SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited 'A' Grade by NAAC / 12B Status by UGC / Approved by AICTE

www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

M.TECH - BIOTECHNOLOGY

UNIT – I – FOOD & NUTRACEUTICALS – SBTA7013

Components of Food

Food is essential for the survival of living organisms. It provides us with energy to carry out daily activities and develop, grow and repair our body parts in case of any damage. Therefore understanding the importance of consuming the right kind of food becomes a necessity.

Furthermore, most of the food items available today are adulterated in many possible ways. The food that we consume comes from numerable sources and the variety is huge. You can think of the last meal you had, and list down sources from which your food was obtained. This helps us understand how food varies in its sources and components.

What Do Different Food Items Contain?

Each dish we eat is prepared using more than one ingredient containing different nutrients along with dietary fibres and water. Some nutrients in our food are fats, minerals, vitamins, proteins and carbohydrates.

- Carbohydrates are present in the form of starch and sugars in our food. The presence of carbs in our food can be tested using a dilute iodine solution, an indication of the presence of starch is shown by black-blue colouration.
- Presence of protein in food is tested using a solution of copper sulphate and caustic soda. If the solution turns violet, proteins are present.
- Presence of fat in food is indicated when food wrapped in paper turns oily after releasing its moisture content.

Also Read: Balanced diet

What Do Various Nutrients Do For Our Body?

- Nutrients play a vital role in determining the health of our body. These nutrients are used up during the process of nutrition by each cell in our body to carry out their basic functionality.
- Fats provide more energy to our body as compared to carbohydrates.
- Proteins are bodybuilding foods.
- Vitamins help our body to fight against diseases and also helps to keep our eyes, gums, bones in a healthy shape. There are different kinds of vitamins such as Vitamin A, B complex, C, D. E, K etc. Vitamin C protects our body against diseases.

Sources of different Vitamins	
Vitamin A	Carrot, Mango, Papaya
Vitamin C	Tomato, Guava, Lemon, Orange
Vitamin B	Rice, wheat

Vitamin D	Liver, fish, milk, butter

- Roughage, also known as dietary fibres, is obtained from plants such as fruits, whole grains etc. They do not provide nutrients but add bulk to our food and are essential to our body as they aid in digestion.
- Water helps absorb nutrients from food and aids in getting rid of wastes in the form of sweat and urine

Components of Food (Tabular Representation)

Food Component	**Functions**
Carbohydrates	These are digested and broken down into glucose and provide energy to the body
Fats	Store energy, protects and insulates the important organs
Proteins	Help in metabolism, act as enzymes, and hormones
Vitamins	These help in maintaining healthy bones, boost the immune system, heal wounds, repair and damage of cells and converting food into energy
Iodine	Formation of thyroid hormone
Calcium	Helps in the proper functioning of the nervous system and maintain healthy bones
Phosphorus	Helps to maintain acid-base balance in the body
Sodium	Controls the blood pressure
Iron	Facilitates the formation of haemoglobin
Fibres	They help in food absorption and prevents constipation
Water	They help in absorbing nutrients from the food and release waste from the body in the form of urine and sweat.

Balanced Diet

A diet, which contains the right amount of nutrients, roughage and water is called a balanced diet. Eating a balanced diet can never lead us to obesity. Consuming the right food is as important as having knowledge of the nutrient content of your food.

Deficiency Diseases

Deficiency diseases occur in our body due to lack of nutrient supply over a long period of time. Listed below are a few deficiencies caused as a result of lack of nutrients.

Name of Nutrient	Disorder caused	Symptoms
Vitamin C	Scurvy	Takes a long time to heal, bleeding gums
Iron	Anaemia	Weakness
Calcium	Tooth decay and bone	Tooth infections, Weak bones

Nutraceuticals

The term Nutraceuticals is a hybrid or contraction of nutrition and pharmaceuticals. Nutraceuticals are products derived from food sources that are purported to provide additional health benefits, in addition to the basic nutritional value found in foods. It is classified into two:

a. Dietary supplements

b. Functional foods

a. Dietary supplements

A product intended to supplement the diet that bears or contains one or more of the following dietary ingredients:

· a vitamin

· a mineral

· an herb or other botanical

· an amino acid

· a dietary substance used by man to supplement the diet by increasing the total dietary intake or

· a concentrate, metabolite, constituent, extract, or combination of any ingredient described above.

Dietary supplements are further defined as products that are labeled as dietary supplements and are not represented for use as a conventional food or as a sole item of a meal or the diet. Supplements can be marketed for ingestion in a variety of dosage forms including capsule, powder, softgel, gelcap, tablet, liquid, or indeed, any other form. Eg. Multi-vitamin capsules.

b. Functional foods

Functional foods are fortified or enriched during processing and then marketed as providing some benefit to consumers. Sometimes, additional complementary nutrients are added, such as Vitamin D to milk. Functional foods are "***Ordinary food that has components or ingredients added to give it a specißc medical or physiological beneßt, other than a purely nutritional e$ect.***" All functional foods must meet three established requirements: Foods should be

· present in their naturally occurring form, rather than a capsule, tablet, or powder

· consumed in the diet as often as daily and

· should regulate a biological process in hopes of preventing or controlling disease.

Classification of functional foods

Functional foods are classified in to

a. Probiotic

b. Prebiotic

c. Synbiotic

d. Phyto chemicals

a. Probiotic

Probiotic is a greek word which means "for life" It was coined by Lilly and Stilwell in1965. Probiotics are living microorganisms which upon ingestion in sufficient numbers, exert health benefits beyond basic nutrition. Probiotics are a viable microbial dietary supplement which uplifts the health of the host.

b. Prebiotic

In 1995, Prebiotics was defined by Gibson and Roberfroid as non-digested food components that, through the stimulation of growth and/or activity of a single type or a limited amount of microorganisms residing in the gastrointestinal tract, improve the health condition of a host. Prebiotics may be used as an alternative to probiotics or as an additional support for them. Prebiotics have enormous potential for modifying the gut microbiota, but these modifications occur at the level of individual strains and species and are not easily predicted a prior.

c. Synbiotic

In 1995, Gibson and Roberfroid introduced the term “synbiotic” to describe a combination of synergistically acting probiotics and prebiotics. As the word “synbiotic” implies synergy, the term should be reserved for those products in which a prebiotic component selectively favours a probiotic microorganism. Synbiotics have both probiotic and prebiotic properties and were created in order to overcome some possible difficulties in the survival of probiotics in the gastrointestinal tract.

d. Phytochemicals

Phytochemicals are plant chemicals that differ from nutrients in some important ways. Phyto is a greek word for plants. Essential nutrient which include protein, fats, minerals, and vitamins are essential for life. Phytochemicals are not necessary for life but they help to promote optimal health by lowering risk for chronic diseases, such as cancer and heart disease. They are found only in plant foods. Fruits and vegetables are among the best sources of these compounds. Phytochemicals are believed to have many health benefits and prevent lifestyle diseases.. Some groups of phytochemicals have been linked to decreased cancer risk also. Following are examples of some phyto chemicals with nutritional importance.

i. Flavanoids

Flavanoids are a special class of phytochemicals that includes hundreds of different compounds. They are excellent

antioxidants and some have hormonal properties. Among some of the most studied flavonoids are allicin, which is found in onions and garlic.

Benefits of Flavanoids

· Longer life span

· Prevents obesity and helps in weight management

· Prevents cardio vascular disease, diabetes, cancer.

· Prevents neuro generative disease

· Slows down ageing process.

ii. Carotenoids

Carotenoids are a group of phytochemicals that act as pigments, giving plants their bright green, orange, yellow, red, and blue colors.

Benefits of carotenoids

· Beta-carotene, found in carrots, sweet potatoes, green leafy vegetables, red peppers, and pumpkin. Beta- carotene from foods has been linked to a reduced risk for lung cancer.

· Lycopene, found in tomatoes and strongly linked to reduced risk for prostate cancer.

· Lutein, foundingreenleafyvegetables and linked to reduced risk for cancer and macular degeneration.

iii. Antioxidants

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. Antioxidants are carotenoids, lycopene, vitamin C,

vitamin A, vitamin E etc..

Benefits of Antioxidants

· Slower signs of aging, including of the skin, eyes, tissue, joints, heart and brain

· Healthier, more youthful, glowing skin

· Reduced cancer risk

· Detoxification support

· Longer life span

· Protection against heart disease and stroke

· Less risk for cognitive problems, such as dementia

· Reduced risk for vision loss or disorders like macular degeneration and cataracts

· Antioxidants are also added to food or household products to prevent oxidation and spoilage

What is Fortification of Food?

Fortification is the addition of key vitamins and minerals such as iron, iodine, zinc, Vitamin A & D to staple foods such as rice, milk and salt to improve their nutritional content. These nutrients may or may not have been originally present in the food before processing.

Why do we need Fortification of Food?

