concentrated.
- High salt concentration in remaining cytoplasm kill the microbial cell by causing mechanical damage to the cell.
- There are two methods of freezing the food i.e. quick freezing and slow freezing.
- Slow freezing is more effective in killing microbial cell but quick freezing is considered more appropriate for preservation of food.
- When food is frozen very slowly, there is sufficient time for cytoplasmic water to diffuse out.
- In this case microbial cell is dehydrated to greater extent.
- Similarly, larger ice-crystal are formed due to continuous addition of diffused water into ice.
- Larger ice crystal gives more mechanical damage to microbial cell.
- However, if larger ice-crystal are formed in food, it gives more mechanical damage (causes cracking of food) to the food.
- Therefore, quick freezing is commonly used for food preservation.
- Although, frozen storage completely prevents microbial decomposition, it brings several undesirable changes in some food.
- Some such examples include:
    - Protein of food e.g. meat may become dehydrated so that the food becomes very tough and rigid.
    - Myoglobin present in meat may be oxidized into brown colored meat-myoglobin.
    - Fat present in food may be hydrolyzed or oxidized.
    - Ice-crystals formed on surface of food evaporate and give mechanical damage on the surface of food. This damage is called freeze-burn.
- **Methods of freezing:**
- **Quick freezing:**
    - In quick freezing, temperature of the food lowered very quickly.
    - Food is frozen quickly by immersing food into solution of refrigerant. For.eg. fish frozen by immersing it into frozen solution of NaCl.
    - By blowing frigid air (at -17.8 to -34.4^{o}C) over the food to be frozen.
- **Slow freezing:**
    - In this process, temperature of the food is lowered very slowly.
    - Food is frozen slowly by placing it inside the mechanical refrigerator.
    - This technique is commonly used to freeze food in home.
    - However, slow freezing forms larger ice-crystal in food and give more mechanical damage.
    - Therefore, quick freezing is preferred over slow freezing.

Differences between slow freezing and quick freezing

Slow freezing	Quick freezing
It is more effective in killing micro-organisms in food.	It is less effective in killing micro-organisms in food.
Larger ice-crystals are formed.	Smaller ice-crystals are formed.
Microbial cell is dehydrated to greater extent.	Microbial cell is dehydrated to lesser extent.
It gives more mechanical damage due to formation of larger ice-crystal.	It gives less mechanical damage due to formation of larger ice-crystal.
Micro-organisms may adopt slowly to lower temperature.	There is no time for adaptation.
Cold shock does not occur.	Microorganisms may be killed by cold shock.

III. Preservation of food by Drying:

- Many spoilage micro-organisms are highly susceptible to drying.
- Some spoilage organisms are killed rapidly by drying and growth of most other organism is lowered or, inhibited by drying.
- Therefore, microbial decomposition of food can be prevented or at least minimized by drying the food.
- There are different processes of drying food.
- Drying is performed by either evaporation of water from the food or by lowering A_w value of food by addition of salt and sugar.
- During drying of food by evaporation, several parameters like time of drying, humidity of air, temperature of the air etc. should be controlled.
- Improper control of this parameter brings several undesirable changes on food.
- For.eg. if food is dried quickly by placing it in very hot environment, moisture is evaporated only from the surface but not from inner layer of food.
- In this case, very hard layer is formed on surface that prevents further dehydration of food.
- This defect is called case hardening.
- **Different methods of drying food include:**

i. Solar drying:

- In this method, food is dried by placing it in hot atmosphere of sun.
- Many foods like grains and chopped pieces of vegetables used for making pickles are dried by this method.

ii. Drying by mechanical drier:

- In this method, food is dried with the help of some mechanical drier.
- Liquid food like milk are dried by spray drying and drum drying method.

- Some liquid foods are dried by placing them in vacuum chamber of lower temperature.

iii. Drying by addition of salt and sugar:

- It is indirect method of drying.
- When salt or sugar is added in food, it binds water in the form of shell of hydration and make it unavailable for micro-organisms.
- Therefore, they decrease the amount of available water (A_w) of food.
- Example of food dried by this method includes preservation of food by adding high salt concentration and preservation of milk by adding sugar.

IV. Preservation of food by Radiation (irradiation):

- Two types of radiation are used in food preservation. They include:

i. Non-ionizing radiation:

- UV light is an example of non-ionizing radiation.
- When microbial cells are exposed to UV light, it is absorbed by nitrogenous bases of DNA.
- After absorption, UV light catalyze the formation of thymine dimer by linking ring of adjacent thymine.
- When such DNA replicates rate of error of replication is very high at the position of thymine dimer.
- Therefore, UV light kills microbial cell by inducing mutation.
- One major limitation of UV light in food preservation is its lower penetration power.
- Therefore, it cannot penetrate into inner layer of solid food and viscous liquid food.
- It is applicable only for clear liquid food like clear fruit juice.
- It can also be used to kill micro-organisms on surface of solid food.
- UV light of wavelength around 254nm has maximum antimicrobial activity.
- In food industry germicidal lamp that emits UV light mainly in the range of 254nm are used as a source of UV light.
- **Application of UV light in food preservation:**
    - UV light is used to kill micro-organisms present in clear liquid foods. For.eg. it is used to kill yeast cells and other micro-organisms in fruit juice.
    - It is used to inhibit growth of yeast and mold on surface of some foods like pickles, vinegar, cheese etc. UV light is commonly used to prevent surface spoilage of various foods including pickles, vinegar etc.
    - UV light is used to disinfect water used for production of beverages.
    - It is used to kill micro-organisms present in air of food processing room.
    - It is also used to kill micro-organisms on various food processing instruments like knife, eating utensils, etc.
    - UV light is used in rapid ageing of meat in meat industries. Ageing is the process of drying meat by hanging pieces of meat in controlled environment. Usually meat is aged by hanging it in room at a temperature of 2.2-3.3^{o}C. At this temperature, ageing occurs in several months. Temperature is maintained lower to prevent growth of micro-

organisms in meat during ageing. If ageing is done at higher temperature micro-organisms grow in meat and spoil it before ageing takes place. In rapid ageing technique temperature of room is maintained around 18^{o}C and UV light is used to inhibit growth of micro-organisms in meat during ageing. At this temperature ageing occurs in 2-3 days.

ii. Ionizing radiation: (Cold sterilization)

- X-ray, γ-ray and cathode ray are examples of ionizing radiation.
- When microbial cells are exposed to ionizing radiation, they cause ionization of cytoplasmic water and generate very toxic compound like H_2O_2, hydroxyl free radical (OH^*) and super oxide ion (O_2^-).
- They are powerful oxidizing agents and kill the microbial cell by oxidizing various cellular materials.
- Some common ionizing radiation used in food industry include:
- **X-ray:**
  - Production of X ray is expensive.
  - Furthermore, its penetration power is lower. Therefore, it is not commonly used in food preservation.
- **γ-ray:**
  - It has good penetration power and it can penetrate upto 20cm even in solid food.
  - γ-ray is emitted in all direction from the source. Therefore it is difficult to make γ-ray to hit the food.
  - So, efficiency of γ-ray in food preservation is only 10-25%.
- **Cathode ray:**
  - Cathode ray is directional and it is emitted in only one direction.
  - Therefore, efficiency of cathode ray in food preservation is high (upto 40-80%).
  - Unfortunately, it has lower penetration power and can penetrate only upto 0.5cm in solid.

Applications of ionizing radiation in food processing:

- Some applications of ionizing radiation in food preservation includes:
- Low level of ionizing radiation is used to kill microorganisms on fresh fruits and vegetables. It is used to kill insect as well as microorganisms on vegetable and fruits.
- High level of ionizing radiation is used to kill microorganisms and insect on dry vegetable and fruits.
- Ionizing radiation are commonly used to preserve meat and meat product. For.eg. irradiation is commonly used to inhibit microorganisms, nematodes and other parasites present in meat and meat products.

Undesirable changes of ionizing radiation:

- In meat, it increases pH, damage glutathione, increase carbonyl compound and generate bad smelling compound like H_2S and methylmercaptan.
- In fatty food, it damages natural anti-oxidant and increases chance of oxidative rancidity.
- It causes loss of several vitamins like pyridoxine, thiamine, vit. C, vit. D, vit. A, vit. B_{12}. Therefore, it reduces nutritional value of food.

Principle of PEF – high voltage pulse

PEF technology involves the use of pulses having higher electric fields for only a few micro to milliseconds with intensity in the range of 10-80kV/cm. The process depends on the number of pulses delivered to the product which is held between two electrodes. These electrodes have a specific gap between them which is known as treatment gap of the chamber. During PEF processing, high voltage is applied that results in the inactivation of microorganisms present in the food sample. The electric field is applied in different forms like as exponentially decaying waves, bipolar waves or oscillatory pulses. The process can also be carried at various temperature ranges such as ambient, sub-ambient and above-ambient temperatures. Food is packed after treatment with PEF and then stored under refrigerated conditions.

The science involved behind the transfer of electric pulses from food is that food contains several ions that provide a definite level of electrical conductivity to the product. This technique is usually preferred for liquid foods because electrical current flows into the liquid food more efficiently and the transfer of pulses from one point to other in liquids is quite easy due to the presence of charged molecules present.[8]

A group of researchers, Zimmermann et al.,[9] stated that mechanism of the functioning of PEF technology is the delivery of pulsing power to the product that is placed between a set of electrodes confining the treatment gap of the PEF chamber. The typical system has a pulse generator that produces high voltage pulses, treatment chamber that handles the product to be treated and associated with controlling and monitoring devices. Food product is placed in the treatment chamber equipped with electrodes connected with each other by a nonconductive material which prevents the flow of electricity from one to the other. High voltage electrical pulses are generated that are transferred to the product. The product placed between the electrodes experiences a force per unit charge which ruptures the bacterial cell membranes.[10] In general, PEF technology is suggested for pasteurization of various food products including milk, juices, yogurt, liquid eggs and soups.[11] Furthermore, combination of PEF with ultrasound, high pressure and ultraviolet light treatments may enhance the process output.

Microbial inactivation by PEF

Several studies have investigated the mode of action of PEF to reduce the microbial load in various food products. Nonetheless, the exact mechanistic approach underlying the microbial inactivation by PEF has not been fully expounded as yet. However, a general mechanism of PEF action involves instability of microbial membranes by the induction of electrical field and electromechanical compression that leads to the pore formation in membrane.[12] Mechanical fickleness of membranes is caused due to critical membrane potential which is formed by electrical field. Electroporation results in a significant increase in the membrane rupture and permeability which is termed as electro permeabilization. Electropermeabilization can be

reversible or irreversible, depending on the degree of membrane organizational changes that results in cell death.[13] Literature explains that membrane permeability is increased in a considerable manner by increasing the strength of electric field. This elevated membrane instability harmonizes with inactivation of microbial cells. In general, spores are claimed as more obstinate to PEF treatment as compared to vegetative cells.[14]

Factors influencing PEF performance to inactivate microorganisms

The capability of PEF to inactivate microbes depends on several factors that can be categorized as process parameters, nature of product and properties of microbial cells.[6] These factors play a dynamic role to attain the optimum performance of PEF treatment. An overview of these parameters is discussed herein.

Process parameters

Several process parameters affect the ability of PEF to reduce microbial population in food such as strength of electric field, pulse length and shape, number of pulses and temperature.[15] On a general node, increased intensity of these factors improves microbial inactivation but their exact relationship with the survival rate of microorganisms is not clear. Therefore, exact measurement of all these parameters is required to acquire reliable results.

Product nature

The administration of PEF is also influenced by nature of product as a vast range of products are being treated by PEF which include fruit juices, liquid eggs, milk and dry herbs. It is investigated through experimental trials that PEF treatment is not much effective for products having particles or special structures, i.e. emulsions.[6] Additionally, physical and chemical properties of the food also affect the rate of microbial decontamination. Various studies have revealed the influence of pH, water activity and electrical conductivity on the efficiency of PEF to inactivate microorganisms.[16]

pH has a significant influence on the inactivation kinetics of microbes. Jeantet et al.,[17] reported that higher inactivation of *Salmonella* was observed in foods having neutral or above neutral pH values. Similarly, Alvarez et al.,[16] testified substantial reduction in L. monocytogenes number in high acid foods such as citrus juices. Likewise, conductivity of the treatment medium has inverse relation with microbial inactivation. It is observed that foods having high electrical conductivity show less inactivation of microorganisms after PEF treatment.[18] On the contrary, water activity has a direct relation with microbial reduction by PEF treatment as confirmed by Min & Zhang.[19]

Characteristics of microbes

Inactivation of microorganism by employing PEF technology also depends on microbial characteristics including type of microorganisms, species and strains.[20] Generally, Gram-positive and Gram-negative bacteria are thought to be more resistant in comparison with yeast cells. Likewise, bacterial and mold spores are also claimed as recalcitrant to PEF treatment.[14] Additionally, cell size and shape also affect the inactivation kinetics due to the

difference in the development of critical membrane potential.[21] PEF treatment affects different bacterial species at altered rate. It is usually proposed that Salmonella and E. coli are more susceptible to PEF as compared to Listeria and Bacillus species. Growth conditions like temperature, growth medium, concentration of nutrients and pH of the treated medium also influence PEF efficiency as well.[6]

Applications in food industry

Application of PEF technology has been extensively demonstrated for the pasteurization of various food products like juices, milk & dairy products, soup and liquid eggs. However, it has several limitations such as product must be free from air bubbles and must have lower electrical conductivity. Additionally, particle size should be less than the gap of the treatment region to ensure appropriate treatment. PEF is generally not suitable for solid foods however several solid products have also been investigated to be efficiently treated by deploying PEF treatment. PEF technology can also be used to enhance the extraction of several bioactive components and sugars from plant cells.[2]

PEF processing has shown its potential to treat less viscous fruit juices having less electrical conductivity such as apple, citrus and cranberry juices. PEF technology also executes beneficial aspects on the quality parameters of fruit juice. Correspondingly, Yeom et al.,[22] compared pasteurized and PEF-treated citrus juice during refrigerated storage (4°C) for a period of 112days and observed less browning in PEF-processed juice comparatively to traditionally pasteurized juice due to conversion of ascorbic acid to furfural. Besides, PEF-treated foods also retained their fresh flavor, textural & functional attributes and extended shelf life in addition with microbiological safety.[23] In recent years, PEF technology has been utilized for various purposes like enhancement of drying efficiency, modification of enzymatic activity, solid food preservation, waste water treatment and extraction.[24]

PEF in fruit processing

PEF processing has promising applications in citrus industry with special reference to the inactivation of microorganisms and prevention of developing off-flavors during the storage.[25] Jemai et al.,[21] noticed that treatment of apple juice with PEF resulted in enhancement of diffusion coefficients of soluble substances. This technology can also be fruitfully applied for the disintegration of biological tissues that enhances the extraction of intracellular compounds from different fruits. For instance, pectin is a very useful component found in many fruits that is traditionally being extracted through enzymatic reaction but this reaction has less yield of pectin due to poor efficiency. Alternatively, PEF treatment is employed as short pulses to avoid excessive heat and undesirable electrolytic reactions that can enhance the extraction rate of pectin from fruit pomace.[26]

Bacterial inactivation in milk by PEF

Inadequately pasteurized milk may cause several health hazards due to the presence of several spoilage causing and pathogenic bacteria particularly *Escherichia coli* and *Listeria* and *Pseudomonas spp*.[27] Elevated concerns regarding the impact of heat

treatment on the quality of milk and consumer demand for fresh-like quality attribute products have encouraged the induction of PEF for milk pasteurization. PEF-induced microbial inactivation is believed to be an effective way of milk preservation without adversely affecting the milk quality.

Studies have demonstrated the effectiveness of PEF processing for microbial reduction in simulated milk ultra-filtrate and skim milk.[28] However, presence of fat and protein moieties limits the adeptness of PEF in whole milk because these molecules protect bacterial cells during treatment.[29] Therefore, it is imperative to validate the worth of PEF treatment to inactivate bacteria in a complex whole milk matrix for a genuine comparison with thermal pasteurization.

Garcia et al.,[30] observed sub-lethal injury in bacterial cells and concluded that PEF treatment can be successfully used in synergy with other hurdles to get more benefits. In another trial, PEF processing of milk was combined with heat treatment up to 55-60°C and a significant reduction was observed in the microbial load.[31] More recently, Sharma et al.[29] employed PEF treatment at different dose, time and temperature ranges to inactivate Gram-positive and Gram-negative bacteria in whole milk and reported 5-6 log reduction in bacterial number at 22-28kV cm^{-1} for 17-101µs at 50°C. On the other hand, some studies did not clearly demonstrate the effects pre-heating, temperature, time & dose variation on PEF efficiency and effects of PEF treatment on milk quality and functional properties. Therefore, it is questionable whether PEF can maintain the integrity of heat-sensitive milk components while inactivating spoilage and pathogenic microorganisms in whole milk and need further exploration.

PEF and meat quality

Meat products have been widely consumed around the globe due to the presence of valuable micronutrients high quality protein.[32] Quality of meat is at prime importance because meat quality is considered as the most vital factor for purchasing decisions of consumers.[33] Pulsed electric field technology has shown its potential for various applications in solid foods with different aims including structural modifications, changing physical quality parameters, extraction of bioactive compounds and preservation. Nevertheless, this technology has limited applications in muscle foods.[34] PEF technology can be employed in meat processing for various purposes including enhancement of cell permeation to increase tenderness, attenuation of microbial load to improve the shelf life and maintenance of volatile profile of meat during storage.[35]

PEF technology can considerably improve enzyme release and glycolysis that are essential for proteolysis for meat tenderization. O'Dowd et al.,[34] studied the effect of PEF on meat quality @ 1.1-2.8kV cm^{-1} and reported no significant improvement in meat tenderness during storage. Later on, Bekhit et al.,[36] found a weighty improvement in beef tenderness by PEF treatment (5-10kV). Likewise,[37] applied PEF (5-10kV) at different frequencies (20, 50 and 90Hz) on beef muscles and revealed that PEF treatment reduced shear force up to 19% & improved tenderness by augmenting the degradation of desmin and troponin-T during a refrigeration storage of 21days.

Consumer acceptance of PEF treated products

Pulsed electric field technology is gaining importance as a non-thermal way to process or preserve foods. Additionally, industrial implementation of this technique and commercialization of PEF-treated food products are also in progress. Conversely, there is a lack of steadiness in terms of terminology, product & process description, product communication with consumer and marketability of the PEF-processed foods. Efficient marketability of an emerging technology needs unambiguous benefits for consumers. Consumer attitude determines the level of acceptance of a novel food product and this attitude depends on the method of introduction of a new product and technology.[38]

PEF-processed foods have limited acceptance by the consumers because of several reasons. The major hitch is that the nature of this technology has not yet fully expressed to consumers as consumers require information about the products they consume. Moreover, PEF-treated products are generally labeled as "minimally processed foods" that induces negative image in the mind of consumer that the food is not well-processed and may cause serious health implications after consumption.[39] Since clear communication is of prime importance for consumers, the description about the technology and terminologies used on the labeling should be explained to them in order to enhance the marketability of PEF-processed foods.

High pressure processing (HPP)

Pascalization, **bridgmanization**, **high pressure processing** (**HPP**) or **high hydrostatic pressure** (**HHP**) **processing**[2] is a method of preserving and sterilizing food, in which a product is processed under very high pressure, leading to the inactivation of certain microorganisms and enzymes in the food.[3] HPP has a limited effect on covalent bonds within the food product, thus maintaining both the sensory and nutritional aspects of the product.[4] The technique was named after Blaise Pascal, a French scientist of the 17th century whose work included detailing the effects of pressure on fluids. During pascalization, more than 50,000 pounds per square inch (340 MPa, 3.4 kbar) may be applied for around fifteen minutes, leading to the inactivation of yeast, mold, and bacteria.[5][6] Pascalization is also known as bridgmanization,[7] named for physicist Percy Williams Bridgman.

Uses

Spoilage microorganisms and some enzymes can be deactivated by HPP, which can extend the shelf life while preserving the sensory and nutritional characteristics of the product.[9] Pathogenic microorganisms such as *Listeria, E. coli, Salmonella,* and *Vibrio* are also sensitive to pressures of 400-1000 MPa used during HPP.[10] Thus, HPP can pasteurize food products with decreased processing time, reduced energy usage, and less waste.[9]

The treatment occurs at low temperatures and does not include the use of food additives. From 1990, some juices, jellies, and jams have been preserved using pascalization in Japan. The technique is now also used there to preserve fish and meats, salad dressing, rice cakes, and yogurts. Furthermore. it preserves fruits, vegetable smoothies and other products such as meat for sale in the UK.[11][12]

An early use of pascalization in the United States was to treat guacamole. It did not change the sauce's taste, texture, or color, but the shelf life of the product increased to thirty days, from three days prior treatment.[5] However, some treated foods still require cold storage because

pascalization obviously cannot destroy all proteins, some of them exhibiting enzymatic activity[13] which affects shelf life.[14]

In recent years, HPP has also been used in the processing of raw pet food. Most commercial frozen and freeze-dried raw diets now go through post-packaging HPP treatment to destroy potential bacterial and viral contaminants, with salmonella being one of the major concerns.[15]

Process

In pascalization, food products are sealed and placed into a steel compartment containing a liquid, often water, and pumps are used to create pressure. The pumps may apply pressure constantly or intermittently.[1] The application of high hydrostatic pressures (HHP) on a food product will kill many microorganisms, but the spores are not destroyed.[9] Pascalization works especially well on acidic foods, such as yogurts and fruits,[3] because pressure-tolerant spores are not able to live in environments with low pH levels.[26] The treatment works equally well for both solid and liquid products.[1]

Bacterial spores survive pressure treatment at ambient or chilled conditions. Researchers reported that pressure in combination with heat is effective in the inactivation of bacterial spores. The process is called pressure-assisted thermal sterilization.[27] In 2009 and 2015, Food and Drug Administration (FDA) issued letters of no objection for two industrial petition for pressure-assisted thermal processing. At this time, there are no commercial low-acid products treated by PATP available in the market.

During pascalization, the food's hydrogen bonds are selectively disrupted. Because pascalization is not heat-based, covalent bonds are not affected, causing no change in the food's taste.[28] This means that HPP does not destroy vitamins, maintaining the nutritional value of the food.[9] High hydrostatic pressure can affect muscle tissues by increasing the rate of lipid oxidation,[29] which in turn leads to poor flavor and decreased health benefits.[30]Additionally, there are some compounds present in foods that are subject to change during the treatment process. For example, carbohydrates are gelatinized by an increase in pressure instead of increasing the temperature during the treatment process.[31]

Because hydrostatic pressure is able to act quickly and evenly on food, neither the size of a product's container nor its thickness play a role in the effectiveness of pascalization. There are several side effects of the process, including a slight increase in a product's sweetness, but pascalization does not greatly affect the nutritional value, taste, texture, and appearance. As a result, high pressure treatment of foods is regarded as a "natural" preservation method, as it does not use chemical preservatives.

MICROWAVE PROCESSING

Introduction

Microwaves are part of electromagnetic spectrum in the frequency range falling between radio and infrared region. Two frequencies have been set aside for exclusive use of microwave heating application namely 915 MHz and 2450 MHz.
Microwave heating is a method that offers technique of heating requiring neither conduction nor convection. Microwave generates heat within the food rapidly raising the temperature to the d*e*-sired extent. Special oscillator tubes called magnetrons and keltron, which generate the microwaves are used. These devices convert low frequency electrical energy into hundreds and thousands of megacycles. The electromagnetic energy at microwave frequency is conducted through a coaxial tube or wave-guide at a point of usage. The microwaves are channeled along a wave guide, then a stirrer or paddle distributes them evenly into cavity. Once they are inside the cavity, three things can happen to the microwaves, i.e. reflection, transmission and absorption.

The microbial inactivation kinetics for microwaves are essentially the same as the inactivation kinetics of conventional thermal processing. Although as many as four separate effects have been proposed -selective heating of micro-organisms, electroporation, cell membrane rupture and cell lyses due to electromagnetic energy coupling are the significant ones. It has also been suggested that microorganism load can be reduced to a greater extent by microwave treatment.

Mechanism of Microwave Heating

Heating with microwave frequency involves primarily two mechanisms dielectric and ionic. Water in the food is often the primary component responsible for dielectric heating. Due to their dipolar nature, water molecules try to follow the electric field associated with electromagnetic radiation as it oscillates at the very high frequency. Such oscillation of trip molecules produces heat. The second major mechanism of heating with microwave frequency is through the oscillatory migration of ions in the food that generate heat under the influence of the oscillating electric field. Kinetic energy is actually imparted to the ions by the electric field so that the field is alternating rapidly heat.

Microwaves penetrate materials and release their energy in the form of heat as the polar molecules (ones with positively and negatively charged ends - such as water) vibrate at high frequency to align themselves with the frequency of the microwave field. The microwaves interact directly with the object being heated. The interaction is related to the chemical properties of the object and it is possible to apply heat in ways that can not be achieved by conventional means: convection heating, conductive heating or radiant heating .

Microwave Generation

The microwaves are generated by special oscillator tubes called "Magnetrons and Kystron". These are devices that convert low frequency electrical energy into hundreds and thousands of megacycles. The electromagnetic energy, at microwave frequency is conducted through a coaxial

tube or wave guide at a point of usage. Both Magnetron and Kystron are electron tubes which generate microwaves.

