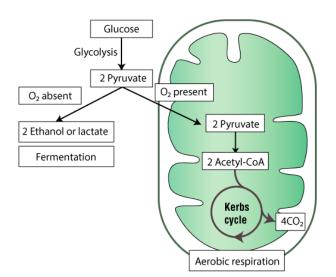
Fermentation

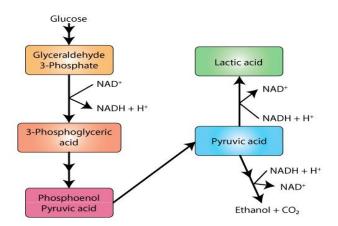
Fermentation is an enzyme catalysed, metabolic process whereby organisms convert starch or sugar to alcohol or an acid anaerobically releasing energy.



The science of fermentation is called "zymology".

Process of Fermentation

Fermentation is an anaerobic biochemical process. In fermentation, the first process is the same as cellular respiration, which is the formation of pyruvic acid by glycolysis where net 2 ATP molecules are synthesised. In the next step, <u>pyruvate</u> is reduced to lactic acid, ethanol or other products. Here NAD+ is formed which is re-utilized back in the glycolysis process



Types of Fermentation

- Homo fermentation: only one type of product formation
- Hetero fermentation: more than one product formed

On the basis of the end product formed, fermentation can be categorized as follows:

1. Lactic Acid Fermentation

Lactic acid is formed from pyruvate produced in <u>glycolysis</u>. NAD+ is generated from NADH. Enzyme lactate dehydrogenase catalyses this reaction. Lactobacillus bacteria prepare curd from milk via this type of fermentation. During intense exercise when oxygen supply is inadequate, muscles derive energy by producing lactic acid, which gets accumulated in the cells causing fatigue.

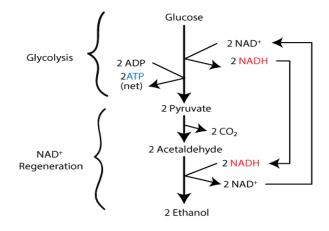
$$C_{6}H_{12}O_{6}(Glucose) \xrightarrow{Lactobacillus} 2CH_{3}CHOHCOOH(LacticAcid)$$

$$Glycolysis \begin{pmatrix} Glucose \\ 2 \text{ ADP} \\ 2 \text{ ADP} \\ (net) \\ 2 \text{ Pyruvate} \\ 2 \text{ NAD}^{+} \\ (net) \\ 2 \text{ NAD}^{+} \\ (net) \\ 2 \text{ Lactate} \\ \end{pmatrix}$$

2. Alcohol Fermentation

This is used in the industrial production of wine, beer, biofuel, etc. The end product is alcohol and CO_2 . Pyruvic acid breaks down into acetaldehyde and CO_2 is released. In the next step, ethanol is formed from acetaldehyde. NAD+ is also formed from NADH, utilized in glycolysis. Yeast and some bacteria carry out this type of fermentation. Enzyme pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions.

$$C_{6}H_{12}O_{6}(Glucose) \xrightarrow{Dehydrogenase}{Decarboxylase} 2CH_{3}CHO(Acetaldehyde) + 2CO_{2} \rightarrow 2C_{2}H_{5}OH(Ethanol)$$



3. Acetic acid Fermentation

Vinegar is produced by this process. This is a two-step process.

The first step is the formation of ethyl alcohol from sugar anaerobically using yeast.

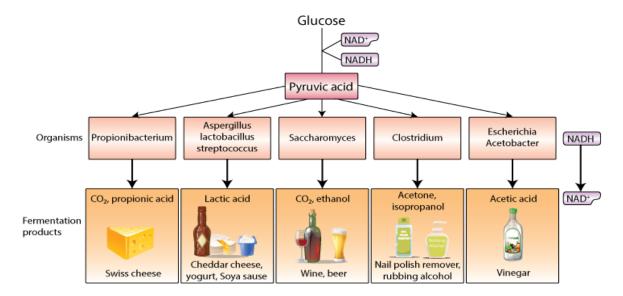
In the second step, ethyl alcohol is further oxidized to form acetic acid using acetobacter bacteria. Microbial oxidation of alcohol to acid is an aerobic process.

