FOOD BIOTECHNOLOGY – Biol. 3132

CHAPTER ONE

THE DEFINITION AND SCOPE OF FOOD BIOTECHNOLOGY

Introduction:

- The use of biotechnology in food production is not new. It has been used for thousands of years. Early examples of biotechnology include the domestication of animals, planting of crops and the use of micro-organisms to make cheese, yogurt, bread, beer and wine. Products can be developed again (formulated), often changing or improving the end product.
- For example: reducing saturated fat, sugar or salt of a product; increasing nutrient availability; improving the taste of a product, etc.
- 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", is 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 fermentation process in food biotechnology offers a method of preservation, e.g. by producing acid which lowers the acidity (converting a perishable food into one that has a longer shelf-life); can be used to change the nutritional value of food products, e.g. converting milk to cheese; can create or improve sensory characteristics of foods (flavour, aroma and texture).
- Future biotechnology is promising as it is estimated that global population is to rise to around eight billion by 2030 and probably to over nine billion by 2050:
  - 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.

Definitions: What is Food Biotechnology?

- Food biotechnology is the evolution of traditional agricultural techniques such as crossbreeding and fermentation.
- It is an extension of the type of food development that has provided nectarines, tangerines and similar advancements.
- Food biotechnology 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.
- Remember… genes are sections of DNA that code for protein.
- Called genetically engineered (GE) or genetically modified (GM) foods, they are a source of an unresolved controversy over the uncertainty of their long-term effects on humans and food chains.
- Nicknamed "Franken foods" by anti-GM food groups.
- Food technology based on biology
    - Ancient food biotechnology:
        - Fermentation by microbes: Cheese, Beer, Wine, Bread, etc
        - Modern food biotechnology
        - Tissue culture,
        - Genetic engineering

Modern food biotechnology:

- According to the definition of the Codex Alimentarius Commission (CAC 2001a) (adapted from the Cartagena Protocol on Biosafety — see Section 3.3), modern biotechnology is defined as the application of (i) in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or (ii) fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers, and that are not techniques used in traditional breeding and selection.
- The application of modern biotechnology to food production presents new opportunities and challenges for human health and development. Recombinant gene technology, the most well-known modern biotechnology, enables plants, animals and microorganisms to be genetically modified (GM) with novel traits beyond what is possible through traditional breeding and selection technologies.

- It is recognized that techniques such as *cloning*, *tissue culture* and *marker-assisted breeding* are often regarded as modern biotechnologies, in addition to genetic modification.
- The inclusion of novel traits potentially offers increased agricultural productivity, or improved quality and nutritional and processing characteristics, which can contribute directly to enhancing human health and development.
- From a health perspective, there may also be indirect benefits, such as reduction in agricultural chemical usage, and enhanced farm income, crop sustainability and food security, particularly in developing countries.
- The novel traits in genetically modified organisms (GMOs) may also, however, carry potential direct risks to human health and development. Many, but not all, genes and traits used in agricultural GMOs are novel and have no history of safe food use.
- Several countries have instituted guidelines or legislation for mandatory premarket risk assessment of GM food. At the international level, agreements and standards are available to address these concerns.
- GMOs may also affect human health indirectly through detrimental impacts on the environment, or through unfavourable impacts on economic (including trade), social and ethical factors.
- These impacts need to be assessed in relation to the benefits and risks that may also arise from foods that have not been genetically modified.
- For example, new, conventionally bred varieties of a crop plant may also have impacts — both positive and negative — on human health and the environment.

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

What Does the Future Hold? Food biotechnology 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?

- Food biotechnology is and will continue to be an important area in science as the world's human population continues to increase and the world's agricultural lands continue to decrease.
- The following are reasons why "we" genetically modify food.

1) Extended Shelf Life

- The first steps in genetic modification were for food producers to ensure larger profits by keeping food fresher, longer.
- This allowed for further travel to and longer availability at markets, etc…

Example: Long Shelf Tomatoes

- These genetically modified tomatoes promise less waste and higher profits.
- Typically, tomatoes produce a protein that softens them after they have been picked.
- Scientists can now introduce a gene into a tomato plant that blocks synthesis of the softening protein
- Without this protein, the genetically altered tomato softens more slowly than a regular tomato, enabling farmers to harvest it at its most flavorful and nutritious vine-ripe stage.

2) Efficient Food Processing

- By genetically modifying food producing organisms, the wait time and quantity of certain food processing necessities are optimized.
- Again this is a money saver.

Example: Rennin Production

- The protein rennin is used to coagulate milk in the production of cheese.
- Rennin has traditionally been made in the stomachs of calves which is a costly process.
- Now scientists can insert a copy of the rennin gene into bacteria and then use bacterial cultures to mass produce rennin.

- This saves time, money, space and animals.

3) Better Nutrient Composition

- Some plants, during processing, lose some of the vital nutrients they once possessed.
- Others are grown in nutrient poor areas.
- Both these problems can be solved by introducing genes into plants to increase the amount or potency of nutrients.
- “Biofortification”

Example: Golden Rice

- Scientists have engineered "golden rice", which has received genes from a daffodil and a bacterium that enable it to make beta-carotene.
- This offers some promise in helping to correct a worldwide Vitamin A deficiency.

4) Efficient Drug Delivery - “Biopharming”

- Inserting genes into plants/animals to produce essential medicine or vaccines.

Many Unpatented Examples

- A cow with the genetic equipment to make a vaccine in its milk could provide both nourishment and immunization to a whole village of people now left unprotected because they lack food and medical help (in progress).
- Bananas and potatoes make hepatitis vaccines (done).
- Making AIDS drugs from tobacco leaves (done).
- Harvest vaccines by genetically altering hydroponically grown tomato plants to secrete protein through their root systems into the water (done).

Potential Problems???

- With every technology there is an associated risk involved.
- The following are some examples of potential problems associated with food biotechnology.

1) Creating “Superbugs”

- Since many of the “vectors” used to introduce genes to plants and animals are bacteria and viruses, it is realistic to think there is a chance they could undergo a mutation and prove harmful or become recombinant like the H1N1 virus and thus more virulent.
- However, the bacteria and viruses used in these procedures are usually non-pathogenic.

2) Negative Effects on Human Health

- Most of these food products undergo testing to see if any adverse health effects occur.
- However, allergies were not thought of in one case where a gene from a brazil nut was transferred to soy bean plants!
- Thankfully a food product was not pursued as someone came to their senses!
- Important to note that not all genes from a potential allergenic food will cause an allergy.

3) 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

CHAPTER TWO

FERMENTATION TECHNOLOGY OF TRADITIONAL ETHIOPIAN FOODS AND BEVERAGES

Introduction:

- There is a huge diversity of foods where microbial activity is an essential feature of their production. Numerous food products owe their production and characteristics to the fermentative activities of microorganisms.
- Many foods such as ripened **cheeses, pickles, yoghurt, beer, sauerkraut, and others** are preserved products in that their shelf life is extended considerably over that of the raw materials from which they are made.

What is fermentation?

- Fermentation is the metabolic process in which carbohydrates and related compounds are oxidized with the release of energy in the absence of any external electron acceptors.
- The final electron acceptors are organic compounds produced directly from the breakdown of the carbohydrates.
- Only partial oxidation of the parent compound occurs, and only a small amount of energy is released during the process.
- In broad context, fermentation is a process in which chemical changes are brought about in an organic substrate through the action of enzymes elaborated by microorganisms.
- The primary benefit of fermentation is in the conversion of sugars and other carbohydrates, e.g., converting juice into wine, grains into beer, carbohydrates into carbon dioxide to leaven bread, and sugars in vegetables into preservative organic acids.
- Fermentation is derived from the Latin Verb - Fervere - to boil
  - Describing the appearance of the action of yeast on extracts of fruit or malted grain.
  - The boiling appearance is due to the production of carbon dioxide bubbles caused by the anaerobic catabolism of the sugars present in the extracts.
  - Two of the most important and commonly occurring pathways of fermentation are:
    - Homolactic acid fermentation and
    - Heterolactic/Alcoholic fermentation.

- Fermentation can occur via many other pathways. The process is used widely to brew beer, make wine and vinegar, make yogurt, etc

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.

Lactic Acid Bacteria - LAB

- Major group of Fermentative organisms.
- This group is comprised of 11 genera of gram-positive bacteria:
  - *Carnobacterium, Oenococcus, Enterococcus, Pediococcus, Lactococcus, Streptococcus, Lactobacillus, Vagococcus, Lactosphaera, Weissells and Leucconostoc*
- Related to this group are genera such as *Aerococcus*, *Microbacterium*, and *Propionbacterium*.
- The lactic acid bacteria can be divided into two groups based on the end products of glucose metabolism.
- Those that produce lactic acid as the major or sole product of glucose fermentation are designated homofermentative.
- Those that produce equal amounts of lactic acid, ethanol and CO_2 are termed heterofermentative.

- All members of *Pediococcus*, *Lactococcus*, *Streptococcus*, *Vagococcus*, along with some lactobacilli are homofermenters.
- *Carnobacterium*, *Oenococcus*, *Enterococcus*, *Lactosphaera*, *Weissells and Lecconostoc* and some Lactobacilli are heterofermenters
- The heterolactics are more important than the homolactics in producing flavour and aroma components such as acetylaldehyde and diacetyl.
- The lactic acid bacteria are mesophiles:
  - ✓ they generally grow over a temperature range of about 10 to 40°C,
  - ✓ an optimum between 25 and 35°C.
  - ✓ Some can grow below 5 and as high as 45 °C.
  - ✓ Most can grow in pH range from 4 to 8. Though some as low as 3.2 & as high as 9.6.

TRADITIONAL ETHIOPIAN FOODS AND BEVERAGES

1. 'INJERA' FERMENTATION

- 'Injera' is sour, leavened pancake-like bread used in Ethiopia. In Ethiopia, 'injera' is made mainly from 'Teff '(*Eragrostis tef*), but other cereals such as wheat, barley, maize and sorghum may be used for the same purpose.
- Some of the commonly identified microorganisms are moulds, *Enterobacteriaceae*, aerobic mesophilic bacteria and the dominant yeasts.
- Fermentation is an acid type and *Enterobactriaceae* initiates it. Lactic acid bacteria are responsible for acid production. Yeasts take part only at late stage.
- The presence of lactic acid bacteria during fermentation not only produce necessary metabolites for flavor and taste, but also inhibit the growth of undesirable microorganisms.
- Addition of Sorbates and Benzoates prior to baking reduces the spoilage.

2. 'KOCHO ' FERMENTATION

- 'Kocho' is a fermented product prepared in Ethiopia from Ensete (*Ensete Vemtricosum*) which, sometimes, is called 'false banana'.
- Fermentation is initiated by *leuconostic mesenteriodes*. In eight days, the pH drops from 6.5 to 5.6 and two *Lactobacillus spp (L.cornyforms and L.plantarium)* dominate to reduce pH to 4.2 within the next five days.
- Spore formers occur during the first fifteen days. The population of yeast reaches to their maximum population between 22-43 days.

3. 'SILJO' FERMENTATION

- 'Siljo' is a fermented condiment made from safflower (*Carthamus tinctorius*) extract and Faba bean (*Vicia faba*) flour.
- Black mustard serves as a source of starter microorganisms. This is because the substrate has *Lactobacillus acidophilus*, *L. plantarum* and *L.* d*elbrueki* to initiate the fermentation and dominate the fermentation process.
- One study reported that *Enterococcus, Bacillus, Lactococcus, Lactobacillus* and yeasts dominate the fermenting' siljo'.
- Studies also indicated that the final pH of 'Siljo' is 4.0 which is dropped from4.5 in 36 hours.

4. 'AYIB' (COTTAGE CHEESE) FERMENTATION

- 'Ayib' is a traditional Ethiopian cottage cheese made from sour milk after the fat is removed by churning.
- Study results indicated that aerobic mesophilic gram positive rods and gram negative bacteria *(Enterobacteriaceae*, *pseudomonas spp*, Lactic acid bacteria) and some yeasts were found in Ayib.

5. AWAZE FERMENTATION

- 'Awaze' is one of the traditional fermented condiments, and consumed with other items. It is a fermented vegetable spice.
- Red pepper (*Capsicum annum*), garlic (*Allum ursinum*), ginger (*zingber officinale*) are the raw ingredients to make 'Awaze'. It is commonly used in central and northern Ethiopia with raw meat.
- Lactic acid bacteria found in high number and *Bacillus* is also dominant flora found in it. Study indicated that aerobic mesophilic bacteria decrease during fermentation and yeast increases.
- Hetrofermentative lactic acid bacteria dominate at the beginning and homofermentative take over the fermentation later.
- Studies have indicated that 'Awaze ' has strong antibacterial activity towards some pathogenic species such as *Salmonella thyphimurium.*

6. TELLA FERMENTATION

- 'Tella' is one of most important Ethiopian drink, which is more or less fermented from barely, wheat maize, millet, sorghum, and other cereals.
- The fermentors are *Lactobacillus* species and yeasts (S*accharomyces* species). Yeasts are the dominant one and present throughout the fermentation.
- The increment in alcohol content is accomplished by yeast growth & decrease the reducing sugar.

7. 'TEJ' FERMENTATION

- 'Tej' is an Ethiopian traditional wine prepared from honey. A mixture of honey and sugar may be used as major fermentable substrates. Tej fermentation is a natural process which relies on the microorganisms present in the substrates.
- Yeast (genus *saccharomyces*) is responsible for the converting of sugars to ethanol in 'Tej'. In addition to yeast, *Lactobacillus*, S*treptococcus*, *Leuconostoc* and P*ediococcu*s are species which have roles in the fermentation.

