Jimma University
College of Natural Sciences
Department of Biology

FOOD BIOTECHNOLOGY – Biol.3132

Cr. Hr = 2

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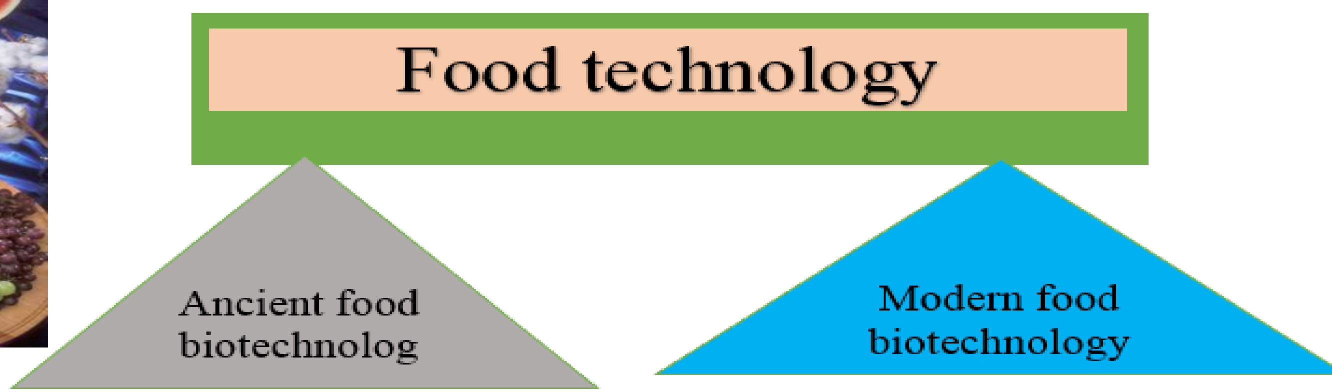
Introduction:

- **Biotechnology**, as the “use of living systems, organisms, or parts of organisms to manipulate natural processes in order to develop products, systems, or environments to benefit people”
- widely used in industry, agriculture and medicine
- It has the potential to improve efficiency of agriculture and allow sustainable food production
- Food ingredients are produced by industrial fermentation of micro-organisms

- ✓ The use of biotechnology in food production is not new
- It has been used for thousands of years
- The fermentation process in food biotechnology offers a method of preservation
    - ❖ e.g. by producing acid
- Future biotechnology is promising:
    - ✓ sustainable, affordable food supply and demand;
    - ✓ stability in food supplies;
    - ✓ achieving global access to food and ending hunger;
    - ✓ Reducing the impact of food production on the world’s environmental systems.

What is Food Biotechnology?

- ➢ is the evolution of traditional agricultural techniques such as crossbreeding and fermentation or;
- ❖ is the application of technology to modify genes of animals, plants, and microorganisms to create new species which have desired production, marketing, or nutrition related properties



Scope of Food Biotechnology

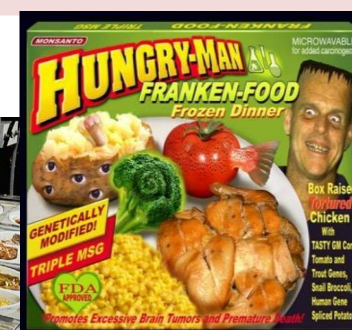
- Food biotechnology employs the tools of modern genetics to:
  - ✓ Enhance its profound positive impact on farming and food security
  - ✓ Reducing the use of pesticides, herbicides, insecticides, chemical feltilizers,
  - ✓ Improving farming efficiency
  - ✓ Reducing farmers production costs
  - ✓ Enhance beneficial traits of plants, animals and microorganisms for food production
  - ✓ Involves **adding** or **extracting** select genes to achieve desired traits.
  - ✓ Advance methods that are currently used to improve many foods.
  - ✓ Improving the nutritional taste & quality of food to attain consumers demand
  - ✓ Simplifying production of foods, allow more food to be produced on less land, etc

Food biotechnology Future Hold

- Has the potential to:
  - Reduce levels of natural toxins in plants
  - Provide simpler and faster ways to locate pathogens, toxins and contaminants
  - Keep products fresher longer
  - Identify ways to eliminate allergens from many foods
  - Increase food supply to support growing world population and decreasing agricultural space

Why genetically modify food?

- Extended Shelf Life
- Efficient Food Processing
- Better Nutrient Composition
- Biopharming



Potential Problems???

- ✓ Creating “Superbugs”
- ✓ Negative Effects on Human Health

- ✓ Other Potential Problems with GE Foods:
  - Antibiotic resistance (significance needs to be determined)
  - Introduction of new proteins into foods (FDA seeks comments)
  - Plants used to make nonfood substances.
  - Special concerns with animal feeds.
  - Unintended “pleotropic” effects
  - Increases of known toxins, decreases in nutrients
  - Activation of dormant pathways, allergens

Controversy over Biotech Foods:

- Against ethical beliefs: regarding cloning.
- GM crops : may bring out harmful effects
- Food safety risk? ....unintended consequences
- Safety risk for environment .....could spread
- Genetically Engineered label ....not required in U.S.A.
- Playing God ....not natural
- Benefits multinational corporations not consumers, not developing nations
- Not equivalent to non-GM
- Labeling indicates process used
- Consumer right to know and choose, Country's right to know and choose

2. FERMENTATION TECHNOLOGY OF TRADITIONAL ETHIOPIAN FOODS AND BEVERAGES

- Ethiopian traditional foods such as ripened cheeses, Wakalim, Borde, Tela, Teji, pickles, yoghurt, beer, sauerkraut, and others

What is fermentation?

Substrate

Process → **Products**

Organism

- ✓ Homolactic acid fermentation and
- ✓ Heterolactic/Alcoholic fermentation

Pathways of fermentation

FERMENTED PRODUCTS - whose production involves the action of m/os or enzymes which cause:

- Desirable biochemical changes and
- Significant modification to the food /substrate/
- Include: Fermented Foods, Organic acids, Antibiotics, Vitamins, SCP, AA, Hormones, Enzymes, Nucleotides, Polymers, etc.

FERMENTED FOODS

- Foods that have been subjected to the action of micro-organisms or enzymes, in order to bring about a desirable change
- Numerous food products owe their production and characteristics to the fermentative activities of microorganisms
- Fermented foods originated many thousands of years ago when presumably micro-organism contaminated local foods

✓ Micro-organisms cause changes in the foods which:
  - Help to preserve the food,
  - Extend shelf-life considerably over that of the raw materials from which they are made,
  - Improve aroma and flavour characteristics,
  - Increase its vitamin content or its digestibility compared to the raw materials.

TRADITIONAL ETHIOPIAN FOODS AND BEVERAGES

- Microorganisms have long played a major role in the production of food and beverages
- As Food directly(Mushrooms, yeasts ,cyanobacterium), SCP or indirectly fermented of d/t food stuff and their enzyme (to improve nutritional value of food)
- Production of beverages including beers, wines and distilled spirits

1. **'INJERA' FERMENTATION**

- Teff, microorganisms (molds, *Enterobacteriaceae*, aerobic mesophilic bacteria and the dominant yeasts).

2. **'KOCHO ' FERMENTATION**

Ensete, *Leuconostic mesenteriodes, Lactobacillus spp*

3. **‘SILJO' FERMENTATION**

Safflower extract and Faba bean flour, *Lactobacillus acidophilus*, *L. plantarum* and *L*. d*elbrueki (initiator)*, *Enterococcus*, *Bacillus*, *Lactococcus*, *Lactobacillus* and yeasts (dominate).

4. ‘**AYIB' (COTTAGE CHEESE) FERMENTATION**

Milk, *Enterobacteriaceae*, *pseudomonas spp*, Lactic acid bacteria and some yeasts

5. **AWAZE FERMENTATION**

Red pepper, garlic, ginger, Lactic acid bacteria found in high number and *Bacillus also* dominant

FOOD BIOTECHNOLOGY

3. BIOTECHNOLOGY IN PRODUCTION OF BEER



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Raw Materials for Brewing of beer

- **Malted Barley ;** Controlled *sprouting and kilning* of the grain.
- The purpose of malting is to develop amylases and proteases in the grain.
- To break down the carbohydrates and proteins in the grain to nourish the germinated seedling
- Malted Grains provide the sugars that are fermented by yeast to produce alcohol and CO2
- Malted grains are a major contributor to the flavor and aroma profiles of beer.

Raw Materials for Brewing of beer

- During malting, barley grains are cleaned/screened (Why)?
- The grains are then steeped in water at 10-15°C but water are changed approximately *at 12-hours intervals* (Why)?
- The grain absorbs water and increases in volume ultimately by about 4%.
- Steeping takes two to three days.
- The grains are then drained of the moisture and may be transferred to a malting floor/revolving drum to germinate
- The plant hormone gibberellic acid is sometimes added to the grains to shorten germination time.

Raw Materials for Brewing of beer

- Production of enzymes is complete in four to five days of the growth of the seedling;
- **kilning,** which consists of heating the 'green' malt in an oven.
- Subsequently the temperature of heating depends on the type of *beer to be produced.*
- The darker Munich beers with a strong aroma drying takes up to 48 hours at 100 – 110°C.
- For beer of the Pilsener type the malt is pale and has no pronounced aroma and kilning takes 20-24 hours at 80–90°C.
- At the end of malting, The rootlets are removed and used as cattle feed.

Raw Materials for Brewing of beer

- Cleaning and milling of malt; the barley malt is cleaned of dirt and passed over a magnet to remove pieces of metals, particularly iron.
- The purpose of milling is to expose particles of the malt to the hydrolytic effects of malt enzymes during the mashing process.
- The finer the particles therefore the greater the extract from the malt.
- Very fine particles hinder filtration and prolong it unduly.

Raw Materials for Brewing of beer

- As a brewing cereal, barley has the following advantages;
- Its husks are thick, difficult to crush and adhere to the kernel.
- Thick husk is a protection against fungal attack during storage.
- Gelatinization temperature (52-59°C), lower than the optimum temperature of α-amylase (70°C) as well as of β-amylase (65°C) of barley malt.
- Barley grain contains very high amounts of beta-amylase

- Hops are the spice of beer.
- Ìts botanical name is *Lupulus humulus*
- It is a temperate climate crop and grows wild in northern parts of *Europe*, *Asia* and *North America.*
- The female flower of the species is normally used in pellets
- hops added is about amounts varying 0.4-4.0 g/liter.
- Resins which provide the precursors of the bitter principles in beer
- Essential (volatile) oils which provide the hop aroma

- The addition of hops has several effects:
- Provide unhopped beer with the characteristic bitterness and pleasant aroma of hops.

Hops

- Hops have some anti-microbial effects against especially against beer sarcina (*Pediococus damnosus)* and other beer spoiling bacteria.
- The tannins in the hops help precipitate proteins during the boiling of the wort, if not removed cause a haze (chill haze)
- Provide colloidal stability and foam head retention of beer

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Brewer's yeasts

- Yeast metabolizes the sugars from the grains and produces alcohol and CO2.
- Yeast produces fermentation by-products such as *phenols and esters* that add significant flavor and aroma character to beer
- Before being used, the packages are kept in cooled environment (2-4°C)
- There are two main types of yeast, typically called *ale and lager*

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Brewer's yeasts

- Ale Yeast; (*Saccharomyces cerevisiae);*
- Generally prefers warmer temperatures and rises to the top of the vessel during fermentation (15-25°C).
- Also known as **top-fermenting yeast.**
- Lager Yeast; (*Saccharomyces uvarum/Saccharomyces carlsbergensis)*
- Generally prefers lower temperatures and settles to the bottom of the tank during fermentation and maturation (4-12°C).
- Also known as **bottom-fermenting yeast.**

WATER

- The mineral and ionic content and the pH of the water have profound effects on the type of beer produced.
- Example; nitrates slow down fermentation, while iron destroys the colloidal stability of the beer.
- It must be drinking water so the water supplied by the distribution network can be used.
- Are realized in order to lower down the quantity of *calcium carbonate and magnesium,* which can cause extreme hard water
- Because of organoleptic reasons; not because of sanitary reasons .