 $C_2H_5OH(ethanol) + O_2 \xrightarrow{acetobacter} CH_3COOH(acetic acid) + H_2O$

4. Butyric acid Fermentation

This type of fermentation is characteristic of obligate anaerobic bacteria of genus clostridium. This occurs in retting of jute fibre, rancid butter, tobacco processing and tanning of leather. Butyric acid is produced in the human colon as a product of dietary fibre fermentation. It is an important source of energy for colorectal epithelium. Sugar is first oxidized to pyruvate by the process of glycolysis and then pyruvate is further oxidized to form acetyl-CoA by the oxidoreductase enzyme system with the production of H_2 and CO_2 . Acetyl-CoA is further reduced to form butyric acid. This type of fermentation leads to a relatively higher yield of energy. 3 molecules of ATP are formed.

$$C_6H_{12}O_2 \xrightarrow[butyricum]{clostridium} C_4H_8O_2 + 2CO_2 + 2H_2$$



Advantages of Fermentation:

Fermentation is suitable for all kinds of environments. It is one of the oldest metabolic processes which is common to prokaryotes and eukaryotes. Fermentation is widely used in various industries.

Using suitable microorganisms and specified conditions different kinds of fermentation products are formed namely:-

- Wine
- Beer
- Biofuels
- Yoghurt
- Pickles
- Bread
- Sour foods containing lactic acid
- Certain antibiotics and vitamins

Fermentation can make food nutritious, digestible and flavoured. There are many benefits of consuming fermented food.

- It improves digestion and helps to maintain intestinal bacteria
- It has an anti-cancer effect.
- Improves immune system
- Reduces lactose intolerance

Other than the food industry, there are many other areas where the fermentation process is used. Methane is produced by fermentation in sewage treatment plants and freshwater sediments

Fermentation is a metabolic process that involves the conversion of organic compounds, typically sugars or carbohydrates, into various products, such as alcohol, acids, or gases, by microorganisms like yeast or bacteria. It has been used for thousands of years in various applications, including food and beverage production, pharmaceuticals, and biofuel production.

Advantages of Fermentation:

- 1. **Preservation of Food**: Fermentation can extend the shelf life of perishable foods by creating an acidic or alcoholic environment that inhibits the growth of spoilage microorganisms. Examples include sauerkraut, kimchi, and yogurt.
- 2. Enhanced Nutritional Value: Fermentation can increase the nutritional value of certain foods. For instance, fermented dairy products like yogurt and kefir are rich in probiotics, which promote gut health.
- 3. **Flavor Development**: Fermentation can lead to the development of complex and unique flavors in food and beverages. Examples include the distinct flavors of beer, wine, and cheese.
- 4. **Natural Preservation**: Unlike some chemical preservatives, fermentation relies on natural microorganisms and does not typically involve the use of synthetic additives, making it a more natural preservation method.
- 5. **Production of Biofuels**: Fermentation is used to produce biofuels such as ethanol and biogas from renewable sources like corn, sugarcane, or organic waste. These biofuels are considered more environmentally friendly than fossil fuels.
- 6. **Medicinal Products**: Fermentation is used in the production of antibiotics (e.g., penicillin), vaccines, and various pharmaceuticals, making it a crucial process in the healthcare industry.

7. **Reduced Waste**: Fermentation can be used to convert organic waste materials into useful products, reducing environmental waste.

Disadvantages of Fermentation:

- 1. **Time-Consuming**: Fermentation processes can be slow and may require a longer time compared to other food processing methods, which can affect production efficiency.
- 2. **Inconsistent Results**: Fermentation outcomes can vary due to factors such as temperature, pH, and the specific microorganisms used. This can lead to inconsistent product quality.
- 3. **Contamination Ris**k: Fermentation processes are vulnerable to contamination by unwanted microorganisms, which can spoil the product or lead to safety issues.
- 4. **Limited Control**: The control over fermentation processes can be challenging, especially in large-scale production. Factors like temperature, humidity, and microbial activity can be difficult to manage.
- 5. **Space and Equipment** Requirements: Fermentation facilities may require a significant amount of space and specialized equipment, which can be expensive to set up and maintain.
- 6. **Energy Intensive**: Some fermentation processes, particularly those used in biofuel production, can be energy-intensive, requiring heating, cooling, and agitation, which can contribute to operational costs.
- 7. **Byproduct Management**: Fermentation byproducts, such as waste materials and carbon dioxide, need to be managed properly to minimize environmental impacts.

GENERAL METHODS OF FERMENTATION

The purpose and importance of food fermentation in our life including food preservation, textural modification and nutritional improvement, has already been highlighted earlier. In recent years, the fermentation industry stands next to information technology (IT) and software industries. It is therefore, pertinent to know general method's used in fermentation for the production of fuels, food and pharmaceutical products and life saving drugs.