8. 'BORDE' FERMENTATION

- 'Borde' is one of the traditional fermented beverages made from maize or wheat .It is a very popular meal in southern parts of Ethiopia and some other parts of the country. Borde' is consumed while in an active stage of fermentation.
- Aerobic mesophilic bacteria, Lactic acid bacteria, *Enterobacteriaceae* and yeast are commonly found in 'Borde' with different concentration.
- Hetrofermentative *Lactobacilli* dominated the lactic flora throughout the fermentation and yeast count increases as fermentation proceeds.

9. 'WAKALIM' FERMENTATION <<<<<<<<<<**ETC** >>>>>>>>>>

CHAPTER THREE

BIOTECHNOLOGY IN THE PRODUCTION OF BEER

Introduction

- Alcoholic beverages have been produced throughout recorded human history.
- They are manufactured worldwide from locally available fermentable materials, which are sugars derived either from fruit juices, plant sap and honey, or from hydrolyzed grain and root starch.
- The term beer is given to non-distilled alcoholic beverages made from partially germinated cereal grains, referred to as malt.
- They include ales, lagers and stouts, which normally contain 3–8% (v/v) ethanol.
- Microorganisms have long played a major role in the production of food and beverages and yeasts are primarily used, either in single or mixed cultures.
- Their fermentation products are ethanol, a range of desirable organoleptic (flavour and aroma) compounds and CO_2 (provides carbonation for some products).
- The yeasts involved in these alcoholic fermentations are mostly strains of *Saccharomyces cerevisiae*, which cannot directly ferment starch.
- They require prior hydrolysis of the polysaccharide to simple sugars and small dextrins (not greater than three glucoseunits).

Enzymatic Reactions:

- The first part of beer manufacture - the enzymatic steps- begins in the malting houses that convert barley into malt.
- It is the malt that serves as the source of the amylases, proteinases, and other enzymes necessary for hydrolysis of large macromolecules, such as starch and protein.
- For most beers, the malt also serves as the substrates for those enzymes (i.e., malt contains the starch and protein hydrolyzed by malt enzymes)

Raw Materials for Brewing

- Generally, the raw materials used in brewing are: barley malt, adjuncts, yeasts, hops, and water.

1. Barley malt

- Two distinct barley types are known.

1. One with **six rows** of fertile kernel *(Hordeum vulgare*) and
2. The other with **two rows** of fertile kernels (*Hordeum distichon*).

- The six-row variety is used extensively in the United States, whereas the two-row variety is used in Europe as well as in parts of the US.
- The six-row varieties are richer in protein and enzyme content than the two-row varieties.

2. Adjuncts

- Adjuncts are starchy materials which were originally introduced because the six-row barley varieties grown in the United States produced malt that had more diastatic power (i.e. amylases) than was required to hydrolyze the starch in the malt.
- For example the term now includes sugars (e.g. sucrose) added to increase the alcoholic content of the beer.
- Since adjuncts contain little nitrogen, all the needs for the growth of the yeast must come from the malt.
- The malt/adjunct ratio hardly exceeds 60/40.
- Adjuncts fall into two categories:
    - **Grains:** corn, rice, wheat, oats or rye
    - **Specialty Ingredients:**
        - sweets – honey or maple
        - fruits – raspberry, cherry or cranberry
        - spices – cinnamon, coriander or clove

3. Hops

- Hops are the dried cone-shaped female flower of hop-plant *Humulus lupulus* (synomyn: *H. americanus, H. heomexicams, H. cordifolius*).
- It is a temperate climate crop and grows wild in northern parts of Europe, Asia and North America.

- It is botanically related to the genus *Cannabis,* whose only representative is *Cannabis sativa* (Indian hemp, marijuana, or hashish).
- Provide the precursors of the bitter principles in beer and the essential (volatile) oils which provide the hop aroma.

Hop flowers are used to:

- MICROBIAL STABILIZATION - hops have antiseptic qualities
- HOP AROMA - fruity flowery characteristics
- BITTERNESS - balances malt sweetness
- FOAM STABILIZATION - enhances head properties

4. Water

- The mineral and ionic content and the pH of the water have profound effects on the type of beer produced.
- In general calcium ions lead to a better flavor than magnesium and sodium ions.
- Water is so important that the natural water available in great brewing centers of the world lent special character to beers peculiar to these centers.
- Water styles can effect flavor:
  - ✓ hard water – helps add crisp cleanness
  - ✓ soft water – adds smoothness

5. Brewer's yeasts

- Yeast is the catalyst of change: one cell micro-organism, produces carbon dioxide and alcohol
- Yeasts in general will produce alcohol from sugars under anaerobic conditions, but not all yeasts are necessarily suitable for brewing.
- Brewing yeasts are able, besides producing alcohol, to produce from wort sugars and proteins a balanced proportion of esters, acids, higher alcohols, and ketones which contribute to the peculiar flavor of beer.
- two types of brewers' yeasts are used to produce beer:
  - the top fermenting yeasts (ale) and
  - the bottom fermenting yeasts (lager)
- There are literally thousands of brewer's yeast that create a variety beer styles

Brewery Processes

- Steps in the process of beer production: the processes involved in the conversion of barley malt to beer may be divided into the following:

1. Malting
2. Cleaning & milling of the malt
3. Mashing
4. Wort boiling
5. Fermentation
6. Storage or lagering
7. Packaging

1. Malting: - Takes place in malt houses, occasionally in a brewery (Coors)

- Malting involves the controlled partial germination of barley grain.
- This modifies the hard vitreous grain into afriable (easily crushed) form containing more readily degradable starch and generates hydrolytic enzymes,
- These enzymes are produced by the germinated barley to enable it to break down the carbohydrates and proteins in the grain to nourish the germinated seedling

2. Cleaning and milling of malt

- The barley malt is cleaned of dirt and passed over a magnet to remove pieces of metals, particularly iron. It is then milled.
- 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.
- However, very fine particles hinder filtration and prolong it unduly.

3. Mashing: - grain and hot water mixture and takes place in a mash mixer

- The purpose of mashing is to extract as much as possible the soluble portion of the malt and to enzymatically hydrolyze insoluble portions of the malt and adjuncts.
- In essence mashing consists of mixing the ground malt and adjuncts at temperatures optimal for amylases and proteases derived from the malt.
- The aqueous solution resulting from mashing is known as wort.

Starch hydrolysis:

- The objective in mashing is to convert as much of the malt and adjunct starch as possible to fermentable sugars.
- Malt enzymes involved in starch degradation are collectively referred to as diastase, consisting of a mixture of β-amylase, α-amylase, limit dextrinase (a debranching enzyme) and α -glucosidase.

Mash separation

- At the end of mashing, husks and other insoluble materials are removed from the wort in two steps. First, the wort is separated from the solids. Second, the solids themselves are freed of any further extractable material by washing or sparging with hot water.

4. Wort boiling

- Wort – clear sugary liquid containing fermentable carbohydrates collected in the boil kettle or just kettle during run off, also called sweet wort
- The wort is boiled for 1-1½ hours in a brew kettle (or copper)
- When corn syrup or sucrose is used as an adjunct it is added at the beginning of the boiling.
- Hops are also added, some before and some at the end of the boiling.
- The purpose of boiling is as follows.

1. To *concentrate* the wort sugar, which loses 5-8% of its volume by evaporation during the boiling?
2. To *sterilize* the wort to reduce its microbial load before its introduction into the fermentor.
3. To *inactivate* any enzymes so that no change occurs in the composition of the wort.
4. Coagulate proteins and
5. Extract hop oil

Pre-fermentation treatment of wort

- The hot wort is not sent directly to the fermentation tanks; it should be cooled.
- The cooled wort is now ready for fermentation.
- It contains no enzymes but it is a rich medium for fermentation.
- It has therefore to be protected from contamination.

5. Fermentation

- The cooled wort is pumped or allowed to flow by gravity into fermentation tanks and yeast is inoculated or 'pitched in' at a rate of 7-15 x 10^6 yeast cells/ml, usually collected from a previous brew.
- Depending on the type of brewer's yeast fermentation can be
    - Top fermentation (ale)
    - Bottom fermentation (lager)

A. Top fermentation

- This is used in the UK for the production of stout and ale, using strains of *Saccharomyces cerevisiae*.
- Wort is introduced by a fish tail spray so that it becomes aerated to the tune of 5-10 ml/liter of oxygen for the initial growth of the yeasts.
- The entire primary fermentation takes about six days.
- Yeasts float to the top during this period,

B. Bottom fermentation

- Wort is inoculated to the tune of 7-15 x 10^6 yeast cells per ml of wort.
- The yeasts then increase four to five times in number over three to four days.
- 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.
- The total fermentation period may last from 7-12 days.

Formation of some beer components

- During wort fermentation in both top and bottom fermentation anaerobic conditions predominate; the initial oxygen is only required for cell growth.

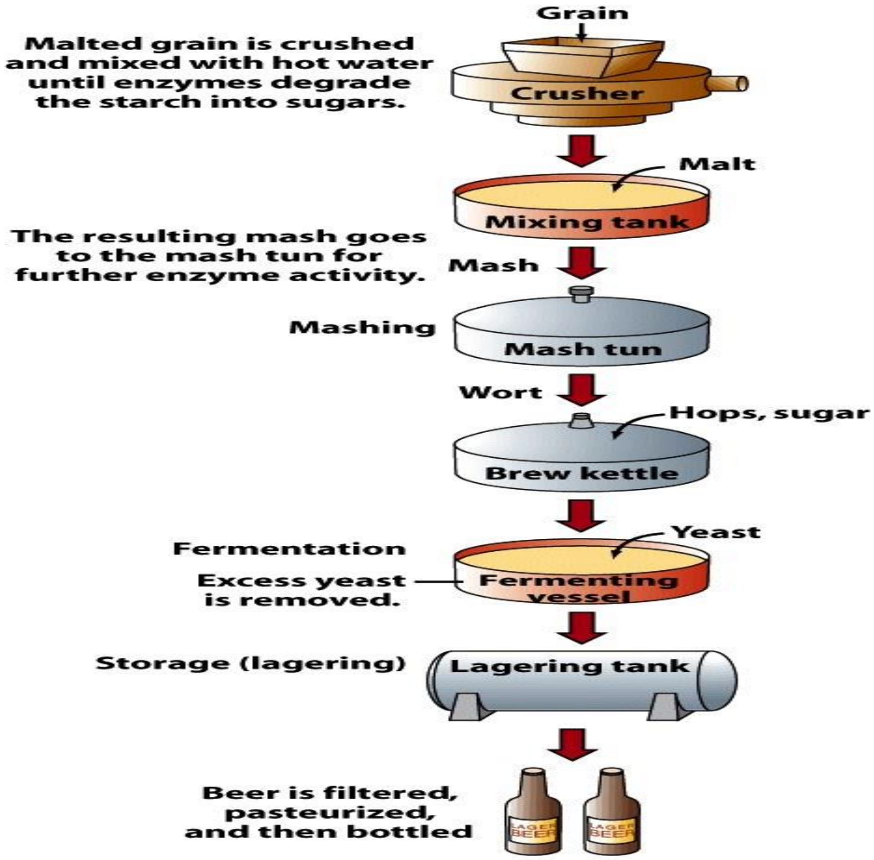
- Fermentable sugars are converted to alcohol, CO_2 and heat which must be removed by cooling.
- Apart from ethanol and CO_2, probably the next most abundant product is glycerol..
- Acids, including acetic, lactic, succinic and caproic, are also excreted, causing the pH to fall to around 4.0 by the end of the fermentation.
- Other minor metabolic products that are important beer flavour compounds are also produced.

6. Storage or lagering

- Stored in containers to allow it to mature. It is then filtered then packaged.
  - Cellars - *beer is stored cold*

7. Packaging

- The beer is transferred to pressure tanks from where it is distributed to cans, bottles and other containers.
- The beer is not allowed to come in contact with oxygen during this operation; it is also not allowed to lose CO_2, or to become contaminated with microorganisms.
- Bottles are thoroughly washed with hot water and sodium hydroxide before being filled.
- The filled and crowned bottles are passed through a pasteurizer, set to heat the bottles at 60°C for half hour.
- The bottles take about half hour to attain the pasteurizing temperature, remain in the pasteurizer for half hour and take another half hour to cool down.
- This method of pasteurization sometimes causes hazes and some of the larger breweries now carry out bulk pasteurization and fill containers aseptically.



Summary;

- The beer manufacture process consists of four distinct stages: **malting**, in which barley is converted to malt; **mashing**, in which enzyme and substrate extraction and reactions occur and a suitable growth medium is prepared; **fermentation**, in which wort sugars are fermented to beer; and **post-fermentation**, in which the beer is made suitable for consumption.