70% of people in India do not consume enough micronutrients such as vitamins and minerals. About 70 percent of pre-school children suffer from anaemia caused by Iron Deficiency and 57 percent of preschool children have sub–clinical Vitamin A deficiency. Neural Tube Defects (NTDs) are the most common congenital malformation with an incidence that varies between 0.5-8/1000 births. It is estimated that 50-70% of these birth defects are preventable. One of the major causes is deficiency of Folic Acid.

Thus, deficiency of micronutrients or micronutrient malnutrition, also known as “hidden hunger”, is a serious health risk. Unfortunately, those who are economically disadvantaged do not have access to safe and nutritious food. Others either do not consume a balanced diet or lack variety in the diet because of which they do not get adequate micronutrients. Often, there is considerable loss of nutrients during the processing of food. One of the strategies to address this problem is fortification of food. This method complements other ways to improve nutrition such as such as diversification of diet and supplementation of food.

What are the benefits of Fortification?

- Since the nutrients are added to staple foods that are widely consumed, this is an excellent method to improve the health of a large section of the population, all at once.
- Fortification is a safe method of improving nutrition among people. The addition of micronutrients to food does not pose a health risk to people. The quantity added is so small and so well regulated as per prescribed standards that likelihood of an overdose of nutrients is unlikely.
- It does not require any changes in food habits and patterns of people. It is a socio-culturally acceptable way to deliver nutrients to people.
- It does not alter the characteristics of the food—the taste, the feel, the look.
- It can be implemented quickly as well as show results in improvement of health in a relatively short period of time.
- This method is cost-effective especially if advantage is taken of the existing technology and delivery platforms.
- The Copenhagen Consensus estimates that every 1 Rupee spent on fortification results in 9 Rupees in benefits to the economy. It requires an initial investment to purchase both the equipment and the vitamin and mineral premix, but overall costs of fortification are extremely low. Even when all program costs are passed on to consumers, the price increase is approximately 1-2%, less than normal price variation. Thus it has a high benefit-to-cost ratio.

Phytonutraceuticals

Phytonutrients are natural chemicals or compounds produced by plants. They keep plants healthy, protecting them from insects and the sun.
They can be found in:

- fruits
- vegetables
- whole grains
- tea
- nuts
- beans
- spices

Phytonutrients also have antioxidant and anti-inflammatory properties that can help support a healthy human body.
There are thousands of phytonutrients found in plants and related foods. Some of the most common phytonutrients are:

- carotenoids
- ellagic acid
- resveratrol
- flavonoids
- phytoestrogens
- glucosinolates

Phytonutrient health benefits

While their antioxidant qualities lead the pack in healthful benefits, phytonutrients are also known for other characteristics:

- **Carotenoids** are beneficial for eye health and immune health. Two of the six more common carotenoids — lutein and zeaxanthin — are found in the retina and can decrease your risk of developing macular degeneration by 43 percent, according to studies.
- **Flavonoids** can protect against cancer and cardiovascular disease. These phytochemicals contribute to healthy cell communication. This can trigger detoxification, decrease inflammation, and reduce the risk of tumors spreading.
- **Glucosinolates** are similar in helping to prevent cancer. Found predominantly in cruciferous vegetables — such as broccoli, bok choy, cauliflower, and brussel sprouts — they help to eliminate toxins in the body.

Types of phytonutrients

Phytonutrients are available in supplement form. However, they are best consumed as nutrient-rich foods.

Supplements don't provide all the necessary nutrients to sustain the body and, in rare cases of high dosage, can be toxic.

Carotenoids

Carotenoids are pigments in plants that are responsible for the bright-colored hues of vegetables and fruits. There are more than 600 carotenoids, and they must be consumed through foods and sources of fat. Some common types of carotenoids include:

- alpha-carotene
- beta-carotene
- beta-cryptoxanthin
- lutein
- lycopene
- zeaxanthin

Carotenoids act as antioxidants, and some can be converted into vitamin A. They support immune system function, eye health, and reduce your risk of cancer. Some foods rich in carotenoids are:

- pumpkins
- carrots
- spinach
- kale
- tomatoes
- oranges
- yams

Ellagic acid

Ellagic acid is a phytochemical known for reducing cancer risk and lowering cholesterol. Ellagic acid has antioxidant and anti-inflammatory properties. The highest levels of ellagic acid are present in raspberries. Other foods rich in this compound include:

- strawberries
- blackberries
- grapes
- pomegranates
- walnuts

- pecans

Resveratrol

Resveratrol is found predominantly in grapes — specifically, the grape skin — and wine. This compound supports cardiovascular and cognitive health. Resveratrol has also been associated with increased cerebral blood flow.

Resveratrol can be found in other foods:

- peanuts
- pistachios
- strawberries
- blueberries
- dark chocolate

Flavonoids

Flavonoids are one of the largest groups of phytonutrients. This compound is rich in antioxidant properties and anticancer activity. There are many subgroups of flavonoids, including:

- flavones
- anthocyanins
- flavanones
- isoflavones
- flavonols

Some foods rich in flavonoid compounds are:

- green tea
- apples
- onions
- coffee
- grapefruits
- legumes
- ginger

Phytoestrogens

These compounds are associated with reducing the risk of cancer, heart disease, and osteoporosis.

Phytoestrogen mimics estrogen in the body, which may be beneficial for women in relieving discomfort from hot flashes and other menopausal symptoms.

However, some studies have shown phytoestrogens may disrupt hormone function.

Be mindful of your intake of phytoestrogens and get to know how they may impact your body, as everyone is different.

Foods rich in phytoestrogen compounds include:

- soy
- broccoli
- oranges
- carrots
- coffee
- legumes

Glucosinolates

Glucosinolates are compounds found predominantly in cruciferous vegetables. They are known for helping to regulate inflammation, metabolic function, and stress responses. Glucosinolates have also been associated with cancer prevention. StudiesTrusted Source in rats and mice found

that the compounds that form from broken down glucosinololates inactivate carcinogens and protect cells from DNA damage. However, this has not been proven in human studies. Common foods rich in glucosinolates include:

- broccoli
- bok choy
- cauliflower
- brussel sprouts
- cabbage
- mustard

Scope involved in the industry, Indian and global scenario

Overview – Nutraceutical Industry

Millions of people were infected with the novel coronavirus (with the number increasing for every second passing), in fighting viruses we need to build immunity which is crucial. And, it is no secret that the role of nutrients in supporting the immune system is extremely important.

With the rising demand for natural immunity-boosting products during the pandemic made the nutraceuticals industry more in demand. There is a rise in demand for functional foods as people believe that it will add exceptional health and wellness benefits. Functional foods created a Positive attitude among the consumers mainly on account of the health benefits offered by nutraceutical products fueled the growth of the market

Changing Lifestyles, increase in ageing population and increasing healthcare costs have supported the overall growth of the nutraceuticals industry. As per the statistics 9 out of 10 adults are consuming 55% minerals & over 50% vitamins in their daily diets.

In the coming ten years the nutraceuticals industry has potential to grow upto the size of $25-30 billion dollars but it requires a lot of regulatory changes to create an organized ecosystem for the industry players across the value chain.

In 2019 global nutraceutical market size valued at $ 382.51 billion dollars and it is forecasted to have 8.3% Compound Annual Growth Rate in coming periods

A study conducted by RNCOS and ASSOCHAM estimated the growth of Indian nutraceuticals market could reach $8.5 billion dollars by 2022 from $2.8 billion dollars in 2015.

Nutraceutical Market Report Scope

Report Attribute	Details
Market size value in 2020	USD 412.7 billion
Revenue forecast in 2027	USD 722.5 billion
Growth Rate	CAGR of 8.3% from 2020 to 2027
Base year for estimation	2019
Historical data	2016 - 2018
Forecast period	2020 - 2027
Quantitative units	Revenue in USD million and CAGR from 2020 to 2027
Report coverage	Revenue forecast, company share, competitive landscape, growth factors and trends
Segments covered	Product, region
Regional scope	North America; Europe; Asia Pacific; Central & South America; Middle East & Africa
Country scope	U.S.; Canada; Mexico; Germany; U.K.; France; Italy; Spain; Russia; China; India; Japan; Australia & New Zealand; Brazil; Argentina; South Africa
Key companies profiled	Cargill; Incorporated; Archer Daniels Midland Company; DuPont; Nestle S.A; Danone; General Mills; Innophos; WR Grace; Amway Corporation
Customization scope	Free report customization (equivalent up to 8 analysts working days) with purchase. Addition or alteration to country, regional & segment scope.
Pricing and purchase options	Avail customized purchase options to meet your exact research needs. Explore purchase options

Insights- Nutraceutical Products

Nutraceuticals are nutritional functional foods or the medicines extracted from a variety of food sources to administer supplementary benefits simultaneously with basic health benefits. Nutraceutical product markets are categorized into functional foods, functional beverages and dietary supplements. The segment of functional beverages led the overall market followed by functional foods and dietary supplements in 2019. Over the years the functional beverage industry will be driven by product development coupled with technological upgradation.

