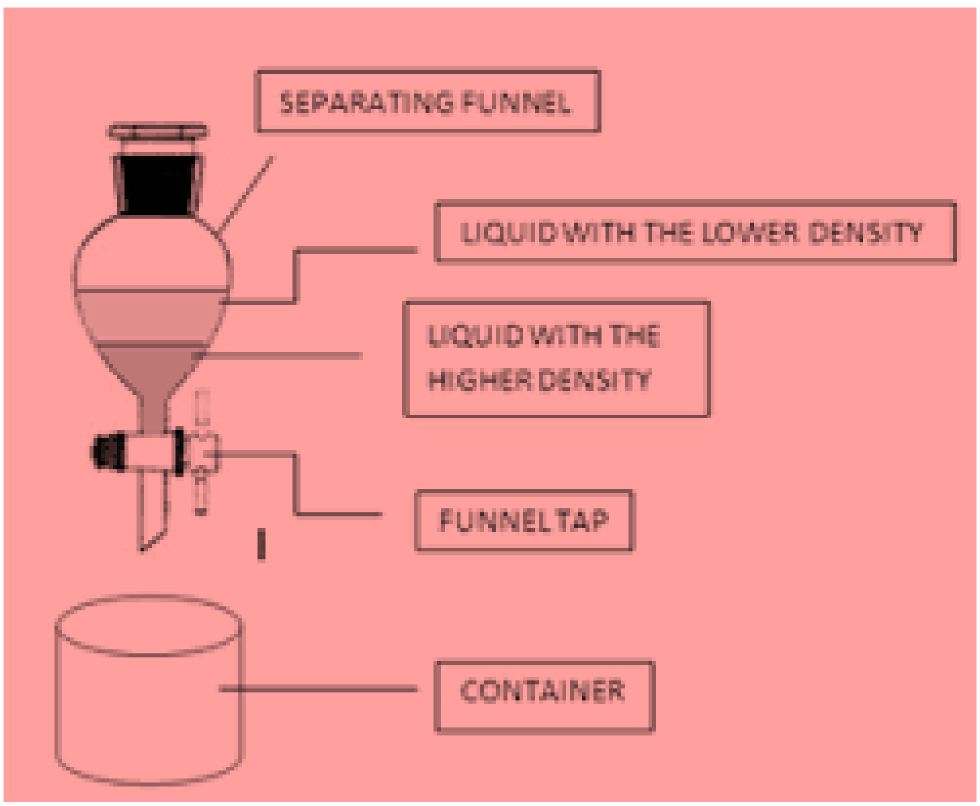
UNIT IV

Liquid-liquid extraction

- It is a separation process that takes the advantage of the relative solubilities of solute in immiscible solvents.
- Solute is dissolved more readily and becomes more concentrated in the solvent in which it has a higher solubility.
- A partial separation occurs when a number of solutes have different relative solubilities in the two solvents used.
- Solvent should be non-toxic, selective, inexpensive and immiscible with broth and should have a high distribution co-efficient for the product.



Electrophoresis

- Electrophoresis is the migration of charged molecules under the influence of an electrical field.
- It was widely used in both analytical and small scale preparative purification of proteins and nucleic acids.
- The component of a sample separate on the basis of their relative electrophoretic mobilities.
- The mobility is a function of charge and molecular weight of the particles.
- There are four basic technique in electrophoresis
  - ✓ Zone electrophoresis
  - ✓ Moving boundary electrophoresis
  - ✓ Isotachophoresis
  - ✓ Isoelectric focusing

Chromatography

- It's done to separate those contaminants that resemble the product very closely in physical and chemical properties.
- It includes affinity, size exclusion, reversed phase chromatography, ion-exchange chromatography.
- Chromatography is a laboratory technique for the separation of a mixture.
- The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase.*
- The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases.
- Types - *Paper chromatography*, *Thin-layer chromatography (TLC), Gas Chromatography*

Crystallization

- Crystallization is a common technique used to purify solids. Two common methods of crystallization are "gradual cooling" and "diffusion".
- Gradual Cooling - Gradual cooling involves dissolving the impure solid in a minimum amount of a hot solvent and allowing the resulting solution to cool slowly to room temperature. During the cooling process, pure (or almost pure) crystals form and are then collected by vacuum filtration.
- Diffusion - Crystallization by diffusion is an alternative to gradual cooling that does not use heated solvents. This crystallization process is preferable if the desired compound degrades at the elevated temperatures of solvent boiling points.

