UNIT 3 INDUSTRIALLY IMPORTANT YEAST, MOLD AND BACTERIA

Structure

3.0 OBJECTIVES

After reading this unit you should be able to:

- state the fermenter, types of microorganisms involved in fermentation and their processes;
- explains the different products made by fermentation; and
- describe the waste from food processing industry and their utilization.

3.1 INTRODUCTION

Microorganism on one hand are responsible for causing a number of diseases, on the other hand they are employed to produce a number of useful products. These useful microorganisms include an array of yeasts, molds and bacteria. Traditionally, the man prepared wine, curd, vinegar and pickles using fermentation. Earlier, the term 'Fermentation' was used for the production of wine but at present it encompasses the foods made by the application of microorganisms including lactic acid bacteria (LAB). Lactic acid fermentation is one of the oldest method of preserving fruits and vegetables. Apart from contributing certain desirable physical and flavour characteristics, it also prolongs the availability and processing period of the products at relatively low cost. Many of the fermented products are made at industrial scale making use of microorganisms. Some of the fermented products and industrially important microorganisms are listed in Table 3.1.

Table 3.1: Production/synthesis of various compounds by micro-organisms

Product	Micro-organism(s) involved
Alcoholic beverages & related products	
Beer	*Saccharomyces cerevisiae*; *S. carlsbergensis*
Bourbon whiskey	*S. cerevisiae*
Cider	*S. cidri*
Palm wine	*Acetobacter s*pp.; yeasts
Sake	*Aspergillus oryzae*; *Lactobacillus* spp.; *Leuconostoc* spp.; *S. cerevisiae*
Scotch whiskey	*S. cerevisiae*
Thumba	*Endomycopsis fibuliges*
Tibi	*Betabacterium vermiforme; S. intermidium.*
Vinegar	*Acetobacter* spp.
Wines	*S. cerevisiae* var. *ellispoideus*
Breads	
Idli	*Leuconostoc mesenteroids*
Rolls, cakes etc.	*S. cerevisiae*
Colour	
β-carotene	*Blakeslea trispora; Rhodotorula spp.*
Astaxanthine	*Phiffia rhodozyma*
Dairy products	
Acidophillus milk	*Lactobacillus acidophilus*
Bulgarian milk	*L. bulgaricus*
Cheeses (brie,	*Streptococcus lactis; S. cremoris*: *camembert, cheddar, S. durans; Penicillium camembertii*: *edam, P. candidum; P. roquefortii; Lactobacillus caseiroqueforte)*
Kefir	*Streptococcus lactis; L. bulgaricus; torula* spp.
Kumiss	*L. bulgaricus; L. leicuhmannii; Torula* spp.
Yoghurt	*L. bulgaricus*
Enzymes	
Amylases	*Bacillus* spp.; *Aspergillus niger; A. oryzae.*
Cellulases	*Trichoderma reesei.*
Glucose oxidases & catalase	*Corynebacterium* spp.
Invertase	*S. cerevisiae*
Lipase	*Saccharomycopsis lipolytica.*
Pectinases	*Aspergillus* spp.
Proteases	*B. licheniformis*; *B. subtilis; Aspergillus* spp.; *S. cerevisiae*

Meat and fishery products

Country cured hams	*Aspergillus; Penicillium* spp.
Dry sausages	*Pediococcus cerevisiae*

Microbial cells as fermented products

Bakers' yeast	*Saccharomyces cerevisiae*
Single cell	*Candida utilis; C. arborea;* protein (SCP) *Methylophilus methylotrophus; Saccharomycopsis lipolytica; Spirulina*
Mushrooms	*Agaricus bisporus*; *Morchella hortensis*

Non-beverage plant products

Miso	*Aspergillus oryzae*
Saurkraut	*Neurospora sitophila*
Sufu	*L. delbrueckii*
Tempeh	*A. oryzae; Rhizopus oligosporus; R. oryzae*

Organic acids

Acetic acid	*Acetobacter aceti; C. aceticum*
Citric acid	*Aspergillus niger; Saccharomycopsis lipolytica*
Lactic Acid	*Lactobacillus delbrueckii*

Polysaccharides

Alginate	*Azotobacter vinelandii; Pseudomonas aeruginosa*
Dextrans	*Leuconostoc mesenteroids; Klebsiella; Acetobacter*
Pullulan	*Aureobasidium; Pullularia* spp.