There are two distinct types of fermentation which are commonly used for the above-referred purpose. These are:

- a) Aerobic fermentation
- b) Anaerobic fermentation

Aerobic Fermentation

These are carried out under the aerobic conditions in the presence of oxygen which is required for the growth and product formation by the microorganisms. Majoirty of the fermentations such as antibiotics, single cell protein, enzymes and amino acids come under this category. As listed above, the key of these fermentations is the microorganism/s and raw materials (ingredients) and cultural (pH, temperature, humidity and water content) and nutritional characteristics influence the product formation significantly. In many of the fruit and vegetable and agricultural commodities fermentations, the microorganisms grow at very low moisture level whereas in others contrary to this, it is carried out at very high levels of moisture in the range of 85-90% with adequate supply of oxygen. The former is termed as the solid-state fermentation (SSF) and the latter is called as the submerged culture fermentation.

Solid-State Fermentation (SSF)

SSF refers to the growth of microorganisms on solid materials without the presence of free liquid. It is considered to be economical since low moisture content is used and also does not require expensive equipments e.g, sophisticated fermentors. It is extensively practiced in the oriental food fermentations (miso, tempeh, soysausce, natto), secondary metabolites, enzymes, organic acids and composting etc. Although solid-state fermentation is a simpler and less expensive process of growing microorganisms, recovery of the final product adds finally to the cost during the down stream processing step. However it is widely followed for food and feed enrichment, food and feed enzymes production and composting and waste minimization.

Submerged Culture Fermentation

This method of fermentation has been practised for several years for the production of life saving drugs, enzymes, amino acids and organic acids by bacteria, yeasts, fungi and actinomycetes etc. Oxygen is supplied by either shaking or bubbling air through the liquid medium. Agitation, aeration and temperature affect the fermentation. Batch culture or continuous culture methods are followed for the production of fermented products - commercially.

Anaerobic Fermentation

These are carried out by strict anaerobes or facultative anaerobes such as bacteria and yeasts in the absence of oxygen. Examples of anaerobic fermentation are:

Ethanol (ethylalcohol) : Saccharomyces ellipsoideus Saccharomyces cerevisiae

Brewing (beer) : Saccharomyces carlsbergensis Saccharomyces cerevisiae

Lactic Acid Food preservation and fruit and vegetable fermentation : Pediococcus spp., Lactococcus spp. Leuconostoc mesenteroides

Acetone and butanol : Clostridium acetobutylicum Clostridium Saccharoaceto butylicum

Biogas (Methane carbon dioxide and other gases) : Strict anaerobic fermentation

TYPES OF FERMENTATION

Fermentations have been classified on the basis of relationship of the formation of product, substrate utilization or the free amount of water as given below:

Solid state fermentation:

Fermentation processes which take place in the absence or near absence of free water in the substrate are termed as solid state fermentation (SSF). It is imperative, however, that the substrate contain enough moisture absorbed in the substrate particles within the substrate. SSF have been used mostly for food fermentation and production of a few enzymes.

Extractive fermentation: There are several industrially important products being catalysed by enzymes which are susceptible to end product or feedback inhibition. Hence, the increased concentration of the product inhibits the enzymes involved in its own synthesis so that the overall rate of conversion of substrate to the desired product is lowered. When the end product or anyone ofthe by-product of fermentation interacts with the enzyme, the synthesis of the final product proceeds sub-optimally and in extreme case may stop altogether. This problem has largely been overcome by using a technique called extractive fermentation. In it there is fast removal of product,

or by-product of a metabolic pathway, so that their subsequent interference with the cellular or medium component is not possible. Hence, it involves all the actions taken for the separation of a product from its producing cell. Separation of the product can be achieved either inside the reactor (internal) or outside the reactor (external).

Submerged fermentation:

Fermentation processes which take place in the presence of free water in the substrate are termed as sub-merged fermentation. Such fermentations have been used mostly to the produce fermented food and beverages.

CLASSIFICATION OF FERMENTED FOODS

It is important to know about different varieties of fermented foods prepared and consumed all over the world depending upon the agricultural and food raw materials produced in that region. These fermented foods can be classified into the following categories:

- i) Fruit and Vegetable products
- ii) ii) Beverages (alcoholic and non-alcoholic)
- iii) iii) Cereal products
- iv) iv) Milk Products
- v) v) Fish Products
- vi) vi) Products from Legumes
- vii) vii) Meat products
- viii) viii)Starch Crop products