Storage and Packaging

- Physical protection – The objects enclosed in the package may require protection from, among other things, mechanical shock, vibration, electrostatic discharge, compression, temperature, etc.
- Barrier protection – A barrier to oxygen, water vapor, dust, etc., is often required. Permeation is a critical factor in design. Some packages contain desiccants or oxygen absorbers to help extend shelf life.
- Containment or agglomeration – Small objects are typically grouped together in one package for reasons of storage and selling efficiency
- Information transmission – Packages and labels communicate how to use, transport, recycle, or dispose of the package or product. With pharmaceuticals, food, medical, and chemical products, some types of information are required by government legislation. Some packages and labels also are used for track and trace purposes.

Reverse osmosis

- Reverse osmosis is a more economical operation for concentrating food liquids (such as fruit juices) than conventional heat-treatment processes.
- Its advantages include a lower operating cost and the ability to avoid heat-treatment processes.
- Reverse osmosis is extensively used in the dairy industry for the production of whey protein powders and for the concentration of milk to reduce shipping costs.
- Reverse osmosis (also described as hyperfiltration) is a separation process where the solvent molecules are forced by an applied pressure to flow through a semipermeable membrane in the opposite direction to that dictated by osmotic forces.
- It is used for the concentration of smaller molecules than is possible by ultrafiltration as the pores are 1–10 angstroms diameter.
- Nano-filtration is a modified form of reverse osmosis that utilizes charged membranes to separate small solutes and charged species based on both charge and size effects.

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Purification

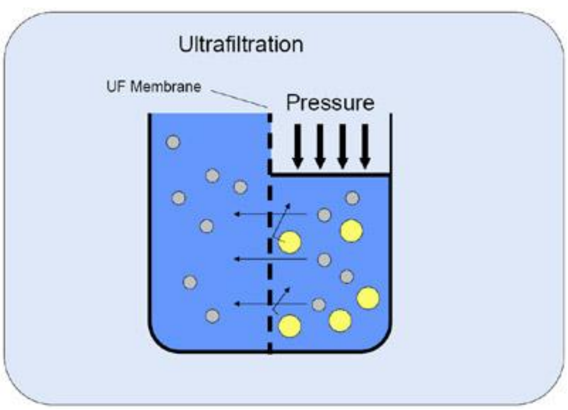
Protein precipitation is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants.

Precipitation by ammonium sulphate

- It is an effect based on the electrolyte–non-electrolyte interaction, in which the non-electrolyte could be less soluble at high salt concentrations.
- It is used as a method of purification for proteins,
- Salting out is the most common method used to precipitate a protein. Addition of a neutral salt,
- Such as ammonium sulfate, compresses the solvation layer and increases protein–protein interactions.
- As the salt concentration of a solution is increased, the charges on the surface of the protein interact with the salt, not the water.

Ultrafiltration

- Ultrafiltration (UF) is a membrane filtration process similar to Reverse Osmosis, using hydrostatic pressure to force water through a semi-permeable membrane.
- The pore size of the ultrafiltration membrane is usually 103 - 106 Daltons.
- Ultrafiltration (UF) is a pressure-driven barrier to suspended solids, bacteria, viruses, endotoxins and other pathogens to produce water with very high purity and low silt density.



- Ultrafiltration (UF) is a variety of membrane filtration in which hydrostatic pressure forces a liquid against a semi permeable membrane.
- Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane.
- Ultrafiltration (UF) is used to remove essentially all colloidal particles (0.01 to 1.0 microns) from water and some of the largest dissolved contaminants.

Treatment of effluent

Liquid waste flowing out of a factory, farm, commercial establishment, or a household into a water body such as a river, lake, or lagoon, or a sewer system or reservoir.

Industrial wastewater treatment

Industrial wastewater treatment covers the mechanisms and processes used to treat waters that have been contaminated by anthropogenic activities.

Source of Industrial waste - Agricultural waste, Iron and steel industry, Mines and quarries, Food industry, Chemical industry, Nuclear industry

Process

1. Pre treatment

   Pre-treatment removes materials that can be easily collected from the raw waste water before they damage or clog the pumps and skimmers of primary treatment clarifiers (trash, tree limbs, leaves, etc.).