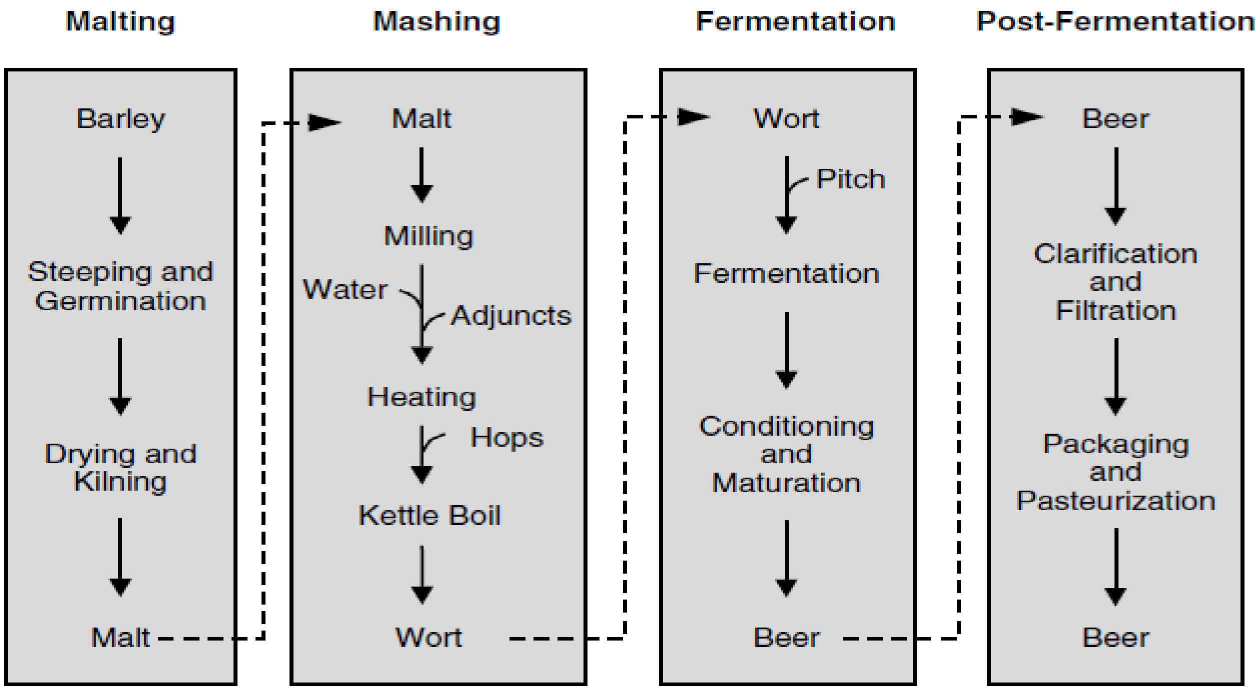


Fig. Manufacture of beer

Microorganisms used in beer fermentations :

- *Yeasts: Saccharomyces bayanus, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces pastorianus, Saccharomyces uvarum,*
- Bacillus Spp; Bacillus subtilis, Bacillus licheniformis, Bacillus stearothermophilus, Bacillus cerus, *Bacillus acidopullulyticus, Lactobacillus species;*
- *Aspergillus nigeger, Aspergillus oryzae, Penicillium emersonii, Penicillium funiculosum, Rhizopus delemar, Rhizopus niveus, Trichoderma reesei, Trichoderma viride,*

Strain improvement:

- The Science and technology of manipulating and improving microbial strains, in order to enhance their metabolic capacities for biotechnological applications, are referred to as strain improvement.
- The success of strain improvement depends greatly on the target product:
    - Raising gene dose simply increase the product, from products involving the activity of one or a few genes, such as enzymes.

- This may be beneficial if the fermentation product is cell biomass or a primary metabolite.

Targets of strain improvement

- Rapid growth
- Genetic stability
- Non-toxicity to humans
- Large cell size, for easy removal from the culture fluid
- Ability to use cheaper substrates
- Elimination of the production of compounds that may interfere with downstream processing
- Increase productivity.
- To improve the use of carbon and nitrogen sources.
- Reduction of cultivation cost; lower price in nutrition, lower requirement for oxygen.
- Production of additional enzymes and compounds to inhibit contaminant microorganisms

What Should We Look for when We Plan a Strain Improvement Program?

- In general, economic is the major motivation.
- Metabolite concentrations produced by the wild types are too low for economical processes.
- For cost effective processes improved strain should be attained.

Genetic characteristics of brewer's yeast strains

- All industrially used brewer's yeast strains belong to the *Saccharomyces*.
- Top-fermenting brewer's yeast (ale yeast) has been assigned to the species *Saccharomyces cerevisiae.*
- Bottom-fermenting (lager) brewer's yeast originally known as *S. carlsbergensis*
- In terms of their genetic constitution, bottom-fermenting (lager) brewer's yeast is most striking among industrial yeast strains.
- Several genetic studies have revealed lager brewer's yeast as being a natural allopolyploid interspecies hybrid between *S. cerevisiae* and a non-*S. cerevisiae* yeast.

Methods of Strain Improvement

Optimization of microbial activity - can be done by

- ✓ Optimizing environmental conditions
- ✓ Optimizing nutrition of microorganisms
- ✓ Genetic Modification: Mutation, recombination, gene cloning

Optimizing environmental conditions

- ❖ Modification of physical parameter (temperature,agitation,etc)
- ❖ Modification of chemical parameter (pH,O_2 concentration)
- ❖ Modification of biological parameter (enzymes)

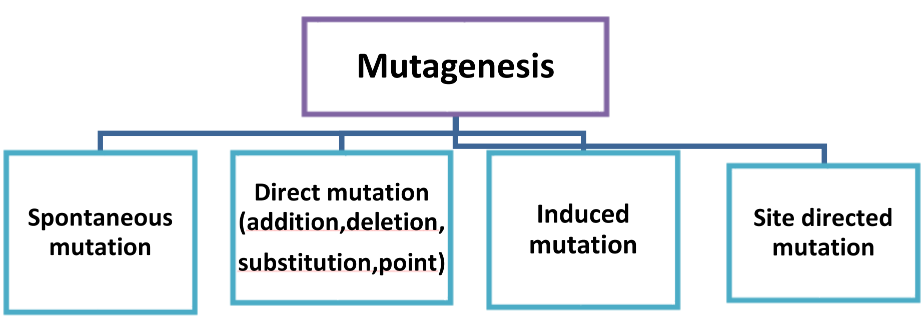
Optimization of nutrition of microorganisms

- ❖ Carbon sources
- ❖ Nitrogen sources
- ❖ Mineral sources and other sources
- ❖ Precursor
- ❖ Enzymes

MUTAGENESIS - Method not involving foregin DNA

- ❖ It is process of mutation in strain improvement of microorganism.
- ❖ Mutagenesis is a process of treatment given to microorganism which will cause an improvement in their genotypic and phenotypic performances.
- ❖ In a balanced strain development program each method should complement the other.

- ❖ Mutations occur in vivo spontaneously or after induction with mutagenic agents.
- ❖ Mutations can also be induced in vitro by the use of genetic engineering techniques.



Spontaneous mutation:

- ✓ Occur spontaneously at the rate of between 10^{-10} and 10^{-15} per generation and per gene; usually the mutation rate is between 10^{-7} and 10^{-6}.
- ✓ The rate of spontaneous mutation depends on the growth conditions of the organism.
- ✓ All mutant types are found among spontaneous mutations, but deletions are relatively frequent.
- ✓ The causes of spontaneous mutations, which are thus far understood, include integration and exclusion of transposons, along with errors in the functioning of enzymes such as DNA polymerases, recombinant enzymes, and DNA repair enzymes.
- ✓ Because of the low frequency of spontaneous mutations, it is not cost-effective to isolate such mutants for industrial development.
- ✓ Spontaneous and induced mutants arise as a result of structural changes in the genome:

MUTAGEN	MUTATION INDUCED	IMPACT ON DNA	RELATIVE EFFECT
Ionizing Radiations-X Rays,gamma rays	Single or double strand bearkage of DNA	Deletion/structural changes	high
UV rays,chemicals	Pyrimidine dymerisation	Trnsversion,deletion,frameshift transitions from GC AT	Medium
Hydroxylamine(NH2OH	Deamination of cytosine	GC A T transitions	low
N-Methyl –N'-Nitro N-Nitrosoguanidine	Methylation of bases and high pH	GC AT transitions	high
Nitrous acid(HNO2)	Deamination of A,C & G	Bidirectional transitions, deletion, AT GC/GC AT	Medium
Phage,plasmid,DNA transposing	Base substitution, breakage.	Deletion,duplication,insertion.	high
Ethylmethane-sulfonate	Alkylation of bases C & A	GC AT transition	high

Induced mutation:

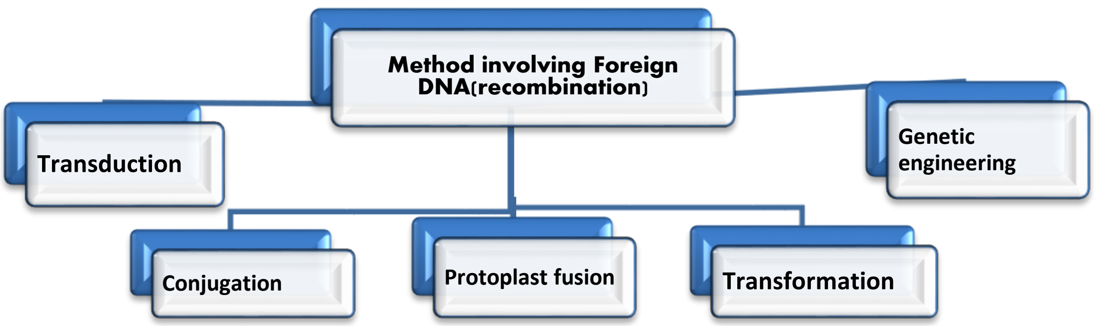
- The rate of mutation can be increased by various factors and agents called mutagens.
- ionizing radiations (*e.g*. X-rays, gamma rays)
- non-ionizing radiations (*e.g*. ultraviolet radiations)
- various chemicals (*e.g*. mustard gas, benzene, ethidium bromide, Nitroso guanidine-NTG)
- Among physical agents, UV is to be preferred since it does not require much equipment, and is relatively effective and has been widely used in industry.
- Chemical methods other than NTG (nitrosoguanidine) are probably best used in combination with UV.
- The disadvantage of UV is that it is absorbed by glass; it is also not effective in opaque or colored organisms.

Gene Technology

- Gene technology includes in vitro recombination, gene cloning, gene manipulation, and genetic engineering.
- Gene technology permits introduction of specific DNA sequences into prokaryotic or eucaryotic organisms and the replication of these sequences; that is, to clone them.
- To carry out these procedures, the following steps are necessary:
- The DNA sequence to be cloned must be available.
- The sequence must be incorporated into a vector.
- The vector with the DNA insert must be introduced by transformation into a host cell, where the vector must replicate the insert in a stable manner.
- The clone, which contains the foreign DNA, must be selectable in some manner.
- The use of gene technology to produce a genetically modified organism may involve:
    - removing a gene;
    - altering a gene;
    - adding extra copies of an existing gene;
    - adding a gene from another organism.
- It is also possible to switch off undesirable characteristics such as the production of a particular protein.
- When DNA from an organism is modified using gene technology, the organism is then referred to as a genetically modified organism (GMO)

Recombination - Method involving Foreign DNA

- The genetic information from two genotypes can be brought together into a new genotype through genetic recombination.
- The disadvantages of genetic recombination are:
- In most cases, the productivity of the recombinants usually is intermediate between the values of the parent strains.



- During strain development process, there is a frequent decline in the increase in yield is observed. This phenomena is overcome by allowing genetic-cross between unfavorable mutant alleles and alleles of one of the parents. Such a procedure is not available during recombination work.
- High-yielding strains can actually increase the cost of the fermentation because of changed physiological properties (greater foaming, changed requirements for culture medium, etc.).
- By crossing back to wild-type strains, high-yielding strains with improved fermentation properties may be formed.
- An effective strain development approach should involve the use of sister-strain, divergent strain, and ancestral crosses at specific intervals, besides use of carefully mutagenesis to ensure the maintenance of genetic variability.

Gene Cloning

Cloning = Asexual Reproduction= Process of producing a new organism from cells or tissues of existing organism. Whole nucleus of any cell type is used. Children are genetically identical to parent. All offspring (children) carry same genetic material.

CHAPTER FOUR

BIOTECHNOLOGY IN DAIRY INDUSTRY

Introduction

- Fermented milks result from the selective growth of specific bacteria in milk.
- These products have evolved around the world over thousands of years and are believed to have originated in the area that is now the Middle East.
- These products probably resulted from the need to extend the shelf life of milk in the absence of refrigeration.
- Storage of raw milk at ambient temperature probably led to growth of lactic acid and other bacteria.
- This bacterial activity produced desirable flavors, and importantly increased the shelf life of milk because of a high acid content.
- Procedures of fermented milk production were subsequently refined; the products became popular, and gradually spread to Asia, Europe, and other parts of the world.
- Consumption is now the highest in European countries, but these products form an important component of the diet in many other countries as well.
- Today yogurt, buttermilk, and sour cream are probably the most widely consumed fermented milk products, but there are many different types of such products that are either manufactured commercially or produced on a small scale, and sometimes in homes, for local consumption.
- In addition to being excellent sources of nutrients, these products have become popular because of potential health benefits.

Milk????

- It is an important part of a balanced diet
- Milk contains: fat, starch, sugar, protein, minerals
- All milk comes from dairy cows and others.
- It is treated in different ways to produce different types.
- Milk is available in forms such as:
  - Skimmed
  - UHT (ultrahigh temperature)
  - Powder
  - Pasteurised
- Milk is a major component of the diet in the world. For certain purpose, it would be desirable to have the chance to change the milk composition.