Fruits and Vegetable Products

You are now fully convinced that the fermentation of food commodities was practiced by the early man and by trial and error many technologies were developed. Vegetables have been preserved throughout the world by fermentation. Examples are cabbage (Sauerkraut, Korean Kimchi), radish, mustard leaf, gherkins and cucumbers, ginger onion, chilli and bambooshoots (Malaysian pickles), carrot, turnips and peppers. In India, relatively very few vegetables are fermented and preserved for consumption. Among fruits, olives are commercially fermented and consumed in European countries as an appetizer.

ii) Beverages (alcoholic and non-alcoholic)

Beverages are produced in large quantities in all regions of the world. We normally find two types of beverages which are common everywhere. The first group comprises of alcoholic beverages in which fermentation plays a major role in contributing the flavour and chemical and physical characteristics of the fermented products.Beer and wine fall under this category. After fermentation further distillation is done and thus a variety of products termed 'spirits' such as whisky, gin, brandy, rum etc., are produced.

The second category of beverages are non-alcoholic e.g., coffee, cocoa and tea. All of them involve fermentation. India is a major producer of tea and coffee. If you look at the worlds map, you find an interesting observation that these are based on the type of agricultural crops cultivated in that particular area depending on the geographical and

climatic conditions. The colder countries of Europe including Britain, Scandinavia, Netherlands and Poland consume beer which is manufactured mostly from barley. The southern countries of Europe grow grapes extensively and produce different varieties of wine. These beverages have spread to many countries wherever the European settled e.g. Northern America (The United States of America, Canada), Australia and South Africa. Rice beer in Indian subcontinent, sorghum beer in Africa, sake (rice) in Japan and a variety of alcoholic beverages are produced in different parts of the world. In Europe and North America apples are used for cider production. In warmer climate in Africa, Asia, Oceania (Australia and New Zealand), the Indian subcontinent and South America the sweet liquid sap of palm trees is fermented to wine. In India, it is known as 'Toddy'. Cashew apple pulp is extensively is used for the preparation of Feni in Goa.

The alcohol content of most of the fermented materials varies between 5- 18%. Its concentration is increased in the range of 35-55% by distillation and thus brandy, whisky rum and gin are manufactured. Consumption of excessive amounts of alcoholic beverages leads to intoxification and loss of body control. Therefore it is prohibited in many countries and also many religions of the world.

In contrast to alcoholic beverages, the non-alcoholic beverages most widely consumed throughout the world are coffee, tea and cocoa and these are largely produced in India especially in southern part of the country. The tea leaves are fermented as such by the natural microorganisms. In the case of coffee and cocoa the pulp surrounding the beans are removed by the natural fermentation. This process contributes to the flavour of the final product. Bacteria, yeasts and moulds are involved in the fermentation of these commodities.

iii) Cereal Products

Cereals are the major staple food in every parts of the world. These are wheat, rice and maize. The most popular fermented cereal product is bread which is consumed in every region. It is done by fermenting wheat flour dough with the yeast Saccharomyces cerevisiae. Lao-Chao is a fermented rice product of China prepared by natural fermentation containing strains of Rhizopus oryzae, Rhizopus chinensis and Endomycopsis species etc., Puto of Philippines, Ang-Kak of China, Ragi of Indonesia, Tape-Ketan of Indonesia, Ogi of Nigeria, Injera of Ethopia and Banku of Ghana are produced extensively and consumed regularly in these countries. In India, mixed fermented preparations of rice and pulses and other commodities are idli, dhokla, khaman, papad and jalebies etc., Ambali, bhatura, kulcha and warri are also Indian fermented foods prepared and used in different parts of the country.

iv) Milk Products

Milk products have been included in our diets since time immemorial. In early days, natural fermentation of milk was the best method of preserving milk. Dahi and Chhanchh (butter milk) are important ingredients of every day's meal all over the country. The other products are youghurt, cultured milk acidophilus milk, cheese, Srikhand, Kefir and Kumiss etc.,

Fermented milk products have therapeutic properties along with their nutritional characteristics, wholesomeness and good flavour and digestibility. These qualities are

introduced in the product by a number of lactic acid producing bacteria e.g. Lactococcus lactis subsp. lactis, Lactococcus lactis sub-sp. cremoris (cultured butter milk, sourcream, cottage cheese, other soft and hard cheeses), Lactococcus lactis sub-sp. diacetylactis (sour cream, butter, cheese, butter milk), Streptococcus thermophilus (yoghurt, Lactobacillus delbrueckii sub-sp. bulgaricus (yoghurt, kefir, kumiss, bulgarian butter milk), Lactobacillus acidophilus (Acidophilus butter milk).