The Brewing Process

- The processes involved in the conversion of barley malt to beer may be divided into the following:
- **Malting**
- **Cleaning and milling of the malt**
- **Mashing**
- **Mash operation**
- **Wort boiling treatment**
- **Fermentation**
- **Storage or lagering**
- **Packaging**

The Brewing Process

- Milling – The first step of the process is crushing the malt. This breaks apart the grains
- Mashing- certain amount of water is mixed with crushed/milled malt
- The mashing takes place in a closed insulated vessel called mash/kettle tun
- This activates naturally occurring enzymes in the grain that convert the grain starches into sugars, like maltose, that yeast can metabolize.
- The aqueous solution resulting from mashing is known as wort.

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The Brewing Process

- The progress of mashing is affected by a combination of *temperature, pH, time, and concentration of the wort.(how ?)*
- Temperature optima of alpha- and beta-amylases

Enzyme	Optimum temperature	Temperature of destruction	Optimal pH
Alpha-amylase	70°C	80°C	5.8
Beta-amylase	60-65°C	75°C	5.4

Lautering (Filtration)

- During Lautering the fermentable sugars are rinsed from the grains.
- As the sugary liquor from the mash, now called wort, is slowly drained from the bottom of the mash tun
- spent grain is unloaded from the lauter tun and can be delivered to a stock-breeder as they are valuable food for many animals (cow, pork, poultry).

Mash Tun

The mash tun is a vessel in which the milled malted barley is mixed with water

Mash

These photos show the milled Malted barley being mixed with Warm water.
The enzymes Convert the starch to maltose and The proteins to amino acids creating What is known as **sweet wort.**

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Lautering (filtering)

The sweet wort
Is separted from
The spent barley
By a filtration step
Known as **Lautering.**
The Barley husks serve As
the primary Filtering
material.

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Mash Tun with used Mash

These are the spent malt that acted as a filtering bed for the sweet wort.

Scraping out the used mash

Used mash heading towards feedlot

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Wort boiling

- ✓ The wort is boiled for **1-1½ hours** in a brew kettle (or copper) which used to be made of copper
- ✓ *Hops are also added, some before and some at the end of the boiling.*
- ✓ The purpose of boiling is as follows;

Wort boiling

- To concentrate the wort, which loses 5-8% of its volume by evaporation during the boiling
- To sterilize the wort to reduce its microbial load before its introduction into the Fermenter
- To inactivate any enzymes so that no change occurs in the composition of the wort.
- To extract soluble materials from the hops, which not only aid in protein removal, but also in introducing the bitterness of hops.

Wort boiling

- To develop color in the beer(oxidation of phenolic compounds, and reactions between amino acids and reducing sugars).
- Removal of volatile compounds: volatile compounds such as fatty acids
- During boiling proteins and tannins are precipitated while the liquid is still warm. Some more precipitation takes place when it has cooled to about 50°C.
- Trub is removed either with a centrifuge, or a whirlpool separator which is now more common.

Wort boiling

- *The cooled wort is now ready for fermentation. It contains no enzymes but it is a rich medium for fermentation.*
- During the transfer to the fermentor the wort is oxygenated ??
- The cooled wort is pumped or allowed to flow by gravity into *fermentation tanks* and yeast is inoculated /pitched
- *During the boiling, agitation and circulation of the wort help increase the amount of precipitation and flock formation.*

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Sweet Wort

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Kettle(wort boiling)

Top fermentation

- Yeast is pitched in at the rate of 0.15 to 0.30 kg/hl at a temperature of 15-16°C.
- The temperature is allowed to rise gradually to 20°C over a period of about three days.
- The entire primary fermentation takes about six days.
- Yeasts float to the top during this period, they are scooped off and used for future pitching.

Top fermentation

- ✓ They are treated in **casks or bottles** in various ways.
- ✓ It is **'primed'** to improve its taste and appearance by the addition of a small amount of sugar
- ✓ The yeasts grow in the sugar and carbonate the beer.
- ✓ It is stored for **seven days or less at about 15°C.**
- ✓ beer is 'fined' by the addition of isinglass(precipitates yeast cells, tannins and protein-tannin complexes)
- ✓ *The beer is transferred to pressure tanks from where it is distributed to cans, bottles and other containers.*
- ✓ The filled and crowned bottles are passed through a pasteurizer, set to heat the bottles at 60°C for half hour.

Bottom fermentation

- Wort is inoculated to the tune of 7-15 x 106 yeast cells per ml of wort.
- Yeast is pitched in at 6-10°C and is allowed to rise to 10-12°C, which takes some three to four days
- It is cooled to about 5°C at the end of the fermentation.
- CO2 is released begins to collapse after four to five days as the yeasts begin to settle.
- The total fermentation period may last from 7-12 days

Bottom fermentation

- ✓**Lagering:** At the end of the primary fermentation above, the beer, known as 'green' beer, is harsh and bitter.
- ✓ The green beer is stored in closed vats at a low temperature (around 0°C), for six months
- ✓ To mature and make it ready for drinking
- ✓ Yeasts are sometimes added to induce this secondary fermentation
- ✓ The secondary fermentation saturates the beer with CO2
- ✓ Any tannins, proteins, and hop resins still left are precipitated during the Lagering period.

Bottom fermentation

✓

✓To reduce Lagering time, beer is stored at high temperature (14°C) to drive off volatile compounds

✓ The beer is then chilled at – 2°C to remove chill haze materials, and thereafter it is carbonated artificially

✓ In this way lagering could be reduced from 2 months to 10 days.

✓ Lagering gives the beer its final desirable organoleptic qualities

✓ The beer is filtered through membrane filters to remove these.

✓ The filled and crowned bottles are passed through a pasteurizer, set to heat the bottles at 60°C for half hour.