- From the dietary and human health point of view:
  - A greater proportion of unsaturated fatty acids in milk fat would be appreciated
  - Possibility to reduce lactose content in milk in order to make it accessible also for persons with expressed lactose intolerance
  - Milk devoid of β-lactoglobulin.
- From technological point of view: Alteration or primary structure of casein helps to improve technological properties of milk. Dairy producers would have the opportunity to choose to:
  - Produce high protein milk
  - Milk destined for cheese manufacturing that has accelerated curd clotting time, increased yield, and more protein recovery
  - Milk containing neutraceuticals
  - Replacement for infant formula

Milk Biota

- The microorganisms in raw cow's milk consist of those that may be present on the cow's udder and hide and on milking utensils or lines. Under proper handling and storage conditions, the predominant biota is gram positive.
- Raw milk held at refrigerator temperatures for several days invariably shows the presence of several or all bacteria of the following genera: *Enterococcus, Lactococcus, Streptococcus, Leuconostoc, Lactobacillus, Microbacterium, Oerskovia, Propionibacterium, Micrococcus, Proteus, Pseudomonas, Bacillus*, and *Listeria*, as well as members of at least one of the coliform genera.
- The biota that is unable to grow at the usual low temperature of holding tends to be present in very low numbers. Studies have revealed the presence of psychrotrophic spore formers (Psychrotrophic clostridia, psychrotrophic *Bacillus* spp.), *Nocardia* spp. and *mycobacteria* in raw milk.
- The pasteurization process eliminates all but thermoduric strains, primarily streptococci and lactobacilli, and spore formers of the genus *Bacillus* (and *Clostridium*, if present in raw milk). Milk is the vehicle for some diseases.

Dairy Enzymes

- **Rennet**

  - Milk contains proteins, specifically caseins, which maintain its liquid form. Proteases are enzymes that are added to milk during cheese production, to hydrolyze caseins, specifically kappa casein, which stabilizes micelle formation preventing coagulation. Rennet and rennin are general terms for any enzyme used to coagulate milk.

- Technically rennet is also the term for the lining of a calf's fourth stomach. The most common enzyme isolated from rennet is chymosin.

Table: Types of rennet - Milk clotting enzymes (rennet) can come from different sources.

Type of rennet	Source	Advantage	Disadvantage
Calf rennet	calves	Original source, used for centuries.	Animals must be killed, risk of disease
Fungal rennet	fungus	Cheap, large amounts, OK for vegetarians.	taste
GM yeast rennet	yeast	No animals involved, OK for vegetarian, same as animal rennet.	Public concern about genetically modified foods

- **Recombinant Chymosin**
  - Bovine chymosin traditionally known as calf rennet has been extensively used as milk coagulant during the manufacture of a variety of cheese all over the world. Cheese industry is also expanding rapidly and hence the demand for calf rennet or its substitutes is also showing a phenomenal increase.
- **Proteases**
  - Milk contains a number of different types of proteins, in addition to the caseins. Cow milk also contains whey proteins such as lactalbumin and lactoglobulin. The denaturing of these whey proteins, using proteases, results in a creamier yogurt product.
- **Lactase**
  - Lactase is a glycoside hydrolase enzyme that cuts lactose into it's constituent sugars, galactose and glucose. Without sufficient production of lactase enzyme in the small intestine, humans become lactose intolerant, resulting in discomfort (cramps, gas and diarrhea) in the digestive tract upon ingestion of milk products.

- **Catalase**
  - The enzyme Catalase has found limited use in one particular area of cheese production. Hydrogen peroxide is a potent oxidizer and toxic to cells. It is used instead of pasteurization, when making certain cheeses such as Swiss, in order to preserve natural milk enzymes that are beneficial to the end product and flavour development of the cheese.
- **Lipases**
  - Lipases are used to break down milk fats and give characteristic flavours to cheeses. Stronger flavoured cheeses, for example, the italian cheese, Romano, are prepared using lipases. The flavour comes from the free fatty acids produced when milk fats are hydrolyzed. Animal lipases are obtained from kid, calf and lamb, while microbial lipase is derived by fermentation with the fungal species *Mucor meihei*.

DAIRY PRODUCTS

- Biotechnology has already made significant contributions in dairy industry. Some of the potential applications and target areas where Biotechnology has already made its impact along with future prospects are given below:

Dairy Production

- Recombinant Vaccines
- Rumen manipulation
- DNA finger printing and RFLP
- PCR based diagnostics (Animal Health care)
- Embryo Transfer Technology
- Animal Cloning
- Gene Pharming and Transgenics
- Recombinant Bovine/Buffalo Somatotropin - rbst

Dairy Processing

- Designing milk through genetic engineering
- Genetically Modified Organisms (GMOs): Starter Cultures & Genetically modified foods
- Food grade Biopreservatives
- Recombinant dairy enzymes/proteins

- Accelerated cheese ripening
- Probiotics
- Functional foods and neutraceuticals
- Dairy waste management and pollution control
- Gene probes and PCR based pathogen detection

Production of cheese

- Cheese is a stabilised curd of milk solids produced by casein coagulation and entrapment of milk fat in the coagulum.
- Cheese, the nature's wonder food and the classical product of biotechnology, is a highly nutritious food with good keeping quality, enriched pre-digested protein with fat, calcium, phosphorus, riboflavin and other vitamins, available in a concentrated form.
- Cheese, the most important category of fermented foods, has been reported to have therapeutic, anticholesterolemic, anticarcinogenic and anticariogenic properties beyond their basic nutritive value.
- The water content (whey) is greatly reduced, in comparison with milk, by the separation and removal of whey from the curd.
- With the exception of some fresh cheeses, the curd is textured, salted, shaped, and pressed into moulds before storage and curing or ripening.
- There are said to be approximately 1,000 named cheeses throughout the world, each produced using a variation on the basic manufacturing process.
- Most of these varieties fit into one of three main categories according to their moisture content, and method and degree of ripening:

1. Soft cheese -- Feta, Cream Cheese

- High moisture (55 - 80%)
- Fresh, unripened (cottage cheese, Ricotta, Quarg, Fromage Blanc, Neufchatel, Mozzarella)
- Surface mould-ripened (Brie, Camembert)

2. Semi -soft / semi-hard cheese -- Cheddar, Gouda

- Moderate moisture (41 - 55%)
- Surface smear ripened (Limburger, Munster, Tilsit)
- Ripened by bacteria (Caerphilly, Lancashire, St Paulin)
- Blue-veined, internally mould ripened (Stilton, Roquefort, Gorgonzola)

3. Hard / Low moisture cheese -- Parmesan, Romano

- Low moisture (20 - 40%)
- Ripened by bacteria, with eyes (Emmental, Gruyere)
- Ripened by bacteria, no eyes (Cheddar, Edam, Cheshire)
- Very hard (Grana (Parmesan), Asiago, Romano)

Basic steps in the production of cheese

1. Pasteurization

- Cheese may be made from raw milk, pasteurised milk, or milk that has undergone a sub-pasteurisation (thermisation) treatment.
- Pasteurisation destroys the vegetative cells of pathogens as well as many spoilage organisms, and some of the enzymes naturally present in the milk.

2. Starter cultures

- The acidification of milk is the key step in the making of cheese.
- Acidification is essential for the development of both flavour and texture; it promotes coagulation; and the reduction in pH inhibits the growth of pathogens and spoilage organisms.
- It is normally achieved by the fermentation of lactose by bacterial starter cultures to produce lactic acid, although some fresh cheeses, such as cottage cheese, may be acidified by the direct addition of acid, and do not require a starter.

3. Curd formation

- In curd cheeses, a coagulant is normally added to the acidified milk.
- This may consist of acid proteinases produced by moulds such as *Mucor miehei*, or chymosin (the most important component of rennet) produced by fermentation using genetically modified bacteria.
- Rennet, in combination with acid from the starter, causes coagulation of the milk curd by precipitating casein as an aqueous gel.
- The curd is then allowed to set for a time depending on the cheese variety.
- For most hard or semi-hard cheeses, this would be approximately one hour.

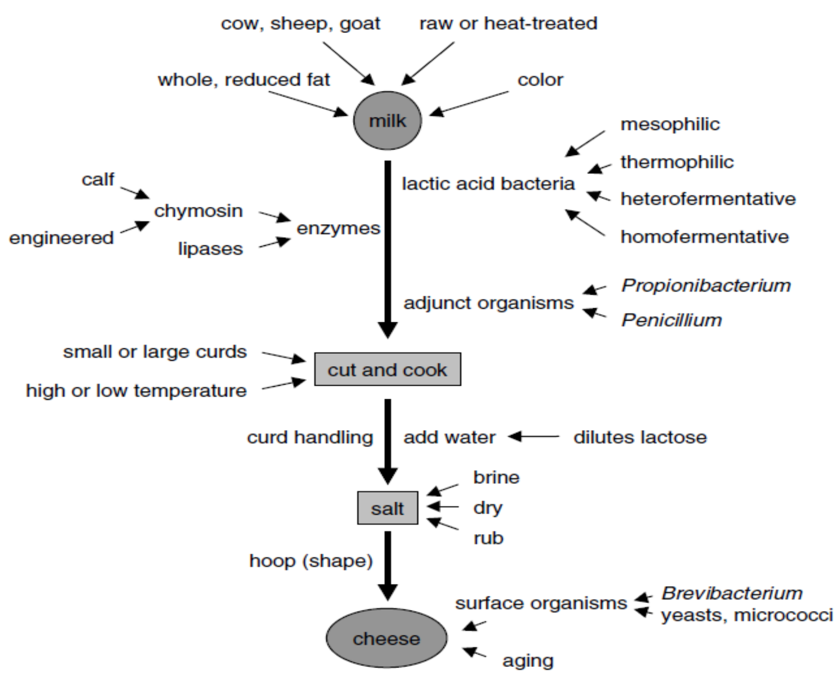
4. Salting/brining

- In the manufacture of **Cheddar**, salt is added to the milled curd before pressing (dry salting) at a concentration of 1.5 - 2% w/w.

- In other varieties, such as **Gouda** and **Camembert**, the moulded cheese is immersed in concentrated brine.
- Some blue cheeses are salted by rubbing dry salt into the surface of the moulded cheese.
- Salting inhibits the growth of the starter culture and other microorganisms, contributes to the flavour, and affects texture.

5. Ripening/Cure Curds

- All but fresh cheeses require some degree of ripening for the full development of flavour and texture.
- During ripening, further moisture loss occurs, and a complex combination of microbial and enzymatic reactions take place, involving milk enzymes, the coagulant, and proteases and peptidases from the starter culture and non-starter organisms, which remain viable although their growth is inhibited. Ripening conditions vary with cheese variety.

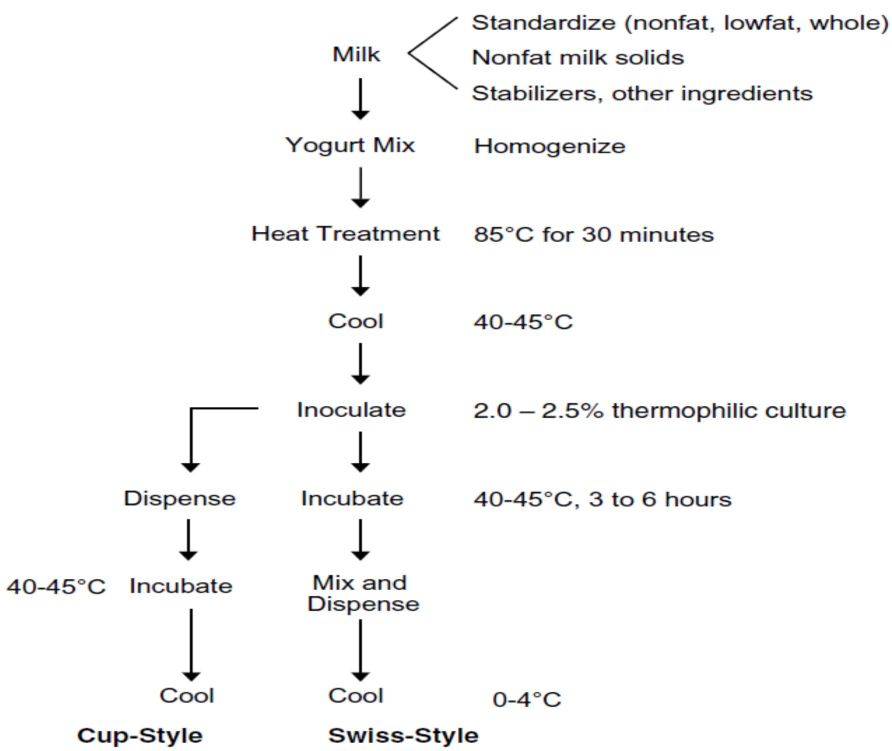


Yoghurt production

- Yogurt (yoghurt) is considered a fluid milk product by the U.S. Food and Drug Administration (FDA) and must be made using pasteurized milk. It can be made from skim (non-fat), reduced fat, or whole milk.
- Yoghurt is a term used to describe a wide range of related products, which may be classified according to legal standards (full-, medium- or low-fat), gel type (set or stirred) and whether or not

they are flavoured (natural, fruit, or flavoured) or if they are subjected to a further process (heating, freezing, drying or concentrating).