Realising the health giving properties of fermented milk products, different varieties of preparations are being marketed all over the world. These are acid alcohol fermented milk products, high acid fermented products, medium acid fermented products, low acid fermented products and whey based beverages.

v) Fish Products

The fermented fish products are popular in some countries. Philippine fish sauce and Vietnamese Nuoc-mam are prepared by fermenting sardines, shrimps and small sea fish etc., Malaysian budu is consumed as a condiment on rice and as a flavouring ingredient in various dishes. Baloa baloa is a fermented rice shrimp mixture of Phillipine and consumed by most of the people.

vi) Legumes Products

Pulses constitute an important component of diet after cereals in Asian countries, especially in India. In India we consume tur or arhar, black gram, green gram, Bengal gram, lentils and a range of beans. Soybeans is also gaining popularity in India. Legumes are rich in proteins, and also in oil and carbohydrates. Unlike cereals, their digestibility is poor and therefore, they are fermented to different products in oriental countries e.g., China, Japan, Indonesia, Malysia, Thailand and several African Countries. Majority of these pulses and beans contain oligosaccharides such as stachyose and verbascose which cause flatus in the intestine. Trypsin inhibitors are also present in these agricultural commodities. Fermentation stimulates these undesirable constituents. Thus the fermented products. Tempeh, sufu, soybean milk, soy sauce, natto, bangkrek, khaman, warries and mixed fermented foods containing cereals and pulses e.g., idli, dosai, dhokla etc., are consumed in different regions of the world.

vii) Meat Products

There are not many fermented meat products. These products are dry and semi-dry sausages. In the United States of America, the commercial sausages are Genoa and Salami. Among the popular European brands (dry) are summer sausage, cervelet, thurunger, and Teewurst. Semi-drysausages are turkey sausage, fermented frankfurter and Frischwurst etc., In Europe and Western World, fermented sausages are preferred whereas in India, their consumption is almost negligible. The lactic acid producing bacteria e.g., Lactobacillus plantarum, L. sake, L. curvatus, Pediococcus acidilactici, P. pentosaceus, Lactococcus lactis are mainly responsible for fermentation of meat. The safety and shelf life of the products are important because these products are contaminated easily and frequently by pathogenic microorganisms.

viii) Starch Crop Products

Products Cassava (tapioca) is a major food crop cultivated in several African Countries. It is a staple food to most of the people. It contains cyanogenic glucosides and therefore it must be processed. During fermentation, the cyanide content is reduced completely. Gari, lafun, fufu, peujeum, poi and tape are some of the products. Maize, Sorghum and millets are used for the preparation of fermented products like ogi, uji, koko fube and chika etc.

PRE-REQUISITES FOR INDUSTRIAL FERMENTATIONS

After knowing these preliminary information about the fermentations, it is necessary to know what are the important points to be considered before starting laboratory or industrial scale fermentation.

a) Microorganism/s is the key for any fermentation whereas fermentor is the heart of the process. The choice of a good medium/substrate/raw-material is virtually as important as selecting a right type of strain or microorganism for the success of fermentation. The medium serves the following purposes:

- i) It supplies nutrients for growth.
- ii) ii) It supplies nutrients for energy. iii) It supplies nutrients for building of cell substance.
- iii) It is required for the production of final product. Nutrients needed for the growth and product formation are:
- iv) Carbon compounds derived mostly from starch, sugar and molasses. Nitrogeneous compounds.
- v) Inorganic salt
- vi) Water
- vii) Vitamins.
- viii) Growth factors.
- ix) Precursors of fermentation products.
- x) Dissolved oxygen and other gases. ix) Buffers.
- xi) Antifoam substances.
- xii) Lysate of dead cells.

Production of wine

The term 'wine' is applied to a beverage made by alcoholic fermentation of grape or grape juice and final production is obtained without distillation. But now-a-days, any fleshy fruit or flower in the new world may be employed for this purpose. Wine was suggested to have been made during the Neolithic period in the near East. These are the part of food of man ever since his settlement in Tigoris Euphorates basins and have also been used as a therapeutic agent.

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- Since 15th century, wine making and consumption has been flourishing and now the modern science and technology has improved the wine production industrially and available worldwide.
- It is believed that the initiation of wine was from the Northern Zagros mountains of Iran at around 4000 B.C. Although a fix date and time of era earlier than this has yet been found.
- Wine is a product of a fruits such as berries, apples, grapes, cherries, palm and rice which is fermented partially or fully depending upon the alcohol content.
- Mostly grapes of *Vitaceae* family species are used in the winemaking: *Vitis labrusca* and *Vitis vinifera* because it contains all the necessary ingredients from pulp, juice and seeds that contains essential acids, sugars, minerals, tannins and vitamines.