Vitamins and Amino acids

Riboflavin	*Eremothecium ashbyi*
Vit. B-12	*Bacillus megaterium; Streptomyces olivaceus, Propionibacterium*
Pro-Vit. A.	*Rhodotorula gravillis.*

3.2 CULTURING OF IMPORTANT MICROORGANISM

The human food supply consists basically of plants and animals or products derived from them, so our food supply can contain microorganisms in interaction with the food. The interactions between microorganisms and our food is beneficial as exemplified by many cultured products developed by fermentation and are consumed and enjoyed by many people (Bread, bear, wine etc). To produce such products microorganisms are added as pure culture or mixed cultures. However, in some cases no cultures may be added if the desired microorganisms are known to be present in sufficient numbers in the original raw material.

Starter culture, pure as well as mixed are usually employed in the manufacture of certain fermented food and dairy products. Cultures for food fermentations are selected primarily on the basis of their stability and their ability to produce desired products. Mother culture is usually prepared daily from a previous

mother culture and originally from the stock culture. The mother cultures can be used to inoculate a large quality of culture medium to produce the mass or bulk culture to be used in the fermentation process.

Bacterial cultures: Most of the bacterial cultures employed as starters are for dairy products. Sausage and bread also use pure or mixed cultures of lactic acid bacteria (LAB) e.g. *Streptococcus lactis* sub sp *lactis, S. mesenteroides* sub-sp. *cremoris* etc.

Yeast cultures: Most yeasts of industrial importance are of the genus *Saccharomyces*. It is used to manufacture wine, beer and other alcoholic products.

Bakers' yeast: Yeast for baker's yeast production: Strains of *S.cerevisiae, S.uvarum* are used.

Wine yeast: *S. cerevisiae var. ellipsoidus* (Plate 1.1)

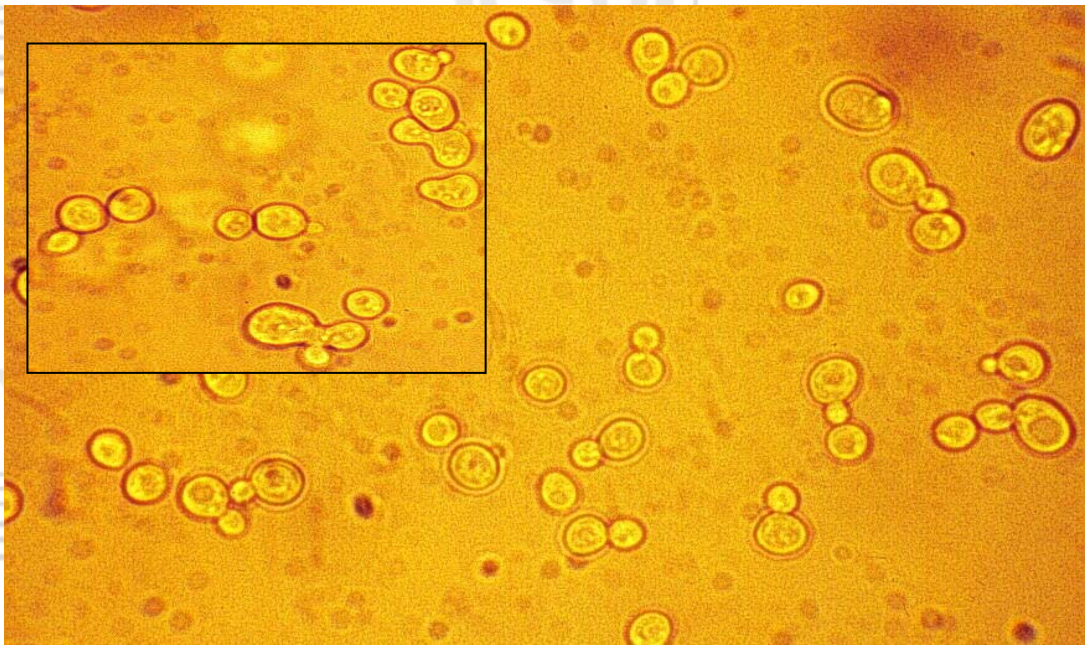

Plate 1.1: Photo micrograph of yeast cells showing budding (inset yeast cells are mating)

Distillers yeast: High alcohol yielding strains of *S.cerevisiae* var *ellipsoidus*.