1. **Magnetron:** It is a cylindrical diode with a ring of resonant cavities that acts as a anode structure. The cavity is the space in the tube which becomes excited in a way that makes at a source for the oscillation of microwave energy . The Magnetron is a vacuum valve in which the electron, emitted by the cathode, turn around under the action of a continuous electric field produced by the power supply and of a continuous magnetic field. The movement produces the electro-magnetic radiation.

2 **Keltron:** It is a vacuum tube in which the oscillation are generated by alternatively slowing down and speeding upon electron beam. This results in periodic bunching of electrons. Keltron uses the transit time between two given points to produce this modulated electron stream which then delivers pulsating energy to a cavity resonator and sustain oscillation within the cavity.

Advantages of Microwave Processing:

The main advantage of a microwave oven over the conventional oven (electric and gas oven) is its high thermal efficiency in converting the energy in electricity into heat in the food. Other advantages are:

1. Speedy: microwave cookers heat food more quickly than any other conventional oven (shortening of processing time often by 70-85% and more).
2. Clean: with microwave cooking there is no risk of the food burning onto the cooker walls or they do not become hot in the way that the surfaces of conventional oven do. In addition, most foods are cooked covered and so remain in their containers (higher quality of product).
3. Smell free: because food is contained within the cooker cavity (and usually also in a covered dish), smells are kept to a minimum.
4. Less washing up: it is often possible to microwave food in serving containers or on the plate from which it is to be eaten. This is reducing the kind of washing up required when saucepans and metal oven dishes are used.
5. Thawing: thawing can be done quickly in a microwave cooker, saving hours in the fridge or kitchen and removing, the need for too much forward planning.
6. Nutritionally sound: many foods retain more nutrients than when cooked conventionally, as cooking time is so short, and there is little or no added water, particular examples are fish, vegetable.
7. Easy to use: once controls and cooking techniques are mastered, microwave cookers are extremely easy to use.
8. Cool: unlike conventional ovens, microwave cookers do not produce external heat and so can be used anywhere that is convenient such as a dining room.
9. Higher capacity: due to shorter residence time
10. Less space requirement by up to 50-90% against other methods
11. Better hygiene of working environment
12. Easier and faster maintenance
13. Savings of electric energy in comparison with conventional methods are frequently within the range of 25-50%.

14. Waste elimination and lower consumption of fossil fuels, causing lowering of environmental stress.

Disadvantages of Microwave processing

1. Because of speed, and the way in which microwave energy cooks, food cooked in a microwave oven will not be brown, so no crust formation or browning in case of bread or meat (in such cases microwave with grilling can be used).
2. High initial cost.
3. Short cooking time does not allow flavors to develop and this makes food unacceptable.

Application of Microwave in dairy and Food Processing

1. Baking: for internal heating microwave, for external heating hot air (electric coil) or infrared for crust formation.
2. Concentrating: concentration of heat sensitive fluids and slurries at relatively low temperature in relatively short time.
3. Cooking: it cooks relatively larger pieces without high temperature gradients between surface and interior (for continuous cooking of meals).
4. Curing: effective for glue-line curing of laminates (as in package) without direct heating of the laminate themselves.
5. Drying: microwave selectively heats water with little direct heating of most solids. Drying is uniform throughout the product, drying at relatively low temperature.
6. Enzyme inactivation (blanching): rapid and uniform heating inactivates enzymes, so it is adapted for blanching of fruits and vegetables without leaching losses associated with hot water or steam and it does not overcook the outside before core enzymes are inactivated.
7. Finish drying: when most of the water has been removed by conventional drying, microwaves remove the last traces of moisture from the interior of the product quickly, and without overheating the already dried material.
8. Freeze drying: the ability of the microwave energy to selectively heat ice crystals in matter makes it attractive for accelerating the final stages of freeze drying.
9. Heating: almost any heat transfer problem can benefit from the use of microwaves because of their ability to heat in depth without high temperature gradient.
10. Pasteurizing: microwaves heat the product rapidly and uniformly without the overheating associated with conventional methods.
11. Precooking: it is well suited for precooking ‘heat and serve’ because there is no overcooking and no cooking losses.
12. Puffing and foaming: rapid internal heating by microwave causes puffing and foaming when the rate of heat transfer is made greater than the rate of vapor transfer out of the product interior. May be applied to puffing of snack foods and other materials.
13. Solvent removal: many solvents other than water are efficiently vaporized by microwave, permitting solvent removal at relatively low temperature.
14. Sterilizing: where adequate temperature may be reached (acid foods), quick, uniform come up time may permit HTST sterilization. Selective heating of moisture containing microorganisms makes possible the sterilization of such materials as glass, and plastic films, which are not themselves heated appreciably by microwaves (it will not destroy bacterial spores)

15. Tempering: microwave heating is roughly proportional to moisture content, so it can equalize the moisture in a product that came from the process of non uniform condition.
16. Thawing: controlled, rapid thawing of bulk items is possible due to substantial penetration f microwaves into frozen materials.

Microwave processing technique has attracted considerable attention in the dairy and food processing area. However, its application in the dairy industry has not aroused as much interest. Some of the applications of microwave in dairy industry include -inactivation of bacteriophage in cheese whey, production of anhydrous milk fat, heat treatment of whey protein concentrates, mass crystallization of lactose in sweetened condensed milk, sterilization of milk, pasteurization of milk (HTST method), in packaging sterilization of yoghurt and tempering of frozen butter. The process can also be used for cooking of cut curd cubes during cheese making, and plasticizing of Provolone and Mozzarella cheese.

Microwave energy is unique energy sources that may allow shorten processing time, saving in energy, labor and space and often better quality products. Advances in technology concentrating, focusing and controlling microwave energy has increased the feasibility of developing microwave processing for the food and dairy industry. Microwave processing is expected to grow beyond our expectation due to increasing consumers demands for newer type of convenience foods having more nutritional value and better sensory quality in the recent years. There is a great potential for the combination ovens because they are more effective than either oven alone in the manufacture of shelf stable packed foods. Advances in microwave oven design and narrowing gap in cost between microwave and thermal processing will provide and incentive for the development of newer microwave processes.

Microwave food processing design development will require additional research on mechanisms of microwave heating of foods, particularly in the areas of energy coupling and propagation modes, and further development of quantitative electro physical and electrochemical models as an aid to microwave process design.

V. Chemical agents used in preservation of food

- Different types of chemical preservative are used to preserve variety of food.
- Chemical preservatives are utilized in food preservation in various ways as given below:
    - Some food preservatives are directly mixed with the food to be preserved.
    - Some chemical preservative are used in wrapper or container of food rather than in food itself.
    - Some chemical preservative are placed in air of storage environment of food.
    - Some chemical preservative are used for treatment of equipment used in food processing.
- Not all food preservative can be used to preserve ah types of food.
- It is because some chemical preservative brings some undesirable changes for.eg. change in taste, smell etc. in some food. For.eg. spices are usually not considered suitable for preservation of milk and milk product because taste of spices in milk product is not considered desirable.
- Some chemical food preservatives used in preservation of food include:
- **a) Organic acids: Examples**
- **i) Propionic acid and propionate:**
    - Propionic acid is naturally found in Swiss cheese as a developed preservative up to a level of 1% .
    - Na and Ca propionate are commonly used to inhibit growth of mold in bakery product like bread.
    - Propionic acid and propionate are effect against mold, with very little or no effect against bacteria and yeast.
    - Therefore they are added in food which usually undergo mold spoilage
- **ii) Benzoic acid and benzoate**
    - Sodium benzoate us used to preserve variety of food like jam, jellies, carbonated beverages, fruits etc.
    - It is most effective at power pH and it's anti-microbial activity decreases with increase in pH.
    - Therefore, it is usually used to preserve acidic and slightly acidic food. At pH of 2.5-4 it is effective against most bacteria, some yeast and mold.
    - Methyl paraben and propyl paraben which are derivative of benzoic acid are also commonly used to preserve variety of food.
    - Their advantages over benzoate is that they are more effective at higher pH.
- **iii) Sorbic acid and sorbates:**
    - Sorbic acid and its Ca, Na and K salt are used as antimicrobial agent to preserve various food.
    - They are effective against yeast and mold but less effective against bacteria.
    - Their antimicrobial activity is high at lower pH.
    - Therefore they are used in slightly acidic food like pickles, fruit juice, syrup and other foods like cheese, bakery product etc. to inhibit growth of mold and yeast.
- **iv) Acetic acid and acetates:**

- Acetic acid and its derivatives like monochloroacetic acid, peracetic acid, dehydroacetic acid and sodium diacetate are used as food preservatives.
- Some examples include;
- Dehydroacetic acid is used to treat wrappers of cheese to inhibit growth of mold on the surface of cheese.
- Acetic acid in the form of vinegar is added in the form of vinegar is added in pickles to inhibit growth of microorganisms. Its activity increases with decrease in pH.
- Sodium diacetate is used in malt syrup. It is also used to treat wrapper of butter.

- **v) Nitrites and nitrates:**
  - Nitrite and nitrate of Na and K are used in curing solution or curing mixture of meat.
  - They have slightly bacteriostatic action, but they are added in meat mainly to fix red colour.
  - In acid environment, nitrite decomposed into nitric acid which reacts with haeme pigment of meat to form nitrosomyoglobin that gives attractive red colour to the meat.
  - Nitrite is a real colour fixative and nitrate is used as a source of nitrite.
  - Nitrite can react with secondary and tertiary amine to form nitrosamine which is carcinogenic.
  - Nitrite and nitrate are mainly used to fix red colour to meat but not to preserve it.
- **b) Smoking (wood smoke):**
  - Smoking is a method of preservation of meat by exposing it to wood smoke.
  - Two main purpose of smoking of meat are to add desirable flavour and to preserve the meat.
  - Some other desirable changes include improvement in colour, drying, and tenderization of meat.
  - Wood smoke contains large no of volatile compounds having bacteriostatic and bactericidal activity that help in preservation of meat.
  - Some such compound include formaldehyde, phenol, cresol, aliphatic acid, acetaldehyde, ketones and others.
  - Wood smoke also contains variety of flavoring compound that give desirable flavor to the meat.
- **c) SO_2 and sulphites:**
  - SO_2 and sulphites are used in wine industry to sanitize equipments and to reduce number of normal flora of grapemust.
  - In aqueous solution, SO_2 and sulphites form sulfurous acid which is anti-microbial.
  - Fume of burning sulfur are used to treat fruits and vegetables.
  - SO_2 is also added in fruit juice, syrup and various wine.
- **d) Sugar and salt:**
  - Sugar and salt are added in variety of food.
  - For.eg. milk is preserved for long time by adding high concentration of sugar.
  - Similarly, foods like pickles, fish etc. are preserved by adding high concentrations of NaCl.
  - Preservative action of salt and sugar include:
  - They bind water and make it unavailable for growth of microorganisms i.e. reduce A_w value of food.
  - Sugar increases osmotic pressure and causes osmotic lysis of microbial cell.
  - They draw out moisture from food and help in drying of the food.

- NaCl ionizes to give chloride ion which is antimicrobial. NaCl reduces the solubility of O_2 in liquid food and hence indirectly interfere with growth of aerobic microorganisms.

- **e) Formaldehyde:**
  - It is highly poisonous and is not permitted in food except as a minor component of wood smoke.
  - In food industry, it is used to sterilize equipment and to kill microorganisms in air of food processing room.
  - It is highly antimicrobial and can kill fungi, bacteria, spore and viruses.
- **f) Alcohol:**
  - Ethanol is anti-microbial.
  - Excellent keeping quality of some food is due to their alcohol content.
  - Ethanol content of soft drinks like beer is not sufficient to completely kill microorganisms but it slows down growth and activity of spoilage organism.
  - Alcoholic content of distilled alcoholic beverages is sufficient to prevent microbial spoilage.
  - Some food products like lemon extract (eg. Jolley) are also preserved by addition of ethanol.
- **g) Ethylene and propylene oxide:**
  - Ethylene and propylene oxide are gaseous sterilizing agents.
  - They are used to sterilize packaged food like dried food.
  - Furthermore, ethyleneoxide is also used to kill microorganisms in air of food processing room.
- **h) Spices and other condiments:**
  - Spices and other condiments added in food for taste and flavour are anti-microbial and help in food preservation.
  - Antimicrobial activity differs with type of spices. For.eg. mustard, flour and mustard oil are effective against like *Saccharomyces* but are less effective against bacteria.
  - Cinnamon and cloves is added in food are very effective against bacteria.
  - Similarly, garlic and onion mixed in food are bacteriostatic or bacteriocidal.
  - However, not all spices can be added in all types of food products because their taste and flavour maybe considered undesirable in certain food.
- **i) Antibiotics:**
  - Use of antibiotics in food preservation is not permitted due to risk of development of antibiotic resistant microorganisms.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC. MICROBIOLOGY

UNIT – IV – FOOD & DIARY MICROBIOLOGY – SMB2202

Yogurt Production

Yogurt Definitions

Yogurt is a fermented milk product that contains the characteristic bacterial cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. All yogurt must contain at least 8.25% solids not fat. Full fat yogurt must contain not less than 3.25% milk fat, lowfat yogurt not more than 2% milk fat, and nonfat yogurt less than 0.5% milk. The full legal definitions for yogurt, lowfat yogurt and nonfat yogurt are specified in the Standards of Identity listed in the U.S. Code of Federal Regulations (CFR), in sections 21 CFR 131.200, 21 CFR 131.203, and 21 CFR 131.206, respectively.

The two styles of yogurt commonly found in the grocery store are set type yogurt and swiss style yogurt. Set type yogurt is when the yogurt is packaged with the fruit on the bottom of the cup and the yogurt on top. Swiss style yogurt is when the fruit is blended into the yogurt prior to packaging.

Ingredients

The main ingredient in yogurt is milk. The type of milk used depends on the type of yogurt – whole milk for full fat yogurt, lowfat milk for lowfat yogurt, and skim milk for nonfat yogurt. Other dairy ingredients are allowed in yogurt to adjust the composition, such as cream to adjust the fat content, and nonfat dry milk to adjust the solids content. The solids content of yogurt is often adjusted above the 8.25% minimum to provide a better body and texture to the finished yogurt. The CFR contains a list of the permissible dairy ingredients for yogurt.

Stabilizers may also be used in yogurt to improve the body and texture by increasing firmness, preventing separation of the whey (syneresis), and helping to keep the fruit uniformly mixed in the yogurt. Stabilizers used in yogurt are alginates (carageenan), gelatins, gums (locust bean, guar), pectins, and starch.

Sweeteners, flavors and fruit preparations are used in yogurt to provide variety to the consumer. A list of permissible sweeteners for yogurt is found in the CFR.

Bacterial Cultures

The main (starter) cultures in yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The function of the starter cultures is to ferment lactose (milk sugar) to produce lactic acid. The increase in lactic acid decreases pH and causes the milk to clot, or form the soft gel that is characteristic of yogurt. The fermentation of lactose also produces the flavor compounds that are characteristic of yogurt. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the only 2 cultures required by law (CFR) to be present in yogurt.

Other bacterial cultures, such as *Lactobacillus acidophilus*, *Lactobacillus subsp. casei*, and Bifido-bacteria may be added to yogurt as probiotic cultures. Probiotic cultures benefit human health by improving lactose digestion, gastrointestinal function, and stimulating the immune system.

General Manufacturing Procedure

The following flow chart and discussion provide a general outline of the steps required for making yogurt. For a more detailed explanation see the literature references by Staff (1998), Tamime and Robinson (1999), Walstra et al. (1999) and the website by Goff, www.foodsci.uoguelph.ca/dairyedu/yogurt.html.

General Yogurt Processing Steps

- Adjust Milk Composition & Blend Ingredients
- Pasteurize Milk
- Homogenize
- Cool Milk
- Inoculate with Starter Cultures
- Hold
- Cool
- Add Flavors & Fruit
- Package

1. Adjust Milk Composition & Blend Ingredients

Milk composition may be adjusted to achieve the desired fat and solids content. Often dry milk is added to increase the amount of whey protein to provide a desirable texture. Ingredients such as stabilizers are added at this time.

2. Pasteurize Milk

The milk mixture is pasteurized at 185°F (85°C) for 30 minutes or at 203°F (95°C) for 10 minutes. A high heat treatment is used to denature the whey (serum) proteins. This allows the proteins to form a more stable gel, which prevents separation of the water during storage. The high heat treatment also further reduces the number of spoilage organisms in the milk to provide a better environment for the starter cultures to grow. Yogurt is pasteurized before the starter cultures are added to ensure that the cultures remain active in the yogurt after fermentation to act as probiotics; if the yogurt is pasteurized after fermentation the cultures will be inactivated.

3. Homogenize

The blend is homogenized (2000 to 2500 psi) to mix all ingredients thoroughly and improve yogurt consistency.

4. Cool Milk

The milk is cooled to 108°F (42°C) to bring the yogurt to the ideal growth temperature for the starter culture.

5. Inoculate with Starter Cultures

The starter cultures are mixed into the cooled milk.

6. Hold

The milk is held at 108°F (42°C) until a pH 4.5 is reached. This allows the fermentation to progress to form a soft gel and the characteristic flavor of yogurt. This process can take several hours.

7. Cool

The yogurt is cooled to 7°C to stop the fermentation process.

8. Add Fruit & Flavors

Fruit and flavors are added at different steps depending on the type of yogurt. For set style yogurt the fruit is added in the bottom of the cup and then the inoculated yogurt is poured on top and the yogurt is fermented in the cup. For swiss style yogurt the fruit is blended with the fermented, cooled yogurt prior to packaging.

9. Package

The yogurt is pumped from the fermentation vat and packaged as desired.

The Preparation of Acidophilus Milk

INTESTINAL toxemia and sour milk therapy are subjects that are far from being on a scientific basis. But it has been demonstrated, at any rate, that milk containing lactic acid is more readily digested than "sweet" milk, whether the acid is a result of bacterial activity or a result of the addition of U. S. P. lactic acid, as is the custom in preparing infant feeding formulas. Furthermore, it has been demonstrated that it is possible under certain conditions to implant the acidophilus type of bacillus in the lower intestinal tract of both man and animal to such an extent that it predominates over all other types. No doubt, too much has been claimed for sour milk therapy; yet there is a preponderance of evidence to support the view that the B. acidophilus culture may be employed in such a way as to have therapeutic value.' In order to have therapeutic value, the culture must contain enormous numbers of bacteria. James2 has shown how the average commercial culture falls down in this respect. A large amount of the culture should be taken daily; and lactose or dextrin or some other carbohydrate that is rather slowly absorbed should be fed at the same time. The kind of culture most satisfactory for supplying the acidophilus organisms in large numbers is a freshly prepared milk culture. Tablets, candies and emulsions have not received the unqualified approval of the Council on Pharmacy and Chemistry,' and any broth culture which has been kept at a refrigerator temperature cannot contain many virulent organisms on account of the fact that they die out rapidly at low temperatures. In a few cities acidophilus milk may be purchased for from $.50 to $1.00 a quart, but it is generally not available at all even at such a price. However, by observing a little care it may be prepared in the home at a very low cost.

The Milk Supply-To start with, the milk must be free from those bacteria which naturally occur in fresh milk. This can be attained by boiling ordinary market milk for a long time, but it has been found that unsweetened evaporated milk is particularly suitable for this purpose. It can be obtained from the grocer at a fair price, can be kept on hand [1105] AMERICAN JOURNAL OF PUBLIC HEALTH always ready for use, and it is certainly sterile. Evaporated milk has been sterilized under rather high steam pressure (about 11 pounds): this is sufficient just to begin to caramelize the milk. Kopeloff has shown that when milk is autoclaved to a "light caramel color" the acidophilus organism grows more rapidly in it than in milk heated to a lesser degree.

The Starter Culture-It is now possible to obtain a pure broth culture of B. acidophilus at, or through, all drug stores. The product of a reliable manufacturer should be specified. One culture which we obtained proved to be B. bulgaricus though it was labeled "B. acidophilus.

" The Control of Temperature-The acidophilus organism grows best at 90° to 105° F. It exhibits less growth at lower temperatures, though it has been found that the organism produces acid at a considerable rate even at room temperature. (Four observations showed an average increase of 0.65 per cent lactic acid in 24 hours at 680 F.)

Milk that has been inoculated can be warmed to 1000 to 1050 F. and transferred to a thermos bottle where it will maintain a temperature high enough to permit very satisfactory growth in 15 to 24 hours. The rate at which the temperature falls off depends upon the quality of the bottle and also, of course, on the temperature of the surrounding air. But it has been found that beginning with a temperature of 1000 F., a quart thermos bottle of average quality will permit the

temperature to drop no more than 100 to 150 in 24 hours. Such temperatures are found quite satisfactory for the rapid production of acidophilus milk.

The Procedure-Carefully clean a quart thermos bottle by allowing it to stand over night full of water containing some washing powder or a little household ammonia; then scald it. Place the cork, a can opener and thermometer in a pan and pour boiling water over them. Wipe the top of a one-pound can of evaporated milk free from dust and pour boiling water over it. Open the can and pour contents into the pan that has been scalded. Fill the can with boiling water and pour into the evaporated milk. Immerse the pan in cool water and stir the mixture with the thermometer until the temperature comes down to about 105° F. Add 2 or 3 ounces of commercial B. acidophilus culture, mix, and transfer to the thermos bottle. (The temperature should now be between 1000 and 1020 F.) Cork and let stand for 24 hours, or until the milk has acquired a pleasantly sour taste. When this is attained, transfer to a clean milk bottle and place in the refrigerator. Succeeding cultures of acidophilus are prepared by using about a teacupful (6 ounces) of milk culture previous made to inoculate the 106 PREPARATION OF AcIDOPHILUS MILK diluted evaporated milk for the next run. Proceeding in such a manner, it will be found that acid is produced at a more rapid rate than when the first quart was prepared using the commercial broth culture as a starter. Thirteen to 17 hours is now quite sufficient. If fermentation is allowed to proceed for a longer time so much acid is developed that the taste becomes unpleasantly sour. After a little experience one may stop the action of the bacteria at any desired degree of sourness. This is effected by merely transferring the milk to a clean glass bottle and placing in the refrigerator. It is perfectly safe to keep the culture at room temperature, but, as has already been mentioned, a considerable increase in acidity may be expected. This is of little consequence, however, if the product is consumed within 24 hours. On the other hand, if the milk is kept in a refrigerator it should be consumed within 48 hours for the reason that the organisms rapidly die out at such low temperatures. One or two tablespoonfuls of lactose or dextrin may be added to each glass of milk consumed, though the pleasant tart taste of the sour milk is to some extent injured by this addition. Most people prefer to take the carbohydrate separately. Typical acidophilus milk has a fine grained curd. It is rather viscous, especially at low temperatures, but not "stringy." It does not develop so high an acidity as is produced by the B. bulgaricus. When a culture becomes rapidly very sour or bitter and is extremely viscous so that when poured, from a bottle it runs out in strings, it is an indication that the culture used was B. bulgaricus. This should be discarded and a new commercial culture obtained from a different source. At any time when souring does not seem to be taking place normally a new start should be made. The nutritive value of this product is exactly the same as that of ordinary cow's milk. Evaporated milk is whole cow's milk evaporated down to one-half its bulk. Therefore, by diluting a can of it with an equal amount of water the composition will be the same as that of normal cow's milk. The evaporated milk flavor, which is objectionable to some people, is scarcely noticeable in acidophilus milk prepared from that product.

Some Experimental Results-A large number of milk cultures have been prepared in thermos bottles on which observations have been made of the influence of (1) the amount of old culture used for inoculation, (2) temperature at beginning of fermentation, and (3) length of time during which fermentation is allowed to proceed.

It has been found that acidophilus milk has its best flavor at 0.8 to 1.0 per cent lactic acid. The result of these observations led to the conclusion that the best results are obtained by the use of about one teacupful of old culture to inoculate one quart of diluted evaporated milk, starting the fermentation in a thermos bottle at 100° to 1020 F. and allowing the fermentation to proceed for 13 to 17 hours. For the first inoculation, however, where the commercial broth culture is used, fermentation should be allowed to proceed for 24 to 27 hours. The fact that the commercial culture is much less active than the prepared milk culture when used for inoculation shows how much greater must be the therapeutic value of the latter.

Method of producing koumiss

The method relates to the dairy industry and can be used in the production of mare's milk products with accelerated (with a single fermentation of mare's milk) and long-term maturation of the koumiss mixture with 2 to 3-fold "rejuvenation" of it with fresh mare's milk.

A known method of producing koumiss (1), including fermentation of mare's milk, making koumiss sourdough, stirring, bottling, cooling, maturation at 16-18 ° C for 1-2 hours and the introduction of a stabilizing additive (apple pectin or agar agar mixed with powdered sugar in a ratio of 1: 5 based on 0.5% of the mass of the milk mixture), followed by spill and high-temperature compensation at 9 ° C for 5 minutes.

A known method of producing koumiss from mare's milk (2), including adding koumiss sourdough milk to the mare, fermenting, kneading the mixture, self-gasing for 2-3 hours without air. Mare's milk is used in dry form, which is pre-mixed with water in a ratio of 1:10, fermentation is carried out until acidity reaches 60-70 ° T, and kneading is carried out for 25-60 minutes depending on the speed of the mixer, followed by exposure for 2 hours moreover, after self-gasification, the mixture is ripened and cooled at a temperature of from 2 to 8 ° C.