Fermentation Tanks

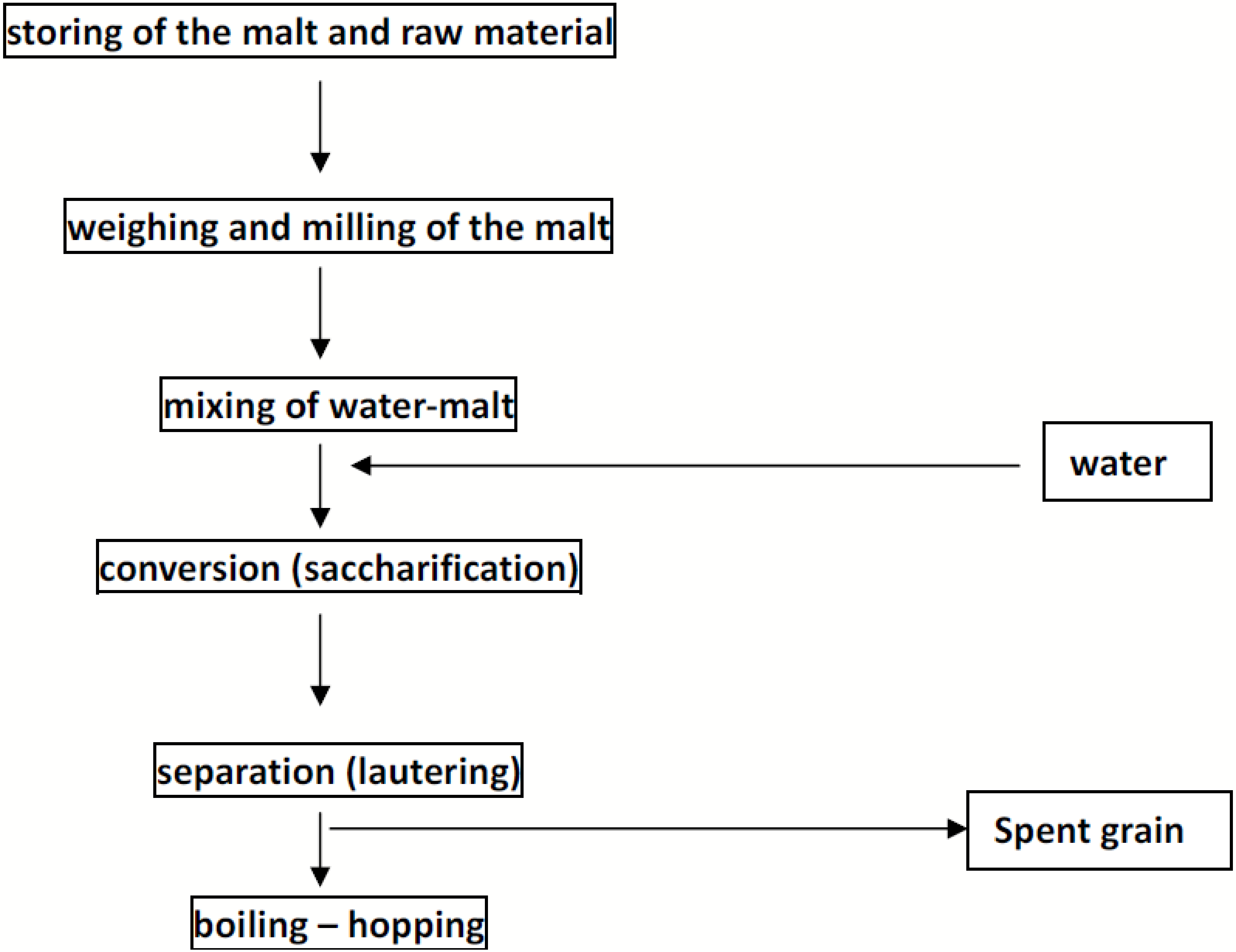
Adding yeast to the fermenter

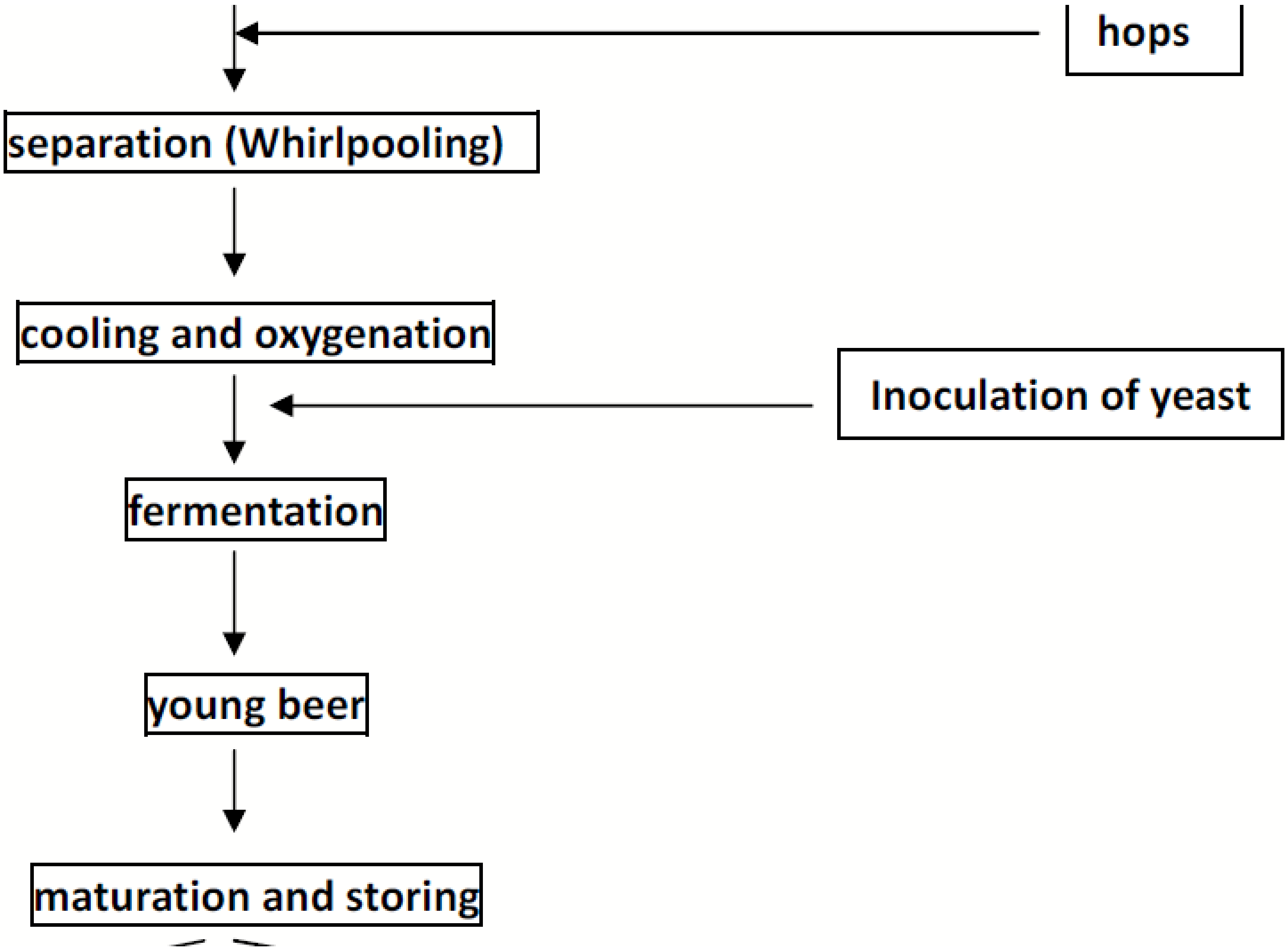
Blow-off hoses on fermentation tanks

Fermentation produces both ethanol and carbon dioxide. The carbon Dioxide is allowed to vent out through these blow-off hoses whose ends Are immersed in a tank of water, producing an air-lock and preventing Oxygen from entering the fermentation tanks.

Cleaning fermentation tanks

Cleanliness is critical in producing Quality beer. Microbial contamination Can result in off flavors and aromas.

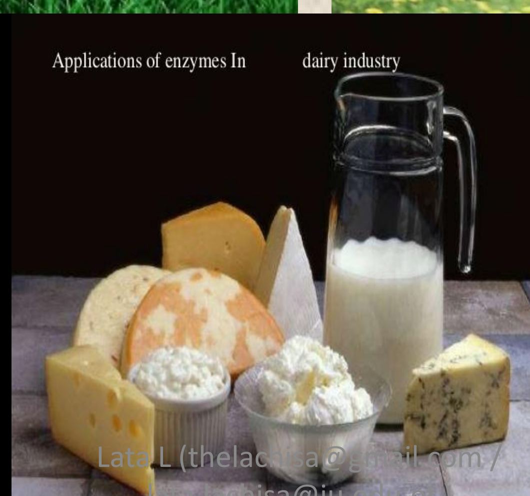

storing of the malt and raw material
weighing and milling of the malt
mixing of water-malt
water
conversion (saccharification)
separation (lautering)
Spent grain
boiling – hopping


hops
separation (Whirlpooling)
cooling and oxygenation
Inoculation of yeast
fermentation
young beer
maturation and storing

FOOD BIOTECHNOLOGY

4. BIOTECHNOLOGY IN DAIRY INDUSTRY



Milk

- **Milk** is the fluid from the mammary glands of animals.
- It is a complex liquid consisting of several hundred components;
- The most important are **proteins**, **lactose**, **fat**, **minerals**, **enzymes**, and **vitamins**
- When fat is removed from milk such as during butter making, the remnant is ***skim milk.***
- On the other hand, when casein is removed such as during cheese manufacture, the remnant is known as ***whey.***
- Whey is high in lactose and its disposal sometimes poses some problem

Milk

- A

- B.

Cheese

- Cheese is a highly proteinaceous food made from the milk of herbivores.
- The scientific study and manipulation of milk for cheese manufacture is however just over a hundred years old.
- Cheese made from the milk of **goat and sheep** has a much stronger flavor than that made from cow's milk.
- This is because the fat in goat and sheep milk contain much lower amounts of the **lower fatty acids**, **caproic**, **capryllic**, and **capric acids**.
- These acids confer a sharp taste

Stages in the manufacture of cheese

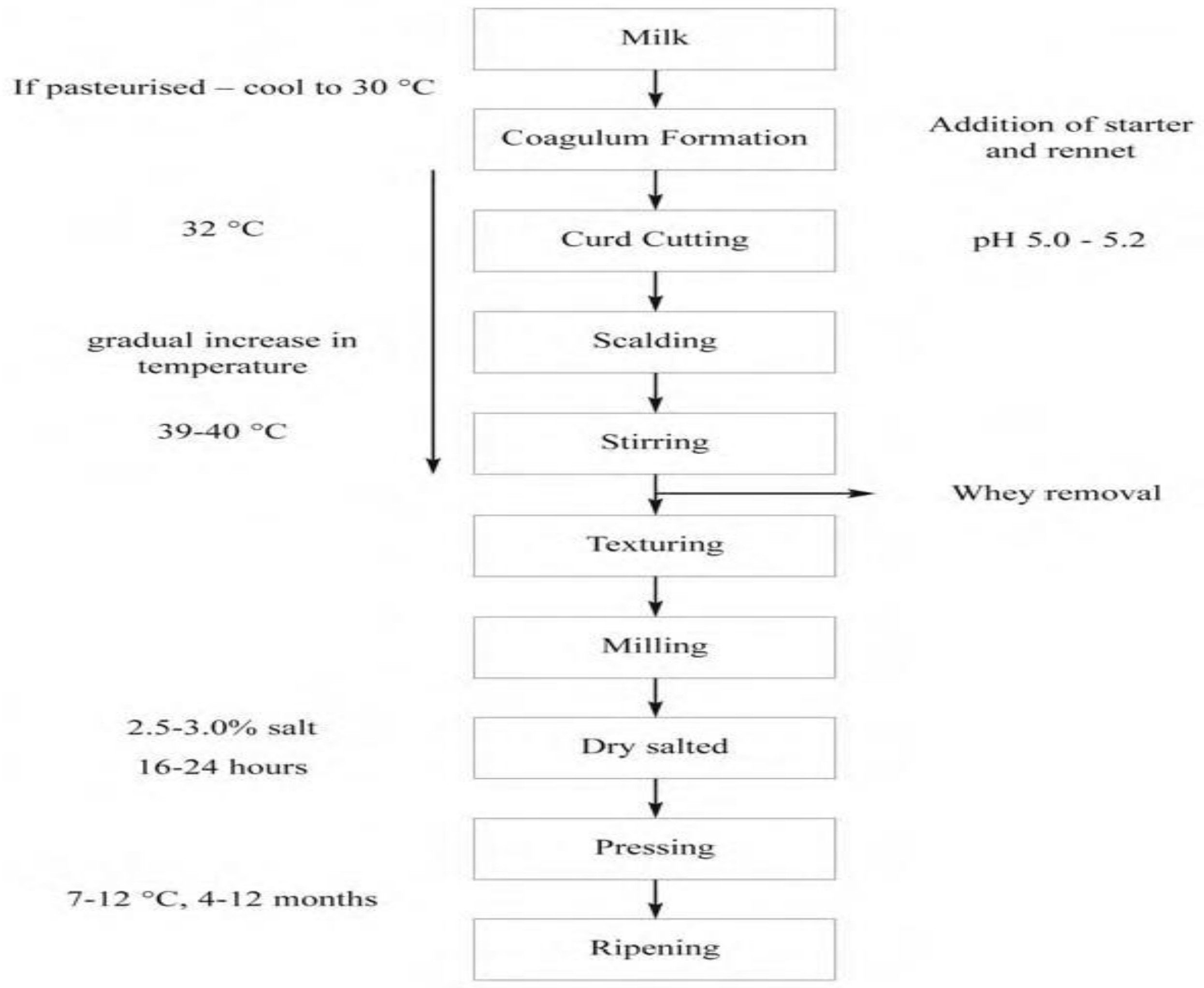
- The manufacture of all the types of cheeses include all or some of the following processes:
- **Standardization of milk:** The quality of the milk has a decided effect on the nature of cheese.
- In the US, **pasteurization** (HTST) must be given to milk, example **cottage** or **cream cheese.**
- For others the milk **need not be pasteurized.** but must be stored at for at least 60 days at 2°C.

Stages in the manufacture of cheese

- **Inoculation of pure cultures of LAB as *starter cultures***
- The choice of starter depends on the type of cheese being produced.
- The temperature of ***scalding/ cooking***, of the curd is an important consideration.
- For cheese prepared at temperatures ***less than 40°C strains*** of *Lactococcus lactis* are used. (mesophilic starters)
- For those prepared at higher ***temperatures (45 - 55 °C),*** the more thermophilic *Streptococcus thermophilus, Lactobacillus bulgaricus and Lact. helveticus are used.*

Stages in the manufacture of cheese

- **Production of Cheddar cheese**



Stages in the manufacture of cheese

- ***Lactic acid has the following effects/significance:***
- It provides a favourably **low pH** for the action of rennin the enzyme which forms the curd from casein in other types of cheeses.
- The low pH eliminates **proteolytic** and other undesirable bacteria.
- Metabolic products from the LAB such as **ketones, esters** and **aldehydes** contribute to the flavor of the cheese.

- **Problems of lactic acid bacteria in cheese-making;**
- ***Attack by bacteriophages***
- ***Inhibition by penicillin and other antibiotics***
- ***Undesirable strains***
- ***Sterilant and detergent residues***

Stages in the manufacture of cheese

- ## Adding of rennet for coagulum formation

- The classical material used in the formation of the coagulum is ‘rennet’ which is derived from the fourth stomach, a bomasum or vell of freshly slaughtered ***milk-fed calves.***
- Rennet is produced by soaking air-dried vells under acid conditions with **12-20% salt.**
- Extracts from young calves contain **94% rennin** and 6% pepsin and from older cows, **40% rennin** and 60% pepsin.
- **Rennin** (chymosin) is the enzyme responsible for the coagulation of the milk.
- The enzymes used in cheese making are now obtained from microorganisms, **mainly fungi.**

- Some commercial microbial rennet and their microbial sources

Commercial Rennet	*Microbial source*
Harmilase	*Mucor miehei*
Rermilase	*Mucor miehei*
Fromase	*Mucor miehei*
Emposase	*Mucor pusillus*
Meito	*Mucor pusillus*
Suparen	*Endothia parasitica*
Surd curd	*Endothia parasitica*
Mikrozyme	*Bacillus subtilis*

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- **Shrinkage of the curd**
- The removal of whey and further shrinkage of the curd is greatly facilitated by heating
- ***Cutting it into smaller pieces***, applying some pressure on it and lowering the pH.
- Expulsion of whey and contraction of curd
- Acid produced by the lactic starters introduce elasticity in the curd, a property desirable in the final qualities of cheese.

