The dried products namely tea leaves, and coffee and cocoa beans are commercially available for beverage preparation.

SWEETENERS

Sweeteners occupy a prominent place in food consumption, by all the people. The most predominantly used ***natural sweetener*** is ***sucrose*** (obtained from sugar cane or beet). Sweeteners are invariably required for the preparation of soft drinks, ice creams, jams, jellies, sauces, bread, confectionery, pickles etc. Hence there is a continuous search for low calorie sweeteners.

Saccharin, a chemically synthesized sweetener (300 times sweeter than sucrose) was used for several years, and its use is now being restricted due to certain adverse health effects. ***High fructose corn syrup*** (***HFCS***), produced from sucrose is sweeter (1.5 times) than sucrose. ***Aspartame*** (aspartyl phenylalanine) is a product of innovative biotechnology. It is about ***200 times sweeter than sucrose*** and is truely a ***low calorie sweetener***. Aspartame is approved for human consumption and is widely used in soft drink and other industries.

THAUMATIN-A SWEET PROTEIN

Thaumatin is a protein extracted from the berries of the *Thaumatococcus daniellii*, a native plant in Africa. Thaumatin is about ***3000 times sweeter than sucrose***. Thaumatin certainly appears to be a good sugar substitute and a low-calorie sweetener. It is in fact being used as a sweetener and flavour enhancer of various foods. At least five different forms of thaumatins with a molecular weights in the range of 20,000–22,000 have been isolated and characterized.

Genetic engineering to produce thaumatin

Natural production of thaumatin is very limited and therefore can never meet the demands of consumption. Biotechnological production of this sweetener is the method of choice for its large scale manufacture.

Thaumatin-encoding mRNA species have been isolated, identified and converted to cDNA and finally to double-stranded DNA. These DNAs were cloned and transferred to *E. coli*. But the yield of thaumatin production was very low. Attempts were also made to express thaumatin genes in yeasts such as *S. cerevisiae* and *Kluyveromyces lactis*. Some success has been reported in this direction.

MONELLIN-A CANDIDATE FOR SUGAR SUBSTITUTE

Monellin is a ***protein*** found in the fruit of an African plant *Discorephyllum cumminsii*. It is about ***100,000 times sweeter than sucrose*** (on molar basis). Monellin is dimer composed of an A-chain (45 amino acids) and a B-chain (50 amino acids). This protein is optimally sweet when both the chains are held together and not when they are separated.

It has been possible to chemically synthesize a gene that encodes both A and B chains as a single polypeptide. This gene was in fact introduced and expressed in tomatoes for the production of monellin. However, the success has been very limited.

FLAVOUR ENHANCERS

Taste and flavour are very important for the acceptability of foods. ***Monosodium glutamate is the most commonly used flavour enhancer***. Its production and other aspects are described elsewhere (Chapter 26). The degraded products of proteins and RNAs also impart flavour. Thaumatin and monellin (described above) are also flavour enhancing agents, besides sweetening.

The other flavouring agents are citric acid (produced by *Aspergillus niger*), acetone and butanol (produced by *Clostridium acetobutylicum*). The lactones formed by some microbial fermentations also enhance flavour e.g. γ-butyrolactone produced in yeast fermentation of wine and beer. Mention must be made about alcohol also which can increase flavour and taste when added at appropriate concentrations e.g. certain medicinal syrups.

ALCOHOLIC BEVERAGES

Alcoholic beverages are produced throughout the world. The type of the beverage and the substrate used for its production mostly depends on the crops grown in the region. For instance, in

TABLE 28.2 Alcoholic beverages along with the common substrates used for their production

Beverage	*Alcohol (%)*	*Substrate(s)*
Beer (ale, lager)	4–8	Barley and other cereals
Wine (red, white)	10–16	Grapes
Sake	10–14	Rice
Champagne	10–12	Grapes
Cidar	7–12	Apple juice
Brandy	35–45	Grapes or other fruits
Whisky	40–55	Cereals (barley, rye, corn)
Rum	50–60	Molasses
Gin, vodka	40–50	Potato, wheat, rye
Chinese brandy	30–40	Rice
Toddy	5–15	Palmyra juice
Arrack	60–75	Rice, jaggery, waste carbohydrates

Poland, Russia and Scandinavia, beers manufactured from barely are consumed while in France, Italy and Greece, wines obtained from palmyra juice are mostly consumed by rural people.