The disadvantages of the described methods are the short shelf life due to the lack of environmentally friendly equipment and the loss of organoleptic characteristics of koumiss, the irrational use of raw materials in the summer, low consumer properties, taste and appearance.

The objective of the proposed method is the manufacture of the target product according to the traditional technology of koumiss making-producing natural koumiss (from mare's milk) due to strict adherence to the technological regime, hygiene and high-quality koumiss sourdough.

The cooking method is as follows:

- Fresh mare's milk, with constant stirring, is poured into the sourdough with an acidity of 120 ° T in a ratio of 1: 3. The mixture is stirred at a temperature of 28-30 ° C and left alone for ripening until the acidity of the mixture reaches 60-70 ° T. Then milk of the next milk yield is added, and after repeated kneading for 1 hour and rest for 2 hours, koumiss fermentation is activated, a large amount of carbon dioxide is released, the surface of the mixture is covered with an even layer of the smallest foam (which is not observed when making the initial koumiss mixture) , a specific koumiss flavor and aroma appears. The number (rejuvenation) of koumiss is carried out depending on the number of milks. With each subsequent addition of milk, koumiss wanders harder - its quality improves. After the last "rejuvenation", koumiss is poured into 0.5 liter bottles, corked with a crown plug. For further ripening, koumiss is placed in a refrigerator with a temperature of 4-6 ° C.

For the production of koumiss, mare's milk must meet the following characteristics:

- acidity 5-7 ° T,

- density 1.029 ° -1.033 ° A
- temperature - 28-30 ° C,
- mechanical pollution - not lower than the first group.

The best production sourdough is the daily left part of koumiss in the active phase of fermentation. Koumiss starter culture, subject to the correct technological regime, maintains its activity and stability for months and even years without requiring replacement, however, if the starter culture acquires undesirable properties (sediment, flakes, excess acidity, is not sufficiently carbonated), it must be replaced. In case of spoilage, you should have insurance leaven selected from a good batch of koumiss, which is stored in the refrigerator. Before use, it must be diluted with mare's milk (rejuvenate) in a 1: 1 ratio and kneaded for 60 minutes, in order to further activate microflora and restore normal koumiss fermentation, the mixture needs to be rejuvenated 3-4 times and after each fresh fresh mare's milk is added, knead 60 minutes. Periodically, every 4-5 weeks, the insurance starter should be replaced with a new, fresh koumiss.

Koumiss sourdough must be introduced in such an amount that the acidity of the mixture is 40-45 ° T.

The amount of yeast introduced is determined by the formula:

$$A_З = \frac{(K_С - K_М) \cdot A_М}{K_З - K_С}$$

where

And $_h$ is the amount of ferment required

And $_m$ is the amount of mare's milk,

K $_{With\ the}$ acidity of the mixture

To $_Z$ - the acidity of the starter culture,

K $_M$ - the acidity of milk.

An example of determining the consumed leaven.

There is 100 l of milk with an acidity of 6 ° T, the acidity of the initial mixture (sourdough + mare's milk) is 45 ° T, and the acidity of the starter is 120 ° T.

The required amount of starter culture is determined by the formula:

$$A_{З=} \frac{(45 - 6) \cdot 100}{120 - 45} = 52\ л$$

KEFIR-MANUFACTURE, COMPOSITION, NUTRITIONAL AND THERAPEUTIC PROPERTIES

Introduction

Kefir is a viscous, slightly carbonated dairy beverage that contains small quantities of alcohol and, like yoghurt, is believed to have its origins in the Caucasian mountains of the former USSR. It is also manufactured under a variety of names including kephir, kiaphur, kefer, knapon, kepi and kippi with artisanal production of kefir occurring in countries as widespread as Argentina, Taiwan, Portugal, Turkey and France. It is not clear whether all kefirs originate from a single

original starter culture, since microbial analyses of kefir samples taken from different locations indicate microflora population differences.

Definition

Although no clear definition of kefir exists, it is a viscous, acidic, and mildly alcoholic milk beverage produced by fermentation of milk with a kefir grain as the starter culture (FAO/WHO 2003). The Codex Alimentarius description of kefir state it as Starter culture prepared from kefir grains, Lactobacillus kefir, and species of the genera Leuconostoc, Lactococcus and Acetobacter growing in a strong specific relationship. Kefir grains constitute both lactose-fermenting yeasts (Kluyveromyces marxianus) and non-lactose-fermenting yeasts (Saccharomyces unisporus, Saccharomyces cerevisiae and Saccharomyces exiguus).

Composition

The composition of kefir will be essentially dependant on the type of milk that was used. The major change caused by fermentation measured in term of acid production and alcohol production may also reflect in the composition.Table-30.1 shows the composition standards prescribed by the Codex

Table 30.1 Codex standard for kefir

Milk protein (% w/w)	min. 2.8
Milk fat (% m/m)	<10
Titratable acidity, expressed as % of lactic acid	min. 0.6
Ethanol (% vol. /w)	not stated
Sum of specific microorganisms constituting the starter culture (cfu/g, in total)	10^7 (minimum)
Yeasts (cfu /g)	10^4 (minimum)

(From Codex Standard for Fermented Milks CODEX STAN 243 – 2003)

Kefir Manufacture

Although commercial kefir is traditionally manufactured from cow's milk, it has also been made from the milk of ewes, goats and buffalos. Moreover, kefir produced using soy milk has also been recently reported. The various steps of kefir manufacture are depicted in Figure 30.1.

Traditionally, kefir is produced by adding kefir grains (a mass of proteins, polysaccharides, mesophilic, homofermentative and heterofermentative lactic acid streptocci, thermophilic and mesophilic lactobacilli, acetic acid bacteria, and yeast) to a quantity of milk. The size of the initial kefir grain inoculum affects the pH, viscosity and microbiological profile of the final product. A grain to milk ratios of 1:30 to 1:50 were found optimum. In some manufacturing procedures, a percolate of the grains from a coarse sieve is used as the mother culture to

inoculate fresh milk. Fermentation of the milk by the inoculum proceeds for approximately 24 hours, during which time homofermentative lactic acid streptococci grow rapidly, initially causing a drop in pH. This low pH favours the growth of lactobacilli, but causes the streptococci numbers to decline. The presence of yeasts in the mixture, together with fermentation temperature (21-23°C), encourages the growth of aroma producing heterofermentative streptococci. As fermentation proceeds, growth of lactic acid bacteria is favoured over growth of yeasts and acetic acid bacteria.

Method of manufacture of kefir

Kefir grains are key to kefir production, and it has been found that the finished product has a different microbiological profile from the grains and therefore cannot be used to inoculate a new batch of milk. Grains have been shown to possess a dynamic and complex flora which is not conducive to commercial production of a uniform, stable product; this has prompted researchers to try to produce kefir from a mixture of pure cultures. Some researchers produced a starter consisting of two bacteria (Lactobacillus helveticus and Lactococcus lactis subsp lactis) and one yeast (S. cerevisiae) isolated from kefir grain and combined with two yoghurt strains (Lactobacillus delbrueckii subsp bulgaricus, and Streptococcus thermophilus). Yeast was added to the starter with sucrose either at the beginning, or after lactic acid fermentation. The two resulting kefirs produced were found to have high numbers of viable cocci and lactobacilli and had chemical and organoleptic properties that were similar to traditional kefir. A commercial kefir is being produced in the United States using a mixture of defined microorganisms rather than kefir grains. This starter culture mixture has been reported to contain Streptococcus lactis, Lb. plantarum, Streptococcus cremoris, Lb. casei, Lactococcus lactis subsp Lactis biovar diacetylactis, Leuconostoc cremoris and Saccharomyces florentinus.

Characteristics of Kefir

The flavour, viscosity and microbial/chemical composition of the final kefir product can be affected by the size of the inoculum added to the milk, the occurrence of any agitation during fermentation, and the rate, temperature and duration of the cooling and ripening stages following fermentation. Natural kefir has a refreshing, yeasty taste and a ‘sparkling’ mouth feel. Modern manufacturing procedures for kefir result in ethanol levels in the finished product of 0.01– 0.1% although kefir with ethanol concentrations as high as 0.25% have been produced from grains in the laboratory. The amounts of ethanol and CO2 produced during fermentation of kefir depend on the production conditions used. CO2 content of kefir has been said to be ‘comparatively low’ in relation to other fermented drinks. The distinctive taste of kefir results from the presence of several flavour compounds which are produced during fermentation. Kefir produced from pure cultures did not receive high sensory evaluation scores. Acetaldehyde and acetoin have received particular attention with regard to their roles during kefir manufacture because of their contribution in the taste; both have been found to increase in concentration during kefir fermentation. During storage, acetaldehyde increases in concentration and acetoin decreases.

Kefir Grains

Kefir grains (Figure-30.2) resemble small cauliflower florets: they measure 1-3 cm in length, are lobed, irregularly shaped, white to yellow-white in colour, and have a slimy but firm texture. Grains are kept viable by transferring them daily into fresh milk and allowing them to grow for approximately 20 hours; during this time, the grains will have increased their mass by 25%. Grains must be replicated in this way to retain their viability, since old and dried kefir grains have little or no ability to replicate. In addition, washing grains in water also reduced viability. It has been recommended that in a commercial operation using grains to produce kefir, grains should be kept viable through daily transfers and should only be replaced if their ability to ferment milk becomes impaired. Low temperature storage appears to be the best way to maintain kefir grains for long periods. Storage of kefir grains at 80° or 20°C for 120 days did not change their fermentation properties compared to grains that had not been stored; however, grains stored at 4°C did not produce acceptable kefir after thawing.

Kefir grains

Microbiology of kefir grains

Bacteria found in kefir grains and kefir

The microbial population (Figure 30.3) found in kefir grains have been used as an example of a symbiotic community. This symbiotic nature has made identification and study of the constituent microorganisms within kefir grains difficult.

Bacteria found in kefir grains and kefir

Lactobacilli *Lactobacillus kefir*	*Lactobacillus delbrueckii*
Lactobacillus kefiranofaciens	*Lactobacillus rhamnosus*
Lactobacillus kefirgranum	*Lactobacillus casei*
Lactobacillus parakefir	*Lactobacillus paracasei*
Lactobacillus brevis	*Lactobacillus fructivorans*
Lactobacillus plantarum	*Lactobacillus hilgardii*
Lactobacillus helveticus	*Lactobacillus fermentum*
Lactobacillus acidophilus	*Lactobacillus viridescens*
Lactococci *Lactococcus lactis* subsp *lactis*	**Enterococci** *Enterococcus durans*

Lactococcus lactis subsp *cremoris*	
Streptococci *Streptococcus thermophilus*	**Leuconostocs** *Leuconostoc mesenteroides*
Acetic acid bacteria *Acetobacter pasteurianus* *Acetobacter aceti*	**Other Bacteria** Bacillus spp, Micrococcus spp. *Bacillus subtilis, Escherichia coli*

Yeasts found in kefir grains and kefir

The yeasts in kefir (Table-30.3) have been less well studied than kefir bacteria, although it is obvious that the yeasts in kefir grains provide an environment for the growth of kefir bacteria, producing metabolites that contribute to the flavour and mouthfeel of kefir. To prevent excessive CO2 production (particularly after fermentation), a two stage fermentation process starting with a non-lactose fermenting yeast such as Saccharomyces cerevisiae can be done. The properties of yeasts found in kefir grains vary. For example, some of the yeast found in kefir grains are capable of fermenting lactose, while some are not. Also, it has been observed that some type of yeasts are located at the surface of the grain, while others inhabit the interior. It may be that yeasts located at different locations in the kefir grains play different roles in the fermentation process. Like kefir bacteria, the profile of yeasts is different in kefir grains when compared to the final kefir product.

Nutritional Significance of Kefir

The composition of kefir depends greatly on the type of milk that was fermented. However, during the fermentation, changes in composition of nutrients and other ingredients have also been shown to occur. L(+) lactic acid is the organic acid in highest concentrations after fermentation and is derived from approximately 25% of the original lactose in the starter milk. The amino acids valine, leucine, lysine and serine are formed during fermentation, while the quantities of alanine and aspartic acid increase as compared to raw milk. Appreciable amounts of pyridoxine, vitamin B12, folic acid and biotin were synthesized during kefir production, depending on the source of kefir grains used, while thiamine and riboflavin levels were reduced. Some workers reported decreases in biotin, vitamin B12 and pyridoxine, and significant increases in folic acid, as compared to non-fermented milk.

Bio Active Ingredients in kefir

Kefir has a long tradition of offering heath benefits. There are several compounds in kefir that may have bioactive properties.

(i) Exopolysaccharides

Exopolysaccharides of differing structures and compositions are produced by a variety of lactic acid bacteria including Lactobacillus, Streptococcus, Lactococcus and Leuconostoc. These cell-surface carbohydrates confer protective and adaptive properties on their bacterial producers; since they are often loosely bound to the cell membrane, they are therefore, easily lost to their environment. In food products, exopolysaccharides often contribute to organoleptic and stability characteristics. A unique polysaccharide called kefiran has been found in kefir grains. Grains may also contain other exopolysaccharides. Kefiran contains D-glucose and D-galactose only in a ratio of 1:1. Kefiran dissolves slowly in cold water and quickly in hot water, and forms a viscous solution at 2% concentration.

(ii) Bioactive peptides

Many organisms possess enzymes (e.g. proteinases and peptidases) that are able to hydrolyse the protein in a medium, thereby supporting growth of the organism by liberating peptides and amino acids. The action of proteinase and peptidase enzymes on milk proteins can theoretically result in a very large number of possible peptides. An analysis of the proteinase activity of kefir grain bacterial isolates has shown that several isolates have high proteinase activities which increases the possibility that bioactive peptides may be present in kefir. Studies on the peptide content of kefir drink have shown that kefir contains a large number of peptides.

Therapeutic Significance of kefir

Kefir has had a long history of being beneficial to health in Eastern European countries, where it is associated with general wellbeing. It is easily digested and is often the first weaning food received by babies.

- It has been proposed that stimulation of the immune system may be one mechanism whereby probiotic bacteria may exert many of their beneficial effects. Peptides formed during the fermentation process or during digestion have also been shown to be bioactive, and demonstrate a variety of physiological activities, including stimulation of the immune system in animal models. Stimulation of the immune system may also occur due to the action of exopolysaccharides found in kefir grains.
- Anti tumour effects of a water-soluble polysaccharide (approximate molecular weight 10,000,00 Da) isolated from kefir grains is reported.
- A water soluble polysaccharide fraction from kefir grains was shown to inhibit pulmonary metastasis of Lewis lung carcinoma, whether the kefir-derived polysaccharide was given orally before or after tumour transplantation.
- Some kefir grains have been shown to possess b-galactosidase activity which remains active when consumed and thus can be beneficial for lactose intolerant people.
- Many lactobacilli are capable of producing a wide range of antimicrobial compounds, including organic acids (lactic and acetic acids), carbon dioxide, hydrogen peroxide, ethanol, diacetyl and peptides (bacteriocins) that may be beneficial not only in the reduction of food borne pathogens and spoilage bacteria during food production and storage, but also in the treatment and prevention of gastrointestinal disorders and vaginal

infections. Fresh kefir grains were found to inhibit the growth of the pathogens Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli. Leuconostoc mesenteroides and Lactobacillus plantarum, isolated from kefir grains, have been shown to produce antimicrobial compounds which can inhibit Gram-positive and Gram-negative bacteria. These antimicrobial compounds are found to be heat stable. But their antimicrobial properties are reduced after exposure to proteolytic enzymes. Lactobacilli isolated from kefir grains had antimicrobial activities against E. coli, Listeria monocytogenes, Salmonella Typhimurium, S. Enteritidis, S. flexneri and Yersinia enterocolitica. Bacteriocins were thought to be responsible for the antimicrobial activities .

Kefir is a microbiologically complex product with a large number of different bacteria and yeast involved in its making . Many of these microorganisms are only now being identified by using advanced molecular biological techniques. The study of kefir is made more difficult, because it appears that many different sources of kefir grains exist that are being used to produce kefir. The production of kefir depends on the synergistic interaction of the microflora in kefir grains. During the fermentation process, the yeasts and bacteria in kefir grains produce a variety of ingredients that give kefir its unique taste and texture. After fermentation, the finished kefir product contains many ingredients that are proving to be bioactive and may be used as functional ingredients.

DAHI PREPARATION METHODS, QUALITY OF DAHI, PACKAGING, SHELF LIFE AND DEFECTS

Introduction

Fermentation is one of the simplest ways of preserving milk constituents for human consumption. Fermentation gives an acid taste to milk which is particularly refreshing in worm climate and also imparts certain therapeutic benefits originally absent in milk. Fermented dairy products have assumed prominent position in human diet in many regions of the world. Fermentation leads to partial breakdown of milk constituents and increases the digestibility of cultured milk products.

In Vedic literature also, we could find many references about fermented milk products some are listed below.

- Prasadjya – Dahi after dilution and churning, carrying butter grains in the liquid mass
- Payasya – Strained curd, when mixed with boiled milk, crystal sugar and fermented herbs
- Shrikarini – Consists of strained dahi, crystal sugar and spices
- Rasala -- Sugar and spiced curd

Some of the popular Indian fermented milk products are Dahi, Lassi, Chakka, Shrikhand, Mishti Dahi and Raita.

Product Description

Dahi is produced from heat treated milks after inoculation with certain species of lactic acid bacteria added to milk in the form of starter culture. Lactic acid bacteria added multiply, grow and produce lactic acid, acetic acid and carbon dioxide by utilizing lactose present in milk. Some bacteria uses citric acid of milk to produce certain volatile organic compounds mainly **diacetyl,** which is mainly responsible for flavor of dahi. Judicious combination of acid producing and flavour producing microorganisms in the starter helps in the production of Dahi with a firm body and good flavour.

Definition of *dahi*

Dahi or curd is a semi solid product, obtained from pasteurized or boiled milk by souring, using harmless lactic acid or other bacterial cultures. Dahi may contain additional cane sugar. It should have the same minimum percentage of fat and solids-not-fat as the milk from which it is prepared. Where Dahi or curd, other than skimmed milk Dahi, is sold or offered for sale without any indication of the class of milk, the standards prescribed for Dahi prepared from buffalo milk shall apply.

Table 23.1 FSSR(2011) and BIS standards of *dahi*

Characteristics	FSSR(2011)	BIS
Acidity % lactic acid	-	0.6 - 0.8
Total Plate count	Not more than 1000000/g	
Coliform count	10 per g max	10 per g max
Escherechia coli	Absent in 1g	
Salmonella	Absent in 25g	
Shigella	Absent in 25g	
Stephylococcus aurius	Not more than 100/g	
Yeast and Mould	100 per g max	100 per g max
Anaerobic spore	Absent in 1g	
Listeria monocytogenes	Absent in 1g	
Phosphatase test		Negative
Other requirements	It should have the same minimum percentage of fat and SNF as the milk from which it is prepared. If no standards declared then standards prescribed for dahi from buffalo milk	Dahi shall conform to the requirements of milk fat and MSNF, as laid down in FSSR, 2011

	shall apply	

Table 23.2 Chemical composition of *dahi*

Components	Whole milk Dahi	Skim milk Dahi
	%	%
Water	85-88	90-91
Fat	5 – 8	0.05 - 0.1
protein	3.2-3.4	3.3-3.5
Lactose	4.6-5.2	4.7-5.3
Lactic acid	0.5-1.1	0.5-1.1
Ash	0.7-0.75	0.7-0.75

23.3 Method of Preparation

23.3.1 Traditional method

In traditional method of dahi preparation, milk is heated intensively to boil for 5 to 10 min and then it is cooled to room temperature. cooled milk is added with previous day's curd or buttermilk, stirred and allowed to set undisturbed usually for overnight.

At halwai's shop milk is considerably concentrated before being inoculated with starter culture. So that the total solid content of milk gets increased, particularly increase in the protein content of milk. Concentration of milk results in custard like consistency of dahi and keeps the product from wheying off.

23.3.2 Industrial method of making *dahi*

23.3.2.1 Selection of raw material

Production of cultured/fermented milk demands high quality raw materials with respect to physical, chemical and microbial standards.

23.3.2.2 Filtration/clarification

Fresh raw milk is heated to 35 to 40°C to aid clarification or filtration process then it is filtered to ensure that, milk is free from extraneous matter.

23.3.2.3 Standardization: Fat: 0 – 5%, SNF: 11 – 13%

Fat is standardized based on type of product ranging from fat free to full fat and SNF level is increased by min. 2% than that of milk. It is common to boost the SNF content of the milk to about 12% with the addition of skim milk powder or condensed skim milk.

Increased SNF inturn increases the protein, calcium and other nutrients and resulted with improved body and texture, custard like consistency. Higher milk solids prevent wheying off of the product during storage.

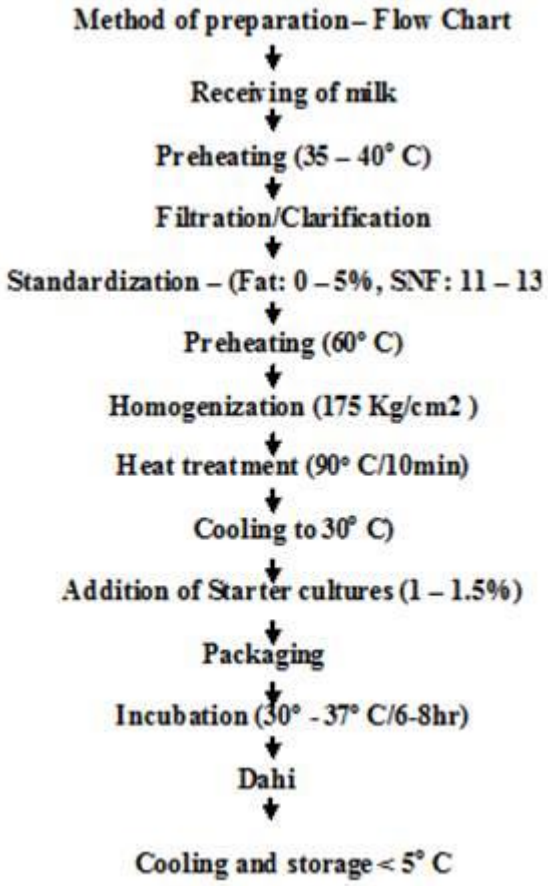


Fig. 23.1 Method of preparation of *dahi*

23.3.2.4 Homogenization: 175 Kg/cm^2

The standardized milk is subjected to homogenization after heating to 60°C to increase the efficiency. Homogenization reduces the cream layer formation during incubation, Single stage homogenization with 175kg/cm^2 pressure would be sufficient to improve texture of dahi.

23.3.2.5 Heat treatment: 9° C/23min

Milk intended for dahi or any other fermented milk product is given severe heat treatment i.e. 90°C for 10min.

Following are the benefits of high heat treatment

- Denatures and coagulates milk albumin and globulins which enhance the viscosity and produce custard like consistency

- Kills contaminating and competitive microbes
- Development of relatively sterile medium
- Removal of air form the medium more conducive for the growth of culture bacteria
- Effective thermal breakdown of protein releasing peptones and sulfhydryl groups, this inturn provide nutrients to starter bacteria

23.3.2.6 Packaging and fermentation

The heat treated product mix is cooled to 37C and it is inoculated with specific dahi culture at the rate of 1 to 1.5%. Starter culture is the most crucial component in the production of high quality fermented milks. Proper selection of culture strains decides the good quality of product. Dairy cultures are available in various forms like freeze dried, liquid and frozen forms. After the product mix is inoculated with dahi culture it is thoroughly mixed and filled into plastic cups, sealed properly to avoid any contamination and spillage of the product. Dahi is packed in food grade polystyrene and polypropylene cups in 100g, 200g and 400g pack sizes. Various packaging machines of upto 400cups/min speed are available to package cultural dairy products in different sizes. The packaged product should be stored at < 5C for extended shelf lifeThus packed product is arranged in cases or crates and transferred to incubation room maintained at 37 to 42C. The product mix is incubated till its pH reaches 4.4 to 4.5 and then it is cooled rapidly to less than 5C by exposing the cups to high velocity cold air.

23.3.2.7 Storage

Dahi is normally stored at 4 5C. Storage area should be maintained clean and tidy to avoid any cross contamination.

Table 23.3 Common defects in dahi

Sl No.	Defect	Probable Cause	Remedy
Flavor defects			
1	Insufficient Flavor	Low citrate level in milk, Low diacetyl content	Add 0.02 0.05% Sodium citrate prior to mixing the starter culture. Cool rapidly after culturing
2	Oxidized flavor	Copper contamination Exposure to fluorescent light Exposure to sunlight	Avoid usage of copper utensils Protect product from direct exposure to Sunlight/ UV light

3	Yeast/cheesy	Contaminating yeast growth	Sanitation check
4	Rancid flavor	Lipolytic activity	Do not mix pasteurized and raw dairy ingredients prior to homogenization
5	High acid	Addition of more culture, Increased incubation time Use of sour milk	Optimum culture addition Blast cool the product immediately after optimum pH is reached Use good quality fresh milk
Body and textural defects			
1	Weak body	Insufficient heat treatment to the mix Too low milk SNF Severe agitation after fermentation	Heat treatment should not be less than 85^{o}C/30min Homogenize the dahi mix prior to homogenization Increase the MSNF content to 11% by adding Skim milk powder
2	Grainy texture	High acidity Improper dispersion of Skim milk powder	Rapidly cool the product to <5^{o}C after attaining optimum acidity Use in line screen/filter
3	Syneresis	Insufficient heat treatment to the mix Improper standardization and too low milk SNF Agitation/disturbances during fermentation	Heat treatment should not be less than 85^{o}C/30min Increase the MSNF content to min. of 11% by adding Skim milk powder Do not disturb the cups during fermentation
4	Ropiness	Contamination of milk with psychotropic microorganisms Culture contamination/impure culture	Proper heat treatment of milk, Avoid cold storage of milk before pasteurization/thermization Use of pure culture

Cheese Production

Cheese Definitions

Cheese comes in many varieties. The variety determines the ingredients, processing, and characteristics of the cheese. The composition of many cheeses is defined by Standards of Identity in the U.S. Code of Federal Regulations (CFR).