Nutraceutical manufacturers extended the product offerings due to ingredient formulation microencapsulation techniques and particle size reduction.

Sports drinks gained popularity among individuals and athletes involved in physical activities. Sports drink market is driven by the millennial generation due to their willingness to pay for health products, high buying capacity and growing inclination towards fitness activities.

Risk of heart diseases are reduced by consumption Omega -3 which reduces heart healthy fats. Expected growth of Omega-3 fatty acids based functional foods industry is USD 38.76 million by 2025 with Compound Annual Growth Rate of 9.3% from 2020 to 2025.

Global Nutraceuticals Market Revenue, by Type, 2025 (USD Million)

Indian Nutraceutical market

Nutraceutical industry in India is expected to hold at least 3.5 percent of global market share by 2023. Indian nutraceutical market amounts to 2 percent of the global market pegged at USD 388 Billion dollars. In India, the pandemic has pushed the people to polarize thoughts on the importance of preventive health. There is clear visibility on the impact created by pandemic on the nutraceuticals industry in India.

The Indian nutraceuticals industry has great prospects. A wide range of products was made available in the Indian nutraceutical market over the last decade, giving an insight into the remarkable growth.

India’s economy is booming as a result of an increase in the overall disposable income of the population on one hand and on the other hand, growing awareness on the importance of diet and nutrition for long- term good health. These conditions have contributed to a favourable market for the nutraceuticals industry in India. With qualified human resources, availability of raw material and world-class R&D facilities will lead India an edge over other countries.

By 2030 the market will touch USD 10 Trillion dollars which is quadrupling growth of the Indian economy. India has over 108 large contract manufacturers in nutraceuticals and most of them operate on the “cookie-cutter” model. In the nutraceuticals market, India’s export is below USD 1.6 Billion dollars and imports in nutraceuticals are of USD 2.8 Billion dollars.

While the global market is growing with 7% Compound Annual Growth rate (CAGR) and with 18% Compound Annual Growth rate (CAGR) The Indian market has been growing much faster for the last three years, driven by functional beverages and food categories.

The Indian nutraceuticals market is dominated by pharmaceuticals and FMCG companies primarily with very few pure-play nutraceuticals companies. Some major companies marketing nutraceuticals in India are Dabur India, GlaxoSmithKline Consumer Healthcare, Cadila Healthcare, Zandu Pharmaceuticals, EID Parrys, Amway, Himalaya Herbal Healthcare, Sami Labs Ranbaxy and Elder Pharmaceuticals.

Challenges faced by Nutraceuticals Sector in India

Nutraceuticals sector is currently regulated by the Food Safety and Standards Authority of India (FSSAI), an authority under the Ministry of Food Processing Industries (MOFPI). Nutraceuticals sector could not achieve full export potential due to the limited attention by FSSAI.

The government needs to seriously think of defining the scope of the nutraceutical sector and product portfolio. There is a need of the hour for creation of a separate regulatory body that can provide prompt resolutions for the grievances, HSN [Harmonized System of Nomenclature] repository and export promotion initiatives by a centralized regulatory body to promote the sector.

Specific financial packages, independent HSN code structure and tax breaks for research, manufacturing and clinical studies will significantly boost the nutraceutical industry and which contributes significantly for public healthcare.

Centralized regulation under the Joint Secretary of the Ministry of Commerce and Industry with the institutionalization of roles and responsibilities can coordinate better decisions.

Public-private partnership (PPP) model can drive the nutraceutical products to penetrate the domestic market and to enhance the nutrition levels among the under-nourished segments of the population.

Global Market of Nutraceuticals

In 2020 Nutraceutical market size was worth USD 382.51 billion. The global nutraceutical market size is estimated to reach a value of USD 722.49 billion by 2027, expanding at a Compound Annual Growth Rate of 8.3% between 2020-27.

The U.S. holds over 27.1% of global nutraceutical market share in 2020 and with USD 63.3 billion nutraceuticals market in the year 2020. The United States will maintain a 6.1% growth momentum in nutraceuticals over a period of 2020-25. China, the world second-largest economy, exhibits the potential to grow with 9.6 % CAGR over a period of 2020-27 and with estimated market size of US$77.2 Billion by the year 2027

Other noteworthy geographic markets like Japan and Canada forecast each to grow at 3.4% and 5.7% respectively over the period 2020-2027. Within Europe, which continues to remain an important element in the world economy, Germany market will grow by 4% CAGR.

Growth and demand for nutraceutical products in emerging countries like Asia-Pacific, Latin America and the Middle East depends on internal market forces and several macroeconomic factors.

Industry Overview: Global v/s Indian Nutraceutical Market

In today's world, Europe, Japan and the USA together hold for more than 90% of the total global nutraceutical market. With compound annual growth rate (CAGR) of 8% the global market should reach USD 336 billion by 2023 from USD 247 billion in 2019. The nutraceutical market has attained maturity in developed countries and now the focus is shifting towards developing economies, especially those across the Asia Pacific including India. Indian nutraceutical market accounted for 2% of the global market share and is expected to be valued at USD 5 billion as of 2019. By 2023 Indian Nutraceutical market is expected to reach USD 11 billion with 2% Compound Annual Growth Rate (CAGR) and expecting to hold at least 3.5% of the global market share.

Conclusion

Expensive treatments have paved the way for nutraceuticals as an alternative to expensive drugs that are assumed to increase in the near future.

Following the pandemic and the consequent lockdown across the globe, health consciousness among people has mounted significantly, leading to a higher demand for products that bolster immunity. Vitamin supplements such as zinc are in huge demand, as these can help patients with reduced respiratory tract infections like SARS-CoV-2. It plays a big role in the body's immune function and can potentially bring down COVID-19 replication. Research and clinical trials are being conducted with full force to analyze the potential of various other vitamin supplements including Vitamin C, Vitamin D, and more in reducing the COVID-19 impact.

Over the years, there has been a sharp rise in vitamin deficiencies because of the growing prevalence of hectic lifestyles, shorter mealtimes, and nutrition loss during cooking as well as food processing. Therefore, consumers are becoming more aware of different deficiencies and the type of vitamin supplement to take, leading to a faster growth rate of the global market.

Given the current market scenario, a key shift in the markets would be that India could be the next big source of raw materials as well as finished goods, as the Chinese trade experiences a slump.

SATHYABAMA

INSTITUTE OF SCIENCE AND
TECHNOLOGY(DEEMEDTOBEUNIVERSITY)
Accredited'A'GradebyNAAC/12BStatusbyUGC/ApprovedbyAICTEwww.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENTOFBIOTECHNOLOGY

M.TECH - BIOTECHNOLOGY

UNIT–II– FOOD &NUTRACEUTICALS–SBTA7013

Secondary plant metabolites

Secondary plant metabolites are classified according to their chemical structures into several classes. In this chapter, the nature of secondary plant metabolites will be discussed as a foundation for a review of the main categories of constituents considered to be of therapeutic importance. Each section includes an overview of a class of the secondary plant metabolites regarding structure, botanical distribution and generalizations about pharmacology, followed by examples of representative molecules. The classes of secondary plant metabolites include:

- Phenolics
- Alkaloids
- Saponins
- Terpenes
- Lipids
- Carbohydrates

2. Phenolics

Phenolics probably constitute the largest group of plant secondary metabolites. They share the presence of one or more phenol groups (Figure 1) as a common characteristic and range from simple structures with one aromatic ring to highly complex polymeric substances. They are widespread in plants where they contribute significantly to the color, taste and flavor of many herbs, foods and drinks. Some phenolics are valued pharmacologically for their anti-inflammatory activities such as quercetin or antihepatotoxic properties such as silybin. Others exert phytoestrogenic activity as genistein and daidzein, while others are insecticidal as naringenin [5]. Many of the phenolic molecules are also effective antioxidants and free radical scavengers, especially flavonoids. Phenolics can be classified according to their structure or biosynthetic origin. According to their structures, phenolics can be classified into:

- Simple phenolics
- Tannins
- Coumarins
- Flavonoids
- Chromones and xanthones
- Stilbenes
- Lignans

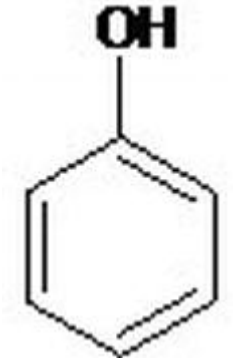


Figure 1.
Phenol.
2.1. Simple phenolics
Phenolic acids are ubiquitous among plants; although free phenols are rare,gallic acid is relatively widespread and is the parent compound of the gallotannins (Figure 2). Gallic acid is well known for its astringent properties but has demonstrated many other activities in vitro, including antibacterial, antiviral, antifungal, anti-inflammatory, antitumor, antianaphylactic, antimutagenic, choleretic and bronchodilatory actions. It also inhibits insulin degradation and promotes smooth muscle relaxation [6]. The phenolic compounds in this group vary according to their functional group, which may be hydroxyl, aldehydic, or carboxylic group; these include eugenol (a phenolic phenylpropane), vanillin (a phenolic aldehyde) and salicylic, ferulic and caffeic acids (phenolic acids). Hydroquinone is also among the most widely distributed of the simple phenols, occurring in a number of plants as the glycoside arbutin. Glycoside formation is common, and the widely distributed glycoside coniferin and other derivatives of phenolic cinnamic alcohols are precursors of lignin [7, 8].