Viticulture is a branch of horticulture that cultivates and harvests wine grapes while enologists study the wine and winemaking process and the science of breeding and fermentation

Types of Wines

Still wines:

These wines retain none of the carbon dioxide produced during the fermentation.

Sparkling wines: These are the wines which have considerable amount of carbon dioxide. Champagne in France is the sparkling wine made in Champagne region.

Dry wines: These wines contain little or no unfermented sugar.

Sweet wines: Wines having either unfermented sugar or with added sugar later on are called sweet wines. Both types of wines generally contain 11 to 14% of alcohol.

Fortified wines: Wines to which distillate of wine called "Brandy" is added and may contain 15 to 21% of alcohol.

Table wines: It is a wine having comparatively low alcohol content (7 to 11%) and little or no sugar.

Sherry: It is produced by special processing technique from wine, containing 18 to 21% alcohol and could be sweet or dry.

Cider: Cider is a low alcoholic beverage obtained from apple by fermentation.

Perry: It is a wine made from pear juice.

Mead: This type of wine was prepared by the Indians from honey.

Vermouth: Wine flavoured with a characteristic mixture of herbs and spices, some of which impart an aromatic flavour and odour while others a bitter flavour. It can be sweet or dry with alcohol content of 15 to 21%.

Toddy: Sweet alcoholic drink, having alcohol content of 4-6%, is made by the fermentation of sap from coconut palm.

Pulque: National drink of Mexico, contains 6-7% alcohol and B-vitamins.

Method of Table Wine Preparation

Grape is the most widely used fruit to make wine but it can be prepared from any fruit having fermentable sugars, optimum acidity, nitrogenous compounds or other growth factors to make wine of acceptable quality. The major difference is in the extraction of sugar from the pulp of some fruits. From grape, red and white wines are produced the world over using black/red coloured and white varieties, respectively.

Preparation of must: For wine preparation, the first step is the preparation of must which is prepared depending upon the type of fruits used and the type of wine to be made. Must is a juice or pulp corrected for sugar, acid/pH, nitrogen source or other requirements for the alcoholic fermentation. To prepare the must, the fruits are trimmed and washed and the must Juice is extracted or fruit is made into pulp. In the preparation of white wine only the free run juice is used while in the red wine, the skin and seeds along with pulp/juice are fermented together for some time to get attractive coloured wine. Proper dilution of fruit pulp is required as fruits like plum and apricot are highly acidic and effect the fermentability besides making the wine unpalatable. The sugar content of the juice or pulp is checked with an instrument called refractometer and is expressed as degree Brix. Sulphur dioxide (S02) is added to the must to control the wild microflora and to allow the yeast to act efficiently to conduct the alcoholic fermentation. Amelioration (or correction) of must for better fermentability with ammonium salt and vitamins like thiamine, biotin is necessary in some fruits.

Preparation of active yeast culture:

An active culture of wine yeast (Saccharomyces cerevisiae var ellipsoideus) is prepared from the stock culture in the juice to be used for wine making.

Fermentation:

After must preparation, activated yeast starter culture is added to the must and fermentation is carried out at a temperature of 20-250 C, till the sugar content or the o Brix stabilizes.

Siphoning/racking

Siphoning or racking is a simple but important process wherein the wine is transferred through a clean pipe into another container, kept at a lower height than the vessel with wine. It is done after completion of fermentation. Two or three rackings are usually done at an interval of 15-20 days to separate the yeast and other settled materials at the bottoms of container.

Maturation

As the newly made wine is harsh and has yeasty flavour maturation (from 6 months upto a year) is allowed to make the wine mellow (It is the term used to signify the sensory quality of wine having smoothness i.e. is devoid of any harsh taste)in taste and fruity in flavour.

Clarification:

Clarification of wine is done by using filter aids such as bentonite, celite and tannin/gelatin using a machine called filter press.

Blending:

Blending is also practiced in some cases to make wine sweet or better flavoured before pasteurization.

Pasteurization:

Wine is generally pasteurized at a temperature of 620 C for 15- 20 min, after bottling.

Storage:

Low temperature storage is preferred for good quality wine.