Mold cultures: Stock of cultures of molds usually are carried in slants of a suitable agar medium and may be preserved as spore stab for a long period by freeze drying (*Penicillium roquefortii).*

3.3 ENZYMES AND KINETICS

Enzymes: Enzymes are biological catalysts possessing extraordinary efficiency, specificity and are mostly protein in nature. Enzyme commission has classified various enzymes on the basis of the type of the reactions catalysed. All the enzymes have been classified into 6 classes.

Class 1 : Oxido-reductase
Class 2 : Transferase
Class 3 : Hydrolase
Class 4 : Lyase
Class 5 : Isomerase
Class 6 : Ligase

Properties of Enzymes

- All enzymes are protein in nature except nucleases.
- All enzymes are specific in their functions.
- Enzymes are sensitive to temperature i.e. they are functional at optimal temperature.
- These are destroyed at higher temperature.
- Enzymes are not destroyed during their use.

Kinetics of Enzyme Reactions

The studies on the kinetics of enzyme reactions must be based on quantitative measurements of the rate of the catalyzed reactions. Main factors which influence the kinetics of enzymatic reactions are as follow:

Enzyme concentration: The velocity of enzymatic reactions is directly proportional to the concentration of the enzyme proteins.

Substrate concentration: When the velocity of the reaction is plotted vs. substrate concentration, classical enzymes give a rectangular curve.

pH: Almost every enzyme exhibits maximum activity at a particular pH which is called optimum pH.

Temperature: Almost every enzyme exhibits an optional temperature at which the enzyme exhibits maximal activity. A graph of enzyme velocity versus temperature, is a bell shaped curve.

Role of enzymes in food processing: The enzymes play a significant role in food processing. Pectinase enzymes are used in juice clarification (apple juice, guava juice), in softening of fruit (apple, tomatoes, peaches, avocadoes), and thereby resulting in increase in yield of juices and pulps extraction of juice from fruits. Proteases (papain) results in clarification and removal of cloudiness in beer and wine. Glucose oxidase enzyme is used in removal of glucose from egg white and thereby, improve, the colour of dehydrated egg powder. Pectinase with cellulase has been employed for extraction of oil from oil containing fruits (olive). Enzyme diastase converts starch to sugar during beer preparation.

3.4 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 of

the 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.

3.5 TYPE OF FERMENTER, CONCEPT OF BATCH AND CONTINUOUS FERMENTATION

Fermenter: The industrial usage of micro-organisms often requires that they be grown in large vessels containing considerable quantities of nutritive media. These vessels are commonly called fermenters. Therefore, fermenter is the basic equipment of fermentation.

Types of fermenters: Some of the types of fermenter are listed below:

1. Shake flasks and bottles
2. Stirred tanks
3. Air-lift fermenters
4. Tower fermenter
5. Rotating disc fermenter
6. Fixed bed fermenter
7. Fluidized bed fermenter

Batch fermentation: In this fermentation, starter culture is added to the medium and the product is withdrawn only after completion of fermentation.

Continuous fermentation: In this fermentation, the substrate is continuously fed to the fermenter and the product is also withdrawn continuously.

3.6 MICROBIAL PRODUCTION OF WINE, VINEGAR, SAUERKRAUT, ETHYL ALCOHOL, BEER, ORGANIC ACID

3.6.1 Wines

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.

Wines are produced by the fermentation of juices/extracts of many fruits such as apple, pear, cherries, most of berries, rhubarb, dandelion, honey, besides bananas, pineapple, cashew nut, pomegranate, lemons, tangerines, oranges, dates and figs. Wines from grapes are classified basically into red and white wines.

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. The generalized flow sheets for wine making from grapes is shown in Figure 3.1.