Salting of the curd and pressing into shape

- Salt is added to most cheese varieties at some stage in their manufacture.
- In the manufacture of **Cheddar**, salt is added to the milled curd before pressing
- **Gouda** and **Camembert**, the moulded cheese is immersed in concentrated brine.
- **Salt** is important not only for the **taste**, but it also contributes to **moisture** and **acidity control.**
- Most importantly however it helps limit the growth of **proteolytic** bacteria which are undesirable.
- The curd is pressed into **shape** before being allowed to **mature.**

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Cheese ripening

- The ripening or maturing: which converts the brittle white curd or raw cheese to the final full-flavored cheese.
- Blue-veined cheese **Roqueforti**, **Gorgonzola**, **Stilton**, conferred by ***Penicillium roquefort***
- **Swiss cheese**, with its characteristic flavor and holes produced by the fermentation products and gases from ***Propionibacterium spp.***
- **Yeasts, micrococci, and *Brevibacterium linens*** impart the characteristic flavor of **Limburger cheese.**

- Ripening conditions vary with cheese variety.
- **Soft, high-moisture** cheeses are ripened for relatively **short periods**, whereas hard, strongly flavoured cheeses may ripen for more **than a year.**
- **Temperature** also varies, Cheddar is normally ripened at approximately 10 °C.
- Blue-veined cheeses are made to have an open texture so that **sufficient oxygen is present** in the cheese to allow the growth of *P. roquefortii* throughout

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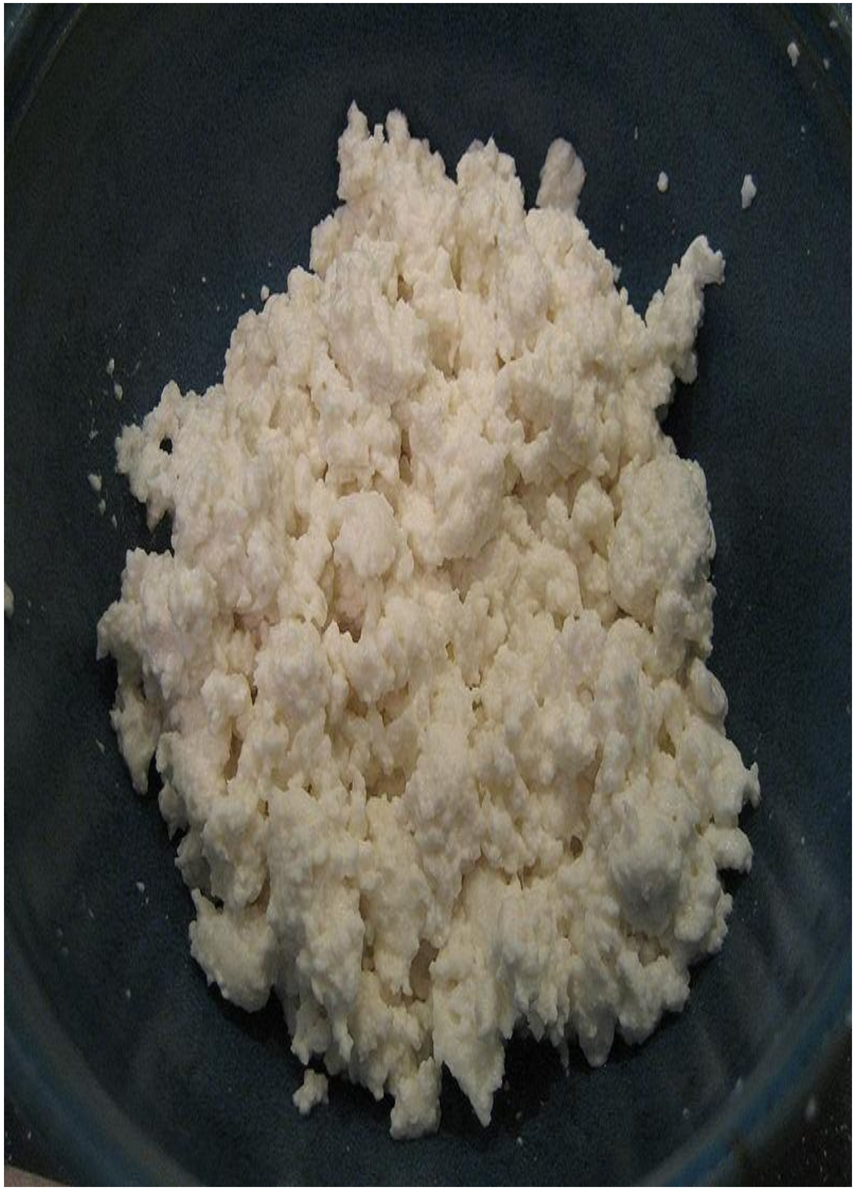
Strain improvement

- Metabolic engineering of lactic acid bacteria
- ***Lactococcus lactis*** is a well-characterized bacterium on the level of physiology and especially, molecular biology.
- This opens the way for application of genetically modified *L. lactis* strains in the very near future.
- The metabolism of *L. lactis* is relatively simple with energy metabolism centered on rapid conversion of ***sugar into lactic acid***

- ✓ Recently a very efficient and tightly-controlled expression system was discovered in *L. lactis*, involving the production of the antimicrobial peptide **nisin.**
- ✓ ***S. thermophilus,*** with a smaller genome size than *L. lactis*, appears to have an even more limited metabolic capacity than *L. lactis*.
- ✓ However, its metabolism has not been studied in such detail as *L. lactis* and its genetics are far less developed.

- Gene expression system from *L. lactis* has been introduced successfully in *S. thermophilus*.


Swiss cheese
Note the characteristic"eyes" (holes)



FOOD BIOTECHNOLOGY

5. BIOTECHNOLOGY OF BAKING INDUSTRY

FERMENTED FOOD FROM WHEAT: BREAD

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Ingredients for Modern Bread-making

- Bread has been known to man for many centuries and excavations have revealed that bakers' ovens were in use by the Babylonians, about ***4,000 B.C.***
- Bread is therefore a major food of the world.
- The basic ingredients in bread-making are **flour**, **water**, **salt**, and **yeasts.**
- In modern bread making however a large number of other **components** and **additives** are used as knowledge of the baking process has grown.
- These components depend on the **type of bread** and **regulations of operating country**.

Ingredients for Modern Bread-making

- They include 'yeast food',
- sugar,
- milk,
- eggs,
- shortening (fat) emulsifiers,
- anti-fungal agents,
- anti-oxidants,
- enzymes,
- flavouring, and enriching ingredients.
- The ingredients are mixed together to form dough which is then baked.

Ingredients for Modern Bread-making

- **Flour;**
- Flour is the chief ingredient of bread and is produced by *milling the grains of wheat,* various species and varieties of which are known.
- For flour production most countries use *Triticum vulgare. A few countries use T. durum*
- The chief constituents of flour are starch (70%), protein (7-15%), sugar (1%) and lipids (1%).
- Flour proteins are of two types;

Ingredients for Modern Bread-making

- The first types account about 15% of the total, are soluble in water and dilute salt solutions and is **non-dough forming.**
- It consists of ***albumins, globulins, peptides, amino acids, and enzymes.***
- The remaining 85% are **insoluble** in aqueous media and are responsible for **dough formation.**
- They are collectively known as **gluten.** It also contains lipids.
- **Gluten** has the unique property of forming an **elastic structure** when moistened with water.

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Ingredients for Modern Bread-making

- Gluten can be easily extracted, by adding enough water to flour and kneading it into dough.
- After allowing the dough to stand for an hour the starch can be washed off under a running tap water leaving a tough, elastic, sticky and viscous material which is the **gluten.**
- It forms the skeleton which holds the starch, yeasts, gases and other components of dough.
- **Gluten** can be classified in to two based on alcohol solubility as(gladilins and glutenins).
- **Q. What is the difference between gladilins and glutenins?**

- Hard wheat with a high content of protein (over 12%) are best for making bread **(Why?)**
- Soft wheat with low protein contents (9-11%) is best for making cakes.
- **Yeast;**
- The yeasts used for baking are strains of ***Saccharomyces cerevisiae.***
- The ideal properties of yeasts used in modern bakeries are as follows;

- Hard wheat with a high content of protein (over 12%) are best for making bread
- Soft wheat with low protein contents (9-11%) is best for making cakes.
- **Yeast;**
- The yeasts used for baking are strains of ***Saccharomyces cerevisiae***
- The ideal properties of yeasts used in modern bakeries are as follows;

- *Ability to grow rapidly at room temperature of about **20-25°C***
- *Ability to produce large amounts of CO_2 rather than alcohol in flour dough*
- *Good keeping quality i.e., ability to resist autolysis when stored at 20°C;*
- *Ability to adapt rapidly to **changing substrates** such as are available to the yeasts during dough making.*
- *High invertase and other enzyme activity to hydrolyze sucrose to higher glucofructans rapidly;*

- Ability to grow and synthesize enzymes and coenzymes under the anaerobic conditions of the dough.
- Ability to resist the osmotic effect of salts and sugars in the dough
- High competitiveness i.e., high yielding in terms of dry weight per unit of substrate used.
- Easy dispersability in water
- The amount of yeasts used during baking depends on the flour type, ***the ingredients used in the baking***, and ***the system of baking used.***

- **Very strong flours** (i.e., with high protein levels) require more yeast than softer ones.
- High amount of components is inhibitory to yeasts e.g., sugar (over 2%), antifungal agents and fat) usually require high yeast additions.
- Baking systems which involve short periods for dough formation need more yeast than others.
- In general however yeast amounts vary from 2-2.75% (and exceptionally to 3.0%) of flour weight.
- The roles of yeasts in bread-making are **leavening, flavor development** and **increased intuitiveness.**

Sugar;

- Significance of Sugar
- To provide carbon nourishment for the yeasts additional to the amount available in flour sugar.

Ingredients for Modern Bread-making

- To sweeten the bread
- To afford more rapid browning (through sugar **caramelization**) of the crust and hence greater moisture retention within the bread.

- **Shortening (Fat)**
- Animal and vegetable fats are added as shortenings in bread-making at about 3% (w/w) of flour in order to yield.
- Increased loaf size
- A more tender crumb and enhanced slicing properties
- Butter is used only in the most expensive breads; lard (fat from pork) may be used
- Vegetable fats especially **soy bean oil,** because of its most assured supply is now common.

- **Emulsifiers (Surfactants)**
- Emulsifiers are used in conjunction with **shortening** and ensure a better distribution of the latter **in the dough.**
- Emulsifiers contain a fatty acid, palmitic, or stearic acid
- Which is bound to one or more poly functional molecules with carboxylic, hydroxyl, and/or amino groups
- Emulsifiers are added as 0.5% flour weight.
- Commonly used surfactants include: calcium stearyl- 2-lactylate, lactylic stearate, sodium stearyl fumarate.

Milk

- Milk to be used in bread-making must be heated to high temperatures before being dried

Significance of milk;

Ingredients for Modern Bread-making

- **To make the bread more nutritious**
- **To help improve the crust color**
- The milk substitutes are added in the ratio of 1-2 parts per 100 parts of flour
- Skim milk and various components including **whey, buttermilk solids**, **sodium or potassium caseinate, soy flour** and/or **corn flour are used**

Ingredients for Modern Bread-making

- **Salt**

- About 2% sodium chloride is usually added to bread. It serves for the following purposes
- *It improves taste*
- *It stabilizes yeast fermentation*
- *As a toughening effect on gluten*
- *Helps retard proteolytic activity, which may be related to its effect on gluten*
- *It participates in the lipid binding of dough.*
- Salt is preferably added at (later stages of dough or at the early stages of baking)

• Water

- Water is needed to form gluten, to permit swelling of the starch, and to provide a ***medium for the various reactions*** that take place in dough formation.
- Water is not softened for bread-making because, as has been seen, calcium is even added
- Water with high sulphide content is undesirable because gluten is softened by the sulfhydryl groups.