2. Grit removal

   Pre-treatment may include a sand or grit channel or chamber where the velocity of the incoming wastewater is adjusted to allow the settlement of sand, grit, stones, and broken glass. These particles are removed because they may damage pumps and other equipment. For small sanitary sewer systems, the grit chambers may not be necessary, but grit removal is desirable at larger plants.

3. Primary treatment

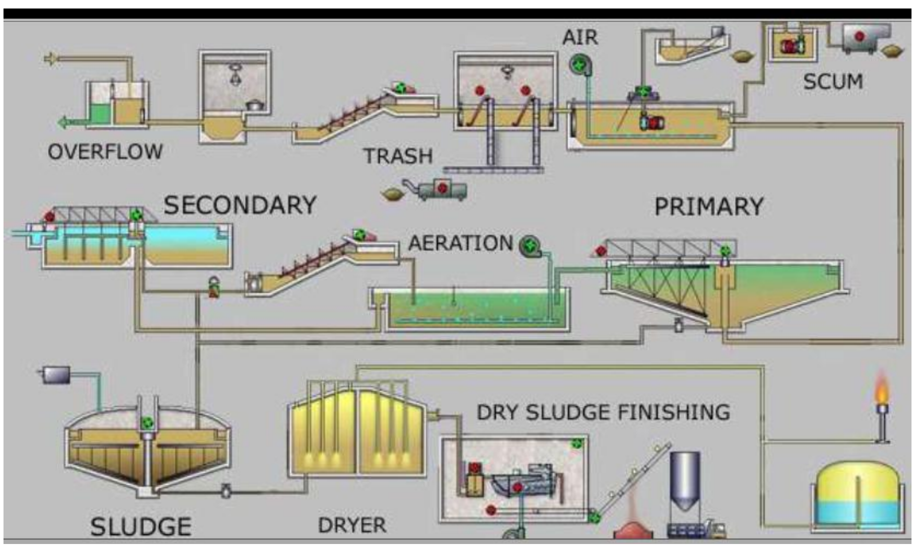
Primary treatment consists of temporarily holding the sewage in a quiescent basin where heavy solids can settle to the bottom while oil, grease and lighter solids float to the surface. The settled and floating materials are removed and the remaining liquid may be discharged or subjected to secondary treatment

4. Secondary treatment

Secondary treatment removes dissolved and suspended biological matter. Secondary treatment is typically performed by indigenous, water-borne micro-organisms in a managed habitat. Secondary treatment may require a separation process to remove the micro-organisms from the treated water prior to discharge or tertiary treatment.

5. Tertiary treatment

Tertiary treatment is sometimes defined as anything more than primary and secondary treatment in order to allow rejection into a highly sensitive or fragile ecosystem. Treated water is sometimes disinfected chemically or physically



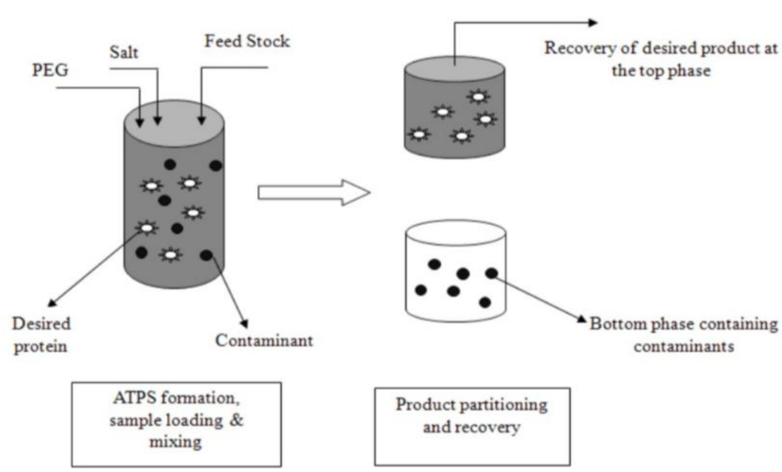
Product Extraction process

Solvent extraction

- Separation process for isolating the constituents of a liquid mixture.
- Liquid–liquid extraction (LLE), also known as solvent extraction and partitioning
- It's a method to separate compounds or metal complexes.
- Based on their relative solubilities in two different immiscible liquids, usually water (polar) and an organic solvent (non-polar).
- It is also widely used in the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel.