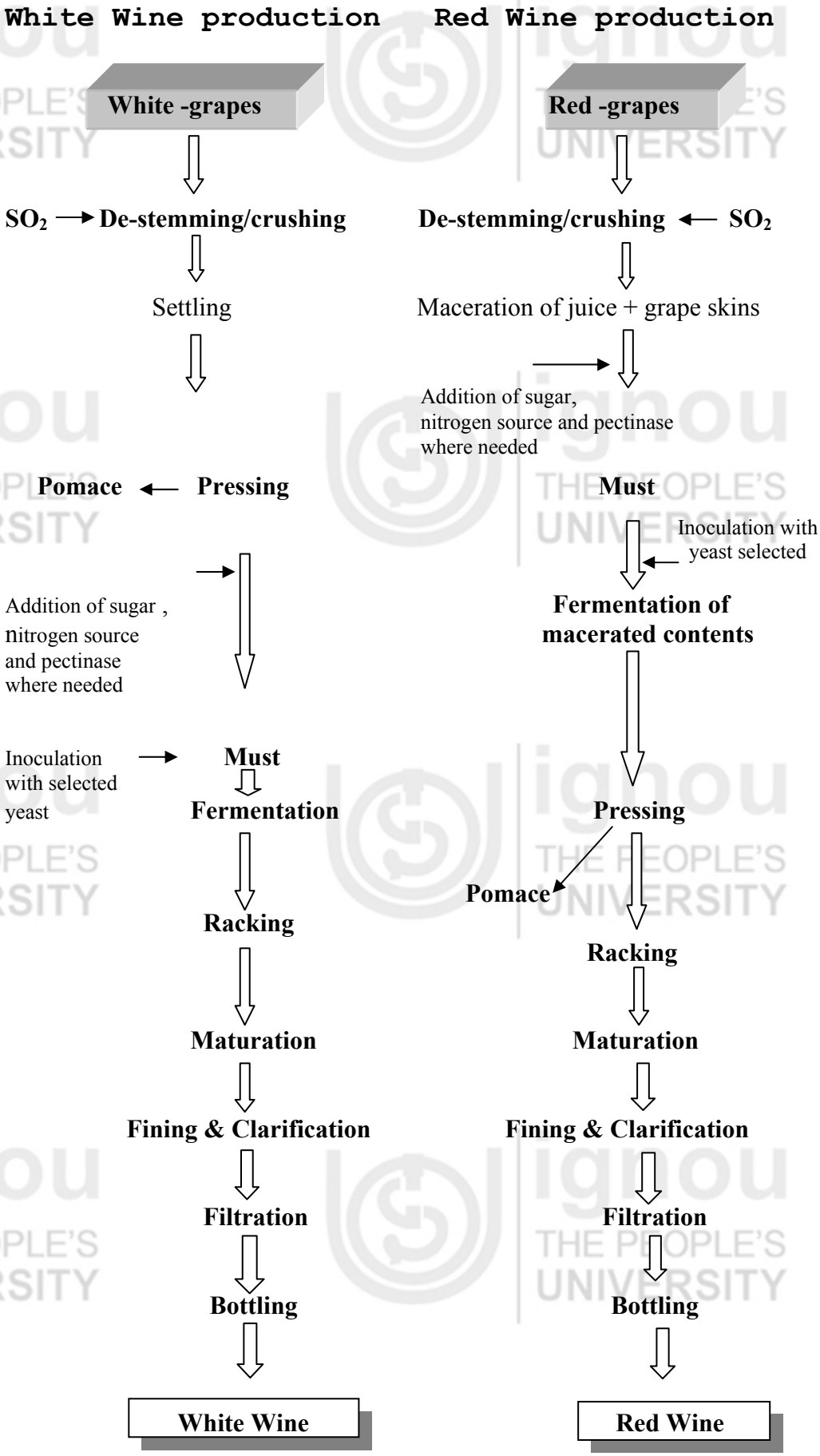

Figure 3.1: Flow–sheet of process to make red and white wine

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 ($S0_2$) 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 20-25^{o}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 62^{0}C for 15-20 min, after bottling.

Storage: Low temperature storage is preferred for good quality wine.

3.6.2 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 14^{o}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 O^{o}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 CO_2 content of about 0.45 to 0.52 per cent, mostly by means of gas collected during the fermentation or by addition of CO_2 from cylinders. Then, beer is cooled, clarified or filtered and packaged in the bottles, cans or barrels.

3.6.3 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,

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:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5 - OH + 55 \text{ Kcal}$$

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:

$$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O + 116 \text{ Kcal}$$

Ethyl alcohol Acetic acid

The optimum temperature of fermentation is 26^{o}C which is achieved by the heat generated in the process.

Process of Vinegar Preparation

Slow process: This process takes a long period and is generally followed in countries like India. The juice kept in the barrels is allowed to undergo alcoholic and acetic fermentations slowly with the passage of time. The bung hole of the barrel is covered with a piece of cloth to screen-off the dust and flies, and the barrel is placed in a damp but warm place. It takes about 5-6 months to complete the whole alcoholic and acetous fermentation to produce the vinegar from the juice. The main drawbacks of this process are: alcoholic fermentation is often incomplete, the acetic fermentation is very slow and the yield is low coupled with an inferior quality vinegar.