Enzymes

- Sufficient amylolytic enzymes must be present during bread-making to breakdown the **starch** in flour into **fermentable sugars.**
- Flour is supplemented during the milling of the wheat with malted barley or wheat to provide this enzyme.
- Fungal or bacterial amylase preparations may be added during dough mixing.
- Bacterial amylase from ***Bacillus subtilis*** is particularly useful because it is **heat-stable** and partly **survives the baking process.**
- Proteolytic enzymes from ***Aspergillus oryzae*** are used in dough making, particularly in flours with excessively high protein contents

- **Mold-inhibitors (antimycotics) and enriching additives**

- The spoilage of bread is caused mainly by the fungi *Rhizopus, Mucor, Aspergillus* and *Penicillium*.
- The chief antimycotic agent added to bread is **calcium propionate.**
- Others used to a much lesser extent are sodium diacetate, vinegar, mono-calcium phosphate, and lactic acid.
- Bread is also often enriched with various **vitamins and minerals** including thiamin, riboflavin, niacin and iron.

Systems of Bread-making

- Large-scale bread-making is mechanized.
- The processes of yeast-leavened bread-making may be divided into:
- **Pre-fermentation (or sponge mixing):**
- A portion of the **ingredients** is mixed with yeast and with or without flour to produce an inoculum.
- **Dough mixing:**
- The balance of the **ingredients** is mixed together with the inoculums to form the dough.

- **Cutting and rounding**:
- The dough formed above is cut into specific weights and rounded by machines.
- **First (intermediate) proofing:**
- The dough is allowed to rest for about ***15 minutes*** at about ***27°C.*** This is done in equipment known as an **overhead proofer**
- **Molding:**
- The dough is flattened to a sheet and then molded into a spherical body and placed in a baking pan which will confer shape to the loaf.

- **Second proofing:**
- This consists of holding the dough for about ***1 hour* at *35-43°C*** and in an atmosphere of high humidity (89-95°C).
- **Baking:**

Systems of Bread-making

- During baking the proofed dough still in the final pan is transferred to the oven where it is subjected to an average temperature of **215-225°C for 17-23 minutes**
- **Cooling and wrapping:**
- The bread is depanned, cool to 4-5°C and wrapped in waxed paper, or plastic bags.

The Three Basic Systems of Bread-making

- There are three basic systems of baking.
- All three are essentially similar and differ only in the ***presence or absence of a pre-***fermentation.
- ***Reading assignment how the three system differ from each other?***
- ***Sponge doughs***
- ***The liquid ferment system***
- ***The straight dough system***

- **Methods of Leavening:**
- Leavening ***is the increase in the size of the dough induced by gases during bread-making***
- Leavening may be brought about in a number of ways.
- Air or carbon dioxide may be forced into the dough

Role of yeasts in bread-making

- Water vapor or steam which develops during baking has a leavening effect
- Oxygen has been used for leavening bread
- Leavening by microorganisms, may be done by any facultative organism releasing gas under ***anaerobic conditions***

Factors which effect the leavening action of yeasts

- The nature of the sugar available
- Osmotic pressure
- Effect of nitrogen and other nutrients
- Effect on fungal inhibitors (anti-mycotic agents)
- Yeast concentration

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Baking

- Bread is baked at a temperature of about 225°C.
- As the baking progresses and temperature rises gas production rises and various events occur as below
- At about **45°C** the undamaged starch granules begin to gelatinize and are attacked by alpha-amylase, yielding fermentable sugars.
- Between **50 and 60°C** the yeast is killed.
- At about **65°C** the beta-amylase is thermally inactivated
- At about **75°C** the fungal amylase is inactivated
- Finally, the **gluten is denatured and coagulates**, stabilizing the shape and size of the loaf

Strain improvement of baker's yeast

- Baker's yeast is responsible for dough leavening by CO_2 production from fermentable sugars
- But also influences the **taste and texture** of bread and other bakery products.
- Wheat flour is composed mainly of starch, but also of maltose, sucrose, glucose, fructose and glucofructans.
- **Maltose** is continuously released from **starch** during the baking process due to the activity of **crop amylases.**
- baker's yeast could be **engineered** to directly use starch, the major carbohydrate in dough.

- **High tolerance** to stresses caused by high sugar concentration (sweet dough), drying and freezing (production of dried yeast and frozen dough, respectively) is necessary.

Strain improvement of baker's yeast

- The production of **baker's yeast** itself is also an important industrial branch

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6. Secondary metabolites for food industry

Q. What is the d/ce b/n primary and secondary metabolite? Examples…

- **Secondary metabolites** are organic molecules that are not involved in the normal growth and development of an organism
- **Primary metabolites** have a key role in survival of the species, playing an active function in the *photosynthesis and respiration.*
- *Examples:* **Primary metabolites**, **ethanol, lactic acid,** and **butanol** and **amino-acids**, **enzymes** and **nucleic acids.**
- **Secondary metabolites** include **antibiotics**, **pigments**, **toxins**, **pheromones**, **enzyme inhibitors**, **pesticides**, **antitumor agents** and **growth promoters** of animals and plants

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CONT…

- ***Q. At w/c growth curve of bacteria does sec met produced?***
- Secondary metabolites are frequently produced at highest levels during a transition from **active growth** to **stationary phase**.
- Secondary metabolism is produced in response to ***a restriction in nutrients.***
- Secondary metabolism appears to be restricted to some species of **plants and microorganisms**
- The ability to produce a particular secondary metabolite, especially in industrially important strains is **easily lost.(How ?)**

CONT…

- Treatment with ***Acridine dyes, exposure to high temperature*** or other treatments known to induce **plasmid loss**
- Secondary metabolic products of microorganism are of immense importance to humans.
- They have a major effect on the **health, nutrition**, and **economics** of our society.
- Different classes of these compounds are bioactive compound in several **medicinal, aromatic**, **colorant**, and **spice plants** and/or **functional foods**.

CONT…

- These compounds are an extremely diverse group of natural products synthesized by **plants**, **fungi**, **bacteria**, **algae**, and **animals.**
- Absence of secondary metabolites does not ***result in immediate death***, but rather in long-term impairment of the organism's survivability
- A simple classification of secondary metabolites includes **three main groups:**

Cont…

A. Terpenes (such as plant volatiles, cardiac glycosides, carotenoids and sterols)

- **Terpenoids** are the largest and most diverse family of natural products, ranging in structure from **linear** to **polycyclic molecules**
- All terpenoids are synthesized through the condensation of **isoprene units**
- **Examples;** *many flavor and aromatic molecules*, such as menthol, linalool, geraniol and caryophyllene

B. Phenolic compounds;

- Phenolic compounds are widely distributed in nature.
- Their chemical structures may vary greatly, including simple phenols such as **hydrobenzoic** acid derivatives and **catechols**
- Long chain polymer with high molecular weight, such as **catechol melanins**, **lignins** and **condensed tannins.**
- **Stilbenes** and **flavonoids** are phenolic compounds with intermediate molecular weight

C. Alkaloids

- Basic compounds synthesized by living organisms containing one or more heterocyclic nitrogen atoms
- Alkaloids constitute a very large group of secondary metabolites, with more than 12,000 substances isolated.
- It seems alkaloid function in plants and animals is linked to **defense mechanisms.**
- **Toxicity** is a good weapon to inhibit the action of **predators**, like **herbivores.**

Cont…

- **Antibiotics**
- Chemical compound produced by a microorganism
- W/C inhibit growth, destroy bacteria and microorganisms in dilute solutions.
- Sir Alexander Fleming first discovered the antibiotic properties of the mould ***Penicillium notatum*** in 1929 at St. Mary's hospital in London
- He noticed that ***Penicillium notatum*** destroyed ***Staphylococcus*** bacterium in culture.
- ***Q. What is the mechanism action of Penicillin on bacteria?***

Cont…

- A large number of antibiotics are produced by ***actinomycetes*** and in particular ***Streptomyces*.**
- They resemble a fungal mycelium in form, but have thinner filaments.
- ***Actinomyces griseus*** was first isolated from soil this bacterium produced a substance;
- That killed many bacteria unaffected by penicillin, including ***Tuburculosis bacillus.***
- The antibiotic was named **streptomycin.**

Production of plant secondary metabolites by plant cell culture

- **Biotechnology;** Offers an opportunity to exploit the cell, tissue, organ or entire organism by;
- Growing them **in vitro and to genetically manipulate** them to get desired compounds
- Plant cell cultures were first established in the late **1930s.**
- In 1956 that Pfizer Inc. filed the first patent for the production of metabolites by **cell cultures.**
- Larger quantities of **visnagin** and **diosgenin** were isolated from cell cultures than from the whole plant.

Increasing productivity of secondary metabolites by plant cell cultures

- Currently; plant cell culture has direct commercial applications as well as value in basic research of ***cell biology, genetics, and biochemistry.***
- In plants, most of the secondary metabolites are produced in **differentiated cells** or **organized tissues.**
- However, **callus** and **cell suspension** culture lack organ differentiation and hence produce low yields of these biochemicals.
- The yield of secondary metabolite by undifferentiated *tissue or cell* cultures can be increased by following techniques:-

Increasing productivity of secondary metabolites by plant cell cultures

- ✓ **Select proper cell line:**
- ✓ The heterogeneity within the cell population can be screened to select lines capable of accumulating **higher level of metabolite.**
- ✓ **Strain improvement** begins with the choice of a parent plant with high contents of the desired products for **callus** induction to obtain high-producing cell lines.
- ✓ In this method, a large population of cells is exposed to a ***toxic*** (**or** ***cytotoxic***) inhibitor or **environmental stress** and only cells that are able to resist the selection procedures will grow.

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Increasing productivity of secondary metabolites by plant cell cultures

✓ **Medium manipulation**:

- Manipulation of the ***culture environment*** must be effective in increasing the product accumulation.

✓The constituents of culture medium like *nutrients*, *phyto hormones* also the culture condition like *temperature*, *light etc* influence the production of metabolites.

✓ Sugar levels , Nitrate levels, *Phosphate levels*, *Growth regulators*

✓For e.g. if sucrose concentration is increased from **3% to 5%,** the production of **rosamarinic acid** is increased by five times.

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Increasing productivity of secondary metabolites by plant cell cultures

- **Elicitors**:
- Compounds that induce the production and accumulation of secondary metabolite in plants are known as **elicitors.**
- Elicitors produced within the plant cells include cell wall derived polysaccharides like pectin, pectic acid, cellulose etc.
- Elicitors of fungal, bacterial and yeast origin, polysaccharides, glycoproteins, inactivated enzymes etc...
- These elicitors when added to medium in ***low concentration*** (50-250ng/l) enhance metabolite production.

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Increasing productivity of secondary metabolites by plant cell cultures

- **Permeabilisation**:
- Secondary metabolites produced in cell are blocked in the vacuole.
- **Cell permeabilization** depends on the formation of pores in one or more of the membrane systems of the plant cell
- By manipulating the permeability of cell membrane, they can be elicited out to media. **How permeabilization applied?**
- Permeabilisation can be achieved by **electric pulse, UV, pressure**, **sonication, heat**.
- Even **charcoal** is added to medium to absorb secondary metabolites.

Increasing productivity of secondary metabolites by plant cell cultures

- **Immobilization:**
- Cell cultures encapsulated in a **garose** and **Calcium alginate gels** or entrapped in membranes are called **immobilized plant cell culture**.
- These immobilized systems effectively increase the productivity of secondary metabolites in number of species.
- **Elicitors** can also be added to these systems to stimulate secondary metabolism.