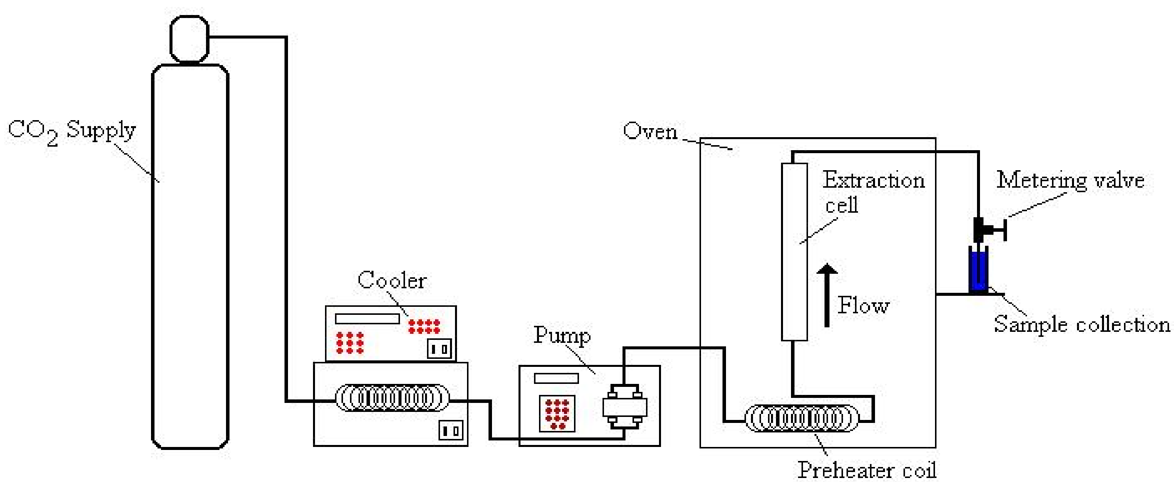
Aqueous two phase separation

- Aqueous two-phase extraction has applications in biochemical processes involving protein separation and purification.
- It includes two different immiscible polymers or polymer and salt systems for protein recovery.
- The two phases are mostly composed of water and non-volatile components, thus eliminating volatile organic compounds.
- PEG (Poly Ethylene Glycol) dextran system – The components is mixed with PEG
- The upper phase is formed by the more hydrophobic PEG, which is lowe density than the lower phase consist of more hydrophilic.
- Its helps to separate the compound easily.



Supercritical fluid extraction

- Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent.
- Extraction is usually from a solid matrix, but can also be from liquids.
- The system must contain a pump for the CO_2, a pressure cell to contain the sample, a means of maintaining pressure in the system and a collecting vessel.
- The liquid is pumped to a heating zone, where it is heated to supercritical conditions.
- It then passes into the extraction vessel, where it rapidly diffuses into the solid matrix and dissolves the material to be extracted.
- The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out.
- The CO_2 can then be cooled, re-compressed and recycled, or discharged to atmosphere



UNIT V

Food ingredients and additives used in fermentation

Food ingredients are any substance that is added to food to achieve a desired effect. Food ingredients are of different types . For example : fermented foods, cereal, cooking oils and so on. Fermented food ingredients are those foods prepared by fermentation process. Fermentation is one of the oldest methods used by Asian people to preserve foods, to destroy undesirable components, to enhance the nutritive value and appearance of the food, also to reduce the energy required for cooking and to make a safer product.

Main functions of food additives:

1. It improves or preserve the nutrient value
2. Maintain the wholesomeness of foods.
3. Control the acidity and alkalinity, and to provide leavening.
4. Provide color and enhance flavor

Fermented food ingredients are those substances which are prepared by fermentation process and are used in many dishes. ♣ Fermentation derived ingredients are being used today in multiple food categories from bakery and meats to soups, sauces and dressing. Some examples of fermented food ingredients – ngari (a type of fermented fish), hawaijar (a type of fermented soyabean), tofu, curd, yogurt, seedle (another fermented fish from Tripura) and so on.

Food ingredients used in fermentation

- Salt – Common table salt contains iodine which act as a best fermenting agent. A combination of salt and water is called brine which is used to ferment foods.
- Water – Purified water was used as a fermenting agent in some cases
- Sugar – Sugar is a best source for microbial growth which enhance the fermentation

Fermented Food Additives

Substances that are added to food to maintain or improve the safety, freshness, taste, texture, or appearance of food are known as food additives. ♣ They are not commonly used as a food itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value. The term does not include contaminants or substances added to food for improving or maintaining nutritional qualities. New additives and new process production of existing of additives are evaluated by the Joint FAQ/WHO Expert Commmittee on Food Additives (JECFA) which normally meets twice a year.