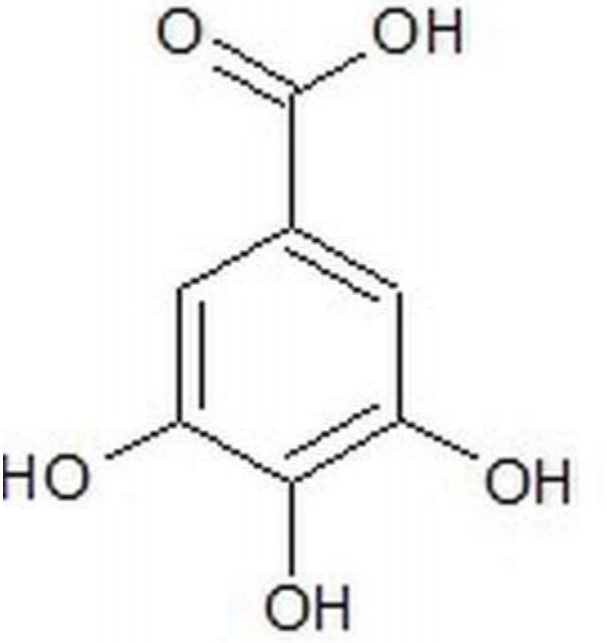


Figure 2.
Gallic acid.
The pharmacological properties of these widely found constituents are probably best demonstrated by the urinary tract antimicrobial arbutin [9] and the anti-inflammatory salicylates [10]. A property shared by all phenols is antimicrobial activity. In fact, phenol itself was the first antiseptic used in surgery [11].
The pharmacological activities of many plants are attributed to simple phenolics among which the antimicrobial and diuretic activities of *Arctostaphylosuva-ursi* were attributed to its phenolic content [12]. *Capsicum* spp. showed circulatory stimulant, rubefacient and analgesic activities due to the presence of capsaicinoids, which are simple phenolic compounds [13]. Moreover, the cholagogue activity of *Cynarascolymus*, the anthelmintic activity of *Dryopterisfilix-mas*, the anti-inflammatory analgesic activity of *Filipendulaulmaria* as well as the anticatarrhal and diuretic activities of *Solidagovirgaurea* are all attributed to the action of simple phenolics [8]. Figure 3 illustrates some examples of simple phenolics.

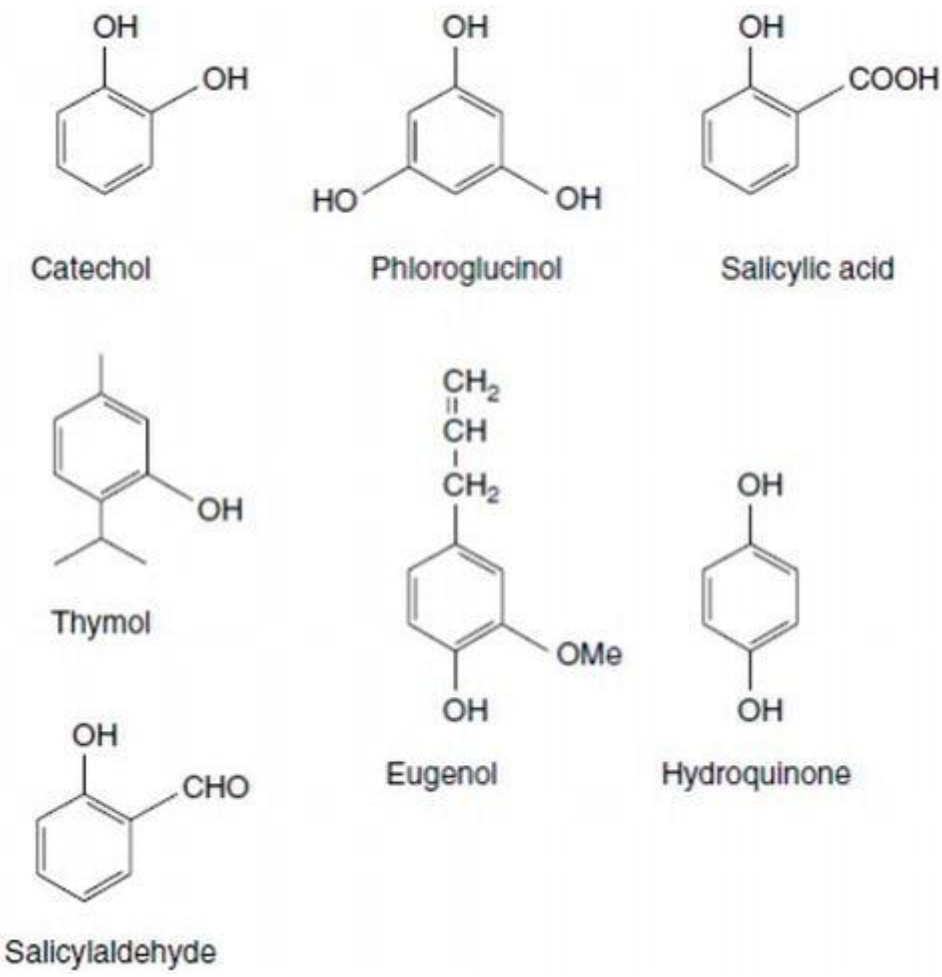


Figure 3.

Examples of simple phenolics.

2.2. Tannins

Tannins are polyphenols which have the ability to precipitate protein. These compounds have been used for decades to convert raw animal hides into leather. In this process, tannin molecules crosslink the protein and make it more resistant to bacterial and fungal attack. Today, however, many substances considered to be tannins by virtue of their structure and biosynthetic origin have limited, if any, ability to make leather [14]. There are two major types of tannins: hydrolyzable tannins and condensed tannins. Hydrolyzable tannins are formed from several molecules of phenolic acids such as gallic and hexahydroxydiphenic acids, which are united by ester linkages to a central glucose molecule. Two principal types of hydrolysable tannins are gallotannins and ellagitannins, which are, respectively, composed of gallic acid and ellagic acid units. Ellagitannins found in plants of medicinal interest and for which structures have been elucidated include geraniin (isolated from *Geranium robertianum* (Herb Robert) and *Geranium maculatum* (American cranesbill) [15]) and tellimagrandins 1 and 2 [16] (isolated from *Quercus alba* (Oak bark), *Punicagranatum* (pomegranate) and *Filipendulaulmaria* (Meadowsweet)) [7].

Condensed tannins, or proanthocyanidins, are compounds whose structures are based on oligomeric flavonoid precursors and vary in the type of linkages between flavonoid units; hydroxylation patterns; stereochemistry of carbons 2, 3 and 4 of the pyran ring and the presence

of additional substituents. Some drugs (e.g., *Camellia sinensis* (tea), *Hamamelisvirginiana* leaves and bark) contain both hydrolyzable and condensed tannins [17].

Tannin-containing drugs act as antidiarrhoeals and have been employed as antidotes in poisoning by heavy metals and alkaloids. Epigallocatechin-3-gallate, the active principal in tea, has been shown to be antiangiogenic in mice. *Vacciniumoxycoccos* (cranberry) juice has long been used as urinary antiseptic [18], which was scientifically proven in a randomized, double-blind, placebo-controlled trial that has been carried out on 153 elderly women [19]. Figure 4 illustrates some examples of hydrolysable tannins.