- **Food additives;**
- contribute to making *foodstuffs palatable* and attractive by enhancing or improving their **flavor, color,** and **texture.**
- Food technologies try to respond to these criteria especially with regard to the **texture**, **taste**, and **aroma** of the foodstuff.
- The need to have the same taste and aroma in order to suit the consumer tastes makes it compulsory to use **additional natural** or **artificial aromas**.

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Cont…

- The most valuable food additives that can be obtained from the plant cell cultures are;
- **Food colorants** (anthocyanins and betalaines),
- **Flavors** (saffron and vanillin),
- **Sweeteners** (steviosides),
- **Pungent food additives** (capsaicin),
- **Anti-bacterial food preservatives** (thiophene).

Cont…

- **Aromas and Fragrances;**
- Natural aromas are a mixture of numerous compounds
- Natural aromas are susceptible to the conservation processes of foodstuffs, such as sterilization, pasteurization, freezing, etc.
- Some aromas are altered by **enzymatic or chemical reactions** and usually disappear if **stored for a long period.**
- Artificial aromas used to be manufactured from ***coal or oil*** derivatives, and they used to be added in ***very low concentrations.***

Cont…

- Aromas from the cell cultures have an advantage of a constant **composition and are independent** on the season.
- The characteristic aromas of cocoa and coffee have been produced by cell cultures of *Tlaeobroma cacao* and *Coffea arabica*, respectively.

Cont…

- **Pigments;**
- The biotechnological methods used for producing natural food colorants consist of growing higher plant cells.
- **Anthocyanins:** are the large groups of water-soluble pigments responsible for many of the bright colors in **flowers and fruit.**
- They are commonly used in acidic solutions in order to impart **a red** color to **soft drinks, sugar confectionary**, **jams,** and **bakery toppings.**
- **Crocin**: the main pigment of *Crocus sativus* stigma is used extensively as **yellow** food colorant.

FOOD BIOTECHNOLOGY

7. Improving food quality through agricultural biotechnology

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INTRODUCTION

- **Agricultural biotechnology** is the term used in crop improvement through ***biotechnology tools***.
- The biotechnology tools that are important for agricultural biotechnology include:
- Conventional plant breeding
- Tissue culture and micro propagation
- Molecular breeding or marker assisted selection
- Genetic engineering and GM crops
- Molecular Diagnostic Tools

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Cont…

➔ The present and future focus is on continuing improvement of agronomic traits such as;

➔ yield and abiotic stress resistance

➔ biotic stress tolerance

➔ improved nutrition and food functionality

➔ plants as production factories for **therapeutics** and **industrial** products.

Improving the nutritional quality of cereal crops

- The FAO estimates that 850 million people worldwide suffer from under nutrition, of which insufficient protein in the diet.
- Most plants have a poor balance of essential amino acids relative to the needs of animals and humans.
- **The cereals** (maize, wheat, rice etc.) tend to be **low in lysine**, whereas **legumes** (soybean, peas) are often deficient in the sulfur-rich amino acids, **methionine and cysteine.**

- Successful examples of improving amino acid balance to date include high-lysine maize, canola and soybeans.

- Free lysine is significantly increased in high-lysine maize by the introduction of the dapA gene (cordapA) from *Corynebacterium glutamicum*

- Consumption of foods made from these crops potentially can help to prevent malnutrition in developing countries, especially among children.

- Genes that code for proteins containing **elevated levels** of the desired amino acid was investigated on sweet potato.

- This was achieved by several investigators **sweet potato** modified with an artificial storage protein (ASP-1) gene
- These transgenic plants exhibited **a two- and fivefold** increase in the total protein content in leaves and roots, respectively
- In order to increase the nutritional quality of cassava storage roots;
- A synthetic ASP1 gene encoding a storage protein rich in essential **amino acids (80%)** was introduced into embryogenic suspensions of cassava

Elimination of allergy causing substances

- ✓ Food allergy is a specific form of intolerance to a food or food component that activates the **immune system.**
- ✓ Food allergy is considered to affect 1–2% of the general population, and up to 8% of children below three years of age.
- ✓ Allergic reactions to food may affect the **skin**, **the gastrointestinal tract and the respiratory system**
- ✓ In principle **genetic modification** provides an exciting opportunity to manipulate the expression and/or sequences of plant proteins to reduce or eliminate their allergenicity.

Cont…

- In practice, this may be difficult to achieve for several reasons.
- *Many plant foods that result in severe allergic reactions contain multiple allergenic proteins*
- It is certainly possible to remove at least some epitopes by protein engineering, as demonstrated for the major **cherry allergen**
- The major dietary allergens in **rice grain** are inhibitors of human α-amylase
- A final important consideration in removing **allergens or epitopes** is that the effects must be permanent without any chance of reversal.

Availability of minerals

- Plant seeds are potentially important sources of minerals for nutrition of humans and livestock
- But a high proportion of the minerals present are unavailable as they are in the form of mixed salts of **phytic acid.**
- Thus, phytate accounts for over 70% of the total **phosphorus** as well as substantial amounts of Mg^{2+}, K^{+}, Fe^{3+}, Zn^{2+}, Ca^{2+} and Cu^{2+}.
- **Phytates** act as storage reserves in the seed and are degraded during germination.
- ***Animals cannot digest phytate and consequently it is excreted.***

Availability of minerals

- The excretion of phosphorus can lead to **eutrophication** of natural waters adjacent to farmland
- The low availability of calcium, iron and zinc in cereals and other plant foods can also contribute to **nutritional deficiency** in humans
- Genetic engineering can be used to digest the **phytin** and increase the mineral availability in seeds, by expression of ***genes encoding phytase.***
- Genes from *Aspergillus* species have been used for this as they express extracellular ***phytase enzymes*** and have been produced commercially.

Availability of minerals

- Feeding studies with transgenic soybean showed a 50% reduction in phosphate excretion by broiler chickens
- Iron deficiency is the most widespread mineral deficiency in humans which has been estimated to affect up to 30% of the total world population
- Improving the release from **phytates** is one possible strategy to increase iron availability.

FOOD BIOTECHNOLOGY

8. Single cell protein (SCP)

INTRODUCTION

- **Single-cell proteins (SCP)** are the dried cells of microorganism, which are used as protein supplement in **human foods or animal feeds**.
- Microorganisms like **algae, fungi**, **yeast** and **bacteria**
- With increase in population and worldwide protein shortage necessitated the use of **microbial biomass as food and feed.**
- **Yeast** was the first microorganism whose importance as **animal feed supplement** was recognized almost a century ago
- **Pruteen;** was the first commercial **SCP** used as animal feed additive.

Cont…

➔Q. What is the shortcoming of SCP production in developing countries?

➔ SCP production has a number of attractive features/significance?

➔ It can be produced through out the year

➔ Microorganisms have a much more **rapid growth** than plants or animals

➔Waste products can be turned into food in the production of SCP

➔ During the production of SCP biomass, certain microbes produce useful byproducts such as **organic acids**

Cont…

➔ Single cell protein high protein and low fat content.

➔ High efficiency substrate conversion

➔ Single cell protiens are good source of vitamin.

➔ Doesn't require sophisticated lab setup for algae and certain other microbes.

Cont…

- The major problem associated with the use of single cell proteins are;
- Many microbes produce various **toxic compounds**
- Single Cell Protein diet suppliments can pose **allergic reaction**.
- Consuming SCP, in-taking higher amount of **Nucleic acids** which can lead to **gastrointestinal problems.**
- SCP production are **expensive** due to the need to maintain high level sterility conditions in the production facility.

Microorganisms used in SCP production

- ✓ Different species of algae, fungi, yeasts and bacteria are used as single cell protein and produced at commercial scale
- ✓ Organisms to be used in SCP production should have the following properties:
- ✓ ***Absence of pathogenicity and toxicity***
- ✓ ***Protein quality and content***
- ✓ ***Digestibility and organoleptic qualities***
- ✓ ***Growth rate***
- ✓ ***Adaptability to unusual environmental conditions***

Substrates for single cell production

- A wide variety of substrates have been used for SCP production and include **hydrocarbons, alcohols,** and **wastes from various sources**.

A. Hydrocarbons as substrate

- Many genera of **yeasts and molds** utilize aliphatic hydrocarbons **(alkanes and alkenes**) for growth and for SCP production
- These microorganisms secrete emulsifying substances during fermentation which increase the solubility of **alkanes** and **alkenes.**
- The **higher lipid** content in these microorganisms **facilitates the hydrocarbon transport.**

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- **Gaseous hydrocarbons**
- Among the gaseous hydrocarbons, **methane** has been most widely studied as a source of SCP.
- Others which have been studied include **propane and butane**
- Perhaps its **greatest advantage** is the absence of **residual hydrocarbon** in the single cell protein produced from it
- One of its major **disadvantages** is that it is **highly inflammable**.
- Single cultures in methane are usually very ***slow growing***.

Liquid hydrocarbons

- The major source of liquid hydrocarbons is **crude petroleum.**
- Hydrocarbons were first studied as a source of microbial vitamins and lipids.
- Most crude petroleum oils are made up of **90-95%** hydrocarbons, which are most often saturated.
- During **petroleum refining,** the crude oil is first distilled at atmospheric pressure in a process known as **'topping'**.
- The petroleum hydrocarbons which have been used to grow SCP are **diesel oil, gas oil, fuel oil, n-alkanes**

Alcohols

- **Methanol**
- Methanol is produced by the oxidation of paraffins in the gas or liquid phase
- The catalysts are mixed zinc and chromium oxides.
- In **methanol fermentation** for SCP production, bacteria are preferred over fungi because of their **fast growth**, **high protein content** and **better yield.**
- **Methanol is suitable as a substrate for SCP for the following reasons:**

Alcohols

- It is highly soluble in water
- The explosion hazard of methanol is minimized
- It requires less oxygen than methane for metabolism by micro-organisms
- It is not utilized by many organisms.

Ethanol

- ✓ Ethanol is produced by the hydration of ethylene which itself is obtained during petroleum refining from coke oven gas
- ✓ Although ethanol can be utilized ordinarily by many bacteria and yeasts, as a substrate for SCP, it is largely used by **yeasts**
- ✓ Fermentation of ethanol for SCP production **requires comparatively less oxygen** and hence releases considerably **less heat**
- ✓ Yeast produced from ethanol is being produced and marketed as **a flavor enhancer in baked foods, pizzas, sauces**, etc., in the United States
- ✓ Disadvantage in using ethanol for SCP production is that it is expensive

Waste Products

- Petroleum-based substrates was increasing rapidly, will be much less used in the future
- Attention is being turned more and more to substrates derived from plants which are renewable during photosynthesis.
- A large number of reports of SCP production from waste material lies scattered in the literature.

- They may be discussed under the following general headings;
- **Plant/wood wastes (**corn cobs, stems, leaves, husks, etc).
- **Starch-wastes:** (rice, potatoes, or cassava)
- **Dairy wastes:(** Whey….. rich in lactose)
- **Wastes from chemical industries:** (A waste mixture of organic acids)
- **Miscellaneous substrates**(Molasses)

Advantages of using Microorganisms (for SCP production)

1. MO grow at very fast rate under optimal conditions
2. Quality and quantity is better than higher plants and animals
3. Wide range of raw materials can be used
4. Culture and fermentation conditions are simple
5. MO can be genetically manipulated easily than plants and animals
6. Have relatively high protein content and nutritional value of the protein is good.
7. Can be grown in vast numbers in relatively small continuous fermentation process, using relatively small land area

Limitations of using SCP/Factors that impair the usefulness of SCP:

1. Nucleic acid content is very high (40% algae; 10-15% bacteria and 5-10% yeast)
2. Presence of carcinogenic and toxic substances
3. Contamination of pathogenic MO
4. Indigestion and allergic reactions
5. Production of food grade SCP is expensive
6. Unacceptable coloration (mainly with algae),
7. Disagreeable flavour (part in algae and yeasts) and
8. The need to kill before consumption.