Example: Vinegar

Vinegar is a liquid consisting of about 5-20% acetic acid (CH3COOH) , water and trace chemicals that may include flavorings. Acetic acid is produced by the fermentation of ethanol by acetic acid bacteria. Commercially, vinegar is produced either by a fast or slow fermentation process. Slow method is usually used as a traditional method of vinegar production. In this method, fermentation proceeds over a few months to a year.

The longer fermentation period allows for accumulation of a nontoxic slime composed of acetic acid bacteria. In the fast method , mother of vinegar (a bacterial culture) is added to the source liquid before adding air to oxygenate and promote the fastest fermentation. In this process, vinegar may be produced in 20 hours up to three days. Generally, vinegar is produced from sugar – rich materials by successive anaerobic and aerobic fermentation. The followings are the steps involved in its production.

Health benefits of white vinegar

It helps weight loss and controls blood sugar level. It may aid reduce cholesterol level. It has antimicrobial properties and so it is useful for treating physical ailments including nail fungus, warts and ear infections. It is also an effective topical treatment for skin infections and burns.

Health benefits of Apple cider vinegar

It is the second most commonly used type of vinegar. This type of vinegar is made from apple cider . They give fruity flavor to the cooking. It is rich in vitamin B and C. It is best for salads, dressings, marinades condiments and so on. And the process begins by crushing apples into juice, adding yeast and letting it ferment in barrels. Apple cider vinegar helps in controlling diabetes and blood sugar level. It also helps to weight loss, stops itch caused by bugs and insects, clears sun burns and pimples, eases sore throat and indigestion. It also has polyphenols, a potential cancer-fighting nutrient.

Health benefits of Rice vinegar

Rice vinegar comes in clear or yellow pale in Japan. In Japan, rice vinegar is very essential to sushi preparation. It is made from the sugars found in rice, and the aged, filtered final product has a mild, clean, and delicate flavor that is an excellent complement to ginger or cloves. Rice vinegar also comes in red and black varieties, which are less common in the United States but very popular in China. Both red and black are stronger than the clear one (often called white) or pale yellow.

Role of Microbes in pickling

Pickling is the process of preserving or extending the shelf life of food by either anaerobic fermentation in brine or immersion in vinegar. In East sia, vinaigrette (vegetable oil and vinegar) is also used as a pickling medium. The pickling procedure typically affects the food's texture, taste and flavor. The resulting food is called a *pickle*, or, to prevent ambiguity, prefaced with *pickled*. Foods that are pickled include vegetables, fruits, meats, fish, dairy and eggs.

A distinguishing characteristic is a pH of 4.6 or lower,[2] which is sufficient to kill most bacteria. Pickling can preserve perishable foods for months. Antimicrobial herbs and spices, such as mustard seed, garlic, cinnamon or cloves, are often added.[3] If the food contains sufficient moisture, a pickling brine may be produced simply by As lactic acid bacteria grow in your pickle crock, they digest sugars in the cucumbers and produce lactic acid. Not only does this acid give the pickles their characteristic sour tang, it controls the spread of spoilage microbes. Also, by gobbling up the sugars, *lactic acid bacteria* remove a potential food source for bad bacteria.

Adding salt to your pickling brine is one important way to help lactic acid bacteria win the microbial race. At a certain salt concentration, lactic acid bacteria grow more quickly than other microbes, and have a competitive advantage. Below this "right" concentration, bad bacteria may survive and spread more easily, possibly out-competing lactic acid bacteria and spoiling your pickles. Too much salt is also a problem: Lactic acid bacteria cannot thrive, leaving your vegetables unpickled. What's more, salt-tolerant yeasts can spread more quickly. By consuming lactic acid, yeasts make the pickles less acidic—and more hospitable to spoilage microbes. adding dry salt.

- *E. aerogens* – produce carbon dioxide which create anaerobic environment in the medium
- *Lactobacillus* sp. – produce lactic acid that softens the substance.
- Salt – inhibits the growth of undesirable microbes

Role of Microbes producing colors and flavors

Flavour is a major component of the sensory characteristics of food and drink, and is integral to the choice of what we consume. The development of flavour is a multifaceted process as it depends on the combination of ingredients used in a product and how our sensory perception of these delivers our flavour experience. Flavour components can include non-volatile compounds which are detected by taste and aroma volatiles which are detected by smell; the combination of these develop the full flavour experience. Some of these compounds are intrinsic to the key ingredients but, for certain food products, the involvement of micro-organisms is essential in producing their characteristic flavours and can deliver complexity and variety.