The common types of alcoholic beverages with their alcoholic contents and substrates used for their production are given in *Table 28.2*.

Historical perspective

Archaelogical evidence indicates that there existed fermentation of grains as early as 4000 B.C. Production of alcoholic beverages is considered to be a part of human civilization. The biological and chemical principles underlying alcoholic beverage production were elucidated by Louis Pasteur in the second half of nineteenth century.

Saccharomyces cerevisiae—the key organism for the production of alcoholic beverages

The most commonly used organism for the production of beverages is the yeast, *Saccharomyces cerevisiae*. This organism is capable of utilizing simple sugars (glucose, fructose) and converting them to ethanol. It is fortunate that *S. cerevisiae* is a unique organism that can ***tolerate*** and grow even at a ***high concentration of alcohol***. This is advantageous for good production yield of alcoholic beverages.

There are two types of *S. cerevisiae* strains—***bottom yeast*** and ***top yeast***. The bottom yeast settles to the bottom whereas the top yeast raises to the top while carrying out fermentation. Top yeast are frequently used for the production of beer while bottom yeast are employed for wine production. However, both strains can be used for beverage production.

Genetically engineered strains of *S. cerevisiae* : By employing recombinant DNA technology, new strains of *Saccharomyces cerevisiae* are constantly being developed. Some of these strains with ***improved production yields of alcoholic beverages***, are in fact approved for use in beverage industries.

ALCOHOLIC BEVERAGE PRODUCTION —GENERAL ASPECTS

The starting materials for beverage production are sugary or starchy materials.

Sugary materials : Fruit juice (or fruits), plant sap and honey that can be directly used.

Starchy materials—various grains and roots : They have to be subjected to hydrolysis to yield simple fermentable sugars. This process, referred to as ***saccharification***, is carried out either by plant materials (barley malt, rye malt or millet malt) or microorganisms (*Aspergillus* sp, *Rhizopus* sp. *Mucor sp*) rich in starch hydrolytic enzymes.

Fermentation process : When subjected to fermentation under ideal conditions, alcohol production ranging from 2 to 16% is usually observed. These alcoholic beverages may be appropriately processed and drunk. e.g. beers. However, for the production of beverages with higher concentration of alcohol (brandy, whisky, rum, gin etc.), distillation has to be carried out to increase the alcohol content.

Brewing is the technical term used for the ***production of malt beverages***. Besides *S. cerevisiae* (described already), *S. carlsbergensis* is also used in brewing industry. The finished products in brewing namely the alcoholic beverages differ from the other industrial fermented products. This is because besides the alcohol content, several other factors are important in finished beverages. These include flavour, aroma, colour, clarity, foam production and stability, and satiety. These factors decide the consumer acceptance and the commercial value of alcoholic beverages. All these beverages can be consumed when freshly produced. However, they are normally kept for storing or aging before consumption. Aging certainly improves flavour, aroma etc.

The commercial production processes employed for the manufacture of beer and wine are briefly described hereunder.

BEER

Beer is an ***undistilled alcoholic beverage*** with alcohol content in the range of 4-8%. It is produced by fermentation of barley or other cereals, by employing the yeasts (most frequently) *S. cerevisiae* or (sometimes) *S. carlsbergensis*.

It is often necessary to add starch — rich materials (wheat, maize, rice) known as ***adjuncts***, to enhance fermentation, besides reducing the cost of raw materials. There are four major steps involved in the production of beer (***Fig. 28.4***).

1. **Malting :** Dried barely are cleaned and soaked in water for a period of two days. The excess water is drained and the soaked barley are incubated for 4-6 days to germinate. Germination process is associated with the formation of enzymes-***amylases*** (starch hydrolysing) and ***proteases*** (protein degrading). The germinated seeds are slowly subjected to increased temperature (up to 80°C) so that the germination process is halted, but the enzymes retain their activities. Malt is prepared by powdering the seeds.