- Thus, SCP is treated with various methods in order to
  - kill the cells,
  - improve the digestibility, and
  - reduce the nucleic acid content.

Some SCPs…

- A large number of algae, yeasts, molds, and bacteria have been studied as SCP sources. Among the most promising genera and species employed include the following:

1. Yeasts

- *Candida guilliermondii, C. utilis, C. lipolytica, and C. tropicalis; Debaryomyces kloeckeri; Candida famata, C. methanosorbosa; Pichia spp.; Kluyveromyces fragilis; Hansenula polymorpha; Rhodotorula spp.; and Saccharomyces spp. Saccharomyces cerevisiae, Pichia pastoris, Candida utilis=Torulopsis and Geotrichum candidum (=Oidium lactis*)),

2. Filamentous fungi

- *Agaricus spp, Aspergillus oryzae, Fusarium venenatum, Penicillium spp, Trichosporon cutaneum, Sclerotium rolfsii, Polyporus and Trichoderma*),

3. Bacteria

- *Bacillus spp.; Acinetobacter calcoaceticus; Cellulomonas spp.; Nocardia spp.; Methylomonas spp.; Aeromonas hydrophila; Alcaligenes eutrophus (Hydrogenomonas eutropha), Mycobacterium sp.; Spirulina maxima, and Rhodopseudomonas sp.*

4. Algae

- *Chlorella spp. Scenedesrnus spp* and *Spirulina*

Single Cell Protein Products

A. Mushrooms

- **Pekilo prossess**
- filamentous fungus **Paecilomyces variotii**
- use of waste from wood processing (monosaccharides + acetate)
- use as animal feed
- **Pruteen**
- methanol (from methane - natural gas) as C1 carbon source
- methylotrophic bacteria (*Methylophilus methylotrophus)* feed protein
- **Quorn**
- fungal mycelium*, Fusarium (mycoprotein) for human consumption*
- processing to resemble meat
- Bacteria, yeasts, and molds can be grown on a wide variety of materials, including food-processing wastes (such as cheese whey and brewery, potato processing, cannery, and coffee wastes), industrial wastes (such as sulfite liquor in the paper industry and combustion gases), and cellulosic wastes (including bagasse, newsprint mill, and barley straw).
- In the case of cellulosic wastes, it is necessary to use organisms that can utilize cellulose, such as a *Cellulomonas* sp. Or *Trichoderma viride.* A mixed culture of *Cellulomonas* and *Alcaligenes* has been employed.

9. FOOD PRESERVATION TECHNIQUES

- Process of treating and handling foods to stop or slow down spoilage (loss of quality, edibility or nutritional value) and thus allow for longer storage
- Usually involves preventing the growth of bacteria, yeasts, fungi, and other microorganisms (although some methods work by introducing benign bacteria or fungi to the food) as well as retarding the oxidation of fats which cause rancidity
  - *The main reasons for food preservation* are to overcome
    - inappropriate planning in agriculture,
    - produce value-added products, and
    - provide variation in diet.
  - In food preservation, the important points that need to be considered are
    - The desired level of quality
    - The preservation length
    - The group for whom the products are preserved

Reasons of food preservation:

- To protect food against microbes and other spoilage agents, - *controlling food spoilage*
- to ensure that food is safe for future consumption and
- to prolong food storage time.

Principles of preservations two general principles are employed in food preservation.

1. Inhibition principles of microbial growth,

i. Reduction of water activity e.g. By drying and salting
ii. Reduction in pH e.g. by fermentation & addition of acids.
iii. Use of preservatives, e.g. sodium benzoate
iv. Use of low temperatures (chilling or freezing)
v. Smoking – which has a drying and preservative effect, etc.

2. Killing principles of microorganisms,

- **Heat treatment**: through pasteurization, canning or sterilization
- **Irradiation** with either ionizing or electromagnetic radiation, e.g gamma rays, cobalt 60 radioactive particles. Radiations kill microorganisms by destruction of DNA and creating toxic reactive compounds in a medium and in microbial cells
- **Use of gases**: by use of ethylene oxide or ozone. The gases destroy both vegetative cells and spores.

Causes of Food Deterioration

- Mechanical, physical, chemical, and microbial effects

Common Food Bourne Pathogens

- **1. Salmonella enteriditus**
  - A common bacteria responsible for severe cases of food poisoning and digestive ailments in humans.
  - Often transmitted through contact with infected animals or ingestion of raw or undercooked egg that has contacted a contaminated shell.
- **2. Campylobacter jejuni**
  - Common bacterial pathogen found in undercooked meats, unpasteurized milk, and other contaminated foods.
  - Responsible for more than 10% of all instances of diarrhea in the US.
- **3. Fungi**
  - A variety of fungi are used in the production of foods, including cheeses and yogurt.
  - Other fungi are contaminants, some even toxic.
  - Often appear on breads with high moisture and sugar content, citrus fruits and grains.
  - Usually spread by airborne spores as opposed.
  - Often appear as fuzzy, blue growth in canned corn or fuzzy, white spots on bread.

Methods of Food Preservation

- Preservation methods start with the complete analysis and understanding of the whole food chain, including
  - growing, harvesting, processing, packaging, and distribution;
- thus an integrated approach needs to be applied.
- It lies at the heart of food science and technology, and it is the main purpose of food processing.
- First, it is important to identify the properties or characteristics that need to be preserved.

Low temperature approach to food preservation

- Low temperatures are desirable for food storage
- Cold slows metabolism and retards microbial growth
- *Listeria monocytogenes* can grow in cold storage
- Found in certain dairy products
- Refrigeration at 5°C retards but does not stop microbial growth
- microorganisms can still cause spoilage with extended time
- growth at temperatures below -10°C has been observed
- Refrigeration/ Freezing
- **Frig**. – few days only – bacteriostatic
- **Freezing** – the placement of a food product in subzero temperatures. also bacteriostatic
  - Softer textures due to ice crystals
  - May enhance thawed food spoilage

High temperature approach to food preservation

- High temperatures desirable to prevent food spoilage, but Proteins and enzymes become denatured. Includes: ***Canning***, ***Pasteurization***, …

Canning - Commonest method, Moist heat under pressure, Balances flavor/safety

- Food heated in special containers to 115° C for 25 to 100 minutes
- Kills spoilage microbes, but not necessarily all microbes in food
- Heat treated to kill microbes
- Low acid: kill most spore formers
- High acid: kill all vegetative bacteria

Pasteurization - Destroys vegetative pathogens

- Kills vegetative pathogens and substantially reduces number of spoilage organisms
- Different pasteurization procedures heat for different lengths of time
  - High Temperature Short Time (HTST) - 72°C, 15 secs
  - Low Temperature Long Time (LTLT) - 70°C, 30 mins
  - Ultra High Temperature (UHT) - 88°C, 3 secs
- Refrigeration not needed

Other approaches to food preservation

1. Radiation

- **Ultraviolet (UV) radiation** - used for surfaces of food-handling equipment, does not penetrate foods
- **Radappertization** - use of ionizing radiation (e.g. gamma ray) to extend shelf life or sterilize meat, seafoods, fruits, and vegetables. It kills microbes in moist foods by producing peroxides from water. Peroxides oxidize cellular constituents
- **Irradiation** – the treatment of food products with low levels of ionizing radiation to kill microorganisms in food.
  - Safe but expensive, X-rays, γ-rays, Public education is needed
  - Aseptic packaging: Pre-sterilized materials assembled into packages and aseptically filled.
  - Gamma radiation kills bacteria, insects, and parasitic worms. peroxides oxidize cellular constituents
  - High-energy electrons

2. Drying - Dessication/Dehydration/salting, preservation methods dependent on the removal of water from food products, providing an inhospitable environment for microorganisms.

- Removal of 90% or more of moisture, Microbes may not be killed,
- Osmotic control with salt/sugar adds bactericidal effects
- Removal of 90% or more of moisture, Microbes may not be killed
- Osmotic control with salt/sugar adds bactericidal effects
  - Sun / Wind / Ovens
  - Meat and Fruit

3. Smoking -

- Dries the Food
- Enhances the Flavor
- Beef, Ham, Bacon, Fish
- Adds Formaldehyde

4. Addition of sugar and other chemicals -

- sugaring/Salting and Pickling
- Rock Salt / Sea Salt / Spiced Salt
- Cheese / Olives / Hams / Eggs

5. Pickling- utilizes a vinegar-based solution to soak foods, creating an environment in which bacteria may not survive.

6. Honeys, etc

7. **Steam Sterilization-** utilizes super-heated water to kill surface bacteria. Often used in the processing of meats

8. Chemical-based preservation

GRAS - chemical agents ―generally recognized as safe‖ includes the following:

1. Benzoic acid (C6H5COOH)

- Sodium benzoate (salt of benzoic acid) - the 1st chemical preservative permitted in foods by the FDA,
- The anti-microbial activity is related to pH.
- Most effective at low pH and ineffective at neutral pH.
- pH of food impacts effectiveness of chemical preservative

2. Sorbic acid (CH3CH=CHCH=CHCOOH)

- Usually used as Calcium, Sodium or potassium salt.

3. Propionates

- The Propionic acid (CH3CH2COOH) and its Ca and Na salts are permitted in breads, cakes, cheeses, etc. as mold inhibitor.

4. Nitrates and Nitrites

- Sodium nitrate (NaNO3) and Sodium nitrite (NaNO2) are commonly used. They are used in meats to stabilize red meat color.
- They inhibit some spoilage and food poisoning organisms and also contribute to flavor development.

5. Acetic acids and lactic acids

6. Anti-fungal agents for fruits

- Benomyl is applied uniformly over the entire surface of fruits.
- Both benomyl and thiabendazole are effective in controlling dry rot caused by *Fusarium* species

7. Antibiotics

- *Nisin* effective against G+ bacteria, primarily, spore formers, but ineffective against fungi and G- bacteria
- *Natamycin* - against yeasts and molds but not bacteria.

FOOD BIOTECHNOLOGY

10. IMPROVING THE INTESTINAL MICOFLORA

- Bacteria, unicellular eukaryotes, and other organisms inhabit the human body in large numbers.
  - It is estimated that the human microbiota contains as many as 10^{14} bacterial cells,
- A number that is 10 times greater than the number of human cells present in our bodies.
- By far, the most heavily colonized organ is GIT which houses a huge microbial ecosystem
  - The microbiota of the human GIT plays a key role in efficient nutrient absorption and thus maintaining human health

- ✓ **Probiotics** are ***live bacteria*** that could exert health beneficial effects upon consumption.
- ✓ **Prebiotics** are indigestible food components that could promote the growth of **beneficial bacteria** including **probiotics.**
- ✓ **Synbiotic** (probiotic + prebiotic) has been found to exert a synergistic effect in improving colon carcinogenesis compared to when both were used individually.

Probiotics

- The word "**probiotic**" (origins: Latin pro meaning "*for*" and Greek **bios** meaning "*life*")
- Was first used in 1954 to indicate substances that were required for a healthy life
- "***Live micro-organisms;*** which, when administered in adequate amounts, confer a health benefit on the host". (FAO/WHO, 2001)
- Probiotic microorganisms must; ***be living*** and ***exert scientifically proven health effects.***

Cont…..