Quick process: In the quick process like generator process alcoholic liquid is in motion and this process is applied mostly to the production of vinegar from spirit (alcohol). Fruit or malt liquors are well supplemented with food for the vinegar bacteria, but to maintain active vinegar bacteria in generator methods using alcohol denatured with ethyl acetate or vinegar, it must be supplemented with a combination of organic and inorganic compounds known as vinegar food. Combinations of substances such as dibasic ammonium phosphate, urea, peptones, yeast extract, glucose, malt, starch, dextrin, salts etc have been made. Materials such as pumice, branches of vines and grape stems for packing the generators are used. Schiizenbach introduced the use of a vat instead of cask for the acetification process and provided mechanical means for the repeated distribution of the acidic liquid over the packing.

Generator: The equipment used is known as "Upright Generator" which in its simplest form is a cylindrical tank that comes in different sizes and is usually made of wood. Its interior is divided into 3 parts:

i) **Upper section:** Here, alcoholic liquid is introduced.

ii) **Large middle section:** In this section, liquid is allowed to trickle down over beech wood shavings, corn cobs, charcoal, coke, or some other material that will provide a large total surface area yet not settled into a compact mass.

iii) **Bottom section:** This section is for the vinegar collection.

The alcoholic liquid is put at the top through an automatic feed trough or a sprinkling device (sparger) and trickled down over the shavings or other material on which a slimy growth of acetic acid bacteria has been developed and the bacteria oxidize the alcohol to acetic acid and the process is called acetification. Air enters through the false bottom of the middle section and after becoming warm, it is exhausted out through a ventilation above. As considerable heat is released by oxidation process, it is necessary to control the temperature below 30^{o}C. It is usually done by using cooling coils, by adjusting the rate of alcoholic liquid, feeding air and by cooling the alcoholic liquid before it enters the generator or by cooling the partially acetified liquid that is returned to the top from the bottom section of the tank for further acetification.

3.6.4 Lactic Acid Bacteria (LAB) and Fermented Foods

Lactic acid bacteria (LAB) are obligate microorganisms producing lactate from sugars as the main end product, besides producing inhibitory substances like organic acids, bacteriocin, hydrogen peroxide which are antagonistic towards other microorganisms. Fermented dairy products are known to be inhibitory to both pathogenic and spoilage causing microorganisms and Yoghurt is the best known fermented milk product (with fruit pulp).Cultured milk and milk products contain lactic acid bacteria that prevent the occurrence of stomach, colon and other cancers.

Traditional fermentations of vegetables were depended upon growth of naturally occurring lactic acid bacteria to metabolise sugars in the vegetables to mainly lactic acid and improve their taste and keeping quality. However, starter cultures are being used now to develop controlled fermentation

It is established that more than one species of lactic acid bacteria are responsible for vegetable fermentation. Lactic acid bacteria responsible for natural fermentation of vegetables are within the genera of *Streptococcus*, *Leuconostoc, Pediococcus* and *Lactobacillus*. Acidity, pH, salt concentration, temperature, naturally occurring inhibitors, chemical additives, exposed brined surface to air and sunlight, amount of fermentable carbohydrates in the vegetables and availability of nutrients in the brine are important factors affecting the lactic fermentation.

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 31^{o}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 82^{o}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 ready-to-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 20^{o}C for a month below 10^{o}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*.

3.6.5 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*.

3.6.6 Enzyme Production

Both submerged fermentation (SF) and solid state fermentation (SSF) are employed for production of enzymes. But SSF is a better method than SF for production of enzymes. Various enzymes have been produced by fermenting food processing waste. Invertase enzyme by fermenting sauerkraut waste with the help of *Canidida utilis* has been produced. This enzyme is widely used in the food processing industry. Subsequently, fungal amylase by using baked bean waste has been produced. Enzymes like cellulase and xylanase are produced by fermenting apple pomace, using *Trichoderma viridae* and *Aspergillus* sp. Pectinase is another enzyme which is produced from wastes like apple pomace.

Table 3.3: Food processing waste used as SCP/animal feed after microbial fermentation

Waste	Microorganisms utilised
Apple pomace	*Saccharomyces cerevisiae* *Candida utilis* *Torula utilis* *Aspergillus niger*
Corn cob	*Aspergillus niger*
Dried citrus peel	*Aspergillus niger*
Fodder beets	*Saccharomyces cerevisiae*
Orange peel and grape stalks	*Pleurotus ostreatus* *Agrocybe aegirata* *Armillarialla mellea*
Sugarcane bagasse	*Polyporus* sp. *Pleurotus* *Trichoderma*
Sugar beet pulp	*Trichoderma reesei* *Tricoderma viridae* *Fusarium oxysporum*

3.6.7 Citric Acid

Citric acid is being produced by fermenting brewery waste with *Aspergillus niger*. Apple pomace is a potential source of citric acid when fermented with *Aspergillus niger* by SSF on various substrates like pineapple juice, molasses, sweet potatoes residue, sugar cane bagasse impregnated with pineapple juice, mandarin orange waste, apple pomace, grape pomace. While production of citric acid by fermenting apple pomace, addition of methanol to the medium increases the yield of citric acid.