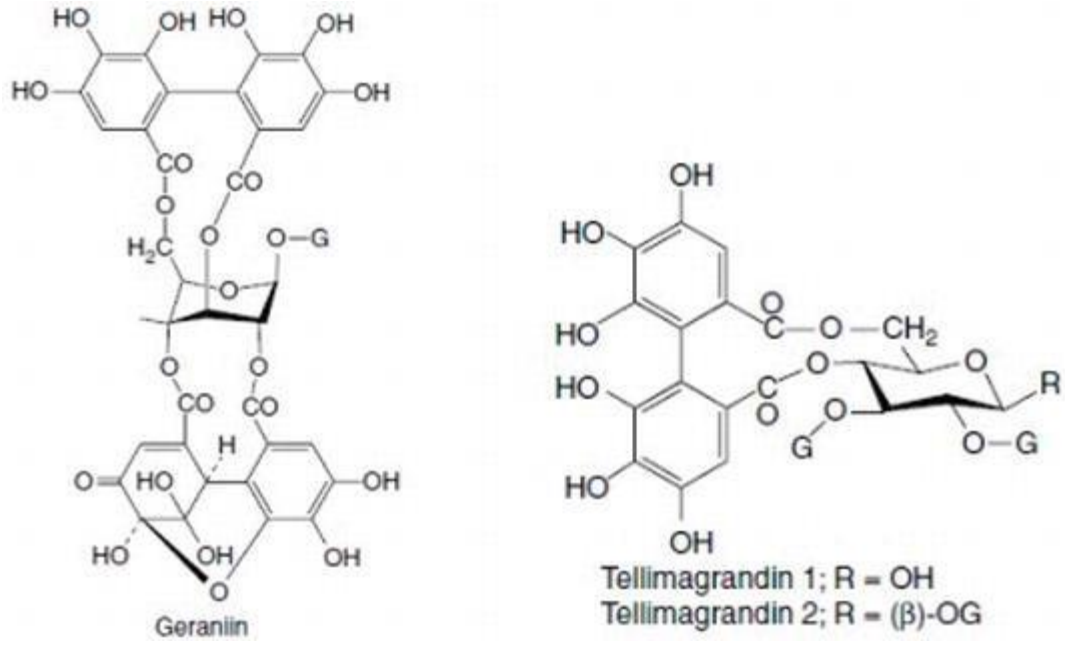


Figure 4.

Examples of hydrolysable tannins.

2.3. Coumarins

Coumarins are derivatives of benzo-α-pyrone, the lactone of *O*-hydroxycinnamic acid, coumarin. Some 1000 natural coumarins have been isolated. Coumarin itself has been found in about 150 species belonging to over 30 different families. The richest sources of coumarin are sweet clover or melilot (*Melilotus* spp.), *Dipteryxodorata* (tonka bean) and *Galiumodoratum* (sweet woodruff) [8]. Aesculetin, umbelliferone and scopoletin are common coumarins present in plants both in the free state and as glycosides. Plants rich in coumarins include *Atropa belladonna*, *Daturastramonium* (Solanaceae), *Daphne mezereum* (Thymeliaceae), *Rutagraveolens* (Umbelliferae) and certain *Aesculushippocastanum* (Horse-chestnut) (Hippocastanaceae) and certain Rosaceae [7]. Anti-inflammatory, anticoagulant, anticancer and anti-Alzheimer's activities are the most important biological activities reported for coumarins [20]. Examples of coumarins are shown in Figure 5.

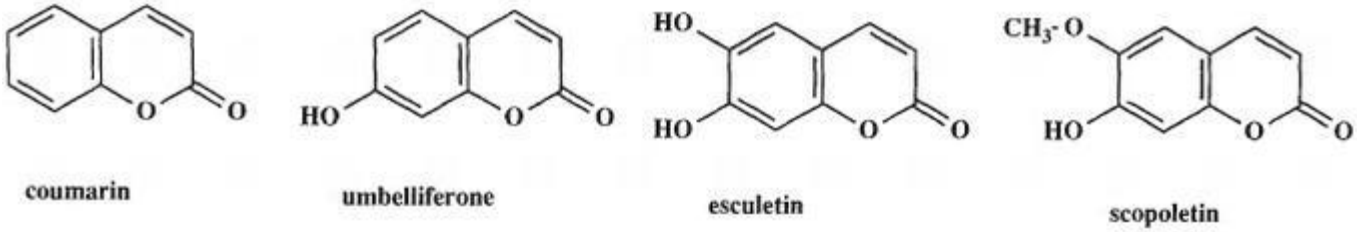


Figure 5.
Examples of coumarins.

2.4. Flavonoids

Flavonoids are the largest group of naturally occurring phenols. More than 2000 of these compounds are now known, with nearly 500 occurring in the free state [7]. The structural skeleton of flavonoids includes a chroman ring bearing an aromatic ring in position 2, 3 or 4. Flavonoids may be divided into various classes according to the oxidation level of the central ring (ring C). The most common of these are anthocyanins, flavones and flavonols. The flavones and their close relations are often yellow (Latin *flavus*, yellow). They are widely distributed in nature but are more common in the higher plants and in young tissues, where they occur in the cell sap. They are abundant in the Polygonaceae, Rutaceae, Leguminosae, Umbelliferae and Compositae. Recent researches have demonstrated the medicinal action of drugs containing flavonoids such as *Glycyrrhizaglabra* (liquorice root), *Chamaemelumnobile* (Roman chamomile) and *Ginkgo biloba* (gingko). A number of flavonoid-containing herbs have now been included in the *British Pharmacopeia*, examples are *Betulapendula* (Birch Leaf), *Calendula officinalis* Flower, *Sambucusnigra* (ElderFlower), *Equisetum ramosissimum* (Horsetail), *Tiliacordata* (Lime Flower), *Leonuruscardiaca* (Motherwort) and *Passifloraedulis* (passion flower). The group is known for its anti-inflammatory and antiallergic effects, for antithrombotic and vasoprotective properties, for inhibition of tumor promotion and as a protective for the gastric mucosa [21, 22]. Examples of flavonoids are shown in Figure 6.

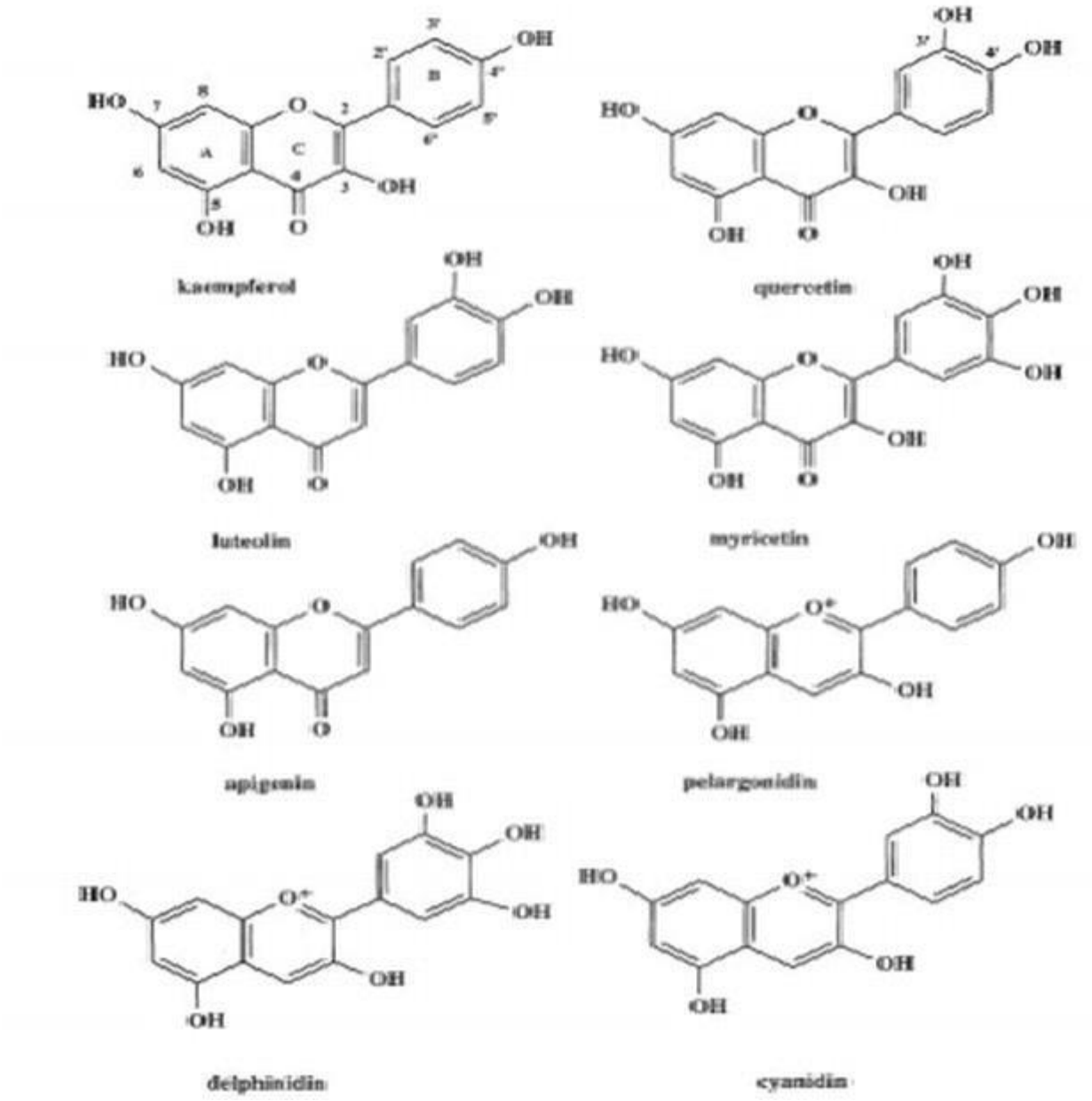


Figure 6.
Examples of flavonoids.