The diversity of products that involve micro-organisms in their production is high. Obvious everyday examples such a bread, yogurt, cheese, salami, wine and beer are well recognised as fermented products, as are products such as kimchi, sauerkraut and kefir, but chocolate, coffee, tea and olives also require the involvement of micro-organisms in their production. In fermented meats like salami, *Staphylococcus carnosus* and *Staphylococcus xylosus* are often added with the lactic acid-producing starter culture. Unusually, these organisms are not very acid tolerant and so do not grow once the pH starts to drop. However, the enzymes they produce are more tolerant and so essentially the bacteria act as bags of enzymes which contribute to fat and protein breakdown, and hence the production of flavour compounds.

The other main groups important in flavour production are yeasts and moulds. Yeasts are of course well known for their alcohol production, but the proteolytic and lipolytic activities of particular species produce a range of flavour compounds. The yeast *Yarrowia lipolytica* breaks down tributyrin, resulting in butanoic acid which has a cheese-like odour, and this is believed to be an important part of the flavour development in a number of cheese varieties. Moulds similarly have proteolytic and lipolytic activity which impart particular characteristics; *Penicillium roqueforti* gives the 'blue' flavour characteristics to cheeses like Stilton and Roquefort.

Cheeses are a good example of a product where the development of sensory characteristics is very dependent on the balance of micro-organisms present. Following the initial fermentation by a starter culture, cheeses undergo a ripening period, the length of which varies with cheese variety. It is during this period that cheeses become complex dynamic ecosystems with the growth of a variety of different micro-organisms contributing to the product's flavour development. For example, in Stilton cheese, *Lactococcus lactis* and *P. roqueforti* are the two starters added by the manufacturer but the final microbiota of a mature cheese after 12 weeks contains many other bacteria and yeasts, some of which have been shown to influence flavour characteristics. As indicated above, *Penicillium* is added to allow development of typical blue cheese flavours, mainly through the production of methyl ketones.

In model cheese studies using controlled flora composition, the inclusion of *Y. lipolytica* with *P. roqueforti* has been shown to increase the development of blue cheese aroma through an increased production of ketones, in comparison to using *P. roqueforti* alone, and resulted in sensory characteristics equivalent to a mature cheese which the mould alone did not. This may result from the yeast's highly lipolytic activity releasing free fatty acids which the mould could then convert to ketones. Thus, the full product characteristics desired by the consumer may rely on this yeast being present. However, this is a species which is present only through chance introduction during processing and hence its presence could be variable from batch to batch, leading to variability in the product.

Many micro-organisms are thus beneficial in contributing to the production of flavour characteristics in many products we consume. However, it should always be remembered that context is key; diacetyl production in dairy products is desirable: in beer it is an off-flavour! One producer's starter culture is another's spoilage agent. Colors have number of beneficial properties like anti cancerous, immune suppressive, antibiotics, anti-proliferative, etc. Microbial pigment are more significant when compare with chemicals. *Staphylococcus aureus* – zeaxanthin; *Monascus roseus* - canthaxanthin

Role of Microbes in alcoholic beverages

Alcoholic beverages are highly produced by microbes in large scale level. Production of alcoholic beverages is a process that involves the active participation of microbe, most often yeast are used. Yeast are majorly used for alcohol production like beer and other alcoholic beverages. *Saccharomyces cerevisiae* were highly used and It ferments the sugar present in the sources. Ex: Wine from grapes, beer from barley.

Yeasts are the main fermenter and alcohol producer in the production of wine, beer and other alcohol drinks. The main yeast species used is Saccharomyces cerevisiae. It ferments the sugars, coming from different sources, e.g., grapes for wine, barley for beer, to alcohol and carbon dioxide. Both wild and cultivated strains are used. The species or strains used in the fermentation play an important role in giving the final taste properties of the drink.