Beer

Beer is an alcoholic beverage primarily prepared from barley besides other cereals in limited quantities and is consumed in large quantities throughout the world. Beer and ale the principal malt beverages made with hops, yeast, water and malt adjuncts. Adjuncts are the malted cereals other than barley, used in minor quantities. Brewing was one of the earliest processes undertaken on a commercial scale and became one of the first process that has developed from an art into a technology. Beer can be differentiated from ale as in beer bottom fermenting yeast is employed while in ale the top fermenting yeast is employed. In the preparation of ale, more hops is used. It is usually pale yellow in colour, tart in taste and have more alcohol content. On the basis of alcohol content beers can be classified as light beer having 3-5% v/v and hard beer having 5-8% alcohol content.

Beer production is divided into four distinct process as described here.

Malting:

It is obtained by soaking followed by germination of barley or other cereals and drying of the germinated cereal. Then, most of sprouts or germs are removed and the malt remains. The malt is crushed before its use in beer making.

Mashing:

It is the process in which extraction of the ground malted barley with water is made. The mashing is done so as to make soluble as much as possible of the valuable constituents of the malt and malt adjuncts. It causes hydrolysis of starches, other polysaccharides and proteins. The insoluble material is then filtered. The liquid so obtained is called wort.

Wort boiling:

Boiling of wort with hops (Hops is the female flowers of hops plant used in beer production to give flavour and bitter taste) is carried out to concentate the wort, inactivate the enzymes, extract soluble substances from the hops, coagulate and precipitate the proteins and other substances, caramelize sugar slightly and to contribute antiseptic substances (Chiefly the alpha resins humulone, co-humulone and adhumulone) to the wort and beer.

Fermentation:

A special beer, bottom fermenting yeast strain Saccharomyces cerevisiae var carlbergensis, is used for the inoculation or pitching of the cooled wort. The wort temperature during the fermentation varies in different breweries but is usually in the range from 3.3 to 140 C. The fermentation is usually completed within 8 to 14 days. During fermentation as the carbon

dioxide is evolved in increasing amounts, the foaming increases; later it decreases to none when the fermentation is finished. At the later stage, the bottom yeast flocculates and settles down.

Aging or Maturation:

The young, green or draft beer is stored or lagered in vats at about Oo C for several weeks to several months, during which period precipitation of proteins, settling of yeast, resin and other undesirable substances takes place and the beer becomes clear and mellowed or matured.

Finishing:

After aging, the lager beer is carbonated to a CO2 content of about 0.45 to 0.52 per cent, mostly by means of gas collected during the fermentation or by addition of CO2 from cylinders. Then, beer is cooled, clarified or filtered and packaged in the bottles, cans or barrels.

Vinegar

The word vinegar is derived from two French words, vin and aigre meaning sour wine but the term is used to denote a condiment prepared from various sugar and starch containing materials by alcoholic and subsequent, acetic acid fermentation. It is one of the several fermented foods prepared and consumed by early man, even today. Earlier, it was used as a beverage, a condiment, a preservative, a household cleansing and medicinal agent. Vinegar mainly consists of a dilute solution of acetic acid in water, also contains colour, 63 Industrially Important Yeast, Mold and Bacteria flavour and extracted substances besides fruit acids, esters and inorganic salts which vary according to its origin. The minimum legal strength for vinegar is 4% acetic acid (w/v).

Types and Composition of Vinegar

1. Synthetic vinegar: This type of vinegar is directly prepared from synthetic acetic acid with the addition of water and finally, it is coloured by caramel.

2. Brewed vinegar: Virtually, anything having enough sugar to produce alcohol can be used to make brewed vinegar. The vinegar usually derives its descriptive name from the material from which it is made such as: cider vinegar is made from apple juice, alegar from ale, malt vinegar from malted grains spirit vinegar from alcohol etc.

Vinegar Preparation

It involves two step fermentations as detailed below:

Alcoholic fermentation:

The first is alcoholic fermentation, mainly carried out by yeast Saccharomyces cerevisiae either by pure culture inoculation or by the natural process of fermentation. The process can be represented by a simplified equation:

 $C6H12O6 \rightarrow 2CO2 + 2C2H5 - OH + 55 \text{ Kcal}$

Glucose Carbon dioxide Ethyl alcohol

In the process, ethyl alcohol is not the only product but small amounts of other compounds like glycerol, succinic acid, amyl alcohol, propyl alcohol etc. are also produced in this fermentation. The fermentation is anaerobic.

Acetous fermentation:

The second fermentation is acetic acid fermentation. It is an oxidative fermentation carried out by acetic acid bacteria like Acetobacter aceti. In the vinegar production, pure culture of acetic acid bacteria is not used, due to more efficiency of mixed cultures. The oxidation reaction can be shown as:

 $\text{C2H5OH} + \text{O2} \rightarrow \text{CH3COOH} + \text{H2O} + 116 \text{ Kcal}$

Ethyl alcohol Acetic acid

The optimum temperature of fermentation is 260 C which is achieved by the heat generated in the process.