- *Yogurt* is produced with a yogurt starter, which is a mixed culture of *S. salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in a 1:1 ratio. The usual starter culture employed to produce yoghurt is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.
- The coccus grows faster than the rod and is primarily responsible for initial acid production at a higher rate than that produced by either when growing alone, and more acetaldehyde (the chief volatile flavor component of yogurt) is produced by *L. delbrueckii* subsp. *bulgaricus* when growing in association with *S. salivarius* subsp. *thermophilus*.
- The coccus can produce about 0.5% lactic acid and the rod about 0.6–0.8% (pH of 4.2–4.5).



Cheese making summary:

1. Homogenize & Pasteurize
2. Add Starter Culture
3. Coagulate Casein
4. Micelles/ curd formation
5. Treat curds to separate whey
6. Ripen/Cure Curds

Yogurt making summary:

- ✓ Heat milk to near boiling
- ✓ Inoculate with Bacteria
- ✓ Coagulate casein micelles
- ✓ Fortify yogurt with extra solids

Microorganisms of importance in dairy industry:

- Alkaligens, Aspergillus, Candida, Micrococcus, LAB, Penicillium, *Streptococcus salivarius* ssp, *thermophilus* and *Lactobacillus bulgaricus*, *L. acidophilus*, *L. casei* and *Bifidobacterium* species, yeasts *(Saccharomyces cerevisiae, Candida colliculosa*, *Candida kefyr, Candida krusei, Candida mycoderma*, *Candida utilis*, *Candida vini*, *Candida zeylanoides*), etc…..

Strain improvement:

1. Metabolic engineering of lactic acid bacteria

- *Lactococcus lactis* is a well-characterized bacterium on the level of physiology and, especially, molecular biology.
- All necessary genetic tools have been developed for this organism, including food-grade cloning vectors and selection markers.
- The metabolism of *L. lactis* is relatively simple with energy metabolism centred on rapid conversion of sugar into lactic acid, and biosynthetic pathways kept to a minimum.
- This makes the organism an attractive model for metabolic engineering activities.

A. Lactose/galactose removal

- Fermented dairy products such as buttermilk and yoghurt contain high amounts of residual lactose.
- In addition, yoghurt contains considerable amounts of galactose, due to the in-ablity of the yoghurt bacteria to ferment this sugar.
- High lactose content is undesirable for all lactose-intolerant human beings, which make up a large part of the world population.
- Too high galactose consumption, especially in combination with ethanol consumption, can lead to accumulation of toxic **galactitol** (major cause of cataract) in human tissue cells.

B. Vitamin production

- A clear example of health-promoting activity by lactic acid bacteria is the ability to produce vitamins. Although most lactic acid bacteria are known as fastidious and often vitamin-auxotrophic bacteria, there are some known exceptions.
- The yoghurt bacterium ***S. thermophilus*** produces the **B-vitamin** folic acid, which is subsequently used, for growth, by the other yoghurt bacterium *L. bulgaricus*.
- By selecting high folic acid-producing strains or by using relative high amounts of *S. thermophilus* compared to *L. bulgaricus*, yoghurt with increased folic acid contents should, in principal, be possible.
- Folic acid is an essential component in human nutrition, is conspicuously absent or present at too low levels in many modern food products and is specifically recommended in the diet of pregnant women.
- *L. lactis* has also the ability to produce folic acid. This B-vitamin seems to specifically involved in the biosynthesis of purines and pyrimidines.
- Interestingly, specific folic acid production was increased by a factor twenty under conditions of reduced growth rates as can be induced in chemostats or by addition of antimicrobials.
- Some of the genes responsible for biosynthesis of folic acid have been cloned and currently attempts are underway to influence, via controlled expression, the production level of folic acid and its m

2. Different methods of strain improvement that we discussed under the previous chapter (chapter 3) can be used to improve the strains utilised in dairy industry.

CHAPTER FIVE

BIOTECHNOLOGY OF BAKING INDUSTRY

Introduction

- Bread is one of the most common, relatively low cost, traditional foods around the world. Yet bread actually has close links with biotechnology, the common denominator being enzymes. For decades enzymes such as malt and fungal alpha-amylase have been used in bread making.
- Due to the changes in the baking industry and the ever increasing demand for more natural products, enzymes have gained real importance in bread formulations.
- The alpha-amylases degrade the damaged starch in wheat flour into small dextrins, thus allowing yeast to work continuously during dough fermentation, proofing and the early stage of baking.
- 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.
- Today, bread supplies over half of the caloric intake of the world's population including a high proportion of the intake of Vitamins B and E.
- Bread is therefore a major food of the world.

Ingredients for Modern Bread-making

- 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 on the practice and regulations operating in a country.
- These include 'yeast food', sugar, milk, eggs, shortening (fat), emulsifiers, anti-fungal agents, anti-oxidants, enzymes, flavoring, and enriching ingredients. The ingredients are mixed together to form dough which is then baked.

1. 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*, but this yellow colored variety is more familiarly used for semolina and macaroni in many countries.
- The chief constituents of flour are starch (70%), protein (7-15%), sugar (1%), and lipids (1%).
- Flour proteins are of two types.
- 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.
- It forms the skeleton which holds the starch, yeasts, gases and other components of dough.

2. 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
  - ✓ Easy dispersability in water
  - ✓ 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 is 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.
- 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.

How do ingredients react to Yeast?

- **SUGAR**: is the food yeast needs to make the carbon dioxide gas which causes the dough to rise. It also helps the crust to brown and adds flavor
- **FAT**: helps to extend the shelf life of the dough. It also helps the dough to stretch easily when forming the crust.
- **FLOUR**: when stirred and kneaded with the yeast mixture, the gluten stretches to form the elastic framework that holds the gas bubbles formed by the yeast. This makes the structure of the crust. The amount and quality of gluten caries depending upon the type of flour used. Flours with the most gluten produce products with the biggest volume. The amount of flour needed for your dough varies slightly depending upon the weather conditions and type of flour used.
- **LIQUID**: all liquids must be at the proper temperature to dissolve the yeast without killing it
- **SALT**: slows down the action of the yeast as well as draws out the flavor of the other ingredients.

3. Yeast food

- The name yeast food is something of a misnomer, because these ingredients serve purposes outside merely nourishing the yeasts.
- In general the foods contain a calcium salt, an ammonium salt and an oxidizing agent.
- The bivalent calcium ion has a beneficial strengthening effect on the colloidal structure of the wheat gluten.
- A well-used yeast food has the following composition: calcium sulfate, 30%, ammonium chloride, 9.4%, sodium chloride, 35%, potassium bromate, 0.3%; starch (25.3%) is used as a filler.

4. Sugar

- To provide carbon nourishment for the yeasts additional to the amount available in flour sugar.
- To sweeten the bread
- To afford more rapid browning (through sugar caramelization) of the crust and hence greater moisture retention within the bread.
- Sugar is supplied by the use of sucrose, fructose corn syrups (regular and high fructose), depending on availability.

5. 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
- While the desirable effects of fats have been clearly demonstrated their mode of action is as yet a matter of controversy among bakery scientists and cereal chemists.
- Butter is used only in the most expensive breads; lard (fat from pork) may be used, but vegetable fats especially soy bean oil, because of its most assured supply is now common.

6. 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 e.g., glycerol, lactic acid, sorbic acid, or tartaric acid.
- Emulsifiers are added as 0.5% flour weight.
- Commonly used surfactants include: calcium stearyl- 2-lactylate, lactylic stearate, sodium stearyl fumarate.

7. Milk

- Milk to be used in bread-making must be heated to high temperatures before being dried; otherwise for reasons not yet known the dough becomes sticky.
- Milk is added to make the bread more nutritious, to help improve the crust color, presumably by sugar caramelization and because of its buffering value.
- Due to the rising cost of milk, skim milk and blends made from various components including whey, buttermilk solids, sodium or potassium caseinate, soy flour and/or corn flour are used.
- The milk substitutes are added in the ratio of 1-2 parts per 100 parts of flour.

8. Salt

- About 2% sodium chloride is usually added to bread.
- It serves the following purposes:
    - It improves taste
    - It stabilizes yeast fermentation

- As a toughening effect on gluten

- Due to the retarding effect on fermentation, salt is preferably added towards the end of the mixing.

9. 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 for reasons already discussed.
- Water with high sulphide content is undesirable because gluten is softened by the sulfhydryl groups.

10. Enzymes

- Sufficient amylolytic enzymes must be present during bread-making to breakdown the starch in flour into fermentable sugars.
- Since most flours are deficient in alpha-amylase, 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 amy1ase 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. Ordinarily however, proteases have the effect of reducing the mixing time of the dough.

11. Mold-inhibitors (antimycotics) and enriching additives

- The spoilage of bread is caused mainly by the fungi *Rhizopus, Mucor, Aspergillus* and *Penincillium*.
- Spoilage by *Bacillus mesenteroides* (ropes) rarely occurs.
- 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.

Process of bread-making

- Large-scale bread-making is mechanized.
- The processes of yeast-leavened bread-making may be divided into:

1. Pre-fermentation (or sponge mixing):

- At this stage a portion of the ingredients is mixed with yeast and with or without flour to produce an inoculum.
- During this the yeast becomes adapted to the growth conditions of the dough and rapidly multiplies.
- Gluten development is not wanted at this stage.

2. Dough mixing: The balance of the ingredients is mixed together with the inoculums to form the dough. This is the stage when maximum gluten development is sought.

3. Cutting and rounding: The dough formed above is cut into specific weights and rounded by machines.

4. First (intermediate) proofing: The dough is allowed to rest for about 15 minutes usually at the same temperature as it has been previous to this time i.e., at about 27°C. This is done in equipment known as an overhead proofer.

5. 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.

6. 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).

7. Baking: 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.

- Baking is the final of the various baking processes.
- It is the point at which the success or otherwise of all the previous inputs is determined.

8. Cooling, slicing, and wrapping: The bread is depanned, cooled to 4-5°C sliced (optional in some countries) and wrapped in waxed paper, or plastic bags.

Bakery products

A. Quick Breads/Muffins

- ***Quick Bread*** = needs no time to rise. These products are made with a leavening agent other than yeast, usually steam or carbon dioxide that is produce with baking powder or baking soda. Ex. muffins, coffee cakes, biscuits etc.
- When making quick breads, DO NOT OVERSTIR, this will develop gluten, producing a tough product.
- All quick breads contain flour, liquid and salt, plus a chemical leavening agent. Depending upon the recipe they may also contain fat, sugar and eggs.

- There are four categories of quick breads depending upon the flour to liquid ratio in the product. The categories include:
  - **Pour batters:** popovers, pancakes, cream puffs._ 1:1 flour liquid ratio
  - **Drop batters:** muffins and some cookies. _ 2:1 flour to liquid ratio.
  - **Soft dough's:** biscuits and scones_ 3:1 flour to liquid ratio
  - **Stiff Dough:** pasta and pie crust._6/8:1 flour to liquid ratio

B. Batters and Dough

Batter: liquid mixture of flour, egg and milk/water

- a liquid mixture of flour, milk, and eggs used in making cakes and pancakes, and for coating foods before frying

Dough: mixture of flour and water

- Refers to a thick, malleable, soft elastic mixture of flour and water/liquid, often with other ingredients such as yeast, oil, butter, salt, and sugar, that becomes bread or pastry when baked.

Pour batters – made with an equal ratio of flour to liquid, usually flour (ex. waffle and pancake)

- Steam is the main leavening agent in pour batters

Drop batters – mixing about two parts flour to one part liquid

- Make muffins and some cookies, baking powder and steam are both leavening agents

Soft dough – three parts flour to one part liquid

- Needs more mixing to develop gluten, example yeast breads, pizza crust and baking powder biscuits

Stiff dough – six to eight times as much flour as liquid

- Drier consistency is needed for some products; example pie crust
- Steam leavens all flour mixtures

Dough leavening:

- Leavening baked goods is a process that causes the product to be light and porous. Leavening involves producing a gas that expands the batter or dough leaving holes as the dough/batter sets during the baking process.

The most widely used leavening agents are:

- Baking soda, Baking powder, Yeast

1. Baking Soda = chemical compound sodium bicarbonate

- Baking soda releases sodium carbonate as well as carbon dioxide when heated
- Sodium carbonate gives food a bad taste and a yellowish color.
- However, baking soda is always used with an acid, which alters the chemical reaction to prevent sodium carbonate from forming
- Some examples of acid are buttermilk, vinegar, lemon juice, molasses, honey, fruits, fruit juices, and cream of tartar.

2. Baking Powder = leavening compound that contain baking soda, dry acids and starch or some other filler.

- The filler in baking powder, usually cornstarch or calcium carbonate, absorbs moisture in the air, which helps prevent a chemical reaction from taking place too soon.
- Baking powder comes in two types: single-acting & double –acting baking powder
- ***Single-acting baking powder*** = as soon as liquid is added, carbon dioxide starts to be released
  - ✓ The quick reaction occurs because the acid in baking powder is soluble in a cold liquid
- ***Double-acting baking powder*** = usually preferred, contains two acids, one that reacts with cold liquid and one that reacts with heat.
- Some carbon dioxide is released as soon as liquid is added, but most is produced as the batter heats in the oven

3. Yeasts

- Microscopic organisms that produce carbon dioxide through fermentation**;** biological reaction that slowly splits complex organic compounds into simpler substances.
- They convert sugar to ethyl alcohol and carbon dioxide. The alcohol evaporates and carbon dioxide causes the product to rise.
- The yeast is killed by the high baking temperatures, which stops fermentation.