3.7 SINGLE CELL PROTEINS

In developing countries like India, deficiency of proteins leads to mal-nutrition. It has necessitated to explore new non-conventional resources of protein production. Amongst the various processes used to supply protein are those based on the microbial growth and microbial biomass especially using the waste material (Table 3.3). Microbial cells used as proteins as single cell protein (SCP) and can be used as protein supplement for feed or food. A number of micro-organisms like yeast, fungi, algae and bacteria can be employed production of SCP and each of them has its advantage and disadvantages. The micro-organism in turn use these substances as starting materials for fermentation and SCP production by assimilation.

The SCP however, is not without limitations also such as high nucleic acids which are metabolized to uric acid and can give rise to articular gout in human beings. Secondly, human being can eat a maximum of 2.0g SCP/kg

body weight/day in their diet. To overcome the nucleic acid levels in SCP various methods have been tried but with a variable success. The success of SCP depends upon economics of SCP production.

3.8 MICROBIAL FERMENTATION FOR UTILIZATION OF WASTE

With the advent of post-harvest technology, the fruits and vegetables are processed for the production of various products. The processing of fruits and vegetables in this way generate a large quantity of bio-degradable waste. The waste from processing of fruits and vegetables include water and various organic substances e.g. simple and complex polysaccharides (Sugars, starch, pectin, etc.), vitamins and minerals. The large quantities of waste generated in this way leads to environmental pollution. In today's environment conscious society, there are regulatory laws for the discharge of industrial effluent under the water conservation and control of Pollution Act 1974 and Environmental Protection Act, 1986 and these are mandatory for the processing industries. The waste from the processing industries can either be disposed-off after necessary treatment as per the directions of the pollution control agencies or it can be utilized by applying suitable technologies as illustrated in Table 3.4.

Table 3.4: Microbial utilization of food processing industry waste

Sl.	Products	Waste
1.	Ethanol	Citrus industry waste, apple pomace, peach waste, cashew apple pomace, pineapple waste, pear cutting.
2.	Biogas	Waste from fruit and vegetable industry as a whole, fermentation (wine and beer) waste.
3.	Single Cell	Apple pomace, peach waste, cashew apple proteins pomace, citrus waste extract, molasses, potato peels, cabbage waste.
4.	Cider, beer and vinegar	Apple pomace.
5.	Pectin	Citrus waste, apple pomace.
6.	Citric acid	Apple pomace.
7.	Baker's yeasts/ industrial yeast	Waste from wine, beer and distillary.
8.	Colour	Apple pomace.
9.	Flavours/Xanthan gum	Fruits and vegetable waste, citrus waste
10.	Animal feed	Apple pomace, peach waste, potato industry waste, olive processing waste.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Define wine, toddy, vermouth, cider, beer and perry.

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2. What is lagering, pitching and draft beer?

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3. Name the yeast used in beer fermentation.

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4. What is role of boiling wort?

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5. Give various steps for wine and beer production.

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6. What is vinegar and SCP?

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7. Name the microorganisms and their sequence in lactic acid fermentation of cabbage.

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8. Name the processes used in vinegar preparation.

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9. Which is commercially available single cell protein source?

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10. Classify enzymes and their role in industry?

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11. Differentiate SF and SSF fermentation.

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12. Name the microorganisms associated with production of following products: Sauerkraut, Beer, Wine, Organic acid (acetic acid) and SCP.

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13. Define batch and continuous fermentation.

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14. Why yeast is preferred as a single cell protein compared to bacteria and algae?

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15. What are different types of fermenters? Enlist the same.