- Certain strain of bacteria haven been discovered over the years to have probiotic properties,
- Mainly consisting of lactic acid producing bacteria;
- (***Lactobacilli,***
- ***Streptococci,***
- ***Enterococci,***
- ***Lactococci,***
- ***Bifidobacteria* & Bacillus)**
- **Fungi such as *Saccharomyces and Aspergillus***

Q. What will be the Selection of probiotic candidates?

- To be active in the colon, probiotics must resist;
- *Salivary enzymes & stomach acid,*
- *Small intestinal secretions of bile and enzymes*
- *The pH changes and chemical milieu of other foods and beverages*
- *Culturability on a large scale & Safe for humans*
- *Genetic stability and maintaining viability in a food product or supplement.*

- ***What are the significance of probiotics:***
- Prevention and reduction of rotavirus & alleviation of complaints due to lactose intolerance.
- Reduction of **cancer-promoting** enzymes and putrefactive (bacterial) metabolites in the gut.
- *Reduction of **Helicobacter pylori*** infection or bacterial overgrowth.
- Prevention or alleviation of allergies and atopic diseases in infants.

Cont…

- ✓ Prevention and alleviation of irregular complaints of the **gastrointestinal tracts** in healthy people.
- ✓ Normalization of passing stool and stool consistency in subjects suffering from **obstipation or an irritable colon.**
- ✓ Prevention or alleviation of **allergies and atopic** diseases in infants.
- ✓ Prevention of **respiratory tract infections** and **treatment of urogenital infections.**

Health relevant effects of probiotics

- ***Control of growth of undesirable organisms in the IT***
- *Lb. acidophilus*, *Lb. casei*, and species of *Bifidobacterium* can inhibit;
- Growth of *Salmonella & Escherichia coli* infections intestinal tract of chickens birds.
- Ingestion of cells of Bi.bifidum reduced shedding of **rotavirus.**
- S. ***Lb. acidophilus*** excreted an antimicrobial substance active against ***Helicobacter pylori***

How probiotic bacteria inhibit the growth of undesirable microorganisms in the intestinal tract?

- Several of these organisms produce antibiotic-like substances **bacteriocins**
- An antimicrobial agent produced by *Lb. reuterii*, has a broad spectrum of activity **(Reuterin)**
- **Competitive exclusion:**
- The ability of **lactobacilli or bifidobacteria** to occupy binding sites on the intestinal wall
- Preventing attachment and growth of ***enteric pathogens.***

Improvement of immune response

- ✓ *Lb. Casei* & *Bi.longum;* stimulate the immune system to control *E. coli* in the gastrointestinal tract.
- ✓ Probiotic microorganisms and their cell-wall components (peptidoglycans, lipopolysaccharides),
- ✓ DNA and metabolites were improve the immune response by modulating the immune response
- ✓ **Example;** stimulating phagocytes/macrophages & natural killer cells

Improving of lactose digestion

- People who lack the ability to digest lactose adequately are classified as lactose intolerance / lactose malabsorption''
- It is associated with the inability to digest lactose into its constituents, glucose and galactose
- Inadequate levels of **β-galactosidase** in the small intestine to hydrolyze ingested **lactose adequately.**
- Inclusion of a purified enzyme **β-galactosidase** in the diet is expensive and survival of the enzyme is minimal.

- Beneficial action results from presence of β-galactosidase in the bacterial cells.
- Being inside the bacterial cells protects the enzyme during passage through the **stomach**
- And active when yogurt reaches the **small intestine**
- Once the yogurt culture reaches the small intestine, it interacts with **bile**
- Increases permeability of the cells of these bacteria & enables the substrate to enter and be hydrolyzed

- The starter cultures used for yogurt manufacture are not bile resistant
- Non-fermented milk containing cells of ***Lb. acidophilus*** also can be beneficial for lactose maldigestors.
- As with yogurt cultures, cells of *Lb. acidophilus* do not lyse in the presence of bile,
- but their permeability is increased permitting lactose to enter the cells and be hydrolyzed.

- **Control of serum cholesterol**
- Several studies have shown that animals consuming milk containing cells of *Lb. acidophilus* had lower serum cholesterol levels
- Some strains of *Lb. acidophilus* can actively assimilate or take up cholesterol during growth in **laboratory media.**
- A portion of the cholesterol is incorporated into the cellular membrane of *Lb. acidophilus*.
- Some of these include *Lb. Casei* and *Bifidobacterium* species. *Bi.longum* removes cholesterol from laboratory media

- **Prebiotic**
- **Prebiotic:** “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of **one** or **a limited** number of bacteria in the colon.
- Sometimes they are termed nondi-gestible oligosaccharides (NDOs).
- Prebiotic activity has been seen in **short-chain** carbohydrates that are resistant to digestion in the upper GI tract but are hydrolyzed and fermented in the colon.

- **Examples of prebiotics;**
- (Inulin,
- Fructo- oligosaccharides,
- Poly dextrose,
- Arabinogalactan,
- Lactulose,
- Lactitol,
- Galacto-oligosaccharide (GOS)

- **Synbiotic;** are products that contain both probiotics and prebiotic.
- These products have the **"good" bacteria** (**probiotics**) and the **non-digestible carbohydrate** source **(prebiotics)** to encourage the growth of beneficial bacteria.
- **Probiotics + Prebiotics = Synbiotics**
- Synbiotic has been found to exert a synergistic effect in improving colon *carcinogenesis* compared to when both were used individually.

- The most common Synbiotic combinations available include;
- **Bifidobacteria and fructo-oligosaccharides(FOS),**
- **Lactobacillus GG and inulin,**
- **Bifidobacteria and Lactobacilli with FOS or inulin**

11. Social and Ethical Considerations Regarding Genetically Engineered Foods

Genetic modification (GM)

- is any process that changes the genetic material of an organism (plant, animal, bacteria or virus) so that it is capable of producing new substances or performing new or different functions.

Examples:

- a genetically modified cloned cow produces milk lacking the whey protein beta-lacto globulin (BLG), a protein to which an estimated 2–6% children are allergic to;
- Genetically engineered bacteria can be used to clean up an oil spill at sea.
- A number of ethical and safety issues need to be considered with genetic modification.
- Some concerns expressed by consumers include fears that the results of genetic modification could harm the environment and pose a danger to humans.

Genetically modified (GM) food

- Foods which have been produced from genetically modified organisms (GMOs) are likely to appear no different from food produced by traditional means. A series of laboratory tests would be needed to show that genes had been changed. Genetically modified (GM) foods can only be authorised in the European Union if they have passed a rigorous safety assessment. In the EU, if a food contains or consists of genetically modified organisms (GMOs), or contains ingredients produced from GMOs, this must be indicated on the label. The GM Food and Feed Regulation lays down rules to cover all GM food and animal feed, regardless of the presence of any GM material in the final product.

- The potential direct health effects of GM foods are generally comparable to the known risks associated with conventional foods, and include, for example, the potential for allergenicity and toxicity of components present, and the nutritional quality and microbiological safety of the food.
- As mentioned above, many of these issues have not traditionally been specifically assessed for conventional food; but in one area — toxicity of food components — there is ample experience related to the use of animal experiments to test potential toxicity of targeted chemical components. However, the intrinsic difficulty in testing whole foods, as opposed to specific components, in animal feeding experiments have resulted in the development of alternative approaches for the safety assessment of GM foods.

Cultural variability and public perception

- Across the world, food is a part of cultural identity and societal life, and has religious significance to people. Therefore, any technological modification, including changes to the genetic basis of crops or animals used for food, may be met with social resistance.

Labelling of GM foods and consumer choice

- In establishing GM food labelling policies to ensure that consumers receive meaningful information, regulatory authorities have had to grapple with a complex array of issues related to GMOs. These have included scientific, health, environmental, political, cultural and economic issues, as well as the appropriate compliance and enforcement requirements.

Two broad regulatory approaches for labelling of GM food exist:

- Voluntary labelling — which is driven largely by market forces, with no legislative requirements to declare the use of GMOs in food production; and
- Mandatory labelling — which requires declaration of characteristics imparted to a food by the use of gene technology (be they health-and-safety and/or process-related), or use of gene technology itself in food production.

Coexistence of different agricultural practices

- The potential risk of outcrossing and contamination by dispersed material from GM plants can pose problems for organic farming, as defined in Codex *Guidelines*. Coexistence of agricultural practices must respect the threshold limits set for contamination of organic products and realize the difficulty of adhering to this goal for certain plants.
- Agricultural practices that include GMOs may need to develop improved agricultural or molecular systems which enable a benign coexistence of GM and GM-free agriculture, in which a limited level of outcrossing is accepted. Otherwise, separation of GM plants with a significant potential for outcrossing from conventional or organic farming may be necessary.
- At present, views on the problems of coexistence as well as management solutions vary from country to country. In various countries, representatives of specific areas are developing strategies for segregation of GM crops and crops of conventional or organic farming.

Economic cost of adopting GM crops

- Numerous reports from organizations either in favour or critical of GM foods have been published, and numerous claims for increased or decreased profitability of agricultural practices including GMOs can be found.
- A review of the United States National Centre for Food and Agricultural Policy concludes that biotechnology is having, and will continue to have, a significant impact on improved yields, reduced grower costs and reduced pesticide use. GM *Bt* cotton seems to have relevant benefits for smallholder farmers in many areas around the world. On the other hand, some report lower yields, continuing dependency on chemical sprays, loss of exports and critically reduced profits for farmers as a consequence of using biotechnology.
- A United States Department of Agriculture report on the economic consequences of GM crops summarized a positive impact of the adoption of *Bt* cotton on net farm returns, but a negative impact in the case of *Bt* maize. An improvement of returns has also been seen with herbicide-tolerant maize, whereas no significant impacts were observed with herbicide-resistant soybean.
- A very detailed study by the European Commission on the economic impact of GM crops on agriculture found that a quick adoption by farmers in the USA was the result of strong profitability expectations. However, there was no conclusive evidence on the farm-level profitability of GM crops.
- The most immediate and tangible ground for farmer utility of GM crops appears to be the combined effect of performance and convenience of GM crops — in particular, herbicide-tolerant varieties. For insect-resistant crops like *Bt* maize, yield losses are more limited than for conventional maize. However, the cost-efficiency of *Bt* maize depends on a number of factors, especially growing conditions.
- In China, a region with a typically high baseline of pesticide use and cases of pesticide poisoning in farmers, a report showed that the use of *Bt* cotton substantially reduced the use of pesticides without reducing the output per hectare or the quality of cotton. This resulted in substantial economic and health benefits for small farmers.

Socioeconomic aspects in the use of GMOs

- Socioeconomic consequences arising from the adoption of GMOs in agriculture require an analysis of consequences for specific groups and interests in society. It has been claimed that there are benefits for large-scale farming, as opposed to small-scale farming, as a result of better adoption of practices associated with GMOs by large-scale farmers, as well as an ability to deal with IPRs. Microenterprises and microcredit schemes are considered by some to be the way to achieve the Millennium Goal of eradicating poverty. Some groups analyzing trade and agriculture feel that the impact of large-scale production and marketing of GMOs would overshadow potential success stories from a few GM products in developing countries.
- Social scientists often discuss the importance of a shift from rural areas with labour-intensive workplaces to areas with 'high-tech' industry. Such shifts could also potentially take place as a result of the introduction of GMOs. An example here could be whether the economies of tropical oil-producing countries could be affected if GM alternatives to palm and coconut oils are engineered and production then moved to other countries.

>>>> THE END <<<<

Assignment for assessment (30%)

1. Describe Downstream processing (including definition, Stages involved, product recovery methods, Ways of purification of products, possible ways of larger scale production of the product and preservation techniques for enhancing shelf life) (10%)
2. How agricultural biotechnology improvement can also improves food security, quality and safety (10%)
3. Define fermentation and list at least five traditionally fermented foods and beverages in Ethiopia. Then, select one food or beverage from your listed; write the Product description, raw materials, involved microbes for fermentation, Procedures, Output (product and final succession microbes), the nature of product (bioactive compound), Preservation methods (Management of product) and traditional knowledge involved (10pts).