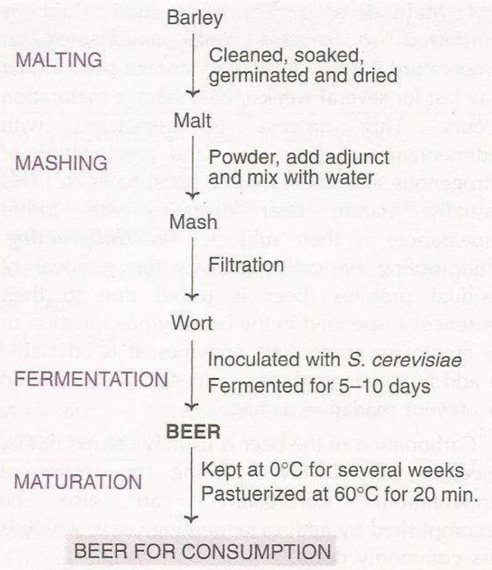

Fig. 28.4 : *Flow chart representing an outline of beer production.*

2. **Mashing :** The powdered malt is mixed with hot water (55–65°C). During the course of mashing, the soluble materials from malt and malt adjuncts are extracted. Mashing is also associated with degradation of starches (by amylases) to produce dextrins, maltose and glucose and hydrolysis of protein (by proteases) to peptones, peptides and amino acids. The temperature and pH influence the activities of enzymes. At the end of mashing, the medium for fermentation which is referred to as ***beer wort*** is developed. This is rich in sugars, amino acids, minerals and vitamins.

Hops are the dried female flowers obtained from hop plant. Addition of hops to wort is often done to provide characteristic flavour, aroma and stabilizing effect to the beer. Besides providing a mild antibacterial activity.

3. **Fermentation :** The beer wort, kept in open or closed bioreactors, is inoculated with pure strains of yeast. *S. cerevisiae* (usually top fermenting strains). The fermentation is carried out at temperature 20–28°C for 5–10 days. In some countries, the bottom fermenting yeast *S. uvarum* is used. For this organism, the ideal temperature for optimal fermentation is 10–15°C.

4. **Maturation :** The fermented fluid is transferred to storage tanks maintained at temperature 0-3°C. During the storage period (that may last for several weeks), cold storage maturation occurs. This process is associated with sedimentation of yeast cells and precipitation of nitrogenous substances, resins, phosphates etc. This partially mature beer (usually with turbid appearance) is then subjected to ***chillproofing***. Chillproofing basically involves the removal of residual proteins (beer is turbid due to their presence) suspended in the beer by precipitation or by employing proteolytic enzymes. It is advisable to add antioxidants during cold-storage maturation to prevent oxidative damage.

Carbonation of the beer is usually carried out by injecting CO_2 (evolved during the course of fermentation). Carbonation can also be accomplished by adding fermenting yeast, which is less commonly done.

The matured and carbonated beer is bottled or canned, and then pasteurized at 60°C for about 20 minutes.

As already stated, there is variation in the raw materials used for beer production. For instance, in Africa it is ***sorghum beer*** that are commonly produced. This is in contrast to the ***barley beer*** produced in most Western countries.

WINES

Wines are originally Middle East and European drinks, although almost every country now produces them. Large scale production of wines is carried out by using ***grapes*** of species *Vitis vinifera*. Grape juice is a good source for wine production because of its high concentration of sugar and other nutrients, natural acidity (that can inhibit unwanted growth of microorganisms) and the capability to produce pleasant aroma and flavour.

Louis Pasteur often used to state ***'wine is the most healthy and most hygienic of beverages'***. There is some recent scientific evidence also in support of this view, since moderate wine consumption reduces the risk for coronary heart disease (CHD).

Production of wines

Quality of the grapes is very important for the production of wines. The grapes are crushed (mechanically or by treading of feet) and the juice extracted. This ***grape juice ready for fermentation*** process is technically referred to as ***must***. It is a practice to add sulfur dioxide to must to inhibit the growth of non-wine yeast and contaminating bacteria. Sulfur dioxide which can kill other organisms can be tolerated by wine-fermenting yeasts. Sometimes, the must may also be subjected to partial or complete sterilization. The must in suitable bioreactors is inoculated with desired strains of the yeast *Saccharomyces cerevisiae*. Initially, oxygen is bubbled through the fermentation medium to promote good growth of yeast cells and gradually anaerobic conditions are established. The wine production normally takes a few days (2-5 days). The fermentation conditions (temperature, time etc.) are actually dependent on the type of wine produced. At the end of fermentation, wines are transferred to storage tanks (or vats) and allowed to age, which may take some months or years. ***Ageing*** of wine is very important ***for the development of characteristic flavour and aroma***. The alcohol content of wines is in the range of 10–16%.