Cheese can be made using pasteurized or raw milk. Cheese made from raw milk imparts different flavors and texture characteristics to the finished cheese. For some cheese varieties, raw milk is given a mild heat treatment (below pasteurization) prior to cheese making to destroy some of the spoilage organisms and provide better conditions for the cheese cultures. Cheese made from raw milk must be aged for at least 60 days, as defined in the CFR, section 7 CFR 58.439, to reduce the possibility of exposure to disease causing microorganisms (pathogens) that may be present in the milk. For some varieties cheese must be aged longer than 60 days.

Cheese can be broadly categorized as acid or rennet cheese, and natural or process cheeses. Acid cheeses are made by adding acid to the milk to cause the proteins to coagulate. Fresh cheeses, such as cream cheese or queso fresco, are made by direct acidification. Most types of cheese, such as cheddar or Swiss, use rennet (an enzyme) in addition to the starter cultures to coagulate the milk. The term "natural cheese" is an industry term referring to cheese that is made directly from milk. Process cheese is made using natural cheese plus other ingredients that are cooked together to change the textural and/or melting properties and increase shelf life.

Ingredients

The main ingredient in cheese is milk. Cheese is made using cow, goat, sheep, water buffalo or a blend of these milks.

The type of coagulant used depends on the type of cheese desired. For acid cheeses, an acid source such as acetic acid (the acid in vinegar) or gluconodelta-lactone (a mild food acid) is used. For rennet cheeses, calf rennet or, more commonly, a rennet produced through microbial bioprocessing is used. Calcium chloride is sometimes added to the cheese to improve the coagulation properties of the milk.

Flavorings may be added depending on the cheese. Some common ingredients include herbs, spices, hot and sweet peppers, horseradish, and port wine.

Bacterial Cultures

Cultures for cheese making are called lactic acid bacteria (LAB) because their primary source of energy is the lactose in milk and their primary metabolic product is lactic acid. There is a wide variety of bacterial cultures available that provide distinct flavor and textural characteristics to cheeses. For a more detailed description of cheese cultures and microbiology, see Fox (2004), Kosikowski and Mistry (1997), and Law (1997).

Starter cultures are used early in the cheese making process to assist with coagulation by lowering the pH prior to rennet addition. The metabolism of the starter cultures contribute desirable flavor compounds, and help prevent the growth of spoilage organisms and pathogens. Typical starter bacteria include *Lactococcus lactis* subsp. *lactis* or *cremoris*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbruckii* subsp. *bulgaricus*, and *Lactobacillus helveticus*.

Adjunct cultures are used to provide or enhance the characteristic flavors and textures of cheese. Common adjunct cultures added during manufacture include *Lactobacillus casei* and *Lactobacillus plantarum* for flavor in Cheddar cheese, or the use of *Propionibacterium freudenreichii* for eye formation in Swiss. Adjunct cultures can also be used as a smear for washing the outside of the formed cheese, such as the use of *Brevibacterium linens* of gruyere, brick and limburger cheeses.

Yeasts and molds are used in some cheeses to provide the characteristic colors and flavors of some cheese varieties. Torula yeast is used in the smear for the ripening of brick and limburger cheese. Examples of molds include *Penicillium camemberti* in camembert and brie, and *Penicillium roqueforti* in blue cheeses.

General Manufacturing Procedure

The temperatures, times, and target pH for different steps, the sequence of processing steps, the use of salting or brining, block formation, and aging vary considerably between cheese types. The following flow chart provides a very general outline of cheese making steps. The general processing steps for Cheddar cheese are used for illustration. For a more detailed explanation see the literature references by Fox (2004), Kosikowski and Mistry (1997), Law (1997), Walstra et al. (1999), and the website by Goff, www.foodsci.uoguelph.ca/dairyedu/cheese.html.

General Cheese Processing Steps

- Standardize Milk
- Pasteurize/Heat Treat Milk
- Cool Milk
- Inoculate with Starter & Non-Starter Bacteria and Ripen
- Add Rennet and Form Curd
- Cut Curd and Heat
- Drain Whey
- Texture Curd
- Dry Salt or Brine
- Form Cheese into Blocks
- Store and Age
- Package

The times, temperatures, and target pH values used for cheddar cheese will depend on individual formulations and the intended end use of the cheese. These conditions can be adjusted to optimize the properties of Cheddar cheese for shredding, melting, or for cheese that is meant to be aged for several years.

1. Standardize Milk

Milk is often standardized before cheese making to optimize the protein to fat ratio to make a good quality cheese with a high yield

2. Pasteurize/Heat Treat Milk

Depending on the desired cheese, the milk may be pasteurized or mildly heat-treated to reduce the number of spoilage organisms and improve the environment for the starter cultures to grow. Some varieties of milk are made from raw milk so they are not pasteurized or heat-treated. Raw milk cheeses must be aged for at least 60 days to reduce the possibility of exposure to disease causing microorganisms (pathogens) that may be present in the milk.

3. Cool Milk

Milk is cooled after pasteurization or heat treatment to 90°F (32°C) to bring it to the temperature needed for the starter bacteria to grow. If raw milk is used the milk must be heated to 90°F (32°C).

4. Inoculate with Starter & Non-Starter Bacteria and Ripen

The starter cultures and any non-starter adjunct bacteria are added to the milk and held at 90°F (32°C) for 30 minutes to ripen. The ripening step allows the bacteria to grow and begin fermentation, which lowers the pH and develops the flavor of the cheese.

5. Add Rennet and Form Curd

The rennet is the enzyme that acts on the milk proteins to form the curd. After the rennet is added, the curd is not disturbed for approximately 30 minutes so a firm coagulum forms.

6. Cut Curd and Heat

The curd is allowed to ferment until it reaches pH 6.4. The curd is then cut with cheese knives into small pieces and heated to 100°F (38°C). The heating step helps to separate the whey from the curd.

7. Drain whey

The whey is drained from the vat and the curd forms a mat.

8. Texture curd

The curd mats are cut into sections and piled on top of each other and flipped periodically. This step is called **cheddarin**g. Cheddaring helps to expel more whey, allows the fermentation to continue until a pH of 5.1 to 5.5 is reached, and allows the mats to "knit" together and form a tighter matted structure. The curd mats are then milled (cut) into smaller pieces.

9. Dry Salt or Brine

For cheddar cheese, the smaller, milled curd pieces are put back in the vat and salted by sprinkling dry salt on the curd and mixing in the salt. In some cheese varieties, such as mozzarella, the curd is formed into loaves and then the loaves are placed in a brine (salt water solution).

10. Form Cheese into Blocks

The salted curd pieces are placed in cheese hoops and pressed into blocks to form the cheese.

11. Store and Age

The cheese is stored in coolers until the desired age is reached. Depending on the variety, cheese can be aged from several months to several years.

12. Package

Cheese may be cut and packaged into blocks or it may be waxed.

Dosa

Dosa or Dose is arguably one of the most popular dishes in India; it is a typical part of the South Indian diet and is gaining popularity all over the world. It is a type of pancake made from fermented batter of rice and blackgram. The literary references to dosa date back to the 1st century AD. The place of origin of dosa is debatable but traditionally accepted to be Udupi, a town in the state of Karnataka. However a popular variant, the thin crust-crispy dosa, was believed to be originated from the Indian state of Tamil Nadu.

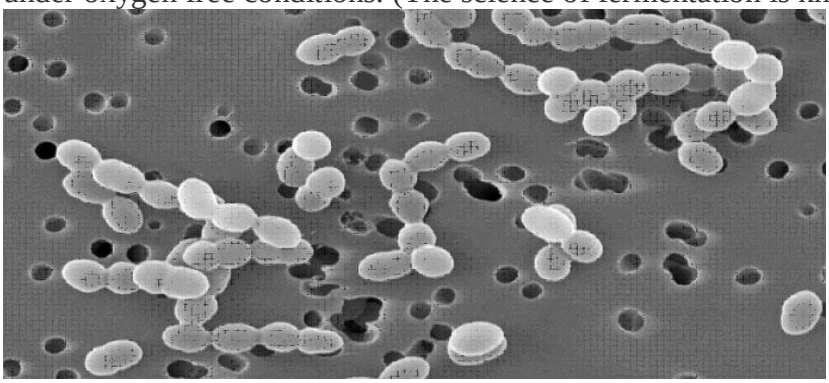
Preparation and serving: Mixture of rice and black grams (usually 2:1) soaked in water overnight is finely ground to form a batter and a pinch of salt is added. The batter is allowed to ferment overnight and then mixed with water to get the desired consistency. The batter is then ladled onto a hot griddle greased with oil or clarified butter. It is spread out evenly with the base of a ladle to form a pancake. Typical dosa is served hot along with vegetable soup (sambar), potato curry and coconut-chilly sauce (chutney) (Fig. 1), but now a day, one can find hundreds of varieties of dosa depending upon their taste and preferences.

Leaving of the batter due to Fermentation

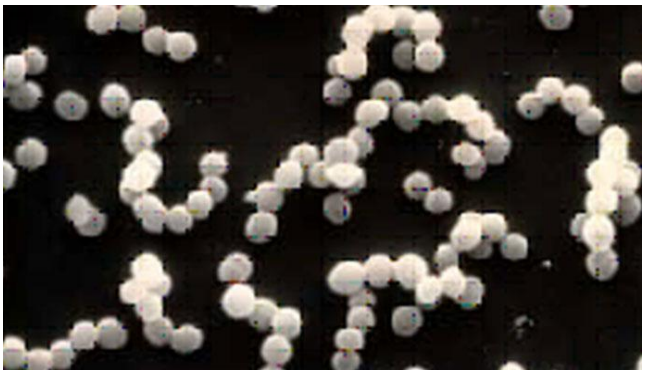
Nutrition: The main ingredients of dosa are rice (Oryza sativa) and blackgram (Phaseolus mungo). White rice, which is normally used for dosa, contains about 90% carbohydrates, 8 percent proteins and 2% fat. It is also a good source of calcium, magnesium, phosphorus, manganese, selenium, iron and vitamins, folic acid, thiamine and niacin.

It has low fiber content and contains pro-inflammatory omega-6 fatty acids. Black gram or Mungo bean is rich in carbohydrates (about 60%) and proteins (about 25%), It also contains about 18% of dietary fiber and is a good source of minerals, potassium, calcium, iron and vitamins, niacin, thiamine, and riboflavin. Black gram has been found to be very useful in controlling cholesterol levels.

Fermentation: Fermentation gives the characteristic texture (leavening), aroma and taste to the dosa batter along with improved digestibility and nutritional value (Fig. 2). Fermentation is the process of converting carbohydrates to alcohol or organic acids with the help of microorganisms, under oxygen free conditions. (The science of fermentation is known as zymology or zymurgy.)



Leuconostoc mesenteroides



Lactococcus lactis

The microorganisms responsible for the fermentation are naturally present in the ingredients of dosa batter, black gram and rice. Some of the fermentation bacteria/microbes are also provided by water and air. A temperature of 25°-30° C is found to be highly favorable for the microorganisms to boost the fermentation process.

Fermentation of dosa batter is carried out mainly by Lactobacillales or lactic acid bacteria (bacteria that convert milk to yogurt), recognized as lactobacillus delbrueckii, L. lactis, Strptociccus lactis, S. faecalis, Leuconostoc mesenteroides and Pedicococcuscerevisiae. Wild yeasts, recognized as Saccharomyces cerevisiae, Debaryomyces hansenii and Trichosporon beigelli, on the other hand, are found to produce flavor compounds and help in the saccharification (hydrolysis) of starch. In the early stages of fermentation, the 'heterofermentative' type bacteria like Leuconostoc mesenteroides (Fig. 3) are found to predominate, producing carbon dioxide and alcohol along with the lactic acid (the mucilaginous property of dosa batter helps to trap the carbon-dioxide evolved during fermentation which results into leavening of the batter).

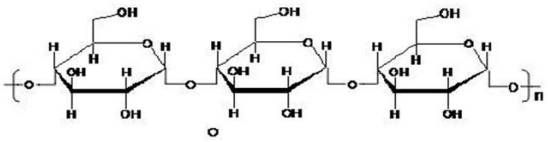


Fig 5 : Amylose (n=5 to 600)

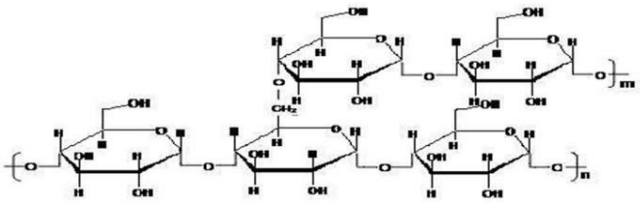


Fig 5 : Amylopectin (m=200 to 2000 ; n=20 to 30)

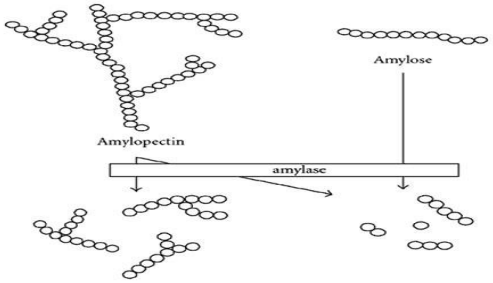


Fig 7 : Hydrolysis of starch by amylase enzymes

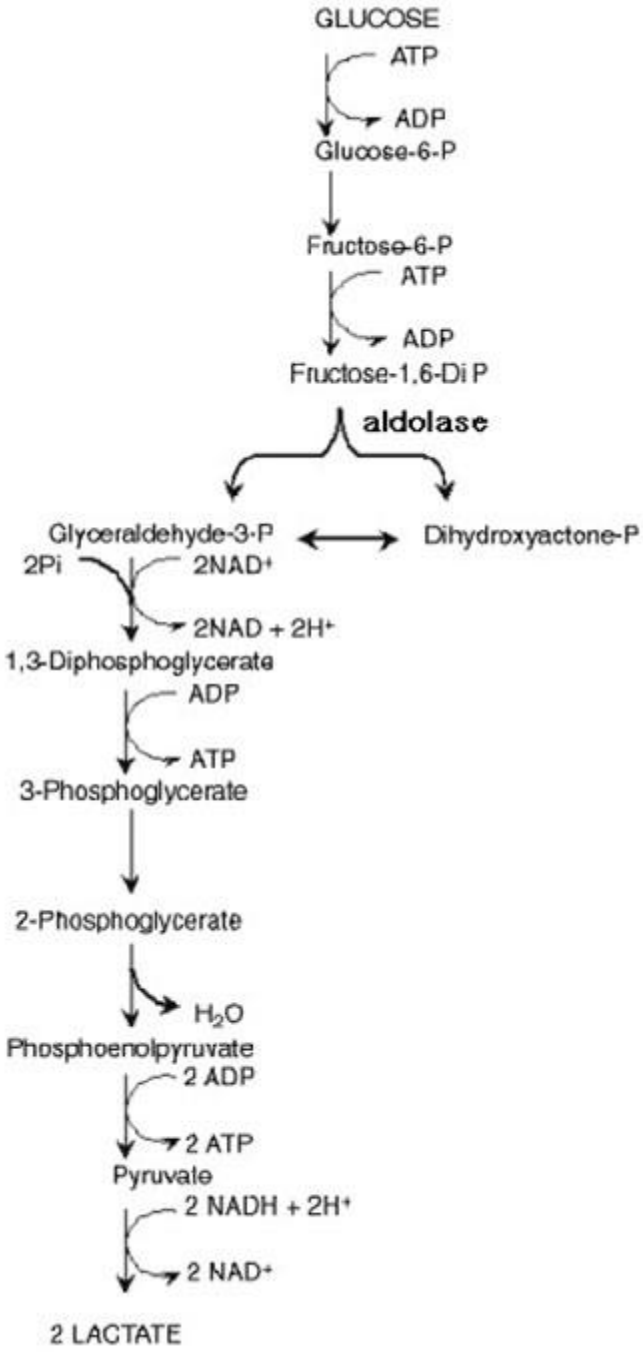
During the later stages of the fermentation the homofermentative lactic acid bacteria like Lactococcus lactis (Fig. 4) dominate and produce only lactic acid. Due to this batter starts turning sour over the time. Starch in the rice and black gram (or in general) contains two types of homopolysaccharides, amylose and amylopectin. Amylose is an unbranched homopolysaccharide consisting of about 5-600 glucose units, linked by α-(1→4) glycosidic bonds.

It forms a helix structure with six glucose units in each helix (Fig. 5). Amylopectin is a branched molecule formed by several glucose units ranging from several hundreds to fifty thousand in a main chain, which are joined by α-(1→4) glycosidic bonds.

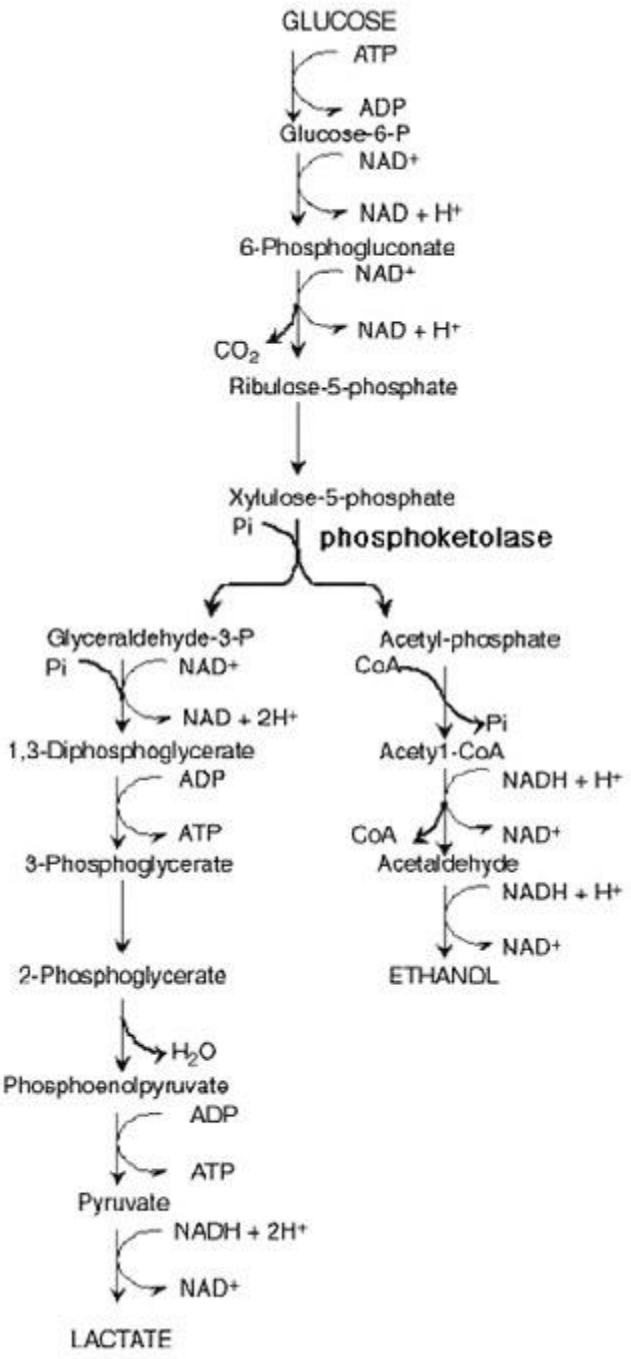
Lateral chains of about 20-30 glucose units are linked to the main chain by a α-(1→6) glycosidic bond. Glucose units on the lateral chain are linked again, joined with themselves by α-(1→4) glycosidic bonds. These branches provide a spongy fiber-like structure to the amylopectin and prevent the formation of a helical structure (Fig. 6). During the fermentation process the starch content of the dosa batter is hydrolysed (broken down) yielding maltotriose and maltose from amylose, or maltose, glucose and limit dextrin from amylopectin, with the help of amylases, the calcium metalloenzymes (Fig. 7). These amylase enzymes (mainly α-amylase, β-amylase and γ-amylase) are provided by the microorganisms (yeasts and bacteria) naturally present in the ingredients of dosa batter and the atmosphere.

The glucose, and other sugar molecules, then undergo a splitting process called glycolysis, a multistep metabolic pathway which involves a sequence of about ten enzyme-catalyzed reactions. Glycolysis can occur either in presence or absence of oxygen. Glycolysis is carried on in two slightly different ways, depending on the microorganisms (enzymes) and conditions

involved in the process. In Embden–Meyerhof–Parnas pathway (homolactic process), glucose is gradually split into two molecules of pyruvate (3 carbon sugar) and yields two molecules of ATP (free energy containing molecule) and two "high energy" electron carrying molecules of NADH.



Scheme 1 : Homolactic fermentation of glucose



Scheme 2 : Homolactic fermentation of glucose

The phosphorylation and transport of glucose usually occurs by an ATP-dependent hexose kinase or a phosphoenolypyruvate (PEP) sugar phosphotransferase system (PTS). In a concomitant step, the 2 electrons that were added to NAD+ in the glycolysis are once again removed from NADH and added back to the pyruvate molecule, regenerating NAD+ and producing lactic acid (or lactate) (Scheme 1). In the phosphoketolase pathway (hetero lactic process), Glucose molecule is converted into glucose-6-phosphate which then dehydrogenates to

give 6-phosphogluconate, upon subsequent decarboxylation it yields pentose-5-phosphate and one molecule of CO2. Pentose-5-phosphate is cleaved into glyceraldehyde phosphate (GAP) and acetyl phosphate. GAP is further metabolized to lactate as in homofermentation, with the acetyl phosphate reduced to ethanol via acetyl-CoA and acetaldehyde intermediates. End-products (CO2, lactate and ethanol) are produced in equimolar quantities (Scheme 2).

Benefits of fermentation: Fermentation process helps to break down the anti-nutrients like phytic acid present in rice and blackgram (phytic acid is known to block the mineral absorption and cause deficiencies). The mocroorganisms involved in the fermentation also produce useful substances like vitamins, folic acid, riboflavin, niacin, thiamin, biotin, vitamin K and some free amino acids as well as some antibiotic and anti-carcinogenic substances, hence increasing the total nutritional value of the dosa. Since dosa batter is predigested by bacteria, it is easier to digest. The lactic acid formed during the fermentation process, along with the various enzymes, aid the digestion of food, especially protein digestion. Lactic acid formed during fermentation not only preserves the food but also promotes the growth of a healthy intestinal flora. Lactobacilli are known to promote digestive health by inhibiting bacteria like Shigella, Salmonella and E.coli.

Role of water: Water is important to maintain the consistency (swelling and gelatinization) of the dosa batter. It acts as a solvent medium for sugars and other substrtates of fermentation process and also for enzymes. Water also acts as a source for the various microorganisms and minerals needed for the fermentation process.

Pinch of salt: Presence of salt helps to controll the fermentation process. It slows down the yeast action and helps the liberation of yeasts enzymes and other useful substances into the batter via osmosis. Since the lactic acid bacteria can tolerate high salt concentrations, the presence of salt gives them an advantage over other less tolerant species and allows the lactic acid fermenters to start the metabolism, which produces lactic acid, which further inhibits the growth of non-desirable organisms. Further addition of salt increases the mineral value of the dosa and the taste.

Introduction to Sauerkraut:

The use of cabbage (Brassica oleracea) as a food antedates known recorded history. Sauerkraut, a product resulting from the lactic acid fermentation of shredded cabbage, is literally acid (sour) cabbage. The antecedents of sauerkraut differed considerably from that prepared at present. At first the cabbage leaves were dressed with sour wine or vinegar.

Later the cabbage was broken or cut into pieces, packed into containers, and covered with verjuice (the juice expressed from immature apples or grapes), sour wine, or vinegar. Gradually the acid liquids were replaced by salt and a spontaneous fermentation resulted.

One may speculate that sauerkraut manufacture comparable to the method used today developed during the period of 1550 to 1750 A.D. although cabbage has been known and used commonly for about 4000 years. Those readers particularly interested in the historical evolution of the sauerkraut fermentation should consult Pederson (1960, 1979) and Pederson and Albury (1969).

Originally sauerkraut was made only in the home because it provided a means for utilizing fresh cabbage which otherwise would spoil before it could be used Now the commercial production of sauerkraut has become an important food industry. Even so, a significant quantity is still produced in the home, particularly in rural and suburban areas where home vegetable gardens still exist.

Cabbage varieties best suited for growth in the major production areas are used early, midseason, and late types are grown. Varieties formerly used such as Early Flat Dutch, Late Flat Dutch, Early Jersey Wakefield, and others have been replaced in part by new cultivars which have been bred to be well-adapted to mechanical harvesting and at the same time inherently contain less water, thus reducing the generation of in-plant liquid wastes. Mild-flavored, sweet, solid, white-headed cabbage is the choice as it makes a superior kraut.