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16. Write 2-3 lines about the following:

Sauerkraut, Kimchi, vinegar

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3.9 LET US SUM UP

Fermentation is an ancient practice, carried out by natural or inoculated microflora. Several microorganisms are important as these are employed to produce the fermented food, additives and products of industrial significance. These include yeast, bacteria and fungi. Fermentation could be classified as solid state fermentation, submerged fermentation or extractive fermentation. Depending upon the mode of operation fermentation could be batch, fed batch or continuous type. At the industrial scale, the fermentationis carried outin the vessel called fermentor or bio reactors. Depending upon the type of fermentation or product, the type of fermentoris employed. Food fermentations include those to produce wine, beer, brandy, whisky, pickles, sauerkraut, kimchi, vinegar, yoghurt additives like citric acid, lactic acid, enzymes, ethanol, single cell proteins being produced commercially using microbial process.

3.10 KEY WORDS

Fermentation : was used for the production of wine but at present it encompasses the foods made by the application of microorganisms including lactic acid bacteria (LAB).

Bakers' yeast : The strains of *Saccharomyces uvarum* used to make bread.

Wine yeast : *S.* cerevisiae *var. ellipsoidus.*

Distillers yeast : High alcohol yielding strains of *S. cerevisiae* var *ellipsoidu* used to higher alcoholic beverages.

Controlled "starter" Culture : pure as well as mixed cultures of microorganisms are responsible for conducting fermentations, employed in the manufacture of certain food and dairy products such as fermented milk, butter, cheese, bread, malt beverages.

Enzymes : Enzymes are biological catalysts possessing efficiency and specificity and are mostly protein in nature.

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).

Submerged fermentation : Fermentation processes which take place in the presence of free water in the substrate are termed as sub-merged fermentation.

Fermenter : The industrial usage of micro-organisms often requires that they be grown in large vessels containing considerable quantities of nutritive media. These vessels are commonly called fermenteors.

Wine : The term 'wine' is applied to the product made by the alcoholic fermentation of grape or grape juice. But any fleshy fruit or flower in the new world may be employed.

Beer : Beer is an alcoholic beverage prepared from barely or other cereals.

Vinegar : The word vinegar is derived from two French words, *vin* and *aigre* meaning sour wine but the term vinegar is used to denote a condiment prepared from various sugar and starch containing materials by alcoholic and subsequent, acetic fermentation.

Acetous fermentation : or acetic acid fermentation which is oxidative fermentation carried out by acetic acid bacteria viz. *Acetobacter aceti*.

Lactic acid bacteria (LAB) : These are the microorganisms that are obligate producing lactate from sugars as the main end product, besides producing inhibitory substances to other microorganisms.

Sauerkraut : It is the product of characteristic flavour, obtained by lactic fermentation of cabbage in the presence of 2-3% of salt.

Kimchi : It is a group of fermented vegetable foods of Korea with cabbage or radish as the main ingredient.

Single cell protein : The microbial biomass used as protein supplement for feed or food is called as single cell protein (SCP).

3.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

1. Your answer should include the following points:

   Wine: It is the product made by the alcoholic fermentation of grape or other fruit or flower.

   Cider: Cider is a low alcoholic beverage from apple juice.

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

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

2. Your answer should include the following points:

   Lagering: It is the process of storage of young, green or draft beer in vats at about O^oC for several weeks to several months, to make it clear and mellow.

   Pitching: It is the process of inoculation of yeast culture in the wort.

   Draft beer: The freshly prepared beer (not matured) is called draft beer.

3. Your answer should include the following points:

   The answer is *Saccharomyces cerevisiae* var *carlbergenesis.*

4. Your answer should include the following points:

   The boiling concentrates, sterilizes the wort and inactivates the enzymes.

5. Your answer should include the following points:

   Various steps are involved in beer production:

   Malting
   Mashing
   Wort boiling
   Fermentation
   Aging or Maturing
   Finishing
   Table wine
   Juice Extraction
   Must preparation
   Fermentation
   Siphoning/racking
   Maturation
   Clarification
   Blending
   Pasteurization

6. Your answer should include the following points:

   Vinegar: Vinegar is the product obtained by acetic acid fermentation of ethanolic liquid of any fruit and contains 3.75 %w/w acetic acid. It is prepared by alcoholic fermentation of fruit juice.

   SCP: Single cell proteins (SCP) are the microbial cells used as a source of proteins. To produce single cell proteins, suitable microorganism and medium is selected. The optimum conditions of growth are also determined and the same are given during the fermentation. The grown biomass is then harvested and the cells are used as a source of proteins.

7. Your answer should include the following points:

   First of all *Pediococus* comes, then *Streptococcus* followed by *Lactobacillus in* cabbage fermentation.