Types of wines

There are hundreds of different types of wines produced in different parts of the world.

Red wine : The red colour of this wine is due the colour extracted from the black grape skins (when crushed totally). Red wines are commonly drunk, by many people in the west, along with lunch and dinner.

White wine : Black grapes after removal of skins or white grapes are used for the production of white wine.

Rose wine : This can be produced with a limited contact to the skins of grapes during fermentation.

Dry wine : It contain relatively higher alcohol content and produced when sugars are completely fermented.

Sweet wine : This is sweet to taste since it contains some residual sugars after fermentation.

Fortified wines : The normal concentration of alcohol in wines is less than 16%. The wines can be fortified with addition of alcohol (usually done by adding brandy or other distilled spirits) to the desired concentration. Some examples of fortified wines are sherry, port and vermouth.

TABLE 28.3 Enzymes used in various food and beverage industries

Industry	*Enzymes*
Dairy	Chymosins (animal/microbial), lipase, lactase, lysozyme
Baking	α–Amylase, protease, phospholipases, xylanase
Starch and sugar	Amylases (α– and β–), glucoamylase, pollulanase, invertase, glucose isomerase, xylanase
Fruit and vegetable processing	Pectin esterase, pectin lyase, hemicellulases, polygalacturanase
Meat processing	Proteases, papain
Animal and fish bone processing	Alkaline phosphatase
Egg white processing	Glucose oxidase, catalase
Brewing production of beer and alcoholic beverage	Amylases (α– and β–), proteases, cellulases, papain, aminoglucosidases, xylanase

OTHER MICROBIALLY-DERIVED FOOD PRODUCTS

There are a large number food products or their ingradients, produced by microorganisms. For full details on them, the reader must refer the corresponding Chapters — single-cell protein and mushrooms (Chapter 29), vitamins (Chapter 27), amino acids (Chapter 26), organic acids (Chapter 24), polysaccharides (Chapter 30), and vinegar (Chapter 24).

ENZYMES IN FOOD AND BEVERAGE INDUSTRIES

Food and beverage biotechnology is very closely linked with the use of enzymes. While a majority of enzymes are microbial in nature, some other sources of enzymes can also be used. In ***Table 28.3***, a selected list of enzymes employed in food processing and beverage industries is given. Some of the aspects of enzymes in dairy, baking and brewing industries are described in the foregoing pages. A few more important ones are described hereunder.

Proteases in food industry

Proteases (proteolytic enzymes) are used ***for tenderisation of meat*** which flavours the meat, besides easy digestion. Proteases are employed for removal of meal attached to bones in the form of a slurry. This can be used for the preparation of soups and canned meats. Partial hydrolysis of certain vegetable proteins also increases their flavour e.g. proteins of soy bean; protein (gluten) of dough in preparing bread.

Lactase in dairy industry

Milk and whey contain about 4-5% lactose. ***Lactose intolerance***, due to a defect in the intestinal enzyme lactase, is ***very common in humans***. It is estimated that half of the world's population suffers from lactose intolerance. These individuals when consume milk and milk products containing lactose (particularly in large quantities) suffer from diarrhea and flatulance (abdominal cramps and increased intestinal motility). Obviously, lactose intolerant persons require milk products with a low lactose concentration.

The enzyme lactase cleaves lactose to glucose and galactose which can be easily assimilated by the body. The lactase enzyme may be obtained from microbial sources e.g. *Kluyveromyces fragilis, Aspergillus niger*. Sometimes, immobilized lactase can also be used to degrade lactose.

For the preparation of flavoured and condensed milks and ice creams, lactase is widely used.

ENZYMES IN THE PREPARATION OF FRUIT JUICES

The turbidity and cloudiness, commonly seen in fruit juices, is due to the presence of pectins and cell debris. Enzyme preparation containing ***pectin esterase, polygalacturonase, pectin lyase*** and hemicellulase is added to the fruit pulp ***for the removal of pectins and other turbid-causing materials***.