Process for Sauerkraut Fermentation:

Properly matured sound heads of cabbage are first trimmed to remove the outer green broken or dirty leaves. The cores are cut mechanically by a reversing corer that leaves the core in the head. Then the cabbage is sliced by power-driven, rotary, adjustable knives into long shreds as fine as 0.16 to 0.08 cm (1/16 to 1/32 inches) in thickness.

In general, long, finely cut shreds are preferred, but the thickness is determined by the judgment of the manufacturer. The shredded cabbage (known also as slaw) is then conveyed by belts or by carts to the vats or tanks for salting and fermentation.

Salt plays a primary role in the making of sauerkraut and the concentrations used are carefully controlled. According to the legal standard of identity the concentration of salt must not be less than 2%, nor more than 3%. As a result most producers use a concentration in the range of 2.25 to 2.5% of salt. Salt is required for several reasons.

It extracts water from the shredded cabbage by osmosis, thus forming the fermentation brine It suppresses the growth of some undesirable bacteria which might cause deterioration of the product and, at the same time, makes conditions favorable for the desirable lactic acid bacteria. Salt also contributes to the flavor of the finished sauerkraut by yielding a proper salt-acid ratio (balance) if the cabbage is properly salted.

The use of too little salt causes softening of the tissue and produces a product lacking m flavor. Too much salt interferes with the natural sequence of lactic acid bacteria, delays fermentation and, depending on the amount of over-salting, may produce a product with a sharp, bitter taste, cause darkening of color, or favor growth of pink yeasts.

Uniform distribution of salt throughout the mass of shredded cabbage cannot be overemphasized. In some factories the slaw is weighed on conveyor belt lines and the desired amount of salt is sprinkled on the shreds by means of a suitable proportioner as it moves along the conveyor to the vat.

In other plants hand-carts are used to carry the shredded cabbage to the vat. Some prefer to salt the weighed cabbage in each cart. Others transport the slaw in carts which are weighed occasionally to check the capacity. The shreds are then dumped into the vat, distributed by forks, and then salted with a specific weight of salt.

The variations of salt concentrations in the brines covering kraut have been thoroughly investigated by Pederson and Albury (1969) and discussed by Pederson (1975, 1979). No mention of recirculation of the brines to gain uniformity in concentration of salt was noted.

It would seem that this method of ensuring uniform salt distribution in sauerkraut brines would be as effective as it is in the olive industry. Only small alterations in tank or vat design would be required to make it possible to completely recirculate the brine, pumping from the bottom and discharging at the surface.

Brine begins to form once the shreds are salted, and the tank is closed once it has been filled to the proper level. Formerly, the slaw was covered with a thick layer of outer leaves and then fitted with a wood cover (head) which was heavily weighted. Within a few hours the brine had formed and the fermentation had started. The head then was fixed in position in much the same manner as with pickle or olive tanks.

Now, however, a sheet plastic cover is used. This cover is much larger in area than the top of the vat or tank itself. The plastic sheeting is placed firmly against the top of the shredded cabbage with the edges draped over the sides of the container to form an open bag. Then enough water or preferably salt brine is placed in this bag so that the weight of the liquid added forces the cabbage shreds down into the brine until the brine covers the surface of the uppermost shreds. Unless the shreds are completely covered with brine, undesirable discoloration together with

undesirable flavor changes will occur. This newer method of covering and weighting provides nearly anaerobic conditions, particularly after fermentation becomes acid and quantities of carbon dioxide are produced. Precautions to avoid pin holes or tears in the plastic are mandatory if aerobic yeast growth is to be avoided.

With the old method of closure film forming yeasts always were a problem and if the scum was not removed at intervals a yeasty flavor was imparted to the kraut. Pichia membranaefaciens yeast strains, in particular, voraciously oxidize lactic acid contained in salt brines. Other genera also may be involved and besides destroying acid also contribute to yeasty flavor.

By the time the tank or vat is filled with the salted shreds and weighted, brine has formed and fermentation has started in a sequence of bacterial species responsible for the lactic acid fermentation.

Microbiology of the Sauerkraut Fermentation:

Although the lactic acid fermentation was described by Pasteur in 1858 and much work had been done in the intervening years with various lactic bacteria from cabbage and cucumber fermentations, it was not established that a definite sequence of bacterial species of lactic acid bacteria were responsible for the fermentation of either vegetable until 1930 when Pederson first described the lactic acid bacteria he observed in fermenting sauerkraut.

Pederson found that the fermentation was initiated by the species Leuconostoc mesenteroides. This species was followed by gas-forming rods and finally by non-gas-forming rods and cocci. Since 1930 additional studies by Pederson and Albury (1954, 1969) have firmly established the importance of Leuconostoc mesenteroides in initiating the lactic fermentation of sauerkraut.

Also they more closely identified the species and sequence of the other lactic acid bacteria involved. Now it is accepted that the kraut, fermentation is initiated by Leuconostoc mesenteroides, a heterofermentative species, whose early growth is more rapid than other lactic acid bacteria and is active over a wide range of temperatures and salt concentrations.

It produces acids and carbon dioxide that rapidly lower the pH, thus inhibiting the activity of undesirable microorganisms and enzymes that may soften the shredded cabbage. The carbon dioxide replaces air and creates an anaerobic condition favorable to prevention of oxidation of ascorbic acid and the natural color of the cabbage. Also carbon dioxide stimulates the growth of many lactic acid bacteria. It also may be that this species provides growth factors needed by the more fastidious types found in the fermentation.

While this initial fermentation is developing, the heterofermentative species Lactobacillus brevis and the homofermentative species Lactobacillus plantarum and sometimes Pediococcus cerevisiae begin to grow rapidly and contribute to the major end products including lactic acid, carbon dioxide, ethanol, and acetic acid. Minor end products also appear.

These are a variety of additional volatile compounds produced by the various bacteria responsible for the fermentation, by auto-chemical reactions, or the intrinsic enzymes of the fermenting cabbage itself. Hrdlicka et al (1967) reported the formation of diacetyl and acetaldehyde, the primary carbonyls formed during cabbage fermentation.

Volatile sulfur compounds are major flavor components of fresh cabbage according to Bailey et al. (1961) and Clapp et al. (1959) and also of sauerkraut. However, according to Lee et al. (1976), the major portion of the volatiles of sauerkraut is accounted for by acetal, isoamyl alcohol, n-hexanol, ethyl lactate, cis-hex-3-ene-l-ol, and allyl isothiocyanate. Of these, only the latter two have been identified as major constituents of fresh cabbage.

These latter authors concluded that although these two compounds define the character of cabbage products (kraut) they do not contribute significantly to the determination of its quality. They further believe that the fresh and fruity odor of such compounds as ethyl butyrate, isoamyl acetate, n-hexyl acetate, and mesityl oxide are probably more important in determining the acceptability of sauerkraut.

Temperature is a controlling factor in the sequence of desirable bacteria in the sauerkraut fermentation at a salt concentration of 2.25%. At the optimum of 18.3°C (65°F) or lower the quality of the sauerkraut is generally superior in flavor, color and ascorbic acid content because the heterofermentative lactic acid bacteria exert a greater effect.

According to Pederson and Albury (1969) an average temperature of about 18°C (65°F) with a salt concentration of 2.25% may be considered normal in the kraut-producing areas of the United States. At (or near) this temperature, fermentation is initiated by Leuconostoc mesenteroides and continued by Lactobacillus brevis and Lactobacillus plantarum, the latter species being most active in the final stages of fermentation.

Under these conditions a final total acidity of 1.7 to 2.3% acid (calculated as lactic acid) is formed, and the ratio of volatile to nonvolatile acid (acetic/lactic) is about 1 to 4. The fermentation is completed in 1 to 2 months, more or less, depending upon the quantity of fermentable materials, concentration of salt, and fluctuations in temperature.

At higher temperatures, as would be expected, they found that the rate of acid production was faster. For example, at 23°C (73.4°F) a brine acidity of 1.0 to 1.5% (calculated as lactic acid) may be observed in 8 to 10 days and the sauerkraut may be completely fermented in about 1 month.

At a still higher temperature of 32°C (89.6°F), the production of acid generally is very rapid with acid production of 1.8 to 2.0% being obtained in 8 to 10 days. As the temperature increased, they observed a change in the sequence of lactic acid bacteria. First, the growth of Leuconostoc

mesenteroides was retarded and Lactobacillus brevis and Lactobacillus plantarum dominated the fermentation. At higher temperatures the kraut fermentation became essentially a homofermentation dominated by Lactobacillus plantarum and Pediococcus cerevisiae.

As a result, the quality attributes of flavor and aroma deteriorated and the kraut was reminiscent of acidified cabbage because of the large quantity of lactic acid and little acetic acid produced by the homo-fermentative species. They also observed that sauerkraut fermented at higher temperatures would darken readily and, therefore, should be canned as quickly as possible after the fermentation was completed.

An extremely important observation they made was that kraut could be successfully fermented even when started at the low temperature of 7.5°C (45.5°F). Leuconostoc mesenteroides can grow at lower temperatures than the other lactic acid bacteria involved in the fermentation. At this low temperature (7.5°C or 45.5°F) an acidity of 0.4% (as lactic acid) is produced in about 10 days and 0.8 to 0.9% in less than a month.

This amount of acidity coupled with saturation of the mass of kraut and brine with carbon dioxide is sufficient to provide the conditions necessary for preservation and later completion of the fermentation provided that anaerobiosis is maintained throughout the period of latency. When the kraut mass warms enough, the fermentation then is completed by the lactic acid bacteria of the genera Lactobacillus and Pediococcus, known to grow poorly if at all at 7.5°C (45.5°F).

Thus, it may require 6 months or more before the fermentation is completed. Such kraut is generally of superior quality because it remains cool and is not subjected to high temperature during-fermentation. In good commercial practice this variation in temperature permits the processor to maintain a supply of new, completely fermented sauerkraut throughout most of the year.

Precedent for the recommendation by Pederson and Albury that sauerkraut be fermented at not over 18.3°C (65°F) had already been recorded by Parmele et al. (1927), Marten et al. (1929), and others.

Defects and Spoilage of Sauerkraut:

Abnormalities of sauerkraut, although varied, with few exceptions can be and generally have been avoided by application of scientific knowledge already available to the industry. For example, the simple expedient of providing anaerobiosis has eliminated most of the problems involving discoloration (auto-chemical oxidation), loss of acidity caused by growth of, molds and yeasts, off-flavors and odors (yeasty and rancid) caused by excessive aerobic growth of molds and yeasts, slimy, softened kraut caused by pectolytic activity of these same molds and

yeasts, and pink kraut caused by aerobic growth of asporogenous yeasts, presumably members of the genus Rhodotorula.

Stamer et al. (1973) described the induction of red color in white cabbage juice by L. brevis while studying the effects of pH on the growth rates of the 5 species of lactic acid bacteria commonly associated with the kraut fermentation. L. brevis was the only species which produced such color formation in white cabbage juice and did so only when the juice was buffered with either calcium carbonate or sodium hydroxide.

No color development occurred when the pH of the juice (3.9) was not adjusted or when the pH of the juice was raised to 5.5 and the juice sterilized by filtration before it was re-incubated. Therefore, red color formation was caused by L. brevis and did not arise as the result of chemical or inherent enzymatic reactions of the juice.

It remains to be seen whether this interesting phenomenon will be observed in industrial kraut fermentations. Since color induction by L. brevis was found to be pH dependent it seems unlikely to be found in normal kraut fermentations but could easily result from accidental addition of alkali to the shredded cabbage during salting.

Slimy or ropy kraut has been observed for many years. It is generally caused by dextran formation induced by Leuconostoc mesenteroides and is transitory in nature. This species prefers to ferment fructose rather than glucose. Therefore, in the fermentation of sucrose, the fructose is fermented leaving the glucose which interacts to form the slimy, ropy, water-insoluble dextrans.

These vary from an almost solid, gelatinous mass to a ropy slime surrounding the bacterial cells. These variations are easily demonstrated by growing L. mesenteroides in a 10% sucrose solution containing adequate accessory nutrients. The fermenting kraut may become very slimy during the intermediate stage of fermentation but with additional time the dextrans are utilized by other lactic acid bacteria. Thus, it is imperative to distinguish between dextran induced slimy kraut and permanently slimy kraut caused by pectolytic activity. The former condition certainly is not a defect but should be considered a normal step in a natural progression.

Raw Materials

Soybeans

Soybeans *(Glycine max)* are also called soya beans, soja beans, Chinese peas, soy peas, and Manchurian beans. They have been referred to as the "King of Legumes" because of their valuable nutritive properties. Of all beans, soybeans are lowest in starch and have the most complete and best protein mix. They are also high in minerals, particularly calcium and magnesium, and in Vitamin B. They have been cultivated since the dawn of civilization in China and Japan and were introduced into the United States in the nineteenth century. In the 1920s and 1930s, soybeans gained popularity in the U.S. as a food crop.

Soybeans are short, hairy pods containing two or three seeds which may be small and round or larger and more elongated. Their color varies from yellow to brown, green, and black. The variety designated yellow #2 are most commonly used for food products. These soybeans get their name from the yellow hilum or seed scar which runs down the side of the pod. The grades of grain allowed for trading are established by the United States Grain Standards which are administered by the U.S. Department of Agriculture. Soybeans are unusual in that, unlike other grains, most are used in processing or exporting, and not much as direct animal feed. This is because soybeans contain "anti-nutritional" factors that must be removed from the beans before they can be of nutritional value to animals. The soybeans used in soy sauce are mashed prior to mixing them with other ingredients.

Wheat

In many traditional brewed recipes, wheat is blended in equal parts with the soybeans. Pulverized wheat is made part of the mash along with crushed soy beans. The nonbrewed variety does not generally use wheat.

Salt

Salt, or sodium chloride, is added at the beginning of fermentation at approximately 12-18% of the finished product weight. The salt is not just added for flavor; it also helps establish the proper chemical environment for the lactic acid bacteria and yeast to ferment properly. The high salt concentration is also necessary to help protect the finished product from spoilage.

American farmers produced surpluses of many agricultural commodities in 1930, but soybeans were not one of them. During the early years of the Great Depression, few farmers raised soybeans, but this changed in just 10 years. In 1929, American farmers produced less than 10 million bushels (352 million L) of soybeans. By 1939 production approached 100 million bushels (3.5 billion L), and in 1995, American farmers raised more than 2.1 billion (74 billion L) bushels of soybeans. No one surpassed Henry Ford as a promoter of soybean production in the 1930s.

In 1929, Henry Ford constructed a research laboratory in Greenfield Village and hired Robert Boyer to oversee experimentation related to farm crops. Ford hired additional scientists to investigate the industrial uses of many agricultural commodities, including vegetables such as

carrots. The greatest success was in soybean experimentation. The researchers developed soy-based plastics and made parts for automobiles out of the products. The scientists manufactured ink made from soy oil, and produced soy-based whipped topping. Many of these processes and products remain in use.

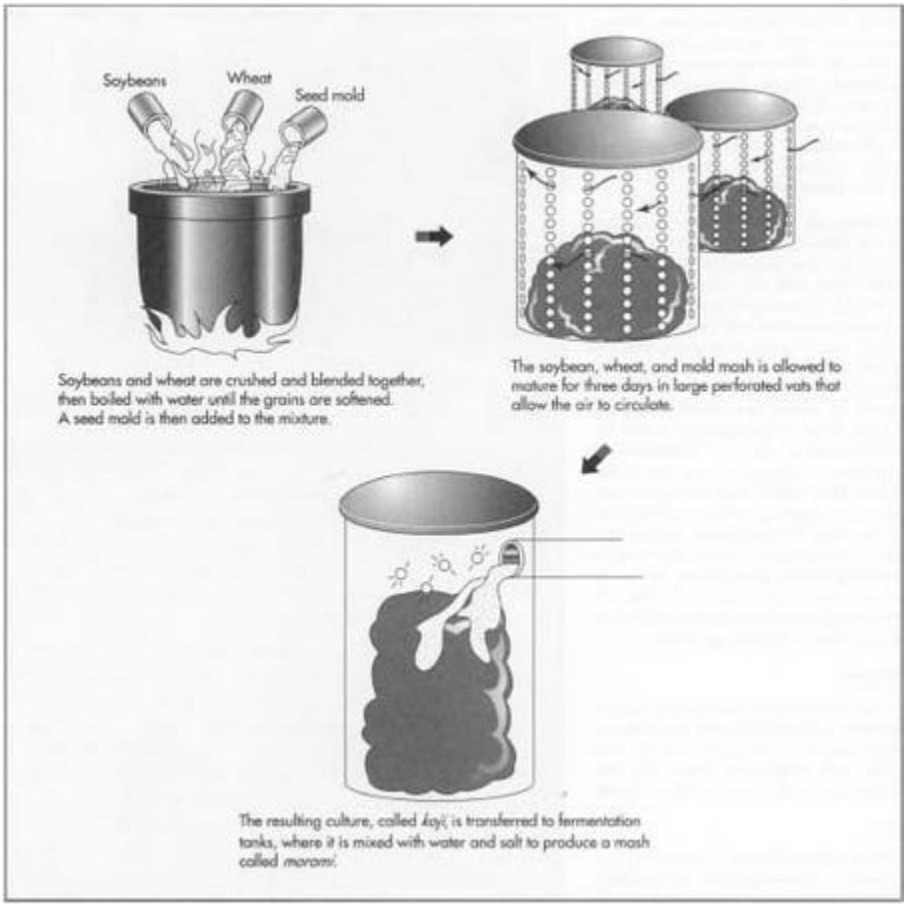
Ford believed that farmers should have one foot on the soil and the other in industry. Ford promoted agricultural production of soybeans through an exhibit in a barn at the Chicago "Century of Progress" World Exposition in 1933. He hosted a meal which included a variety of soybean items and supported the publication of recipe booklets full of soybean-based recipes.

Henry Ford wished to see farmers to produce soybeans on their farms and process them for industrial purposes. Though his vision was not realized, the importance of soybeans in American agriculture came to fruition. Soybeans are one of most important crops raised in America, and provide American farmers millions of dollars in income.

Leo Landis

Fermenting agents

The wheat-soy mixture is exposed to specific strains of mold called *Aspergillus oryzae* or *Aspergillus soyae*, which break down the proteins in the mash. Further fermentation occurs through addition of specific



bacteria *(lactobaccillus)* and yeasts which enzymatically react with the protein residues to

produce a number of amino acids and peptides, including glutamic and aspartic acid, lysine, alanine, glycine, and tryptophane. These protein derivatives all contribute flavor to the end product.

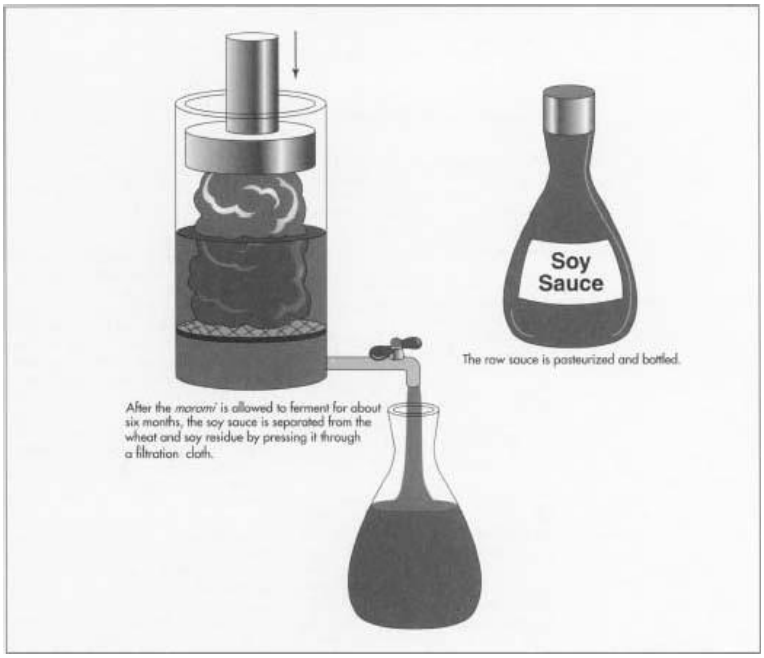
Preservatives and other additives

Sodium benzoate or benzoic acid is added to help inhibit microbial growth in finished soy sauce. The non-brewed process requires addition of extra color and flavor agents.

The Manufacturing Process

Traditional brewed method

Brewing, the traditional method of making soy sauce, consists of three steps: *koji* -making, brine fermentation, and refinement.



Koji-making

- 1 Carefully selected soybeans and wheat are crushed and blended together under controlled conditions. Water is added to the mixture, which is boiled until the grains are thoroughly cooked and softened. The mash, as it is known, is allowed to cool to about 80°F (27°C) before a proprietary seed mold *(Aspergillus)* is added. The mixture is allowed to mature for three days in large perforated vats through which air is circulated. This resulting culture of soy, wheat, and mold is known as *koji*.

Brine fermentation

- 2 The *koji* is transferred to fermentation tanks, where it is mixed with water and salt to produce a mash called *moromi*. Lactic acid bacteria and yeasts are then added to promote further fermentation. The *moromi* must ferment for several months, during which time the soy and wheat paste turns into a semi-liquid, reddish-brown "mature mash." This fermentation process creates over 200 different flavor compounds.

Refinement

- 3 After approximately six months of *moromi* fermentation, the raw soy sauce is separated from the cake of wheat and soy residue by pressing it through layers of filtration cloth. The liquid that emerges is then pasteurized. The pasteurization process serves two purposes. It helps prolong the shelf life of the finished product, and it forms additional aromatic and flavor compounds. Finally, the liquid is bottled as soy sauce.

Non-brewed method (chemical hydrolysis)

Instead of fermenting, many modern manufactures artificially break down the soy proteins by a chemical process known as hydrolysis because it is much faster. (Hydrolysis takes a few days as compared to several months for brewing.)

1. In this method, soybeans are boiled in hydrochloric acid for 15-20 hours to remove the amino acids. When the maximum amount has been removed, the mixture is cooled to stop the hydrolytic reaction.
2. The amino acid liquid is neutralized with sodium carbonate, pressed through a filter, mixed with active carbon, and purified through filtration. This solution is known as hydrolyzed vegetable protein.
3. Caramel color, corn syrup, and salt are added to this protein mixture to obtain the appropriate color and flavor. The mixture is then refined and packaged.

Sauces produced by the chemical method are harsher and do not have as desirable a taste profile as those produced in the traditional brewed manner. The difference in taste occurs because the acid hydrolysis used in the non-brewed method tends to be more complete than its fermentation counterpart. This means that almost all the proteins in the non-brewed soy sauce are converted into amino acids, while in the brewed product more of the amino acids stay together as peptides, providing a different flavor. The brewed product also has alcohols, esters, and other compounds which contribute a different aroma and feel in the mouth.

In addition to the brewed method and the non-brewed method, there is also a semi-brewed method, in which hydrolyzed soy proteins are partially fermented with a wheat mixture. This method is said to produce higher quality sauces than can be produced from straight hydrolysis.

Quality Control

Numerous analytical tests are conducted to ensure the finished sauce meets minimum quality requirements. For example, in brewed sauces, there are several recommended specifications. Total salt should be 13-16% of the final product; the pH level should be 4.6-5.2; and the total

sugar content should be 6%. For the non-brewed type, there is 42% minimum of hydrolyzed protein; corn syrup should be less than 10%; and carmel color 1-3%.

In the United States, the quality of the finished sauce is protected under federal specification EE-S-610G (established in 1978) which requires that fermented sauce must be made from fermented mash, salt brine, and preservatives (either sodium benzoate or benzoic acid). This specification also states that the final product should be a clear, reddish brown liquid which is essentially free from sediment. The non-fermented sauce is defined as a formulated product consisting of hydrolyzed vegetable protein, corn syrup, salt, caramel color, water, and a preservative. It should be a dark brown, clear liquid.

The Japanese, on the other hand, are more specific in grading the quality of their soy sauces. They have five types of soy sauce: *koikuchi-shoyu* (regular soy sauce), *usukuchi-shoyu* (light colored soy sauce), *tamari-shoyu*, *saishikomi-shoyu*, and *shiro-shoyu*. These types are classified into three grades, Special, Upper, and Standard, depending upon sensory characteristics such as taste, odor, and feel in the mouth, as well as analytical values for nitrogen content, alcohol level, and soluble solids.

Byproducts/Waste

The fermentation process produces many "byproducts" that are actually useful flavor compounds. For example, the various sugars are derived from the vegetable starches by action of the *moromi* enzymes. These help subdue the saltiness of the finished product. Also, alcohols are formed by yeast acting on sugars. Ethanol is the most common of these alcohols, and it imparts both flavor and odor. Acids are generated from the alcohols and sugars, which round out the flavor and provide tartness. Finally, aromatic esters (chemicals that contribute flavor and aroma) are formed when ethanol combines with organic acids.

Chemical hydrolyzation also leads to byproducts, but these are generally considered undesirable. The byproducts are a result of secondary reactions that create objectionable flavoring components such as furfural, dimethyl sulfide, hydrogen sulfide, levulinic acid, and formic acid. Some of these chemicals contribute off odors and flavors to the finished product.