2.5. Chromones and xanthones

These compounds are structural derivatives of benzo-γ-pyrone, and although not of great pharmaceutical importance, a few compounds are worthy of mention; eugenin is found in the clove plant and khellin from mustard seeds [7]. More complex are the furanochromones, the active constituents of the fruits of *Ammivisnaga*. Xanthones are found mainly in the Gentianaceae and Guttiferae, otherwise scattered sporadically throughout the plant kingdom as in the Moraceae and Polygalaceae. *Polygala nyikensis* is used by the highlanders of Malawi and bordering countries to treat various skin problems of fungal origin. The root of the plant was recently shown to exert its antifungal activity owing to the presence of xanthones [23].

2.6. Stilbenes

Stilbenes are a relatively small, but widely distributed, group of plant secondary metabolites found mostly as heartwood constituents in a heterogeneous assembly of plant species. They are especially important in the heartwood of trees of the genera *Pinus* (Pinaceae), *Eucalyptus*(Myrtaceae) and *Madura* (Moraceae) [1]. The *para-hydroxylated* compound, resveratrol, is the most widespread stilbene in nature. Resveratrol possesses estrogen-like activity and occurs in *Picea*, *Pinus*, the Fabaceae, Myrtaceae and the Vitaceae [24].

2.7. Lignans

Lignans are dimeric compounds formed essentially by the union of two molecules of a phenylpropene derivative reported from the members of Asteraceae (e.g., *Achillealingulata* [25]), Pinaceae (e.g., *Cedrusdeodara* [26]) and Rutaceae (e.g., *Fagaraheitzii*) [27]. Four major subtypes occur: dibenzylbutane derivatives, dibenzylbutryolactones (lignanolides or derivatives of butanolide), monoepoxylignans or derivatives of tetrahydrofuran and bisepoxylignans or derivatives of 3,7-dioxabicyclo(3.3.0)-octane. Many of these compounds showed antimicrobial and antifungal activities [1], while others showed cytotoxic activities such as wikstromal, matairesinol and dibenzylbutyrolactol from *Cedrusdeodara* [26].

3. Alkaloids

Alkaloids are organic compounds with at least one nitrogen atom in a heterocyclic ring. Their definition is problematic, as they do not represent a homogeneous group of compounds from any standpoint, whether chemical, biochemical, or physiological. Except for the fact that they are all nitrogen-containing compounds, no general definition fits all alkaloids. Alkaloids can be divided according to their basic chemical structure into different types. The following are basic types of alkaloids: acridones, aromatics, carbolines, ephedras, ergots, imidazoles, indoles, bisindoles, indolizidines, manzamines, oxindoles, quinolines, quinozolines, phenylisoquinolines, phenylethylamines, piperidines, purines, pyrrolidines, pyrrolizidines, pyrroloindoles, pyridines and simple tetrahydroisoquinolines [28].

Although plants containing alkaloids have been used by man for at least 3000 years as medicines, teas and potions, the compounds responsible for activity were not isolated and characterized until the nineteenth century [1]. Alkaloids are not common in lower plants. Lysergic acid derivatives and sulfur-containing alkaloids, e.g., the gliotoxins, are detected in fungi. Concerning the pteridophytes and gymnosperms alkaloids reported for their medicinal uses include the lycopodium, ephedra and *Taxus* alkaloids. Alkaloids are unevenly distributed among the angiosperms. The following are the orders reported to be rich in alkaloids: Centrospermae (Chenopodiaceae), Magnoliales (Lauraceae, Magnoliaceae), Ranunculales (Berberidaceae, Menispermaceae, Ranunculaceae), Papaverales (Papaveraceae, Fumariaceae), Rosales (Leguminosae, subfamily Papilionaceae), Rutales (Rutaceae), Gentiales (Apocynaceae, Loganiaceae, Rubiaceae), Tubiflorae (Boraginaceae, Convolvulaceae, Solanaceae) and Campanulales (Campanulaceae, sub-family Lobelioideae; Compositae, subfamily Senecioneae). However, there is no report for the presence of alkaloids in Salicales, Fagales, Cucurbitales and Oleales dicot orders till the present time [7].

Alkaloids demonstrate a diverse array of pharmacological actions including analgesia, local anesthesia, cardiac stimulation, respiratory stimulation and relaxation, vasoconstriction, muscle relaxation and toxicity, as well as antineoplastic, hypertensive and hypotensive properties. The

activity of alkaloids against herbivores, toxicity in vertebrates, cytotoxic activity, the molecular targets of alkaloids, mutagenic or carcinogenic activity, antibacterial, antifungal, antiviral and allelopathic properties have been reported in literature. Many alkaloids are sufficiently toxic to animals to cause death if eaten. Several (e.g., nicotine and anabasine) are used as insecticides [1, 8].

Examples of some alkaloids:

3.1. Nicotine

Nicotine is found in the tobacco plant (*Nicotianatabacum*) and other *Nicotiana* species; it has tranquilizing properties and is the addictive component of tobacco. It is also extremely toxic, causing respiratory paralysis at high doses (Figure 7). Nicotine is a ganglion cholinergic-receptor agonist with complex pharmacological actions, including effects mediated by binding to receptors in the autonomic ganglia, the adrenal medulla, the neuromuscular junction and the brain [29].

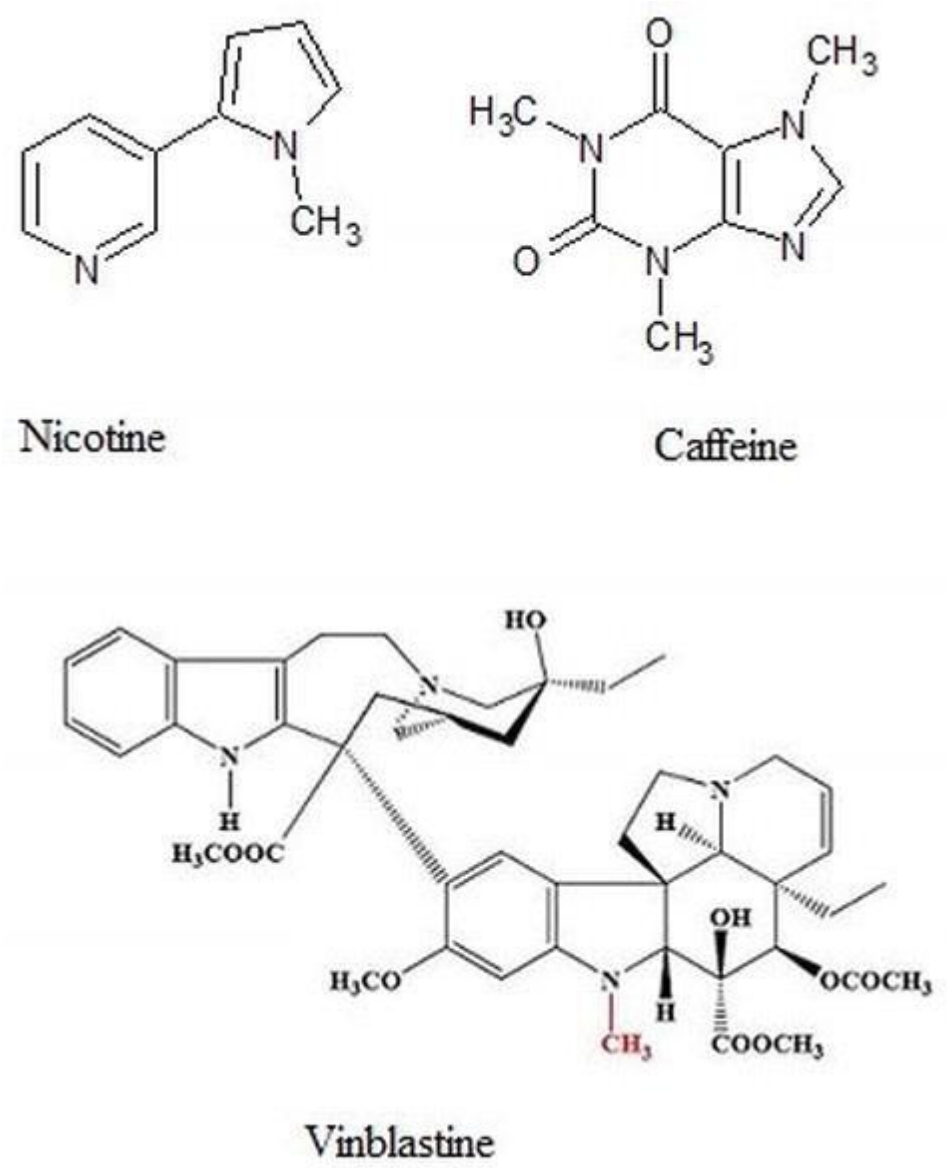


Figure 7.