Microorganisms used in bakery industry

- *Bacillus*, *Candida exiguus*, *Candida milleri*, *Candida valida*, *Lactobacillus acidifarinae*, *Lactobacillus bucheri*, *Lactobacillus crispatus*, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Lactobacillus homohiochii*, *Lactobacillus jensenii*, *Lactobacillus johnsonii*, *Lactobacillus mindensis*, *Lactobacillus mucosae*, *Lactobacillus namuresis*, *Lactobacillus nantesis*, *Lactobacillus panis*, *Lactobacillus parabuchneri*, *Lactobacillus paralimentarius*, *Lactobacillus pontis*, *Lactobacillus reuteri*, *Lactobacillus rossiae*, *Lactobacillus sanfranciscensis*, *Lactobacillus secaliphilus*, *Lactobacillus siliginis*, *Lactobacillus spicheri*, ***Saccharomyces cerevisiae***,

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.
- Efficient fermentation of dough sugars, in particular maltose, by yeast cells is crucial for dough leavening.
- Moreover, it has been regarded as favorable if baker's yeast could be engineered to directly use starch, the major carbohydrate in dough.
- The production of baker's yeast itself is also an important industrial branch.
- In this case, the fast propagation of baker's yeast is desired with high biomass yields related to the carbon sources available in the growth medium, which has mainly been molasses.
- Moreover, a high tolerance to stresses caused by high sugar concentration (sweet dough), drying and freezing (production of dried yeast and frozen dough, respectively) is necessary.

1. Utilization of polysaccharides (starch and dextrins) for improved dough leavening

- Starch is the major carbohydrate in dough.
- Dextrins represent degradation products of starch, i.e., a mixture of large fragments. These starch degradation products result from partial hydrolysis.
- Neither baker's nor brewer's yeasts are able to hydrolyze starch or dextrins, as they do not excrete starch-decomposing enzymes such as α-amylase (which cleaves α-1,4 glycosidic bonds) and isoamylases (debranching enzymes that hydrolyze α-1,6 glycosidic bonds).
- Therefore, α-amylases are often added to dough in the baking industry. These practices could be omitted if bakers were provided with starch/dextrin-decomposing enzymes.

2. Melibiose-utilizing strains

- Molasses, a commonly used raw material for the production of baker's yeast, contains up to 8% raffinose in addition to sucrose.

- This trisaccharide (fructose/glucose/galactose) is hydrolyzed by yeast invertase to fructose and the disaccharide melibiose.
- Bakers' yeast cannot utilize melibiose, because it does not have α-galactosidase (melibiase), the enzyme responsible for the hydrolysis of melibiose into the fermentable sugars, galactose and glucose.
- However, the α-galactosidase enzyme is found in bottom-fermenting brewers' yeast strains. The *MEL1* gene encoding this enzyme has been cloned and transferred into bakers' yeast strains.
- Then these strains expressing *MEL1* on multicopy plasmids or integrated into the *LEU2* locus have been reported to secrete significant amounts of α-galactosidase. All available melibiose was utilized in a beet molasses medium, resulting in higher yeast yields.
- Recombinant *MEL+* bakers' yeast strains devoid of plasmid sequences have been developed by Gasent-Ramirez. These strains exhibit an 8% increase in biomass yield without alteration of the growth rate.

3. Maltose utilization

- Maltose utilization by yeast requires a maltose permease and a maltase enzymes and both are induced in the presence of maltose.
- A major factor limiting the dough fermentation rate is the repression of the synthesis of maltose-utilizing enzymes and the inactivation of the maltase enzyme by glucose.
- Low concentrations (1–2%) of free sugars (mainly glucose and fructose) in the dough repress maltose utilization, causing a lag phase in carbon dioxide production.
- To avoid this lag phase, maltose-utilizing enzymes have been de-repressed by replacing the native promoters of the maltase and maltose permease with constitutive promoters.
- Baker's yeast strains display intrinsic differences in their rates of maltose utilization.
- Non-lagging bakers' strains are characterized by rapid maltose fermentation, unlike lagging strains, which exhibit a lag phase.
- Therefore, expression of a *MALX3* gene isolated from a non-lagging strain in a lagging strain might improve maltose metabolism in unsweetened dough
- Also refer to other methods of strain improvement discussed under the third chapter of this course.

CHAPTER SIX

SECONDARY METABOLITES FOR FOOD INDUSTRY

Introduction

- Secondary metabolites are organic molecules that are not involved in the normal growth and development of an organism. While primary metabolites have a key role in survive of the species, playing an active function in the photosynthesis and respiration, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, often playing an important role in plant defense. These compounds are an extremely diverse group of natural products synthesized by plants, fungi, bacteria, algae, and animals.
- Most of secondary metabolites, such as terpenes, phenolic compounds and alkaloids are classified based on their biosynthetic origin. Different classes of these compounds are often associated to a narrow set of species within a phylogenetic group and constitute the bioactive compound in several medicinal, aromatic, colorant, and spice plants and/or functional foods.
- Secondary metabolites are frequently produced at highest levels during a transition from active growth to stationary phase. The producer organism can grow in the absence of their synthesis, suggesting that secondary metabolism is not essential, at least for short term survival.
- A second view proposes that the genes involved in secondary metabolism provide a ''genetic playing field" that allows mutation and natural selection to fix new beneficial traits via evolution.
- A third view characterizes secondary metabolism as an integral part of cellular metabolism and biology; it relies on primary metabolism to supply the required enzymes, energy, substrates and cellular machinery and contributes to the long term survival of the producer.
- A simple classification of secondary metabolites includes three main groups: terpenes (such as plant volatiles, cardiac glycosides, carotenoids and sterols), phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignin) and nitrogen containing compounds (such as alkaloids and glucosinolates).

6.1. Secondary metabolites from plant cell culture:

- Plants produce secondary metabolites; they are biosynthetically derived from primary metabolites. They are more limited in distribution being found usually in specific families.

- They are not necessary for growth and development, but may serve as pollination attractants, environmental adaptations, or protection.
- Secondary metabolism
  - The metabolisms which are not directly related to maintaining life, are known as secondary metabolisms.
  - The products formed by secondary metabolism are called secondary metabolites.
  - Secondary metabolite plays a role in reinforcement of tissue and tree body (e.g. cellulose, lignin, suberin), protection against insects, diseases, and plant regulation (plant hormones).

1. Alkaloids

- Alkaloids are defined as basic compounds synthesized by living organisms containing one or more heterocyclic nitrogen atoms, derived from amino acids (with some exceptions) and pharmacologically active.
- The class name is directly related to the fact that nearly all alkaloids are basic (alkaline) compounds.
- Alkaloids constitute a very large group of secondary metabolites, with more than 12,000 substances isolated.
- A huge variety of structural formulas, coming from different biosynthetic pathways and presenting very diverse pharmacological activities are characteristic of the group.
- Archeological evidence has demonstrated the use of alkaloids (plant parts or extracts) since 4000 B.C., and they continue to be very important today (Roberts & Wink, 1998). *Poppy* (*Papaver somniferum*) and *opium* have been known and used since antiquity by Sumerians, Arabs, Persians, Egyptians and Greeks.
- Morphine, obtained from the poppy latex, was the first crude drug isolated. It was named after Morpheus, the god of dreams, one of the sons of Hypnos, the god of sleep, in Greek mythology (Wink, 1998). Morphine is legally used nowadays as an analgesic for severe pain.
- Alkaloids are associated with a wide range of pharmacological activities. Many are toxic and can cause death, even in small quantities. Some have antibiotic activities and others interfere with behavior patterns, such as antidepressants (reserpine) and hallucinogens (mescaline).
- 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.

2. Phenolic compounds/ *phenolics* *(including polyphenols and tannins)*

- Phenolic compounds are widely distributed in nature. Their chemical structures may vary greatly, including simple phenols (C6), such as hydrobenzoic acid derivatives and catechols, as well as long chain polymers with high molecular weight, such as catechol melanins (C6)6, lignins (C6-C3)n and condensed tannins (C6-C3-C6)n. Stilbenes (C6-C2-C6) and flavonoids (C6-C3-C6) are phenolic compounds with intermediate molecular weight that present many pharmacological and biological activities.
- Flavonoids, including anthocyanins, flavonols (such as quercetin and myricetin), isoflavones (such as daidzein and genistein) and others are formed by multiple biosynthetic branches that originate from chalcone.
- Phenolic compounds have been widely fractionated in medicinal, aromatic and food plants using CC. Repeated silica gel, sephadex-LH20, RP-18, RP-8, MCI-gel, diaion and toyopearl chromatography columns have been used to fractionate simple phenolics, flavonoids and tannins from kernels and nuts (Zhang et al, 2009; Karamac, 2009), fruits such as apples, *Morus nigra*, *Punica granatum* (Lee et al, 2010; Pawlowska et al, 2008); olive oil (Khanal et al, 2011), tea (Gao et al, 2010; Liu et al, 2009), seeds such as lentils (Amarowicz & Karamac 2003), medicinal species, including *Ulmus davidiana* and *Tridax procumbens*; and aromatic plants, including mint and sage.

3. Terpenoids

- Terpenoids are the largest and most diverse family of natural products, ranging in structure from linear to polycyclic molecules and in size from the five-carbon hemiterpenes to natural rubber, comprising thousands of isoprene units.
- All terpenoids are synthesized through the condensation of isoprene units (C5) and are classified by the number of five-carbon units present in the core structure.
- Many flavor and aromatic molecules, such as menthol, linalool, geraniol and caryophyllene are formed by monoterpenes (C10), with two isoprene units, and sesquiterpenes (C15), with three isoprene units. Other bioactive compounds, such as diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) show very special properties.

Establishing a plant cell culture for secondary metabolite production is a complex problem

- *Not all cell types produce the desired metabolite*

Root cultures are often better than cell cultures

- Roots often secrete the metabolites into the surrounding medium, making it easy for collection.

- Charcoal can be added to the medium, the metabolites are absorbed by the charcoal, and this stimulates even higher production of the metabolite.

Some secondary metabolites produced in cell and root culture

- ***L-DOPA:*** a precursor of catecholamines, an important neurotransmitter used in the treatment of Parkinson's disease
- ***Shikonin*****:** used as an anti-bacterial and anti-ulcer agent
- ***Anthraquinone*****:** used for dyes and medicinal purposes
- ***Opiate alkaloids*****:** particularly codeine and morphine for medical purposes
- ***Berberine*****:** an alkaloid with medicinal uses for cholera and bacterial dysenterry
- ***Valepotriates***: used as a sedative
- ***Ginsenosides*****:** for medicinal purposes
- ***Rosmarinic acid*****:** for antiviral, suppression of endotoxin shock and other medicinal purposes
- ***Quinine*****:** for malaria
- ***Cardenolides*** or ***Cardioactive glycosides*****:** for treatment of heart disease

6.2. Secondary metabolites from microorganisms:

- Microbial secondary metabolites include *antibiotics*, *pigments*, *toxins*, *effectors of ecological competition and symbiosis*, *pheromones*, *enzyme inhibitors*, *immunomodulating agents*, *receptor antagonists and agonists*, *pesticides*, *antitumor agents and growth promoters* of animals and plants.
- They have a major effect on the health, nutrition and economics of our society. They often have unusual structures and their formation is regulated by nutrients, growth rate, feedback control, enzyme inactivation, and enzyme induction.
- Regulation is influenced by unique low molecular mass compounds, transfer RNA, sigma factors and gene products formed during post-exponential development. The synthases of secondary metabolism are often coded by clustered genes on chromosomal DNA and infrequently on plasmid DNA.
- Unlike primary metabolism, the pathways of secondary metabolism are still not understood to a great degree and thus provide opportunities for basic investigations of zymology, control and differentiation.
- Secondary metabolism is brought on by exhaustion of a nutrient, biosynthesis or addition of an inducer, and/or by a growth rate decrease.

6.3. Use of secondary metabolites as food additives:

- Enzymes, amino acids, vitamins, organic acids, polyunsaturated fatty acids and certain complex carbohydrates and flavouring agents used in food formulations are currently produced using genetically modified microorganisms (GMMs).
- The production of food additives or processing aids using GMMs, where the microorganism is not a part of the food, has become an important and generally well-accepted technology, with a significant number of such products on the market.
- Experience with the purification of proteins in the biomedical field suggests that well-standardized purification protocols are of central importance for the safety of these products.

Definition of food additives

- Food additives are substances added intentionally to foodstuffs to perform certain technological functions, for example to colour, to sweeten or to preserve.
- In European Union (Community) legislation, food additives are defined as "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods" (EC Regulation 1333/2008 Article 3).
- Moreover, food additives are natural or manufactured substances, added to foods for a variety of reasons - to restore colours lost during processing (i.e. colours), to provide sweetness in low-sugar products (i.e. sweeteners), to prevent deterioration during storage and to guard against food poisoning (i.e. preservatives).
- Food additives are divided into categories or functional classes according to their technological function, for example preservatives, anti-oxidants, sweeteners, colours, etc.
- Foodstuffs are also divided into categories, and specific conditions are laid down regarding which additives or groups of additives may be used in the different food categories.
- The use of food additives is generally not permitted in certain food categories, e.g. unprocessed foods and foods for infants and young children, including dietary foods for infants and young children for special medical purposes except where specifically provided for in the legislation.

6.4. Improvement of strains for increase production of secondary metabolites ………………………… *Reading Assignment*……………………..…………..