Sauerkraut

It is the clean, sound product of characteristic flavour, obtained by full fermentation, chiefly lactic of properly prepared and shredded cabbage in the presence of not less than 2% nor more than 3% of salt. It contains, upon completion of the fermentation not less than 1.5 per cent of acid expressed as lactic acid. To prepare sauerkraut rough outer leaves of fully mature solid cabbage heads are removed. Head are quartered, the cores are removed and then, shredded the quarters into thin strips which are mixed with salt. About 2.25 to 2.5% of salt by weight should be added to the shredded cabbage to obtain kraut of the best quality. Pack the cabbage loosely in a jar, place a wooden board on the top. In order to press out juice from the cabbage, a heavy stone is placed on the wooden board. The jar is kept at a warm place (24 to 310 C) for 8 to 12 days to allow fermentation to complete. The brine is separated from the cabbage, boiled and poured hot over the cabbage shreds in the jars. Sauerkraut can be packed in cans also. The cans are filled with the hot juice, exhausted and processed till the temperature at the centre of can reaches 820 C.

Prominent bacteria that attain appreciable number early in fermentation are Enterobacter cloacea and Erwinia herbicola and contribute some flavour. However, Leuconostoc mesenteroides bacteria begins to outgrow all organisms and continue acid production upto 0.7 to 1% (as lactic acid). Next, Lactobacillus plantarum, a non-gas forming lactobacilli continues the production of acid and can raise the acidity to 1.5 to 2.0%. These bacteria produce chiefly lactic acid in their fermentation of sugars. A final acidity of 1.7% as lactic acid is most desirable and fermentation can be stopped at this stage by canning or refrigerating the sauerkraut.

Kanji

Carrots of deep purple variety are fermented in Northern India and Pakistan to make a readyto-serve beverage /drink called as Kanji. It is a popular beverage and is considered to have cooling and smoothing properties besides nutritional content. To prepare it, the carrots are washed, grated finally. For every Kg of grated carrot, 7Kg of water, 200g of salt, 40 g of crushed mustard seeds and 8g of hot chillies are added followed by placing the mixture in a glazed earthenware, leaving a tiny whole for the release of gases produced during fermentation. The mixture is fermented for 7-10 days. It is strained through a muslin cloth. The final product is acidic in taste with an attractive purple red colour and is usually consumed within 3-4 days.

Pickles from Vegetables

Vegetables like cucumber are pickled whole or in slices after washing in potable water. For every one Kg of cucumber, 15g salt is added which results in the formation of brine. It is followed by lactic acid fermentation. Depending upon the ambient temperature it takes one to four weeks. The fermented cucumbers are stored in clean capped jars after pasteurization.

Radish can also be pickled in a manner similar to sauerkraut as discussed earlier.

Kimchi

It is a fermented food of Korea with cabbage or radish as the main ingredient. Cucumbers can also be added. Cabbages are cut and brined in 5 to 7% salt solution for 12 hr or in 15% brine for 3 to 7 hr. Then, brined cabbage is rinsed and mixed with 10% seasoning ingredients i.e. garlic, green onions, peppers, ginger, mustard, parseley, sesame grains and fermented shrimp. This mixture is allowed to ferment in jars which takes a few days at temperature of more than 200 C for a month below 100 C. 'Kimchi' has a pH value of 4-4.5 and lactic acid content of 0.4 to 0.8%. The main organisms responsible for fermentation of 'kimchi' are Leuconostoc mesenteroides and acidifying microorganism is Lactobacillus plantarum.

Ethanol Production

The material rich in sugar can be converted into ethanol. The fermentation is carried out using yeast like Saccharomyces cerevisiae. The sugars like glucose is converted into ethyl alcohol and carbon dioxide, anaerobically. Ethanol is a liquid fuel or liquid fuel supplement and is used as a solvent in many industries.

The waste from fruits and vegetable processing industries being rich in polysaccharides (cellulose, hemicellulose and lignin) has been subjected to SSF for the production of ethanol. The cellulose and hemicellulose present in the processing waste like apple pomace are readily fermented by anaerobic bacteria. For ethanol production, the waste from processing industries has to be pre-treated due to presence of lignin. A SSF process has been used for production of ethanol from apple pomace by using Saccharomyces cerevisiae. Apple, pear, orange peel and cherry wastes have also been utilized for production of ethanol by fermentation with Saccharomyces cerevisiae.