Examples of alkaloids.

3.2. Caffeine

Caffeine occurs in a number of botanically unrelated species, including coffee (*Coffea* spp.), tea (*Camellia sinensis*), mate (*Ilex paraguariensis*), guarana (*Paulliniacupana*) and kola (*Cola acuminata*) (Figure 7). Caffeine is bound to chlorogenic acid in raw coffee beans. The roasting process liberates the caffeine and other compounds that contribute to the aroma of coffee. Caffeine is a diuretic and has stimulant effects on the respiratory, cardiovascular and central nervous systems [30].

3.3. Vinblastine

Vinblastine is isolated from *Catharanthusroseus* G. (Figure 7) and has been used to treat diabetes and high blood pressure and as disinfectant. Nevertheless, Vinblastine is so important for being cancer fighters. It is used along with the other vinca alkaloids vinorelbine, vincristine and vindesine, which are in clinical use in the United States and Europe [31].

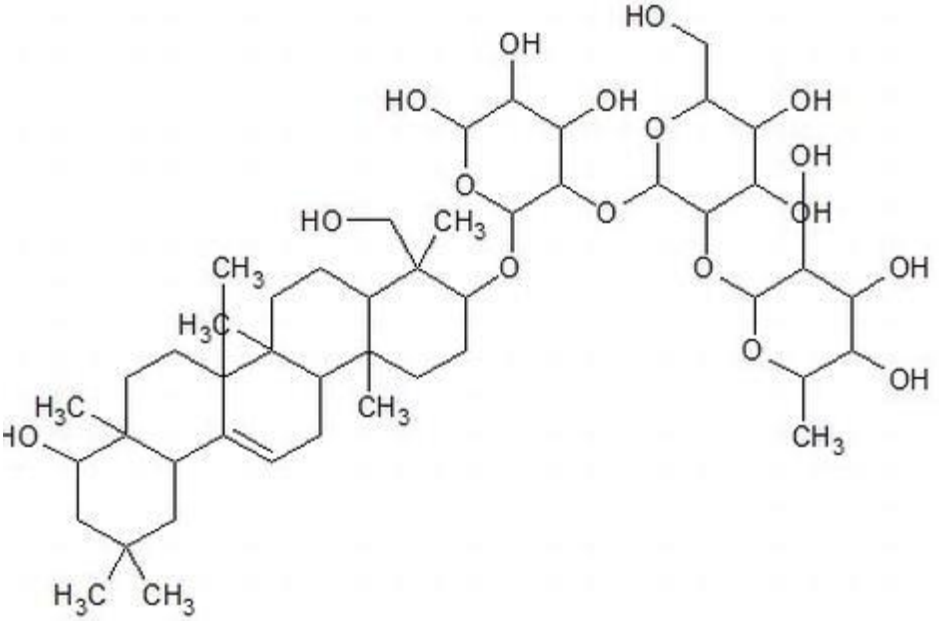
Ranked highest to lowest by country's compensation of specialists

	Specialists		General Practitioners	
	Average Compensation	Ratio to per Capita GDP	Average Compensation	Ratio to per Capita GDP
Netherlands	$253,000	6.0	$117,000	3.6
Australia	$247,000	7.6	$91,000	2.9
United States	**$230,000**	**5.7**	**$161,000**	**4.1**
Belgium	$188,000	6.0	$61,000	2.0
Canada	$171,000	5.1	$107,000	3.4
United Kingdom	$150,000	4.9	$118,000	3.9
France	$149,000	5.0	$92,000	3.1
Ireland	$143,000	4.0	—	—
Switzerland	$130,000	3.8	$116,000	3.4
Denmark	$91,000	2.9	$109,000	3.4
New Zealand	$89,000	3.6	—	—
Germany	$77,000	2.7	—	—
Norway	$77,000	1.9	—	—
Sweden	$76,000	2.5	$66,000	2.2
Finland	$74,000	2.5	$68,000	2.3
Greece	$37,000	3.1	—	—
Portugal	$34,000	3.5	$61,000	3.5
Czech Republic	$35,000	1.7	$32,000	1.7
Hungary	$27,000	1.7	$26,000	1.6
Mexico	$25,000	2.4	$21,000	2.1
Poland	$20,000	1.6		
Average	**$113,000**	**3.7**	**$83,000**	**2.9**
Average excluding U.S.	$107,000	3.6	$78,000	2.8
Median	**$83,000**	**3.3**	**$80,000**	**3.0**

4. Saponins

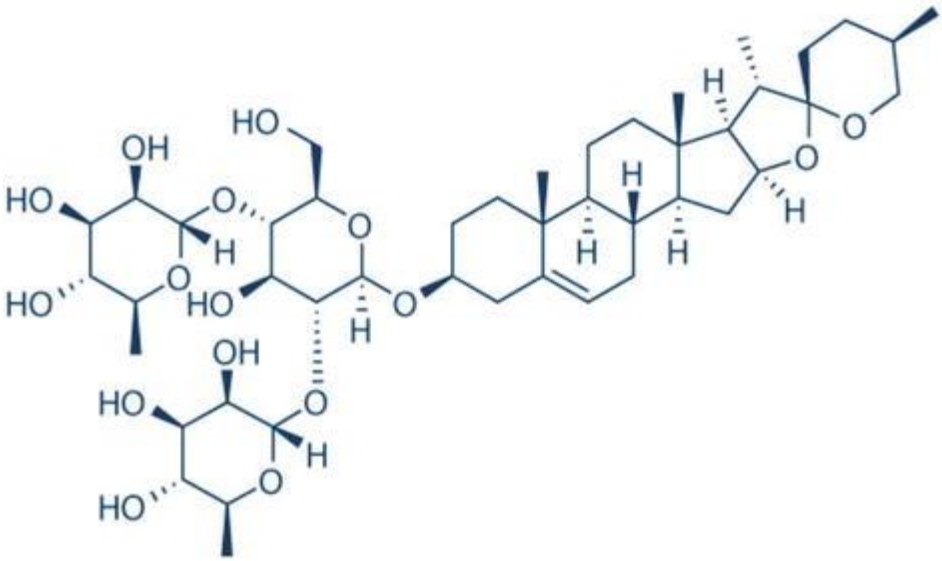
Saponins are compounds that possess a polycyclic aglycone moiety with either a steroid (steroidal saponins) or triterpenoid (triterpenoidalsaponins) attached to a carbohydrate unit (a monosaccharide or oligosaccharide chain) (examples illustrated in Figures 8 and 9). These sugar units are composed variously of pentoses, hexoses, or uronic acids. This hydrophobic-hydrophilic asymmetry means that these compounds have the ability to lower surface tension and are soap-like. They form foam in aqueous solutions and cause hemolysis of blood erythrocytes in vitro. The aglycone portion of the saponin molecule is called the *genin* or *sapogenin*. Saponins are widespread among plants, having been reported from more than 500 plants from at least 90 different families; these substances have been isolated from all parts of plants: leaves, stems, roots bulbs, flowers and fruits, although they tend to be concentrated in the roots of many species such as *Digitalis purpurea* (*foxglove*), *Dioscoreavillosa* (*wild*

yam), *Eleutherococcussenticosus* (*Siberian ginseng*), *Gentianalutea* (*gentian*), *Glycyrrhiza* spp. (*licorice*) and *Panax ginseng* (*Korean ginseng*) [32].



Soyasaponin I

Figure 8.
Example of triterpenoidalsaponin.



Dioscin

Figure 9.
Example of steroidal saponin.

Saponins have demonstrated numerous pharmacological properties. Some saponins have antitumor, piscicidal, molluscicidal, spermicidal, sedative, expectorant and analgesic properties. Glycyrrhizin from *glycyrrhizae radix* (from *Glycyrrhizaglabra*, Fabaceae) is useful as expectorant and antitussive agent. It is also used to treat chronic hepatitis and cirrhosis. Some saponins have anti-inflammatory properties as the saponins

from *Bupleurumfalcatum* (Apiaceae). *Phytolaccaamericana* roots are reputed to possess anti-inflammatory properties in Korean medicine. Similar properties have been demonstrated for a number of other saponins, for example aescin, from horse chestnut (*Aesculushippocastanum*), has been shown to be 600 times more effective than rutin in reducing rat paw edema [33].

5. Terpenes

Terpenes are the largest and most diverse group of plant secondary compounds. The name "terpene" is derived from the word "turpentine," which in turn comes from the old French *ter(e)bintb*, meaning "resin." They are all derived chemically from 5-carbon isoprene units assembled in different ways [8]. Terpenes are classified according to the number of isoprene units in the molecule; a prefix in the name indicates the number of terpene units as follows.