8. Your answer should include the following points:

   Slow process: This process takes long period wherein the juice is allowed to undergo alcoholic acetic fermentations. It takes about 5-6 months to complete the fermentation to form the vinegar from the juice.

   Quick process: In quick processes like generator process alcoholic liquid is kept in motion and this process is applied mostly to the production of vinegar from spirit (alcohol). The alcoholic fermented liquors is well supplemented with food for the vinegar bacteria, such as a combination of organic and inorganic compounds. The process needs additional supply of oxygen.

9. Your answer should include the following points:

   Spirullina which is used as a SCP commercially.

10. Your answer should include the following points:

    All the enzymes have been classified into six classes.

    Class 1 – Oxido-reductase e.g. dehydrogenases, peroxidases.

    Class 2 – Transferase

    Class 3 – Hydrolase

    Class 4 – Lyase

    Class 5 – Isomerase

    Class 6 – Ligase

    Uses of enzymes in food processing: Pectinase enzyme are used in clarification of juices (apple juice, guava juice), lemon juice etc. Pectinase enzyme result in softening of apple fruit, tomatoes, peaches, avocadoes and thereby resulted in increase in yield of juice and pulp during processing. Pectinase enzyme result in easy extraction of juice from fruits. Proteases resulted in clarification and removal of cloudiness in beer and wine. Glucose oxidase enzyme is used in removal of glucose from egg white and thereby, improve, the colour of dehydrated egg powder. Pectinase with cellulase enzymes are also used for extraction of oil from oil containing fruits. Beer cloudiness can be removed by use of proteases e.g. papain. Enzyme diastase resulted in conversion of starch to sugars during beer preparation.

11. Your answer should include the following points:

    The submerged fermentation (Smf) makes use of free liquid while in that of solid state fermentation (SSF) no free water is available.

12. Your answer should include the following points:

    The microorganisms used for the products are listed as below:

    Sauerkraut: Lactic acid bacteria

    Beer: *Saccharomyces cerevisiae* var *carbergenesis*

    Wine: *Saccharomyces cerevisiae* var *ellipsoideus*

    Organic acid (Acetic Acid): *Acetobacter aceti*

    SCP: *Spirullina, Saccharomyces cerevisiae, Candida utilis*

13. Your answer should include the following points:

    Batch fermentation: Here the starter culture is added to the medium and the product is withdrawn after completion of fermentation.

    Continuous fermentation: Here the substrate is continuously fed to the fermenter and the product is also withdrawn continuously.

14. Your answer should include the following points:

    1. Yeasts have nutritive value especially proteins and vitamins.
    2. Able to grow on a variety of carbon and nitrogen source.

3. Has faster growth and high yield, ability to grow at low pH.
4. Can grow on a large number of waste including that from processing industries.

15. Your answer should include the following points:

Types of Fermentors:

1. Shake flasks and bottles
2. Stirred tanks
3. Airlift fermenters
4. Tower fermenter
5. Rotating disc fermenter
6. Fixed bed fermenter
7. Fluidized bed fermenter

16. Your answer should include the following points:

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.

Kimchi: It is a group of fermented vegetable foods of Korea with cabbage or radish as the main ingredient with or without cucumbers.

Vinegar: The word vinegar is derived from two French words,*vin* and *aigre* meaning sour wine but the term vinegar is used to denote a condiment prepared from various sugar and starch containing materials by alcoholic and subsequent, acetic fermentation.

3.12 SOME USEFUL BOOKS

1. Green, J.H. and Kramer, A. (1979) Food Processing Waste Management AVI Publishing Company Westport CT. p.663.
2. Joshi, V.K., Pandey, A. and Sandhu, D.K. (1998) Food Factory Waste Management Technology. In: Biotechnology Food Fermentation Vol. II (eds.) V.K. Joshi and Ashok Pandey. Educational Publishers and Distributors, New Delhi.
3. Joshi, V.K., Sharma, Somesh, Bhushan, Shashi and Attri, Devender (2004) Fruits based alcoholic beverages. In: Concise Encyclopedia of Bioresource Technology, Ashok Pandey (eds.) p. 335-345. The Howorth Press, Inc., New York.
4. Kharatyan, G.S. (1978) Microbes as food for humans. Ann. Rev. Microbiol. 32: 301.
5. Verma, L.R. and Joshi, V.K. (Ed.) (2000) Post-harvest Technology of Fruits and Vegetable – Handling, Processing, Fermentation and Waste Management. Vol. I & II. Indust Publishing Co., New Delhi.