UNIT SEVEN

IMPROVING FOOD QUALITY THROUGH AGRICULTURAL BIOTECHNOLOGY APPROACH

INTRODUCTION

- Plants are the primary source of food for humans and feed for livestock. Through domestication and agricultural activities of breeding and selection, plants were developed into food crops that serve as the major source of dietary carbohydrates, lipids, proteins, vitamins and minerals for humans and livestock directly or indirectly.
- The level and composition of food nutrients vary significantly in different food crops. As a result, individual plant foods are often deficient in certain nutrient components. For example, while root and tuber crops are rich in carbohydrates, they are low in protein; legumes are usually high in protein, but deficient in essential amino acids methionine; and milled rice is rich in starch but contain little essential amino acid lysine, iron, and no provitamin A (ß-carotene).
- Relying on a single food crop as source of nutrients thus will not achieve a balanced diet and results in malnutrition and deficiency diseases, especially in the developing countries, due mainly to poverty.
- The development and application of biotechnology offers opportunities and novel possibilities to enhance the nutritional quality of crops, particularly when the necessary genetic variability is not available.
- While initial emphasis of agricultural biotechnology has been placed on input traits of crops such as herbicide tolerance, insect resistance and virus resistance, increasing effort and promising proof-of-concept products have been made in output traits including enhancing the nutritional quality of crops since 1990s.
- Advancements in plant transformation and transgene expression also allow the use of plants as bioreactors to produce a variety of bio-products at large scale and low cost. Many proof-of-concept plant-derived healthcare products have been generated and several commercialized.

WHAT IS AGRICULTURAL BIOTECHNOLOGY?

- Agricultural biotechnology is a collection of scientific techniques used to improve plants, animals and microorganisms.
- Based on an understanding of DNA, scientists have developed solutions to increase agricultural productivity. Starting from the ability to identify genes that may confer advantages on certain crops,

and the ability to work with such characteristics very precisely, biotechnology enhances breeders' ability to make improvements in crops and livestock.

- Biotechnology enables improvements that are not possible with traditional crossing of related species alone.
- Crops improved through agricultural biotechnology have been grown commercially on a commodity scale for over 12 years. These crops have been adopted worldwide at rates exceeding any other advances in the history of agriculture.
- Agricultural biotechnology can help solve the global food crisis and make a positive impact on world hunger. According to the United Nations, food production will have to rise by 50 percent by the year 2030 to meet the demands of a growing population.
- Agricultural biotechnology has been shown to multiply crop production by seven- to tenfold in some developing countries, far beyond the production capabilities of traditional agriculture, and the global community is taking notice.
- In 2007, 12 million farmers in 23 countries – 12 developing and 11 industrialized – planted 252 million acres of biotech crops, primarily soybeans, corn, cotton and canola. Eleven millions of these were small or resource-poor farmers in developing countries.
- Farmers earn higher incomes in every country where biotech crops are grown. When farmers benefit, their communities benefit as well.

Biofortification and Biotechnology

- While ordinary food fortification refers to addition of supplements to food during processing to make it more nutritious, biofortification involves the development of crops that produce these micronutrients while they are growing.
- Biofortification is a sustainable agricultural strategy for reducing micronutrient malnutrition since rural poor populations have limited access to commercially fortified foods. It consists of breeding new varieties of staple foods that have higher mineral and vitamin content.
- Biofortification can be described as a complementary, rural-targeted micronutrient program strategy for better reaching remote regions, which often comprise the majority of the malnourished vulnerable populations.
- Biofortification can be attained through either of two procedures: conventional breeding or biotechnology. In breeding, plants that are naturally high in nutrients are cross-bred with high yielding varieties to produce high yielding and nutritious plants.

- Agricultural biotechnology often involves genetic improvement of crops by the insertion of gene sequences that confers desirable characteristics to a crop.
- Biotechnology is one tool that has proved to be most valuable in developed countries which have attained food sufficiency.
- Biotechnology has been used in the development of many insect resistant crop varieties using a protein from the soil bacteria *Bacillus thuringiensis* (Bt).
- Now we have Bt cowpea, Bt cotton, Bt maize and also herbicide resistant crops which have revolutionized agricultural productivity of many developed countries. These crops are also known as first generation biotechnology crops.
- In more recent times, second generation biotechnology crops include those that have been biofortified with higher levels of vitamins and minerals such as vitamin A, iron and protein.
- Other examples include the high omega 3 soybean and low trans-fat Vistine III soybean plants.
- Examples of biofortification projects using biotechnology include high vitamin A rice, Golden Rice (GR), the African Biofortified Sorghum (ABS), high vitamin A bananas and nutritious cassava (BioCassava Plus) all enriched with higher contents of vitamin and minerals. These crops were created not only for producer benefits but also for considerable consumer benefits.
- The farmers benefit by increased productivity as a result of increased yield, improved resistance to pests and diseases and tolerance to environmental stresses such as drought.
- The processors benefit by improved quality of food, animal feed or energy source. The consumers benefit by eating healthier foods.

Genetically Engineered Plants

- Agronomic traits: BT Corn, Roundup Ready Soy, Disease Resistance
- Food quality, Nutrition, Metabolic products, Vaccines

Bt Corn

- Natural insecticide from *Bacillus thuringiensis*
- Non-toxic to humans
- Target insect: Corn borer, root worm, Boll worm
- reduces insecticide use - reduces mycotoxins in corn
- 47% U.S. Corn crop Bt (2007); 59% U.S. Cotton crop (2007)

Bt Concerns

- Bt pollen harms non-target species?

- Bt crops select for resistant insects
- Bt pollen can drift to organic fields
- Food system failed to keep BT Starlink corn out of human food products

Herbicide Resistance

- Roundup Ready soy, corn, canola, cotton
- Allows post-emergence herbicide spraying
- Increases yield
- Facilitates no-till farming
- Cross-pollinates weeds
- Fosters dependence on Agrochemcial companies

Disease Resistance

- Canola, Cantaloupes, Cucumbers, Corn, Rice, Papaya, Potatoes, Soybeans, Squash, Tomatoes, Wheat

Health and Nutrition

- **Golden Rice**
  - Vitamin A and Iron enhanced
  - Seeds given to the poor for free
- **Improved Amino Acid Balance**
  - Soy (needs Methionine)
  - Maize (needs Lysine)
- **Banana Vaccines**

IMPORTANT GENETICALLY ENGINEERED CROPS IN 2003

- ***Herbicide-tolerant soybean*** was the dominant transgenic crop, grown commercially in seven countries in 2003 (USA, Argentina, Canada, Mexico, Romania, Uruguay and South Africa).
- ***Insect-resistant maize*** was the second most dominant crop, planted in nine countries (USA, Canada, Argentina, South Africa, Spain, Philippines, Honduras, Uruguay and Germany).
- ***Herbicide-tolerant canola*** was the third most dominant crop, planted in two countries, Canada and the USA.
- ***Bt cotton*** is increasingly important in countries such as China, India, and South Africa.

7.1 Improving the nutritional quality of cereal crops (e.g. essential amino acids, etc,)

- Advancement in agricultural biotechnology has allowed the exploration and development of technologies to correct the deficiency and improve the nutritional quality of food crops.

Biotechnology Provides Nutritional Benefits

- Since the early efforts of biotechnology, scientists have planned to use the technology to make more nutritious foods to benefit consumers around the world.
- As the technology developed, the first generation of agricultural biotechnology products were focused more on input traits, which means these modifications made insect, virus and weed control easier or more efficient for farmers. These first products have been rapidly adopted by U.S. farmers, and now account for the majority of soybeans, cotton and corn grown in the U.S.
- Agricultural biotechnology varieties focused on consumer benefits are often called output traits. These products spent much more time in development, but are moving towards commercial availability.
- Many of these would fit into the category of "functional foods" because they provide added nutrition compared to their conventional counterparts. Following are some examples of trait improvements in the pipeline.

Proteins and essential amino acids

- Plant proteins contribute about 65% of the per capita supply of protein on a worldwide basis, with cereal grains and food legumes as the most important suppliers.
- However, as source of dietary protein, plant proteins are often deficient in certain essential amino acids (EAAs) which are nutritionally indispensable under all conditions.
- For example, cereal proteins are generally low in lysine (Lys) (1.5-4.5 vs. 5.5% of WHO recommendation) while legume, root, tuber and most vegetable proteins are deficient in the sulphur-containing amino acids [methionine (Met) and cysteine (Cys), 1.0-2.0% vs. 3.5% of the WHO reference protein].
- Several molecular approaches have been attempted and developed to correct such deficiency, **either**
- by **increasing the content of protein bound EAAs** in crops, through:
    A. modifying the protein sequence of a major crop protein to contain higher content of a desired EAA;
    B. producing a synthetic protein rich in a target EAA;
    C. expressing a heterologous protein with high content of the desired EAA;
    D. manipulating the expression of a homologous protein for desired EAA; **or**
- by **increasing the pool of a specific free EAA** through metabolic engineering.

Vitamins and Minerals

- Vitamins and minerals are essential food components for human health. Deficiency in dietary micronutrients such as vitamin A, iron, iodine, or zinc will result in micronutrient malnutrition and various deficiency diseases.
- Biofortification through transgenic technology is an innovative and promising approach. A good example is the fortification of rice with provitamin A through metabolic engineering.
- The first generation of provitamin enriched rice, *Golden Rice 1* (*GR1*) was generated by expressing the *psy* gene from daffodil and the *crtI* gene from bacteria in rice to achieve a yield of 1.6μg provitamin A/g in the endosperm.
- High vitamin A rice, Golden Rice (GR), the African Biofortified Sorghum (ABS), high vitamin A bananas and nutritious cassava (BioCassava Plus) all enriched with higher contents of vitamin and minerals.

Carbohydrates, lipids and other compounds

- Food components such as starch, oils, fatty acids, and secondary compounds have also been demonstrated as feasible to manufacture and modify in transgenic plants.
- Our understanding of starch, lipid and secondary compound metabolisms in plants allows application of biotechnology to manipulate the genes participating in the pathways for altered structure, property, level and function of these bio-products, so as to achieve desired quality, for food and healthcare utilization.
- For example, transgenic potatoes that contain only amylopectin and devoid of amylose was first demonstrated in 1991; highlauric acid (40-50% in) canola oil produced by transgenic technology was first commercialized in 1995; and transgenic tobacco plants containing protoalkaloid tryptamine as high as 260-fold over the control plants was obtained in 1990.

7.2 Elimination of allergy causing substances

- A food allergy is any adverse reaction to an otherwise harmless food or food component that involves the body's immune system.
- To avoid confusion with other types of adverse reactions to foods, it is important to use the term "food allergy" or "food hypersensitivity" only when the immune system is involved in causing the reaction.
- Food allergies or hypersensitivities are adverse reactions to foods triggered by the immune system. Within the different types of reactions involved, non-immunological intolerances to food and reactions involving components of the immune system need to be differentiated.

- There are several different types of adverse reactions involving the immune system, which helps the body resist disease.
- In the case of food allergy, "immediate hypersensitivity" is the most clearly understood. This reaction involves three primary components: food allergens, immunoglobulin E (IgE), and mast cells and basophils.
- A food allergen is the part of a food that stimulates the immune system of food-allergic individuals. A single food can contain multiple food allergens, the majority of which are likely to be proteins, not carbohydrates or fats.
- People with food allergies produce increased amounts of IgE, which is an antibody in the immune system.
- When allergic individuals eat certain foods, their immune systems are stimulated by the food allergens to make IgE specific to that food.
- Millions of IgE antibodies then circulate in the blood; they bind to blood cells called basophils and enter body tissues where they bind to mast cells.

Removing allergens and antinutrients.

- The allergenic protein in rice has been reduced by modifying its biosynthetic pathway. There is also work to reduce allergenicity in wheat.
- This work involves inserting a thioredoxin- biosynthesis gene to break the disulfide bonds in the offending protein but without interfering with the functionality of the wheat proteins.
- Cassava roots naturally contain high levels of cyanide. As they are a staple food in tropical Africa, this has led to high blood-cyanide levels which have harmful effects.
- Application of modern biotechnology to decrease the levels of this toxic chemical in cassava would reduce its preparation time.
- In potatoes, insertion of an invertase gene from yeast reduces the natural levels of glycoalkaloid toxin.

7.3. Improved baking quality and Availability of minerals ……… *(Reading Assignment)*

CHAPTER EIGHT

SINGLE CELL PROTEIN PRODUCTION

- **Single cell protein** is a protein extracted from cultured algae, yeasts, or bacteria and used as a substitute for protein-rich foods, especially in animal feeds or as dietary supplements. Many types of animal feeds contain single cell proteins. 60-80% dry cell weight; contains nucleic acids, fats, CHO, vitamins and minerals. Rich in essential amino acids (Lys-Met).
- Microbes can be used to ferment some of the vast amounts of waste materials, such as straws; wood and wood processing wastes; food, cannery and food processing wastes; and residues from alcohol production or from human and animal excreta.
- Single-cell proteins develop when microbes ferment waste materials (including wood, straw, cannery, and food-processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta).
- The problem with extracting single-cell proteins from the wastes is the dilution and cost. Found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation, and filtration, or the use of semi-permeable membranes.
- The "single cell protein" refers to microbial biomass used as food and feed additives.
    - Either the isolated cell protein or the total cell material may be called SCP.
- Protein quality and quantity are the goals of SCP production
- Microorganisms produce proteins much more efficiently than any farm animal.