The Future

The future of soy sauce is constantly evolving as advances are made in food technology. Improved processing techniques have already allowed development of specialized types of soy sauces, such as low-sodium and preservative-free varieties. In addition, dehydrated soy flavors have been prepared by spray drying liquid sauces. These powdered materials are used in coating mixes, soup bases, seasoning rubs, and other dry flavorant applications. In the future, it is conceivable that advances in biotechnology will lead to improved understanding of enzymatic reactions and lead to better fermentation methods. Technology may someday allow true brewed flavor to be reproduced through synthetic chemical processes.

Tempeh Fermentation Process

What happens during the fermentation of soybeans into tempeh? Generally it is agreed that not only is the flavor, aroma, and texture of plain cooked soybeans enhanced during the fermentation process, but there are several nutritional benefits derived in the process as well to make the beans more easily assimilated by the human body. This is done by the main mold Rhizopus oligosporus, and other minor organisms or their enzymes hydrolizing proteins, carbohydrates, and fats to create smaller and more digestible units. This process makes the protein in tempeh more digestible and usable.

A. Changes in Lipids

Free fatty acids increased from 0.5% in the unfermented control to 21.0% in the dehydrated tempeh (with the same moisture content). During fatty acid synthesis, Rhizopus sp. produced only gamma-linolenic acid (GLA) instead of alpha-linoleic acid. GLA is a prostaglandin and leukotriene precursor. It is used therapeutically to decrease the cholesterol and triglyceride content in blood. It is not found in soybean (3).

B. Changes in Carbohydrates

During fermentation, the principal changes in carbohydrates are the rapid decrease of hex-oses and the slow hydrolysis of stachyose, the flatulence factor in beans (4). This makes tempeh a more socially acceptable soybean product.

C. Changes in Proteins and Amino Acids

Steinkraus (5) summarized the biochemical analysis of tempeh. The most significant changes are in the proteins and vitamins. Ammonia (% of total nitrogen) increased from 0.1 to 1.7. Percent nitrogen soluble in water increased from 6.5 to 39.0. Percent nitrogen soluble in trichloroacetic acid increased from 5.9 to 28. There were no significant changes in the amino acid patterns between soybeans and tempeh (6). It is likely that there is no de novo synthesis of amino acids, but only a degradation and consumption of soy protein by the fungi.

D. Changes in Vitamins

Steinkraus (5) also summarized reported work on changes in vitamins during tempeh fermentation. Riboflavin increased by 2-47 times, niacin increased by 2-7 times, and vitamin B12 by 33 times. Thiamin, unfortunately, decreased. Panthothenic acid has been reported to stay the same or increased by 2-4 times. Pyridoxine increased by 4-14 times. Biotin and total folate

compounds were respectively 2.3 and 4-5 times higher in tempeh than in unfermented soybeans. The variations in reported changes may be due to the way tempeh was made in various laboratories and locations, as well as the beans and microorganisms associated with them. For example, according to this author's experience, the production of vitamin B12 fluctuated considerably even under similar fermentation conditions in the same location.

E. Presence of Antioxidants and Antibiotic in Tempeh

An isoflavone identified as 6,7,4'-trihydroxy isoflavone (called Factor 2) has been reported (6,7). The antioxidative effect of factor 2 on retinol was about the same as DL-alpha-tocopherol, and three times that of genistein. Other isoflavones were later reported (8,9).

In Indonesia, tempeh is widely used to ween babies off mother's milk and to help patients recover strength from dysentery and other ailments of the intestinal tract (1). R. oligosporus NRRL 2710 was reported to produce an antibiotic active compound against a number of gram-positive bacteria including S. aureus and B. subtilis, as well as the gramnegative K. pneumoniae (10). It was demonstrated later that K. pneumoniae and R. oligosporus NRRL 2710 grows well together in tempeh fermentation. There was no evidence of K. pneumoniae inhibition by the mold (11,12). This may help explain why tempeh is provided to patients with dysentery and other ailments of the intestinal tract.

F. Reduction of Phytate

Phytate is considered to exacerbate mineral deficiency in human by hindering absorption in the gut. Reduction of phytate was reported to be 22% during tempeh fermentation due to phytase active in R. oligosporus (13). This again demonstrated the benefits of tempeh.

IV. TEMPEH FERMENTATION IN INDONESIA (1) A. A Small Tempeh Manufacturer in Denpassar, Bali

A home-based tempeh factory can be operated by a small family group of four adults and several small children. Fifty kilos of tempeh are made each day in the house. Cleaned soybean is cooked in the early morning. At mid-morning, the cooked beans are cooled manually in a large bamboo colander placed on the floor. The tempeh from the day before is incubated in small 3" x 3" perforated plastic bags, each of which weigh about 3 ounces. These small cakes are incubated for 2 days on wooden slats in a dark room. A bicycle-powered mill, the colander, and an aluminum cooking pot and heat sealer are the only pieces of equipment. The 3-ounce cakes are sold to restaurants and in the market for 150 rupiah (about 6 US cents).

B. A Medium-Scale Manufacturer in Denpassar, Bali

A substantial medium operation produces 750 kilos of tempeh, 7 days a week, 30 days a month. The manufacturer employs 10 young men in this operation.

The soybeans are cooked in delidded 55-gallon drums placed over propane burners. The hulls are skimmed off the beans manually with plastic colanders by two persons. Two others cool and package cooked beans in perforated plastic bags in another room. The beans are piled not on the floor but on a piece of white canvas. Tempe Murni sells 250 grams of finished tempeh for 400 rupiah (U.S. 160) to their distributors, who sell it in the market for 500 rupiah (U.S. 210).

C. A Large Tempeh Manufacturer in Yogyakarta, Java

Yogyakarta has always been viewed as the cultural capital of Indonesia, it has the highest per capita consumption of tempeh in all of Indonesia. On a weekly basis, the average person in Yogyakarta consumes nearly 200 grams of tempeh versus only 75 grams per Balinese citizen. This shows in the local market which is totally inundated by a large variety of tempeh products—both raw cakes and tempeh prepared in various sauces. These products are supplied mainly by one large tempeh maker. This innovative plant produces 5000 pounds of product each day from a 1500 square foot area. This shop does have a gas-fired boiler and copper kettle for cooking the beans but everything else is done with the same level of technology witnessed elsewhere (bamboo colanders on the floor for mixing and packaging, etc.). What is unique about this shop is the ingenious barter system that exists here. The main operation is run by a paid staff of 24 people but the filling is almost all done manually by local workers, They seal the inoculated beans in plastic bags, sometimes melting the plastic by running it near the open flame of a cloth wick stuck into a coke can filled with kerosene! After the bags are sealed, the workers pack them up into the cloth sacks the beans came in, load them onto bicycles, and pedal the load home. There they incubate the beans for several days, and when the beans are white and ripe, take them to the local market for sale. In Yogyakarta, small, 1 ounce packages of tempeh incubated for an extra long time in banana leaves are available and sold for 25 rupiah (about U.S. 10).

In Indonesia, the same basic processing steps for tempeh making takes place. Figure 3 presents a generalized flow chart on these procedures followed in Indonesia (1,14). The steps are as follows:

1. Beans are soaked overnight in what is known as the prefermentation.

2. Early the next morning the beans are dehulled and split, using anything from mills to hands and feet.

3. Beans are cooked in open kettles where more dehulling takes place.

4. Cooked beans are placed in a large woven colander about 3 feet across and placed in front of fans to dry.

5. Cooled beans are inoculated with the culture. Most culture comes from the same source, a local Indonesian lab, and is a type of Rhizopus that is incubated at around 30 °C for 48 hr.

6. Inoculated beans are now scooped into perforated plastic bags or banana leaves. Whereas the perforated plastic bags were the most common way of incubating tempeh, most cooks preferred tempeh incubated in banana leaves. Observations in the market bore this out as tempeh incubated in the leaves was consistently of higher quality than that in the plastic bags.

Soak beans overnight (Pre-fermentation) Dehull and split beans Cook beans in open kettle

Inoculate beans at room temperature (30°C) for 2 days Pack beans in plastic bags or banana leaf

Marketing final product Figure 3 Flowchart of tempeh manufacturing in Indonesia.

7. Incubation takes place in a room or closet where inoculated beans sit out at room temperature on wooden slats.

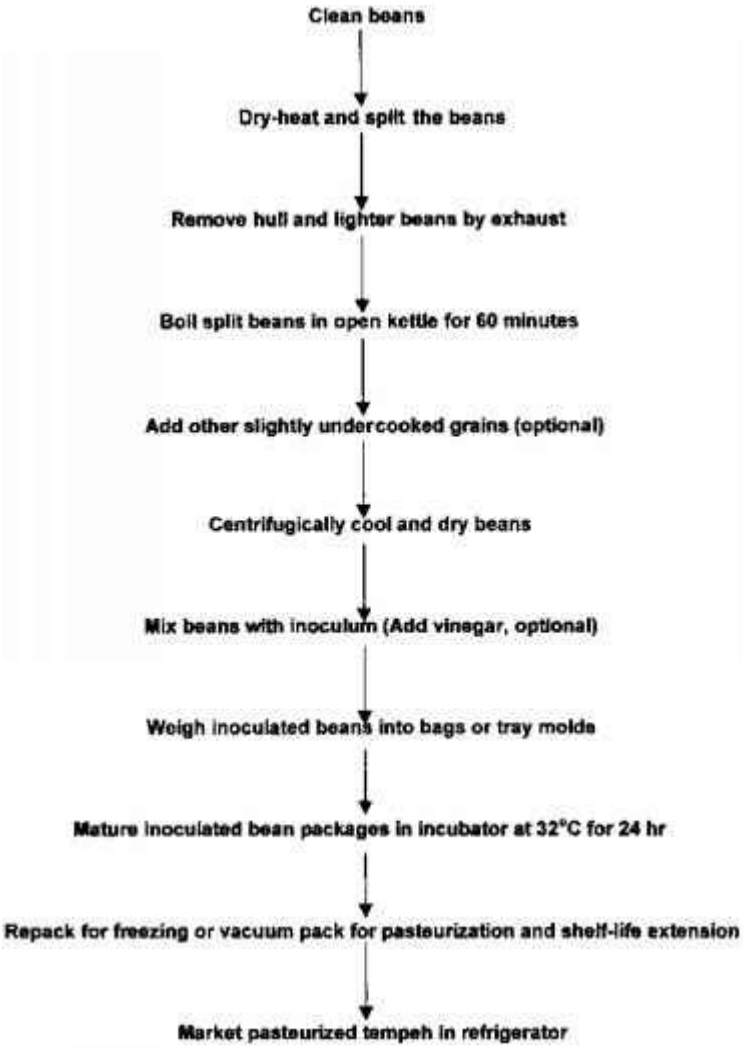
8. After 2 days the tempeh is sold in its perforated incubation bag or banana leaf in the marketplace.

V. TEMPEH MAKING IN NORTH AMERICA, CA 2000

In the year 2000, only about seven shops existed in North America capable of making a thousand pounds of tempeh or more per day. These were Lightlife Foods in Massachusetts, Turtle Island Foods in Oregon, White Wave in Colorado, Northern Soy in New York, Cricklewood Foods in Pennsylvania, Surata Soyfoods in Oregon, and 21st Century Foods in Massachusetts. Even the smallest of these would be viewed as a sanitary modern factory compared to Indonesian methods. Most shops follow a flow chart or processing scheme (Fig. 4) similar to the following:

1. Whole soybeans are heated and split dry in a mill.

2. The split beans and hulls fall into a tube where the lighter hulls are sucked out from the top by connecting it to an exhaust fan. The heavier beans fall to the bottom of the tube into a collection bucket.



Market pasteurized tempeh in refrigerator Figure 4 Flowchart for tempeh manufacturing in the United States.

3. The split beans are cooked at a boil for 60 min in an open steam jacketed stainless steel kettle.

4. Other grains may be added toward the end of the cooking time. These grains are always slightly undercooked. The rule here is to cook the grains for about half as long as one normally would if one were preparing them to eat at our own supper table.

5. Cooked beans are now placed into a centrifuge. These have stainless steel baskets and the beans are spun rapidly for a short period of time to cool and remove excess water.

6. Cooled beans are placed in a horizontal mixer where they are mixed with the innoculant and other grains. Some people acidify the beans with a small amount of vinegar at this point.

7. Beans are now either placed in a mechanical scale of some kind or weighed out by hand into perforated bags or tray molds for burgers.

8. Bags of inoculated beans are now laid flat on perforated trays and placed inside an incubation room where they are heated at 32°C (89.6) for 24 hr.

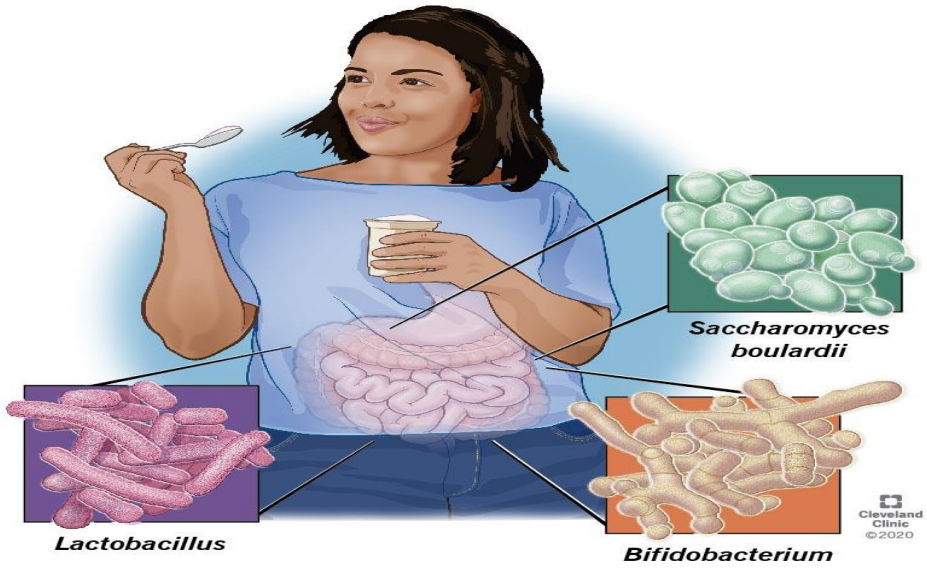
9. At this point tempeh is now bound into a firm, fragrant white cake. Some workers slip an outer bag over the tempeh at this point and freeze the product.

10. Most commonly at this point, finished tempeh is now vacuum packaged and then steam or hot water-pasteurized to extend the shelf life.

11. Tempeh is now cooled down and sold refrigerated it has a shelf life of approximately 3 months.

Probiotics

Probiotics are made of good live bacteria and/or yeasts that naturally live in your body. You constantly have both good and bad bacteria in your body. When you get an infection, there's more bad bacteria, knocking your system out of balance. Good bacteria helps eliminate extra bad bacteria, returning the balance. Probiotic-supplements are a way to add good bacteria to your body.



Probiotics are made of both bacteria and yeast. Common probiotic bacteria can include lactobacillus and bifidobacterium. The most common yeast found in probiotics is saccharomyces boulardii.

What are probiotics?

Probiotics are a combination of live beneficial bacteria and/or yeasts that naturally live in your body. Bacteria is usually viewed in a negative light as something that makes you sick. However, you have two kinds of bacteria constantly in and on your body — good bacteria and bad bacteria. Probiotics are made up of good bacteria that helps keep your body healthy and working well. This good bacteria helps you in many ways, including fighting off bad bacteria when you have too much of it, helping you feel better.

Probiotics are part of a larger picture concerning bacteria and your body — your microbiome. Think of a microbiome as a diverse community of organisms, such as a forest, that work together to keep your body healthy. This community is made up of things called microbes. You have trillions of microbes on and in your body. These microbes are a combination of:

- Bacteria.
- Fungi (including yeasts).
- Viruses.
- Protozoa.

Everyone's microbiome is unique. No two people have the same microbial cells — even twins are different.

For a microbe to be called a probiotic, it must have several characteristics. These include being able to:

- Be isolated from a human.
- Survive in your intestine after ingestion (being eaten).
- Have a proven benefit to you.
- Be safely consumed.

Where do beneficial probiotics (microbes) live in my body?

Though the most common place linked to beneficial microbes is your gut (mostly large intestines), you have several locations in and on your body that host good microbes. These locations are in contact with the “outside world" and include your:

- Gut.
- Mouth.
- Vagina.
- Urinary tract.
- Skin.
- Lungs.

How do probiotics work?

The main job of probiotics, or good bacteria, is to maintain a healthy balance in your body. Think of it as keeping your body in neutral. When you are sick, bad bacteria enters your body and increases in number. This knocks your body out of balance. Good bacteria works to fight off the bad bacteria and restore the balance within your body, making you feel better.

Good bacteria keeps you healthy by supporting your immune function and controlling inflammation. Certain types of good bacteria can also:

- Help your body digest food.
- Keep bad bacteria from getting out of control and making you sick.
- Create vitamins.
- Help support the cells that line your gut to prevent bad bacteria that you may have consumed (through food or drinks) from entering your blood.
- Breakdown and absorb medications.

This balancing act is naturally happening in your body all of the time. You don’t actually need to take probiotic supplements to make it happen. Good bacteria is just a natural part of your body. Eating a well-balanced diet rich in fiber every day helps to keep the number of good bacteria at proper levels.

What are the most common types of probiotic bacteria?

Though there are many types of bacteria that can be considered probiotics, there are two specific types of bacteria that are common probiotics found in stores. These include:

- *Lactobacillus*.
- *Bifidobacterium*.

Probiotics are also made up of good yeast. The most common type of yeast found in probiotics is:

- *Saccharomyces boulardii*.

Can I use probiotics to help with medical conditions?

There is currently a large amount of research happening around the idea of what probiotics can do for your body. Even though there are a lot of possibly positive outcomes, researchers are still working to find definitive answers about how probiotics can help with various conditions.

However, there are some medical conditions where probiotics may help. This can vary between people meaning that what works for one person may not work for another. These can also vary based on the certain probiotic that is taken.

Some of the conditions that might be helped by increasing the amount of probiotics in your body (through food or supplements) include:

- Diarrhea (both diarrhea caused by antibiotics and from *Clostridioides difficile* (C. diff) infection).
- Constipation.
- Inflammatory bowel disease (IBD).
- Irritable bowel syndrome (IBS).
- Yeast infections.
- Urinary tract infections.
- Gum disease.
- Lactose intolerance.
- Eczema (atopic dermatitis).
- Upper respiratory infections (ear infections, common cold, sinusitis).
- Sepsis (specifically in infants).

Can I take or eat something to increase the good probiotics (microbes) in my body?

You can increase the amount of good microbes in your body through foods, drinks and supplements. You may already have certain foods in your daily diet that contain probiotics. Fermented foods in particular (yogurt and pickles, for example) are home to a host of good bacteria that benefit your body. There are also fermented drinks like kombucha (fermented tea) or kefir (fermented dairy drink) that introduce extra probiotics into your diet.

Apart from food, you can add probiotics to your diet through dietary supplements. These aren't drugs, so they do not need to be approved by the Federal Drug Administration (FDA). It's important that you always talk to your healthcare provider before starting any kind of supplement or major change to your diet.

Can I get probiotics from food?

You can absolutely increase beneficial microbes in your body from the foods you eat. Certain foods have probiotics (good bacteria) in them and can benefit the health of your microbiome.

These foods can be introduced into your diet at any point of the day. You may even be regularly eating them now and not realize that they contain probiotics. You will want to check the food label for "live and active cultures." A few suggestions for just some of the probiotic-rich foods you can add to your diet and some times to try them include:

For breakfast, try:

- Yogurt.
- Buttermilk.
- Sourdough bread.

For lunch, try:

- Cottage cheese.
- Kombucha.
- Tempeh.

For a snack, try:

- Fermented pickles.

For dinner, try:

- Fermented sauerkraut.
- Kimchi.
- Miso soup.

Make sure you are still creating a balanced and healthy meal each time you sit down to eat. Though adding probiotic-rich foods into your diet won't hurt you, balance is still key. Adding too much of just one food prevents your body from reaping the benefits of other food groups.

How do I take a probiotic supplement?

There are several ways you can take a probiotic supplement. They come in a variety of forms, including in:

- Foods.
- Drinks.
- Capsules or pills.
- Powders.
- Liquids.

Probiotic supplements may be combined with a prebiotic. Prebiotics are complex carbohydrates that feed the microorganisms in your gut. Basically, prebiotics are the "food source" for the good bacteria. They help feed the good bacteria and keep it healthy. Prebiotics include inulin, pectin and resistant starches.

When you have a supplement that combines a probiotic and prebiotic, it's called a synbiotic.

How effective are probiotics?

Researchers are currently unsure how effective probiotic supplements are for treating conditions. There's constant research on the topic. While many research studies have had positive results on the impact of probiotic supplements, more research is still needed.

It's also important to keep in mind that unlike medications, dietary supplements do not need to be approved by the FDA. This means that manufacturers can sell supplements simply with "claims" of safety and effectiveness.

Always talk with your healthcare provider (or pediatrician) before taking a supplement or giving one to your child. Supplements might interfere with medicines you may be taking. If you are pregnant or breast feeding, check with your provider before taking any supplement.

Are there any storage instructions for probiotics?

Several probiotic strains are very fragile and need to be protected from heat, oxygen, light and humidity. The probiotics might start to break down or die if they are exposed to these elements. Because of this, you may need to refrigerate your probiotics or store it in a particular place. Refrigerating certain probiotic strains ensures that they're still viable when you go to use them and will still provide the full benefit of the probiotic. Always read the labels on any probiotic product you purchase to make sure you store it correctly and use it within the expiration date.

How safe are probiotics?

Because microbes used as probiotics already exist naturally in your body, probiotic foods and supplements are generally considered safe. They may trigger allergic reactions, and may also cause mild stomach upset, diarrhea, or flatulence (passing gas) and bloating for the first few days after starting to take them.

There are certain people who need to use caution when using probiotic supplements. There is a risk of infection in some people. These people include those who have:

- A weakened immune system (those going through chemotherapy for example).
- A critical illness.
- Recently had surgery.

Caution should also be used when giving probiotics to very sick infants.

Always talk to your healthcare provider before starting a probiotic supplement.

Can probiotics hurt me?

For most healthy people, probiotics don't cause any harm. They are generally considered safe and are often "given a try" to see if they could help with various medical conditions. There's a lot of research around the topic of probiotics. Scientists are trying to determine when and how they should be used, as well as how effective they are. Talk to your healthcare provider before starting a probiotic supplement because there are some cases where you shouldn't be taking them. It's always best to have the conversation first before starting a new supplement.

Are there any risks related to probiotics?

Probiotics are generally considered safe. However, there are some risks linked to the supplements. These risks are increased if you have a medical condition that weakens your immune system, have recently had surgery or have other serious medical conditions.

Unlikely, but possible, risks can include:

- Developing an infection.
- Developing a resistance to antibiotics.
- Developing harmful byproducts from the probiotic supplement.

Should I give probiotics to my kids?

Probiotics can be beneficial for both adults and kids. If your child has an illness that requires an antibiotic medication for treatment, taking a probiotic can help shorten symptoms. Probiotics can also be used to help relieve constipation, acid reflux, diarrhea, gas and eczema in children.

Introducing probiotics into your child's diet through food is typically a safe way to give them probiotics. Foods like yogurt and cottage cheese are often part of a balanced diet and can add in good bacteria without much risk.

There are commercially available probiotic supplements specifically designed for infants and children. However, it is important to talk to your child's pediatrician before giving them any probiotic supplement or changing the child's diet to include probiotic-rich foods.

Do I need to take probiotics after I take antibiotics?

Antibiotic medications are often needed to fight an infection. However, while antibiotics are killing the bad bacteria, they are also knocking out the good bacteria in your body. Some people develop conditions like diarrhea after taking an antibiotic. In other people, this may allow for really bad bacteria to take over and populate the gut, such as with C. diff. Some research has shown a positive connection between taking probiotics after an antibiotic and relief from diarrhea. This hasn't been proven yet and doesn't work for everyone.

The thought behind adding probiotics back into your body after taking an antibiotic is that it can repopulate the good bacteria that was destroyed by the antibiotics and re-boot your system. The extra good bacteria helps repopulate your gut and fight off any remaining bad bacteria. Many people feel that adding in probiotics won't hurt, might help you feel better a little faster and prevent diarrhea.

Should I try probiotics?

If you are interested in adding probiotics to your diet, it's worth a conversation with your healthcare provider. Many providers may suggest giving them a try to see if they help with your general health. It is important to remember that not all probiotics behave the same way and have the same effects. Each has their own individual benefits. They generally don't cause harm. One easy way to start can be by simply introducing probiotic-rich foods into your diet, like yogurt.

Before you start any supplements, make sure you talk to your healthcare provider. Your provider may be able to point you in the right direction, helping you figure out the best probiotic to take, how much to take and when to take it. A conversation is always worth the time when it concerns your health.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC. MICROBIOLOGY

UNIT – V – FOOD & DIARY MICROBIOLOGY – SMB2202

Food-Borne Infections and Intoxications:

Listeria monocytogenes

Campylobacter jejuni

Clostridium botulinum

Clostridium perfringes

Bacillus cereus

Background and History -

Great moments in food spoilage and food poisoning

Preservation by drying, adding salt, development of acid fermented foods, coating foods with honey, clay, olive oil.

Development of dietary laws -- India 1000 BC -- unclean foods included meat cut by sword, dog meat, human meat, meat of carnivorous animals, locusts, camels and hairless or excessively hairy animals. Rice which had turned sour, dishes which had been sniffed by a dog or a cat.