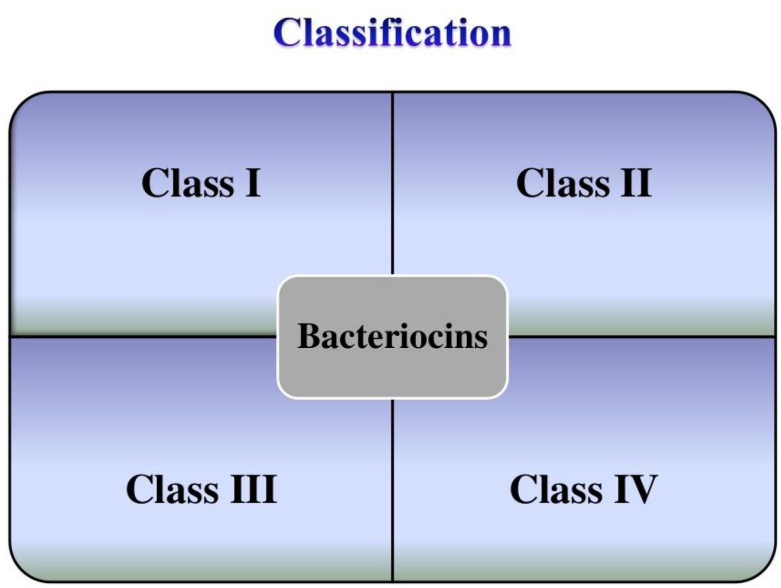
Example: Wine production

Wine is made from grapes or other fruit. The grapes are first cleaned of leaves and stems and the fruit is crushed into must that is ready for fermentation. The yeasts used for the fermentation grow a film on the fruit or in the environment. These wild strains play an important role in the final properties of the drink. However, cultivated strains of *Saccharomyces cerevisiae* are often added to improve the consistency of the final product. There are hundreds of commercially available yeast strains for wine fermentation.

In the fermentation process, energy that is converted to heat is produced as well. It is important to keep the temperature in the fermentation vessel lower than 40ºC to keep the yeasts alive. To improve yeast growth, additional nutrients, like diammonium phosphate, are sometimes added in the fermentation step. When making red wine, there is an additional fermentation step after alcoholic fermentation. Malic acid, naturally present in grape juice, can be converted to lactic acid by lactic acid bacteria naturally found in wineries or added artificially.

Bacteriocins production from Lactic acid bacteria

Bacteriocins are ribosomally synthesized proteins by bacterial strains during their stationary phase. It is secondary metabolites. In 1925, the first bacteriocin called colicin was originally identified asa an antimicrobial protein produced by *E. coli*. **Types –** Class I, Class II, Class III and Class IV. **Isolation –** Isolated from milk products, serially diluted, isolation done by plating methods (pour plate, streak plate, allow to grow in differential media).



Clasifications

- **Class I** - the lantibiotics
- **Class II** - the small heat stable non lantibiotics/unmodified peptides
- **Class III** - large heat labile proteins
- **Class IV -** complex peptides carrying lipid or carbohydrate moieties.

Isolation of Bacteriocins

Fig. 05: Milk products

Collect the **MILK PRODUCTS**

⇩

Make the appropriate dilutions

⇩

Incubate aerobically on de Mann Rogosa Sharpe (MRS) agar

⇩

Isolate the bacteriocins producing bacteria using pour plate technique

⇩

transfer colonies to the MRS broth, obtain the supernatant by centrifugation

⇩

Obtain the precipitates containing bacteriocins

Role of Bacteriocin in food preservation

- There is a continuous raise of awareness in the public regarding the amount of chemical intake by their body as food preservatives.

- In view of the above problem, bio-preservatives are in very high commercial demand at the present, in the form of protective cultures or their metabolites i.e. enzymes and bacteriocins.

- As there is an increase in demand for natural, minimal processed, micro-biologically safe products, bacteriocins provide the consumers with high health benefit.

Advantages of using bacteriocins

i. Safer preservatives than chemicals.

i. More accurate efficacy.

i. Good acceptance from consumers.

i. To overcome various drawbacks such as toxicity of chemicals and nutritional alteration of food.

Applications of using bacteriocins

- Inhibition of pathogenic bacteria.
- Natural preservatives in food industries.
- Agricultural applications – in disease control.
- Pharmaceutical applications.

a. Lantibiotics – treat inflammation, allergies, skin infections, peptic ulcers, etc.
b. Colicins – treat Haemorrhagic colitis and haemolytic uremic syndrome.
c. Microcins – treat Salmonellosis.