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.
- For starchy materials, a combination of *Saccharomycopsis fibuligera* and a *Candida* sp. such as *C. utilis* has been employed, in which the former effects hydrolysis of starches and the latter subsists on the hydrolyzed products to produce biomass.

CHAPTER NINE

FOOD PRESERVATION TECHNIQUES

What Are Foods?

- Foods are materials, raw, processed, or formulated, that are consumed orally by humans or animals for growth, health, satisfaction, pleasure, and satisfying social needs.
- Generally, there is no limitation on the amount of food that may be consumed (as there is for a drug in the form of dosage).
- This does not mean that we can eat any food item as much as we want.
- Excessive amounts could be lethal, for example, salt, fat, and sugar.
- Chemically, foods are mainly composed of water, lipids, fat, and carbohydrate with small proportions of minerals and organic compounds.
- Minerals include salts and organic substances include vita- mins, emulsifiers, acids, antioxidants, pigments, polyphenols, and flavor-producing compounds.
- The different classes of foods are perishable, nonperishable, harvested, fresh, minimally processed, preserved, manufactured, formulated, primary, secondary derivatives, synthetic, functional, and medical foods.

Food Preservation?

- **Food preservation** is the 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.
- Preservation 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.

Why Preservation?

- Another important question is *why food needs to be preserved. ?*
- *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

- It is important to know for whom the preserved food is being produced.
- Nutritional requirements and food restrictions apply differently to different population groups.
- Food poisoning can be fatal, especially in infants, pregnant women, the elderly, and those with depressed immune systems.
- The legal aspects of food preservation are different in case of foods produced for human and for animal consumption.
- Thus, it is necessary to consider the group for whom the products are being manufactured.

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,
2. Killing principles of microorganisms,

1. Inhibition principles

- In this principle, food preservation is achieved by inhibition of growth and multiplication of MOs.
- **The inhibition principle can be achieved by any of the following methods:**
  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

- In this principle, spoilage microorganisms are destroyed (Killed) in the food, and
- The food protected against subsequent contamination by being enclosed in an air tight container.

Methods employed to achieve the killing principle

- **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*** and etc.

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, seafood's, 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 - Desiccation/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.

UNIT TEN

IMPROVING THE INTESTINAL MICOFLORA

INTRODUCTION

- 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 the gastrointestinal tract (GIT) which houses a huge microbial ecosystem
- The microbiota of the human gastrointestinal tract plays a key role in efficient nutrient absorption and thus maintaining human health.
- Under certain circumstances such as:
  - diet, medication, stress, age and general living conditions,
- this balance may be altered leading to the colonization of pathogenic species which is manifested as different gut disorders like inflammatory bowel disease, colonic cancer etc.
- Therefore, knowledge of the gut microflora and its interactions may lead to the development of dietary strategies that serve to sustain or even improve normal gastrointestinal microbiology.
- Probiotics, prebiotics and synbiotics are the new concepts that have been developed to modulate the target gastrointestinal microflora balance.
- Over recent years **three** scientific rationales have been put forward as a means of modulating the human gut microflora towards improved host health.
  1. **Probiotics** have been defined as live microbial food supplements, which have a beneficial effect on the intestinal balance of the host animal.
  2. **Prebiotics** on the other hand, are non-viable food components, which evade digestion in the upper gut, reach the colon intact and then are selectively fermented by bacteria seen as beneficial to gastrointestinal health, namely the bifidobacteria and/or lactobacilli.
  3. The **synbiotic** approach combines both probiotics and prebiotics and aims to stimulate the growth and/or activity of indigenous bifidobacteria and lactobacilli, while also presenting proven probiotic strains to the host.
- All three have received much scientific and commercial interest in recent years and a range of such microbially active food products are now available.

Definitions used by the international scientific associations for probiotics and prebiotics

- **Probiotics**
  - Live microorganisms that confer a health benefit on the host when administered in adequate amounts. E.g., *Lactobacillus acidophilus*, *L. casei* and *Bifidobacterium bifidum*
- **Prebiotic**
  - Selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health
- **Synbiotics**
  - Products that contain both probiotics and prebiotics

Probiotic Microorganisms

- The majority of probiotic microorganisms belong to the genera
  - *Lactobacillus* and
  - *Bifidobacterium*.
- However, other bacteria and some yeast may have probiotic properties as well.
- *Lactobacilli* and *Bifidobacteria* are Gram positive lactic acid-producing bacteria that constitute a major part of the normal intestinal microflora in animals and humans.

Lactobacilli:

- Non-spore forming rod-shaped bacteria.
- They have complex nutritional requirements
- Anaerobic, Acidophilic.
- Found in a variety of habitats where rich carbohydrate-containing substrates are available

Bifidobacteria:

- Constitute a major part of the normal intestinal microflora in humans
- Nonmotile, Non-sporulating rods with varying appearance.
- Most strains are strictly anaerobic.

Health relevant effects of probiotics

- Many strains of probiotic bacteria have been shown
  - (1) to modulate (temporarily) the intestinal microflora and/or
  - (2) to inhibit colonization of the gut by (potential) pathogens, as well as
  - (3) translocation of pathogenic bacteria through the intestinal wall and the infection of other organs.
- Suggested, but unconfirmed mechanisms for these effects include:

  - Reduced intestinal pH,
  - production of bactericidal substances (e.g. organic acids, H_2O_2 and bacteriocines),
  - agglutination of pathogenic microorganisms,
  - strengthening barrier function of the intestinal mucosa,
  - absorption and metabolization of potentially pathogenic, toxic, or cancerogenic metabolites and enzymes,
- **Control of growth of undesirable organisms in the intestinal tract**
  - *Lb. acidophilus, Lb. casei*, and species of *Bifidobacterium* can inhibit growth of undesirable microorganisms
  - Most of the older reports dealing with this type of control focused on a therapeutic approach;
    - in that cultured products made with these organisms were used to treat infections of various types.
  - The newer approach is to provide consumers with products containing the probiotic organisms for use as preventive treatment in controlling intestinal infections.
- **Improvement of immune response**
  - Enhancement of the body's immune response by consuming cells of certain lactobacilli increases resistance of the host to intestinal infections.
  - Of the lactobacilli, *Lb. casei* seems to be the primary one involved.
  - *Bi. longum* also can stimulate the immune system to control *E. coli* in the gastrointestinal tract.
- Probiotic microorganisms improve the immune response **by**
  - Modulating of the systemic and secretary immune response;
  - Increasing proliferation in organs of the immune system(Spleen) ;
  - Stimulating phagocytes/macrophages and natural killer cells;
  - Increasing release of cytokines (IFNα, IFNγ , INFα),
  - Increasing production of specific antibodies
- **Improving of lactose digestion**
  - People who lack the ability to digest lactose adequately are classified as lactose maldigestors. ("lactose intolerance')
  - The problem results from inadequate levels of β-galactosidase in the small intestine to hydrolyze ingested lactose adequately.

- Once a lactose maldigestor consumes sufficient lactose, results in symptoms of cramps, flatulence, and diarrhea.
- These symptoms often follow consumption of milk by such individuals.
- to hydrolyze lactose in the small intestine, the possibility exists for providing such an enzyme via the diet.
- Incorporation of *Lb. acidophilus in* milk

- **Treating inflammatory intestinal diseases**
  - Studies in experimental animals give a clue about the potential application of *lactobacilli, bifidobacteria*, *Lactococcus lactis* or non-food probiotics, particularly nonpathogenic strains of *Escherichia coli* (e.g. strain Nissle 1917) to prevent or treat colitis.
  - Likewise, patients with inflammatory bowel diseases (Crohn's disease and ulcerative colitis, necrotizing entero-colitis, diverticulitis or inflammation of an ileal pouch) responded positively too.
- **Anti-carcinogenic actions**
  - Several mechanisms have been suggested as a cause of these effects and have been investigated in vitro and in animal experiments:
  - Inhibition of tumor-growth and proliferation of tumor cells by glycopeptides and cytotoxic metabolites of lactobacilli.
    - Reduction of (pro) carcinogenic,
    - mutagenic and genotoxic substances (aflatoxines, nitrosamines;
    - and cancer-promoting enzymes (nitro-, azoreductase, β-glucuronidase)
  - in the colon due to :
    - modifications of the gut flora,
    - a decrease in pH,
    - chemical modification, and
    - absorption by the bacteria
- **Control of serum cholesterol**
  - Several studies have shown that animals consuming milk containing cells of *Lb. acidophilus* had lower serum cholesterol levels than did animals that did not receive milk containing the lactobacilli.
  - There may be other probiotic organisms that can help control serum cholesterol levels.
  - Some of these include *Lb. casei* and *Bifidobacterium* species.
- **Probiotics in controlling allergy and atopic diseases of children**

  - Probiotic bacteria modulate immune reactions in persons with allergies and atopic diseases or in at-risk infants.

Probiotic food

- Probiotic microorganisms in foods have to fulfill a lot of other conditions.
- These include a sufficient stability during production and storage,
- Recently, these probiotic organisms are attracting lot of attention as food grade biotherapeutics and have led to the development of a new concept of functional foods
- Survival and bacterial counts of probiotic microorganisms in the food, and the maintenance of its probiotic activity depend on the production process,
- These include chemical composition, water activity, oxygen concentration, pH value,
- A number of companies are now venturing into the development of a variety of probiotic formulations.
- One such company Probiotics International are leading manufacturers and suppliers of nutritional health care products which include the natural live microbial supplements that are known to be beneficial in the treatment of a variety of digestive disorders and diseases associated with stress.
- A few of these products are listed below and commercially available:
  - Protexin-Natural Care Tablets (Maintains healthy nervous & immune system)
  - Protexin Natural Care Powder (Maintains healthy nervous & immune system)
  - Protexin-Vital (Adults) (Maintains healthy intestinal flora in adults)
  - Protexin-Vital (Child) (Maintains natural healthy flora in children)
  - Protexin - Travel (Diarrhoea and sickness during travel)
  - Protexin -Relief (Relieves constipation in elderly)
  - Protexin -Lacta (Lactose intolerance)
  - Protexin-Restore (Restores intestinal flora in children)
- Japan is the world leader in probiotic and prebiotic products.

Prebiotics are dietary fibers

- Prebiotic carbohydrates are dietary fibers, as they are not digested by human enzymes but fermented by the flora of the large intestine.
- Thus, they
  - increase biomass, feces weights, and feces frequency,
  - have a positive effect on constipation and on the health of the mucosa of the large intestine.

- Positive effects of pre- and synbiotics on the intestinal flora,
    - i.e. growth-promotion of potentially protective bacteria (bifidobacteria and in part also lactobacilli) and/or
    - the inhibition of potentially pathogenic microorganisms, as well as
    - stabilization of the intestinal environment by
        - lowering the pH and
        - release of short-chain organic acids,
    - Cancer prevention
    - Production of short-chain fatty acids during fermentation of prebiotic carbohydrates.
        - A more acidic pH and modulations of the intestinal flora,
    - Butyric acid supports the regeneration of the intestinal epithelium
    - Immune modulation
- **Stimulation of mineral adsorption and bone stability**
    - Lowering the pH in the gut improves the absorption of calcium, 20 iron, and magnesium in the large intestine, probably due to an increased mineral solubility.
    - Beneficial effects on calcium absorption and bone mineralization were also demonstrated in pigs and humans.

Prebiotic and synbiotic food

- Prebiotic carbohydrates, mostly inulin and oligofructose, have been added.
- Very often prebiotics are added to probiotic foods, whereby their concentration in the product is typically below 10%.
- For this combination, the term "synbiotic" has been coined.
- For example, two European companies from France and the Netherlands, respectively, launched combinations of *L. acidophilus* strains with fructooligosaccharides (FOS) or inulin, respectively, in the market, claiming to lower blood cholesterol.

Anumber of desirable features have been identified for the selection of efficacious probiotic strains:

1. The probiotic should be of host animal origin. It is likely that the most effective and best adopted probiotic strains will be those originally isolated from the gastrointestinal tract of the target animal.
2. The probiotic strain must be safe. The probiotic strains should not cause disease, be associated with disease states or even related to recognized bacterial pathogens.
3. Probiotic strains must be amenable to industrial processes.

4. To persist to the colon, probiotic strains must show a degree of resistance to gastric acid, mammalian enzymes and bile secretions.
5. To maximize the colonization potential of a probiotic strain, the ability to adhere to humanmucosal cells may be an advantage. The ability of certain probiotic strains to hinder adhesion of pathogens, or their toxins, to human cells has been proposed as one possible mode of probiotic action.
6. The ability of the probiotic strain to persist in the gut has been identified as one important prerequisite of probiotic efficacy.
7. Some probiotics produce anti-microbial agents targeting important gastrointestinal pathogens which are a desirable characteristic. Many lactic acid bacteria and bifidobacteria have been shown to produce bacteriocin-like moleculeswith different spectrums of activity.
8. Certain probiotic strains have been shown to stimulate the immune system in a beneficial, non-inflammatory manner. Such strains have also been shown to relieve the symptoms of allergic conditions such as atopic eczema and bovine milk protein intolerance in human feeding studies.
9. Efficacious probiotic strains should also impact on metabolic activities such as cholesterol assimilation, lactase production and vitamin production.

UNIT ELEVEN

SOCIAL AND ETHICAL CONSIDERATIONS REGARDING GENETICALLY ENGINEERED FOODS

Genetic modification

- 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.

11.1. 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.

11.2 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.

11.3. 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.

11.4. 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.

11.5. 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.

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