Jews and Muslims -- many dietary laws carrying the weight of religious sanctions but which are really laws of simple hygiene.

About 900 AD, Emperor Leo VI of Byzantium -- edict forbidding the making and eating of blood sausage prepared in pig stomachs and smoked --- threat of losing all property and exile. Chief magistrate of the city of the offense would be fined 10 pounds of gold --- equivalent to about $35,000 in todays value.

Spoiled grains recognized from at least Roman time.....over 40,000 deaths due to ergot poisoning (St. Anthonyâs fire) alone in France in 943 AD.

Canning discovered in 1805 by Francois (Nicholas) Appert induced by a 12,000 franc reward for the discovery of a practical method of food preservation --- preserved meats in glass jars kept in boiling water for varying periods of time.

50 years later Louis Pasteur demonstrated the role of microorganisms in the spoilage of beer and wine. Who also demonstrated that the souring of milk was due to microorganisms. This of course led to his use of heat to destroy these spoilage organisms --- the discovery of pasteurization.

1. Listeria monocytogenes

Major public health concern because:

- Severe, non-enteric nature of the disease:
- Meningitis
- Septicemia
- Abortion
- High case fatality rate can be as high as 20-30%
- Long incubation time
- 10 hours to 20-30 days after ingestion

Risk groups

- Pregnant women and neonates
- Elderly
- Immunocompromised or debilitated people:
- Malignancy, antineoplastic treatment, immunosuppressed, chronic liver disease, collagen diseases (lupus), diabetes, AIDS

Properties of the Organism:

- Habitat and sources -- widely distributed!
  - Can survive and grow in water and soil
  - Silage and decaying vegetation have an important role in transmission to animals
  - Sewage sludge is notorious in contaminating vegetables. Can persist in sewage treated soil for months and probably years.
  - Plant material and raw food of animal origin are carried into food processing plants by workers. Organisms can then colonize any moist environmental sites and will grow at low temperatures.
  - Commonly contaminated foods:
    - 2-5% of raw milk samples have been found to be contaminated -- probably from feeds, environment and carrier cows.
    - Cheeses, especially soft cheeses made with raw milk. Growth occurs in spite of low pH associated with this food.
    - Meats -- just about every type, from raw to cured. Hot dogs and sausages are frequent culprits.
    - Vegetables -- widely distributed. Prepared vegetables such as cole slaw are a hazard. Tomatoes and carrots on the other hand are not implicated.
    - Fish-- the organism is often found here too. Smoked fish especially salmon has been implicated -- prevalence here can be as high as 25%

        - Bottom line ý Many foods have been implicated but foods marketed as **refrigerated and ready to eat** are the ones that have been associated with most of the outbreaks.
    - Human Carriage
        - A healthy carrier state exists. Approximately 2-6% of healthy humans excrete the organism in their feces. Oropharyngeal and vaginal carriage is associated with disease states.

- Growth and Laboratory Characteristics
- Facultative anaerobe which grows best aerobically or microaerophilically. The organism is related to Staph/Strep/Clostridium/Bacillus
- The organism is b -hemolytic, it is typically first isolated on blood agar where it can be confused with streptococci.
- It is catalase + which helps to distinguish it from the streptococci also.
- It is a gram positive rod, sometimes growing in short chains -- again allowing confusion with strep.
- It is psychotropic, it can grow well at refrigerator temperatures. This property is sometimes used as an enrichment procedure for these organisms.
- The organism is motile and capable of invading a number of different host cell types.

The Disease Entity:

Minimum infective dose is unclear:

contaminated foods responsible for epidemic and sporadic cases indicate levels of at least **100 cfu per gram of food.** But this is controversial some investigators say 10,000 to 100,000.

Entry and crossing of Host tissues

1. Crossing the intestinal barrier

the organism has to withstand stomach environment

there is some evidence that antacids and H2 blockers increase the risk for Listeriosis

organisms then enter the lumen of the small intestine

if the infectious dose was high, might experience gastroenteritis with febrile symptoms. (There is a question if organism actually multiplies in the lumen).

There are no gross lesions associated with the enteritis.

Translocation/Invasion of intestinal mucosa -- occurs quite rapidly -- in rats (ileal loop model) deep tissue invasion occurs within minutes-- which also implies that there is no multiplication necessary for this process.

Invade the apical epithelial cells of the villi and also the M-cells

And the organisms are quickly found within the macrophages in the stroma and in the peyer's patches.

2. Multiplication in the liver

Organisms are carried by lymph and blood to lymph nodes, spleen and liver. Some estimate that 90% end up in the liver.
This begins to happen within minutes of intestinal traversion.

Many localize to Kupffer cells and most organisms appear to die within these cells. (Induction of antigen specific T-cells and cell mediated immunity.) In most people, this is probably where the infection stops.

If some organisms survive this compartment, they begin to multiply within the Kupffer cells over the next 2-5 days.

During the course of the evolving infection, the organisms spread to hepatocytes -- which becomes the principle site of bacterial multiplication. Organisms spread cell-to-cell and never come in contact with humoral immunity.

Polys accumulate at distinct microabcesses around foci of infected hepatocytes over the next several days. These are gradually replaced by mononuclear cells and lymphs to form granulomas. Infection may clear at this point due to IFN-g activated macrophages and CD-8 antigen specific lymphs.

If infection not controlled here, will next get release of bacteria into circulation to get septecemia, multiple organ infections, with particular tropism for gravid uterus and CNS.

3. Colonization of gravid uterus and fetus

This has been reproduced in a variety of experimental animals including sheep, cattle, guinea pigs, rats and mice -- hematogenous penetration of the placental barrier:

Inflammatory infiltration of placental villi with microabcess formation and some necrosis. Eventual translocation across the endothelial barrier in the trophoblast and invasion of the fetal bloodstream.

Mother may be assymptomatic or exhibit flu-like symptoms. Mother is not at particular risk for systemic infection.

Get generalized infection, fetal death or premature birth of severely infected neonate.

Humans and other animals seem to be most susceptible late in pregnancy and it appears to be related to late pregnancy high physiological levels of estrogens -- these have been shown to depress aspects of cell mediated immunity.

4. Invasion of the brain

Organism has a well described predilection for the neural tissue. Seen most clearly in ruminants where disease presents as encephalitis (in humans its primarily a meningitis). In ruminants, often see focal infections in the pons, cerebellum, medulla oblongata and spinal cord.

How the organisms get into the brain is controversial. Some say its hematogenous - since the organisms are known to be able to invade endothelial cells and could hence be able to invade through the brain microvasculature. Other investigators have shown that the organisms can invade neurons and that intra-axonal transport is possible. Ruminants may get infected through the trigeminal nerve after infection of the oral mucosa.

Intracellular Infectious Cycle and Virulence Factors:

In addition to professional phagocytes such as macrophages, these organisms can invade a number of cell types:

Epithelial cells

Fibroblasts

Hepatocytes

Endothelial cells

Neurons and possibly other neural cells

Internalization:

Organism adheres to surface of target cells and sinks into the target cell through a zipper-like mechanism. Doesn't involve the spectacular ruffling of Salmonella or any of the other membrane structures seen with other bacteria ie., pedestals in E. coli.

A variety of target cell receptors seem to be able to play a role including complement receptors, fibronectin and integrin, and E-cadherin

The most important Listeria adhesins appear to be Internalin A and Internalin B. -- 800 aa membrane anchored, cell surface proteins which play role in the internalization process.

Internalin A binds to E-cadherin (a calcium dependent intercellular adhesion glycoprotein whose cytoplasmic domain can trigger actin cytoskeleton rearrangements via a - and b -catenins)

Internalin B has recently been shown to have affinity for two eucaryotic surface proteins (1) Met - a receptor tyrosine kinase which normally functions as the receptor for hepatocyte growth factor. Binding to Met signal actin rearrangements through phosphatidylinositol-3 (PI3); and (2) globular C1-q receptor -- though this doesn't appear to have a signal domain so its significance is ??

p60 is a 460 aa, 60 kDa extracellular protein found associated with the cell wall and also in culture supernatants. It maybe a murein hydrolase important for proper cell division. The protein does bind to intestinal epithelial cells and mutants defective in the protein production are not invasive

Other adhesins: Ami, Lap, fibronectin binding protein (24.6 kDa).

Vacuole formation, proliferation and spread:

Listeria becomes engulfed into a phagosome and the vacuole becomes acidified soon after uptake and there is evidence that the phagosome is prevented from fusing with lysosomes.

Within thirty minutes of entry, the phagosomal membranes begin to lyse, a step essential to bacterial survival (and virulence) within the cell and a step mediated by hemolysin in combination with phospholipases:

The Listeria hemolysin Hly (also known as listeriolysin or LLO) is a streptolysin O related, cholesterol dependent, pore forming cytolysin. Mutants without Hly are avirulent · again underscoring its essential nature to the intracellular survival of this organism.

Active only at low pH. Has a narrow range: pH 4.5 - 6.5. This apparently insures that the toxin will lyse the phagosome but will not affect cell membranes once the bacterium is free in the cytoplasm. This hemolysin is noted for not being particularly cytotoxic.

Listeria phospholipases: PlcA and PlcB (phospholipase C A and B):These are responsible for the lecithinase activity also correlated with virulence. These also have a role in escape from the phagosome and also in escape from the double membrane vacuole which forms when cells spread cell-to-cell. Also there is evidence that these subvert host signalling pathways mediated by phospholipid hydrolysis products such as diacylglyceride, ceramide and inositol phosphates -- lipid metabolites that play key roles in important cell processes such as growth, apoptosis and the synthesis of cytokines and chemokines.

Once in the cytoplasm the bacteria multiply with a doubling time of ca. 1 hour.

Intracytoplasmic bacteria are immediately surrounded by a cloud of fine, fuzzy, fibrillar material composed of actin filaments which rearranges into a tail of up to 40 um at one pole of the bacterium. -

The polar assembly of the actin tail propels the bacterium in a random fashion through the cell.

Actin assembly is mediated by one protein, ActA, a 610 aa surface protein - distributed assymetrically on the bacterial surface.

Motile force is believed to be due to continuous deposition of actin monomers at the bacterial cell surface -- between the bacterial surface and the growing actin meshwork immobilized in the cytosol. The tangled actin meshwork, containing many actin cytoskeleton binding, crosslinking and regulatory proteins -- such as a -actinin, tropomyosin, talin, vinculin, fimbrin, filamin, villin, gelsolin, ezrin/radixin, cofilin, frofilin, coronin, Rac, CapZ, Arp3, and VASP.

Some bacteria eventually reach the periphery, contact the cell's membrane, and push it out. This leads to finger-like protrusions which penetrate neighboring cells and become phagocytosed by them. Resulting in a secondary phagosome with a double membrane. Bacteria escape from these quickly presumably using hemolysin and phospholipases.

2. Campylobacter jejuni

Campylobacter jejuni is the leading cause of gastroenteritis in the US and probably world-wide. .

- 46% of laboratory confirmed cases of gastroenteritis are attributable to this organism
- estimated over 2 million people per year in the US (approximately 1% of the population is affected each year, estimated incidence of about 1000/100,000)
    - Actual reported US incidence is approximately 41/100,000
- Usually mild and self limiting, however a serious consequence can result in the form of Guillaine-Barre syndrome
- Hawai'i has the highest incidence in the country about 900 reported cases a year with an incidence of 75/100,000, but the thinking is that infections are grossly underreported.
- Incidence in developing countries can be much higher, ie., Mexico and Thailand probably run about 40,000/100,000 per year.

Family Campylobacteriaceae includes 20 Campylobacter species and 4 Arcobacters -- (Arcobacters grow at 15^{o} C and Campylobacters do not).

Properties of the organism

- Curved s-shaped or spiral gram (-) rods, motile with a single polar flagellum at one or both ends.
- Old cultures form spherical, coccoid bodies -- this phenotype is associated with the "viable but not culturable" state.

- Has respiratory metabolism, not fermentative, and it is microaerophilic. Have rather rigorous atmospheric requirements:
    - Grow with 10% CO_2 / 5% O_2. Some species / strains require additional H_2 in the atmosphere
    - C. jejuni will grow at 42° C and this is used as a selection criterion.
- The organism is unusually thin (0.2 - 0.9 m), thus some labs use a membrane filtration technique to isolate the organism.
- Environmental sensitivity:
    - Won't grow below 30° C
    - However, can survive 3-5 weeks in 4° C water and feces. Survival in refrigerated foods is probably similar.
    - Nothing peculiar about thermal sensitivity -- killed by ordinary cooking temperatures.
    - Killed by pH < 2.3

Reservoirs and epidemiology

- Many animals carry the organism -- including domestic farm animals
- Human cases are associated with:
    - Poultry - especially eating chicken out
    - Pets - especially young puppies
    - Water supply
    - Raw milk
- Most cases occur in the summer months -- late spring to early autumn -- this is also true in Hawai'i.
- Slightly higher incidence in males
    - If one looks at incidence as a function of age, one sees two peaks of infection:
        - Very early childhood between 1 to 4 months
        - Young adults -- some people talk about the "second weaning"

Pathogenesis and Disease Characteristics

- Infectious dose is very low -- 500 organisms
- Two distinct disease entities:
- **diarrhea** - copious watery discharge - this is noninflammatory and is commonly associated with Cj infections in developing countries, or,
- **dysentery** - inflammatory colitis with fever, tenesmus, malaise, myalgia, headache, severe abdominal cramps, and frequent, small volume rectal discharges of mucus, inflammatory cells and necrotic tissue containing streaks of blood.
    - Possibility of bacteremia -- 1.5/1000
- Most alarming sequel is Guillaine-Barre syndrome at 0.5 - 1/1000 cases of symptomatic Cj infection.

Virulence factors

- Intestinal biopsies of patients, experimentally infected model animals as well as tissue culture studies have shown that Cj can invade intestinal epithelial cells.
    - Intestinal mucin is chemotactic for Cj. Organisms have been shown to move towards it and then bury themselves into it.
    - Flagella protein is essential both for motility and apparently for binding to epithelial surface.
    - Cj secretes a number of novel proteins upon cultivation with enterocytes. One of these CiaB share amino acid similarity with type III secreted proteins involved in cell invasion in other bacterial species.
    - A plasmid, pVir, present only in some strains of Cj appears to be important for invasion.
    - Invasion is sensitive to microtubule inhibiting agents (like colchicine) but not to actin depolymerizing agents (like cytochalasian D). This is opposite to what is seen with Shigella and Listeria.
    - Immunofluoresence and EM studies show a reorganization of microtubules around the area of attached Cj. The microtubules aggregate into finger-like protrusions with Cj at the tips.
    - Cj cells are then endocytosed, possibly through a coated-pit mechanism since inhibitors of coated pit maturation interfere with internalization.
    - Cj apparently stays within the vacuole and gets exocytosed on the basolateral side of the cell.
- There are reports of a adenyl cyclase activating cholera toxin-like enterotoxin (may play a role in the diarrheal manifestation) and a cytotoxin capable of causing hemorrhage in rat ileal loop experiments, The significance of these is still under investigation.

Autoimmune sequellae

- Evidence has accumulated associating Cj with acute demylinating neuropathy or Guillaine-Barre syndrome (GBS) -- A rapidly progressive form of polyneuropathy characterized by muscular weakness and mild distal sensory loss.
    - Up to 40% of patients with GBS have culture or serologic evidence of Cj infection when neurologic symptoms begin and it has been estimated that between 0.5 - 1 to 1000 Cj infections may be complicated by Guillaine-Barre.
    - Penner serotypes O:1, O:2, O:8, O:17, O:19, and O:41 are disproportionately represented inGBS cases
        - (Two serotyping schemes exist: Penner - based on soluble heat stable antigens; Lior - based on heat labile antigens

      LPS core oligosaccharides from Cj serotypes associated with GBS have been shown to be both structurally related and serologically cross-reactive with human gangliosides associated with motor neurons.

3. Clostridium botulinum

Disease botulism named in 1793 after 13 people in Wildbad, Germany ate a large sausage and became ill --> 6 died. Botulus is sausage in Latin.

Organism isolated by Van Ermagen in 1896 after an outbreak of disease in members of a music club who had eaten salted ham --> 23 got sick and 3 died.

Gram positive rod

anaerobic

spore former

seven types based on serologic specificity of neurotoxin

named A through G

A, B, E and sometimes F --> causes of human botulism

C and D ---> animal botulism, contaminated feed.

G ---> no clear association with disease

The species is also divided into 4 groups :

I = all A's and the proteolytic B and F's - these are all proteolytic

II = all E's and non-proteolytic B and F's - these are all non-proteolytic

III = C and D - don't cause human botulism but will in animals

IV = G (which is also called C. argentinense) - rarely form spores

refrigeration -- although growth can occur at low temp, toxin production is reduced

thermal inactivation (group I is most heat resistant)

pH -- acid foods donât allow growth

salt concentration -- affects water activity (a_w)

(I) tolerates up to 10% salt = 0.94 a_w

(II) tolerates up to 5% = 0.97 a_w

These organisms need an anaerobic environment to grow but reducing substances in food will allow growth in the presence of O_2.

Reservoirs

Widely distributed in nature and in food

A and B producing strains often found on fruits and vegetables and honey.....Honey is the only food ever associated with infant botulism even though other foods fed to infants may have spores. Note that spore level in honey can be very high.

Food Outbreaks

Northern climes --- fish and seafood associated....especially in traditional native dishes and fermented fish products (muktuk -- fermented blubber, skin and meat of beluga whale), fermented salmon eggs. These are usually "E". These foods are high protein low carbohydrate.

Home preserved vegetables --- "A and B"

Home cured meats --- "B"

Commercial products --- garlic in oil (now has to be acidified as an additional safety feature.) Roasted eggplant in oil responsible for several outbreaks.

Disease Characteristics:

Symptoms hit 12-36 hours after ingestion (sometimes sooner, sometimes weeks later!)

nausea and vomiting (B and E)

visual impairment: blurred, ptosis, dilated pupils

loss of mouth and throat function (A and B)

dry mouth, throat, tongue, sore throat

fatigue and loss of coordination

respiratory impairment

abdominal pain and either diarrhea or constipation

(in general , cranial nerve first affected and then descend. With GBS its just the opposite --> extremities first and then ascend.)

Death would be due to respiratory failure....10% fatality rate

Can be confused with CO poisoning and GBS

Infant Botulism:

(may occur in adults after antibiotic and/ chemotherapy)

constipation --- days to week after onset

generalized weakness and weak cry

poor feeding and sucking reflex

lack of facial expression

floppiness

respiratory arrest may occur although death is rare.

Infectious Dose

No tolerance for the neurotoxin in food

0.1 ng/kg is lethal for mouse

active log "A" strains can produce 10^6 mouse lethal doses per ml.

Virulence Factors

Neurotoxin (BoTox)

water soluble

produced as a single polypeptide --- 150,000 MW (progenitor)

cleaved by a protease to form two polypeptides which then become S-S bonded : 100,000 and 50,000 MW

There are differences in serotypes:

A=dimer, trimer, and can be larger

E= monomer and dimer

B= dimer

A,B,E, F are chromosomally encoded

C, D are phage encoded

G is plasmid encoded

These exist in association with a number of nontoxic proteins (hemagglutinin) which may protect the toxin from low stomach pH an proteases.

Toxin action:

All serotypes block the exocytic release of acetylcholine from synaptic vessicles at perpheral motor nerve terminals.

H-chain binds to motor neurons, probably through glycoprotein receptor.

Receptor mediated endocytosis internalizes the toxin.

H-chain forms channel in endosome and L-chain dissociates and passes into cytoplasm

L-chain acts as zinc-dependent endopeptidase which reacts with components of the synaptic vessicle docking and fusion complex:

synaptobrevin (VAMP, vessicle associated membrane protein)

SNAP-25 (synaptosomal associated protein of 25 kDa)

syntaxin (HPC-1)

4. Bacillus cereus

Causes two types of foodborne illness:

Diarrheal disease - food infection mediated by the production of enterotoxin within the small intestine (first recognized in an hospital outbreak caused by contaminated vanilla sauce in Oslo Norway.)

Emetic disease - food intoxication caused by toxin released into food.

Properties of the organism:
Gram positive large (width > 1 um) rod,

spore former -- central spore or paracentral

grows aerobically and anaerobically

beta hemolytic

usually motile

may be present in stools of healthy individuals

grown out of food samples after heat shock --> treat sample at 70^{o} C for 10 minutes; or after ethanol shock --> mix 1:1 with absolute ethanol for 1 hour

Widely disseminated in nature ---> soil and growing plants ---> spread to food is easy.

easy to cross contaminate meats

Rice, spices and dairy products are widely contaminated with this organism....

it doesn't compete well with other organisms...forms spores and so heat treatment, as is normal with cooking rice or pasteurizing milk, kills off competing flora allowing B. cereus to flourish when temperature returns to ambient.

Disease entities:

Two types of food borne illness:

Emetic --- emetic toxin, food intoxication

Characterization of toxin was elusive until realized that it could induce vacuolization in HEp-2 cells.

Turns out that it is a circular peptide, 1.2 kDa, called cereulide --- closely related to the potassium ionophore valinomycin.

(Like valinomycin, cereulide can disrupt mitochondrial function and at least one group has proposed using boar spermatozoa as a bioassay for the toxin --- the toxin uncouples ox-phos in the sperm stopping motility.) -- the mitochondrial toxicity may be related to reported liver toxicity of this toxin.)

Stimulates the vagus nerve leading to the emetic response.

Diarrheal --- enterotoxin, food infection

Norwegian guy, Hauge, 1950, studied the hospital outbreak cited above. Isolated the organism and drank 200 ml of a broth culture at 4 x 10^{6}, experienced symptoms of diarrhea and cramps at 13 hours and these lasted 8 hours.

At least three enterotoxins have been described, one of them has hemolytic activity the others do not.

Staphylococcal (Staph) Food Poisoning

What is Staph food poisoning?
Staph food poisoning is a gastrointestinal illness caused by eating foods contaminated with toxins produced by the bacterium *Staphylococcus aureus* (Staph) bacteria.

About 25% of people and animals have Staph on their skin and in their nose. It usually does not cause illness in healthy people, but Staph has the ability to make toxins that can cause food poisoning.

How do people get Staph food poisoning?

People who carry Staph can contaminate food if they don't wash their hands before touching it. If food is contaminated with Staph, the bacteria can multiply in the food and produce toxins that can make people ill. Staph bacteria are killed by cooking, but the toxins are not destroyed and will still be able to cause illness.

Foods that are not cooked after handling, such as sliced meats, puddings, pastries, and sandwiches, are especially risky if contaminated with Staph.

Food contaminated with Staph toxin may not smell bad or look spoiled.

What are the symptoms of Staph food poisoning?

- Staph food poisoning is characterized by a sudden start of nausea, vomiting, and stomach cramps. Most people also have diarrhea.
- Symptoms usually develop within 30 minutes to 8 hours after eating or drinking an item containing Staph toxin, and last no longer than 1 day. Severe illness is rare.
- The illness cannot be passed from one person to another.

How do I know if I have Staph food poisoning?
You can suspect Staph food poisoning based on the type of symptoms and their fast resolution. Although laboratory tests can detect toxin-producing Staph in stool, vomit, and foods, these tests are usually not ordered except during an outbreak. If you think you might have Staph food poisoning and are experiencing severe symptoms, contact your health care provider.

How is Staph food poisoning treated?
The most important treatment is drinking plenty of fluids. Your healthcare provider may give you medicine to decrease vomiting and nausea. People with severe illness may require intravenous fluids.

Antibiotics are not useful in treating this illness because the toxin is not affected by antibiotics.

How can I prevent Staph food poisoning?
The best way to avoid food poisoning by Staph is to prevent food from being held at an unsafe temperature (between 40°F and 140°F) for more than 2 hours.

Bacteria can multiply rapidly if left at room temperature or in the "Danger Zone" between 40°F and 140°F. Never leave perishable food out for more than 2 hours (or 1 hour if it's hotter than 90° F outside).
Remember to always follow these food safety tips:

- Use a food thermometer and cook foods to their safe minimum internal temperature Keep hot foods hot (140°F or hotter) and cold foods cold (40°F or colder).
- Store cooked food in wide, shallow containers and refrigerate within 2 hours (or 1 hour if it's hotter than 90° F outside).

The following tips that are part of the four steps to food safety – clean, separate, cook, and chill – also can help protect you and your loved ones from food poisoning:

- Wash your hands for 20 seconds with soap and water before, during, and after preparing food, and before eating.
- Do not preparing food if you are ill with diarrhea or vomiting.
- Wear gloves while preparing food if you have wounds or infections on your hands or wrists.

Mycotoxins:

- Mycotoxins are toxic chemical produced by some pathogenic strain of fungi.
- Many strains of fungi contaminate the food and produce potent mycotoxin in food.
- Disease resulting from ingestion of mycotoxin in food is called mycotoxicosis. E.g. mushroom poisoning.
- One important characteristic of most mycotoxin is that they are heat stable.
- Therefore, if mycotoxin is produced in food, it is not damaged easily during normal cooking of food.
- Most of the mycotoxin cause mutation and are associated with various types of cancer.
- Mycotoxins are secondary metabolites of fungi.
- Metabolites produced by microorganisms are divided into two types i.e. primary and secondary metabolites.
- Metabolites produced by certain microorganisms that serve as a growth factor for other microorganisms are called as primary metabolites. E.g. vitamins, amino-acids, carbohydrate etc.
- Primary metabolites are produced during log phase.
- Metabolites produced by certain microorganisms which are not needed for growth if other organisms are called secondary metabolites. E.g. toxin, antibiotic etc.
- Secondary metabolites are produced during stationary phage of growth.

Types of mycotoxin:

1. Aflatoxin:

- It is produced by *Aspergillus flavus, A. parasiticus* and some *Penicillium* spp.
- It is located from variety of mold contaminated food like peanuts, rice etc.
- Two major types of toxin include B_1 and G_1 that show blue and green fluorescence when exposed to UV lights.
- Other types of aflatoxin include B_2, G_2, M_1, M_2, and P_1 which are derivatives of B_1 and G_1.
- Among them B_1 is most toxic.
- After ingestion, aflatoxin binds to chromosomal DNA and causes point mutation and frame-shift mutation.
- By inducing mutation, they cause cancer of liver.
- Aflatoxin is toxic to wide variety of animals like cat, chicken, cattle, human beings etc.

2. Patulin:

- It is produced by wide variety of mold including *Penicillium expansum, P. patulum, P. melini, P. equinum, Aspergillus clavatus, A. terreus* etc.
- It is isolated from many molds contaminated food like bread, sausages, fruits etc.
- It is white crystalline solid.
- It is sensitive to SO_2 and alkali but resistant to acid.
- Patulin binds to NH_2 and -SH functional group of biomolecules and causes chromosomal aberration.

- It affects wide variety of animals and plants as well as bacterial cells like *E. coli* and *Staphylococcus aureus.*
- Therefore, originally it was classified as antibiotics.
- Tissue damage caused by patulin include oedema of brain, hemorrhage of lungs, damage of blood capillary, spleen and kidney.

3. Penicillic acid:

- It is produced by *Penicillium roqueforti, P. cyclopium, P. morteneii, Aspergillus flavus, A. ochraceus.*
- It is isolated from many molds contaminated food like tobacco.
- Penicillic acid binds to –SH and $–NH_2$ group of biomolecules forming covalent bond.
- It is carcinogenic and affects wide variety of animals including rat.

4. Citrinin:

- It is produced by *Penicillium citrinum, P. viridicatum* and other species.
- It is isolated from many mold contaminated foods like- polished rice, bread, meat and meat products
- It is carcinogenic to wide variety of animals.

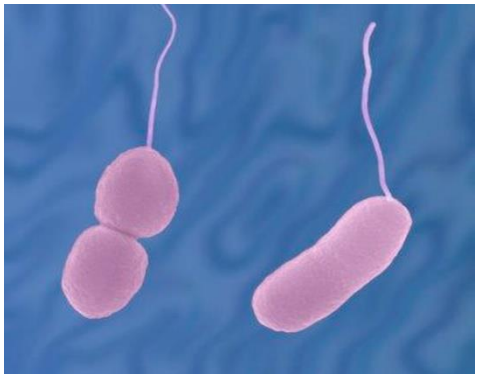
5. Ochratoxin:

- There are atleast seven types of structurally related ochratoxin, of which type A is the most common and most toxic.
- It is produced by many *Aspergillus* and *Penicillium* species like- *A. ochraceus, A. alliaceous, A. mellis, P. viridicatum, P. cycloplum.*
- Like other mycotoxin, it is heat stable and is not damaged during cooking of food.
- When ochratoxin is ingested it induces mitosis and cause cancer of kidney.

6. Sterigmatocystin:

- It is produced by *Aspergillus versicolor, A. nidulus, A. regulosus* and other.
- It causes cancer of liver by inhibiting DNA synthesis.

WHAT IS VIBRIO PARAHAEMOLYTICUS?



Vibrio parahemolyticus is a gram negative enteric bacterium, from the same family that causes cholera found abundantly along the coastal waters all over the world.

WHAT DISEASES ARE CAUSED BY VIBRIO PARAHAEMOLYTICUS?

Vibrio parahaemolyticus is an intestinal infection that is characterized by lower gastrointestinal distress such as diarrhea and cramps. In some cases, nausea, vomiting, fever and headache may also be present.

Occasionally, this disease may manifest itself as a dysentery-like illness with bloody or mucoid stools, high fever and a high white blood cell count, but normally the disease has a duration of only two to three days. Illness with Vibrio parahaemolyticus is most common during the summer months.

WHAT FOOD PRODUCTS ARE COMMONLY ASSOCIATED WITH VIBRIO PARAHAEMOLYTICUS FOOD POISONING?

This disease is most often associated with eating raw or inadequately cooked seafood or any food contaminated by handling raw seafood or contaminated water. This disease is primarily associated with the consumption of raw oysters.

WHAT OTHER VIBRIOS CAN CAUSE DISEASE?

There are several other bacterium from the vibrio family that may cause diarrheal disease including: V. chloerae (of serogroups other than 01), V. fluvialis, V. furnissii and V. hollisae. Sepiticemic disease associated with wound type infections have been associated with V. hollisae. V. alginolyticus and V. damsela.

INCUBATION PERIOD

The incubation period for Vibrio parahaemolyticus is usually between 12 - 24hours, but can range from 4-96 hours with a mean of 15 hours.

EPIDEMIOLOGY OF VIBRIO PARAHAEMOLYTICUS

This organism is not communicable from person to person. Cases of Vibrio parahaemolyticus usually occur during the summer months, due to the fact that the organism can be found floating free in coastal waters and in fish and shellfish. During cooler months, the organism is commonly found in silt or mud on the bottom of marine environments.

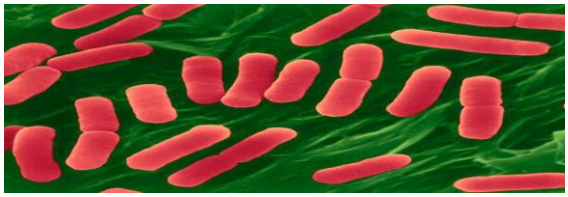
DIAGNOSIS

Vibrio parahaemolyticus is diagnosed by isolating the Kanagawa Vibrio, which is halophilic and is one of the characteristics that produce the hemolytic reaction known as the "Kanagawa phenomenon". There are twelve separate "O" antigen groups and approximately sixty different "K" antigen types that have been identified with this organism. Diagnosis can be determined by the presence of the Kanagawa vibrios in the patient's stool culture or in implicated food.

TREATMENT

This disease is self limiting and best treated with plenty of water replenishment. Antibiotics are usually not necessary but in very sever cases tetracycline, ampicillin or ciprofloxacin could be used.

WHAT IS ESCHERICHIA COLI O157:H7 - E. COLI O157:H7



Escherichia coli (E. coli) is a gram negative bacterium that is commonly present in the intestines of humans and animals.

WHAT MAKES E. COLI O157:H7 SO DANGEROUS?

Most strains of E. coli are harmless, but O157:H7 is a key exception because this strain causes severe diarrhea leading to renal damage and other serious complications including death.

E. coli O157: H7 also has the ability to cause disease at a very low dose, survive at low temperatures and under acidic conditions.

WHO IS MORE SUSCEPTIBLE TO INFECTION FROM E. COLI O157:H7?

People of all age groups are susceptible to these bacteria; however immunocompromised, elderly and young children are at a higher risk.

WHAT DISEASES ARE CAUSED BY E. COLI O157:H7?

Infection with Escherichia coli O157:H7 can range from being asymptomatic to having mild to severe gastrointestinal symptoms. The most common symptoms are abdominal cramping and diarrhea, which or may not be bloody. In an uncomplicated case, the illness should recover in less than 5-10 days.

Complications: Hemolytic uremic syndrome (HUS) is one of the complications following E. coli O157:H7 infection especially in children below the age of 5. Hemolytic uremic syndrome is characterized by acute renal failure, microangiopathic hemolytic anemia, fever, and thrombocytopenia. Indeed, HUS is one of the most common causes of acute renal failure in children. One-third of children diagnosed with HUS do not recover completely, resulting in persistent renal failure and the need for long-term dialysis.

EPIDEMIOLOGY OF E. COLI O157:H7

The disease etiology is universal and can be foodborne or environmental amongst others. E. coli O157:H7 can be transmitted by:

a. Eating uncooked/ undercooked ground beef
b. Consumption of contaminated sprouts, lettuce, salami, unpasteurized milk
c. Swimming in or drinking sewage contaminated water
d. Fecal-oral transmission through an infected person to a healthy individual due unhygienic practices.

INCUBATION PERIOD

The incubation period is usually 2-10 days with a median of 3-4 days.

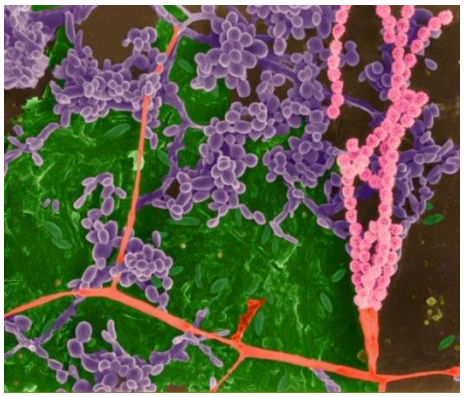
DIAGNOSIS

An infection with E. coli O157: H7 can be diagnosed by isolating the bacteria from stool samples of infected patients.

TREATMENT

Many of the patients recover without any antibiotic treatment within 5-10 days. However when the disease does progress to a life threatening complication such as hemolytic uremic syndrome, the patient must be hospitalized and treated supportively in an intensive care unit. Blood transfusions and renal dialysis is often required for such patients.

WHAT IS SALMONELLA?



Salmonella is a genus consisting of many species of gram negative bacteria, most of which are motile, and are present in animal reservoirs and in the environment.

WHAT DISEASES ARE CAUSED BY SALMONELLA?

Members of the Salmonella genus cause a variety of diseases such as enteric fever, gastroenteritis, and septicemia. Reactive arthritis involving swelling, pain and inflammation of the joints, is a complication following salmonella enteritidis. Salmonella has also been implicated in cases of osteomyelitis in children with co-existant sickle cell anemia.

WHAT SPECIES OF SALMONELLA ARE KNOWN TO CAUSE GASTROINTESTINAL ILLNESS?

The top 4 Salmonella isolates that cause gastrointestinal illness are Salmonella typhimurium, Salmonella enteritidis, Salmonella Heidelberg and Salmonella newport. Other prominent members of the salmonella species that are implicated in gastrointestinal illness are Salmonella javiana, Salmonella poona and Salmonella montevedio.

WHAT FOOD PRODUCTS ARE COMMONLY ASSOCIATED WITH SALMONELLA FOOD POISONING?

Raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressing, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa, and chocolate (from the bad bug book).

WHO IS MORE SUSCEPTIBLE TO INFECTION FROM SALMONELLA?

People of all age groups are susceptible to these bacteria; however immunocompromised, elderly and young children are at a higher risk. Patient's who are HIV positive and who have fully developed AIDS suffer from Salmonella infections more frequently. Incubation period The Incubation period for food borne salmonellosis is 12-72 hours.

EPIDEMIOLOGY OF SALMONELLA

Salmonella is transmitted to humans via the feco-oral route. An infected individual sheds the bacteria in his feces, and the bacterium is viable for months in the environment in water, soil, and manure.

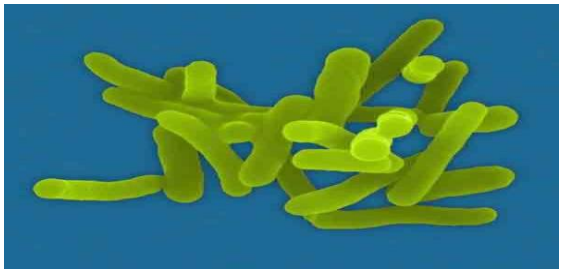
DIAGNOSIS

Salmonellosis can be diagnosed by isolating and culturing the bacteria from the stool or blood of the infected person.

TREATMENT

Most people often recover from a bout of salmonellosis without a course of antibiotic treatment. Treatment for such individuals is only supportive, with intravenous or oral fluids, adequate nutrition and rest. Often times when the illness does get complicated a course of broad spectrum antibiotics might be necessary.

WHAT IS SHIGELLA?



Shigella is a genus of Gram-negative, non motile, rod shaped bacterium that are commonly implicated in food borne illness and diarrheal diseases. The Shigella genus consists of 4 species or serogroups: S. sonnei, S. boydii, S. flexneri and S. dysenteriae

WHAT IS SHIGELLOSIS?

Shigellosis is an infectious disease caused by Shigella species of bacteria. The disease is characterized by diarrhea, fever and abdominal cramps. Typically the stools may contain blood and mucus, and is called Shigella induced dysentery.

WHO IS MORE SUSCEPTIBLE TO INFECTION FROM SHIGELLA SPECIES?

Susceptibility is general, but in endemic areas, infants, young children and the elderly, are more susceptible. Debilitated, immunocompromised and malnourished adults are also at a higher risk.

EPIDEMIOLOGY OF SHIGELLA SPECIES

The only significant reservoir of these bacteria is humans. Fecal-oral route is the most common mode of transmission of the disease. Infection may also occur after ingestion of contaminated food and water. Food handlers who demonstrate poor personal hygiene are often responsible for outbreaks. Outbreaks are also common in conditions of over crowding and poor sanitation such as prisons, refugee camps, as well as, third world nations with poor sewage disposal.

INCUBATION PERIOD

Usually 1-3 days, but can range from 12 to 96 hours. Incubation period for S. dysenteriae could be up to 1 week.

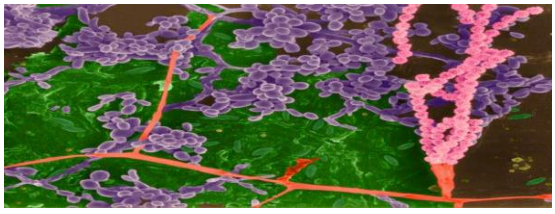
DIAGNOSIS

Shigella can be identified in the stool of the infected person. A culture and sensitivity pattern is important to establish the species of Shigella and the right antibiotic treatment.

TREATMENT OF SHIGELLA

Shigellosis is treated with antibiotics and supportive care. Patients with mild infections can recover with only supportive care. Ampicillin, trimethoprim/ sulfamethoxazole or ciprofloxacin are usually the antibiotics of choice. If the patient is severely dehydrated, he needs to be rehydrated by intravenous fluids.

WHAT IS YERSINIA ENTEROCOLITICA?



Yersinia is a gram negative bacterium that causes an acute bacterial enteric disease characterized by a febrile diarrhea, enterocolitis and acute mesenteric lymphadenitis that mimic's appendicitis.

WHAT DISEASES ARE CAUSED BY THE YERSINIA SPECIES?

Yersinia pseudotuberculosis causes a zoonotic disease of wild and domesticated birds and mammals. Human beings are considered to be incidental hosts and may transmit the disease. Human cases of the disease have been due to contact with household pets, particularly sick puppies and kittens. This bacterium is of concern since it does have the ability to multiply rapidly under refrigeration and low oxygen conditions. Yersinia pestis has been implicated as the causative agent in plague.

HOW DOES THE ENTERITIS CAUSED BY YERSINIA ENTEROCOLITICA MANIFEST?

Yersinia causes an acute bacterial disease that causes diarrhea and/ or vomiting. Fever and abdominal pain are also classic symptoms and the disease may mimic appendicitis. In up to ¼ of the cases, bloody diarrhea is reported. The bacteria may also cause infections of other sites such as wounds, joints and the urinary tract.

INCUBATION PERIOD

The most common incubation period is between 3 to 7 days and generally under 10 days.

EPIDEMIOLOGY OF YERSINIA

Yersinia enterocolitica is transmitted via the fecal-oral route and is most often transmitted by eating or drinking food or water that has been contaminated by contact with infected animals or people. This organism is most commonly found in raw pork or pork products such as chitterlings (pig intestines), but there have also been outbreaks associated with tofu and pasteurized milk that was cross contaminated. Nosocomial transmission has been reported and there have also been reports of transmission by transfusion of stored blood from asymptomatic donors.

DIAGNOSIS

Yersiniosis is usually diagnosed by a stool culture; however, yersinia can also be cultured in vomitus or in standard blood media.

TREATMENT

This organism is resistant to penicillin and its derivatives. The best course of treatment seem to be from the tetracyclines, however, the newer quinolones such as ciprofloxacin may also be effective.

Hazard Analysis and Critical Control Point (HACCP)

- HACCP stands for Hazard Analysis and Critical Control Point.
- Originally developed in the 1960's by NASA and a group of food safety specialists.
- According to US Food and Drug Administration, "HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product".
- HACCP is designed for use in all segments of the food industry from growing, harvesting, processing, manufacturing, distributing, and merchandising to preparing food for consumption.
- It is the means of securing food safety from harvesting to consumption.
- Tool to identify the hazards and applying the major for the food safety.
- HACCP can be applied in every step of food processing.

Principles of HACCP:

Principle 1: Conduct a hazard analysis.
Principle 2: Determine the critical control points (CCPs).
Principle 3: Establish critical limits.
Principle 4: Establish monitoring procedures.
Principle 5: Establish corrective actions.
Principle 6: Establish verification procedures.
Principle 7: Establish record-keeping and documentation procedures.

1. Conduct a hazard analysis:

- Hazard analysis is the very first step.
- All the potential hazards are identified in this step
- The hazard is defined as the any physical, chemical and biological agent that possess the possible risk or threat to the health.
- The basic physical hazards are metal contamination, presence of inedible items. Chemical hazards include the presence of toxins or any unwanted chemicals and biological hazard denote the presence of pathogens.
- Any method that can control these hazards needs to be adopted.

2. Determine the critical control points (CCPs):

- CCP stands for critical control point.
- At this step the control measures can be applied.
- Determination of CCP refers to identifying the point at which the control measures can be applied to eliminate the hazard that has been previously identified.
- CCP is essential to prevent or eliminate hazard or to reduce it to an acceptable level.
- Examples of CCPs may include: Cooking, chilling, metal detection, setting into suitable temperature.

3. Establish critical limits:

- Once the CCP is identified, critical limit points needs to be assigned for each CCP.
- There can be one or more critical limits for each CCP
- Critical limits are defined as the maximum and/or minimum value of CCP such that the hazards are controlled and food safety is assured.
- Critical limits must be defined on the scientific grounds and basis
- Critical limits are usually based on factors such as: temperature, time, physical dimensions, humidity, moisture level, water activity (a_w), pH, regulatory levels, etc.

4. Establish monitoring procedures

- Generally, refers to the planning and carrying out monitoring for CCP
- Monitoring should be done on regular basis
- Monitoring technique may differ based on the CCP
- Monitoring is necessary to identify the deviation and apply the effective measures
- Monitoring is also necessary for future purpose and verification as monitoring process is documented.

5. Establish corrective actions

- Corrective action is taken when the critical limit is not met
- Corrective actions are pre-decided for each CCP
- These actions make sure that no any harmful product reaches the market for consumption

6. Establish verification procedures

- HACCP plan must be validated.
- For testing the validity of the plan several steps can be taken such as checking out the random samples, reviewing the process, confirming that the CCP are under control.

- Verification activities can be carried out by the external hired officers or the internal members.
- No matter who performs the verification, it should be unbiased and fairly carried out.

7. Establish record-keeping and documentation procedures

- Record keeping is must in HACCP
- Records maintained should have the records or information regarding HACCP plan, CCP, critical limits, monitoring, corrective action, all the procedures including the verification procedures.
- Recording keeping is necessary of validation and proper application of HACCP.

Benefits of HACCP:

- Ensures the consumer regarding the safety of the product
- Prioritizes food safety and works to eliminate any kind of hazard
- Necessary for the consistent quality products
- Provides the framework to produce foods safely and to prove they were produced safely.
- Prevents from the possible health outcomes that could have occurred due to mishandling during food production steps
- HACCP is also necessary for obtaining validation.

Index and Indicator Microorganisms

A compilation describing coliforms, Enterobacteriaceae, and E. coli and their role as indicator organisms of possible pathogen contamination.

Three groups of microorganisms are commonly tested for and used as ***indicators*** of overall food quality and the hygienic conditions present during food processing, and, to a lesser extent, as a marker or ***index*** of the potential presence of pathogens (i.e. food safety): coliforms, *Escherichia coli (E. coli*; also a coliform) and *Enterobacteriaceae.*

Index Microorganisms

Microbiological criteria for food safety which defines an appropriately selected microorganism as an index microorganisms suggest the possibility of a microbial hazard without actually testing for specific pathogens. Index organisms signal the increased likelihood of a pathogen originating from the same source as the index organism and thus serve a predictive function. Higher levels of index organisms may (in certain circumstances), correlate with a greater probability of an enteric pathogen(s) being present. The absence of the index organism does not always mean that the food is free from enteric pathogens.

Indicator Microorganisms

The presence of indicator microorganisms in foods can be used to: assess the adequacy of a heating process designed to inactivate vegetative bacteria, therefore indicating process failure or success; assess the hygienic status of the production environment and processing conditions; assess the risk of post-processing contamination; assess the overall quality of the food product.

A number of factors must be considered before testing for a particular indicator organism or group of organisms: the physio-chemical nature of the food; the native microflora of the food (fresh fruit and vegetables often carry high levels of *Enterobacteriaceae* and/or coliforms as part

of their normal flora); the extent to which the food has been processed; the effect that processing would be expected to have on the indicator organism(s); the physiology of the indicator organism(s); the physiology of the indicator organism(s) chosen.

Enterobacteriaceae

The taxonomically defined family, *Enterobacteriaceae,* includes those facultatively anaerobic gram-negative straight bacilli which ferment glucose to acid, are oxidase-negative, usually catalase-positive, usually nitrate-reducing, and motile by peritrichous flagella or nonmotile.

The *Enterobacteriaceae* group does include many coliforms, with the addition of other microorganisms which ferment glucose instead of lactose (i.e. *Salmonella*). Common foodborne genera of the Family *Enterobacteriaceae* include *Citrobacter, Enterobacter, Erwinia, Escherichia, Hafnia, Klebsiella, Proteus, Providencia, Salmonella, Serratia, Shigella,* and *Yersinia.* Psychrotrophic strains of *Enterobacter, Hafnia,* and *Serratia* may grow at temperatures as low as 0C.

If the meat ecosystem favors their growth, genera in the family *Enterobacteriaceae* may be important in muscle food spoilage. Conditions allowing growth of *Enterobacteriaceae* include limited oxygen and low temperature. Members of this family produce ammonia and volatile sulfides, including hydrogen sulfide and malodorous amines, from amino acid metabolism.

The *Enterobacteriaceae* have been used for years in Europe as indicators of food quality and indices of food safety. The use of coliforms as indicators of food quality or insanitation in food processing environments is based upon tradition in the United States. This practice arbitrarily bases judgment on food quality or manufacturing plant insanitation upon recovery of those

members of the *Enterobacteriaceae* group (i.e. coliforms) which ferment lactose, thus ignoring the presence of non-lactose fermenting members.

E. coli

A gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some, such as serotype O157:H7 can cause serious food poisoning in humans. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K, and by preventing the establishment of pathogenic bacteria within the intestine. *E. coli* are easily destroyed be heat, and cell numbers may decline during freezing and frozen storage of foods.

E. coli is the only member of the coliform group that unquestionably is an inhabitant of the intestinal tract and it has become the definitive organism for the demonstration of fecal pollution of water and food not undergoing any processing which would kill the organism.

In cases where it is desirable to determine whether fecal contamination may have occurred, at present, *E. coli* is the most widely used indicator of such, the presence of which implies a risk that other enteric pathogens may be present in the food. In many raw foods of animal origin, small number of *E. coli* can be expected because of the close association of these foods with the animal environment and the likelihood of contamination of carcasses from fecal material, hides, or feathers during slaughter-dressing procedures. The failure to detect *E. coli* in a food, however, does not assure the absence of enteric pathogens.

However, criteria involving *E. coli* are generally not useful for detecting likely fecal contamination for foods that have been processed sufficiently to destroy this bacterium.

E. coli are not always confined to the intestine; they have the ability to survive in the food processing plant environment and re-contaminate processed foods (*E. coli* found in environmental swabs is a good indication of fecal contamination). Hence, the presence of *E. coli* in a heat-processed food does not necessarily indicate fecal contamination, but indicates either process failure or, more commonly, post-processing contamination from equipment or employees or from contact with contaminated raw foods.

Dairy microbiologists use *E .coli* as a true indicator organism to assess post-pasteurization contamination in milk. The presence of *E. coli* in pasteurized milk may indicate inadequate pasteurization, poor hygienic conditions in the processing plant, and/or post-processing contamination because proper pasteurization inactivates levels of *E. coli* anticipated in raw milk.

Coliform

The coliform group is defined on the basis of biochemical reactions, not genetic relationships, and thus the term "coliform" has no taxonomic validity. Coliforms are aerobic and facultatively anaerobic, gram negative, non-sporeforming rods that ferment lactose, forming acid and gas within 48 hours at 35C.

In the case of refrigerated ready-to-eat products, coliforms are recommended as indicators of process integrity with regard to reintroduction of pathogens from environmental sources and maintenance of adequate refrigeration. The source of coliforms in these types of products after thermal processing is usually the processing environment, resulting from inadequate sanitation procedures and/or temperature control.

Coliforms are ubiquitous in nature, therefore a number of factors should be considered when testing for a particular indicator organism such as the native microflora of the food, the extent to

which the food has been processed, and the effect that processing would be expected to have on the indicator organisms.