5.1. Hemiterpenes

They consist of *a single isoprene* unit. Isoprene itself is considered the only hemiterpene, but oxygen-containing derivatives such as angelic acid isolated from *Angelica archangelica* and isovaleric acid from *Vacciniummyrtillus* are hemiterpenoids [1].

5.2. Monoterpenes

They consist of *two isoprene* units and have the molecular formula $C_{10}H_{16}$ (see Figure 10). They are important components of plant essential oils or volatile oils. Monoterpenes tend to occur in members of certain plant families, such as Lamiaceae, Pinaceae, Rutaceae and Apiaceae, from which many essential oils are commercially produced. Some of these compounds, such as geraniol, are almost ubiquitous and can be found in small amounts in the volatile secretions of most plants. Monoterpenes are further classified into unsaturated hydrocarbons (e.g., limonene), alcohols (e.g., linalool), alcohol esters (e.g., linalyl acetate), aldehydes (e.g., citronellal) and ketones (e.g., Carvone). Monoterpenes and other volatile terpenes have a number of widespread medicinal uses. Compounds such as camphor and menthol are used as counterirritants analgesics and anti-itching agents. Many monoterpenes have been used as anthelmintics. A series of monoterpene glycosides appear to have vasodilation effect on coronary vessels and the femoral vascular bed [16].

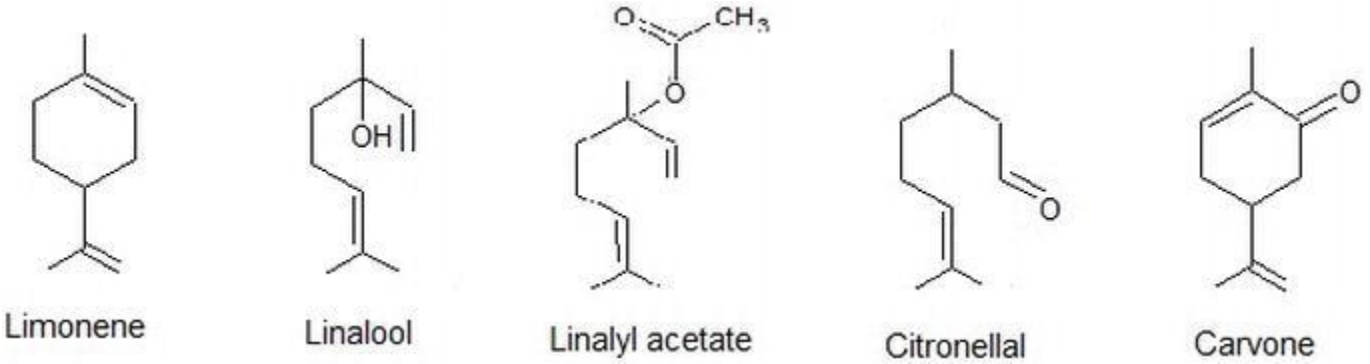


Figure 10.

Examples of monoterpenes.

5.3. Sesquiterpenes

They consist of *three isoprene* units and have the molecular formula $C_{15}H_{24}$ (see Figure 11). Based on biogenetic origin, there are more than 200 different structural types of sesquiterpenes, and several thousand such compounds are known. These compounds can be conveniently classified into three main groups according to structure: acyclic (e.g., farnesol), monocyclic (e.g., bisabolol) and bicyclic (e.g., caryophyllene). A number of sesquiterpene lactones show

antibacterial, antifungal and antiprotozoan activities. Sesquiterpenes from *Vernoniacolorata* inhibit *Entamoebahistolytica* at concentrations comparable to metronidazole, an antiamoebic drug. Helenalin and a series of related compounds are responsible for the cardiotonic properties of *Arnica montana* flowers. *Atractylodisrhizoma*, from *Atractylodismacrocephala* (Asteraceae), is clinically used as diuretic, analgesic and anti-inflammatory. The activity is related to the presence of active compounds including eudesma-4(14)-7(1 l)-dien-8-one and atractylenolide I. Several related medicinal plants are also used for the same purposes due to the presence of sesquiterpenes [1, 34].

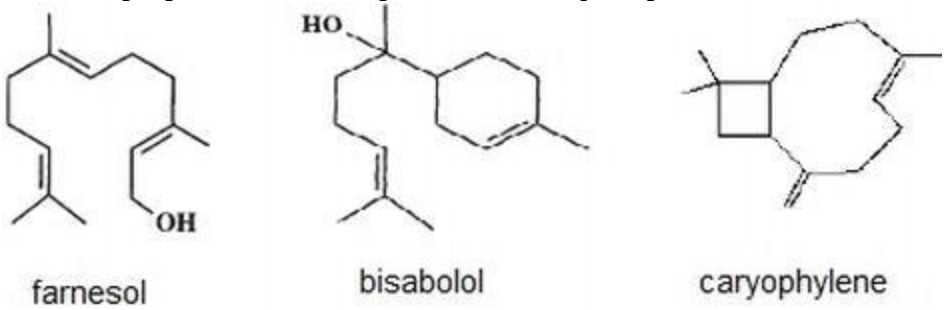
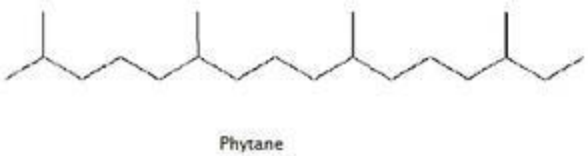


Figure 11.

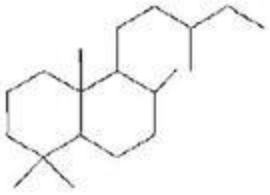
Examples of sesquiterpenes.

5.4. Diterpenes

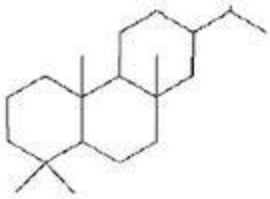
They are composed of *four isoprene* units and have the molecular formula $C_{20}H_{32}$ (see Figure 12). Diterpenes are classified into acyclic and macrocyclic compounds. Moreover, macrocyclicditerpenes are classified according to the number of ring systems present. Diterpenes may be 6-membered ringed structures or they may have fused 5- and 7-membered ringed structures. In addition, many diterpenes have additional ring systems. These occur as side substitutions as esters or epoxides [8]. Diterpenoids constitute the active constituents of a number of medicinal plants. Vitamin K1, an antihemorrhagic compound, first discovered in plants in 1929, is a diterpene. Vitamin A, a diterpenoid, is referred to, together with the related compounds, as "carotenes." The bitter principles of *Jateorhizapalmata* (columba root) belong to furanoditerpenes. *Teucriumchamaedrys* (wall germander) and *T. scorodonia* (wood sage) family Labiatae, both produce diterpenes of the neoclerodane type. They are used in herbal medicine as diaphoretics and antirheumatics [35]. Like all groups of terpenes, diterpenes have demonstrated a range of pharmacological properties including: analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic and antiprotozoal activities [8]. Some diterpenes from *Kalmia latifolia* (Ericaceae) have antifeedant properties with respect to the gypsy moth. The gibberellins, first obtained from fungi of the genus *Gibberella* but also found in higher plants, are diterpenoid acids, which have a marked effect on growth of seedlings [7].



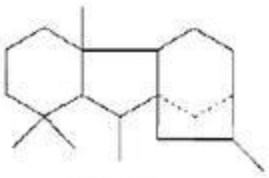
Phytane



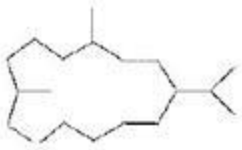
Labdane



Abiatane



Gibberellane



Cembrane

Figure 12.

Examples of diterpenes.

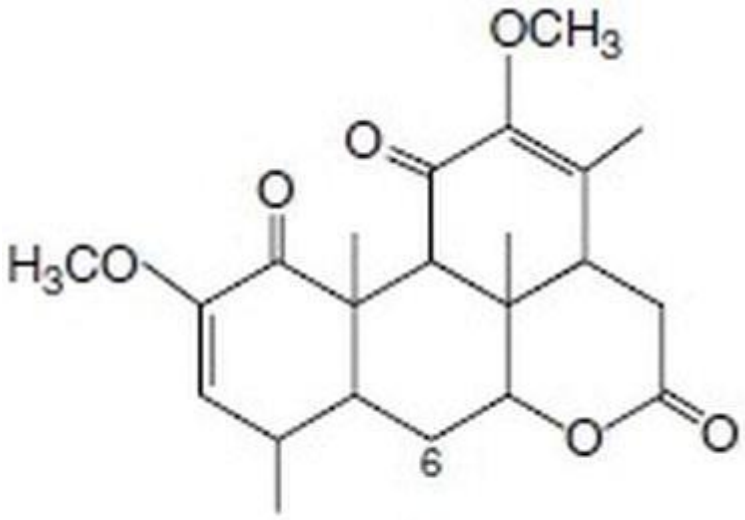
5.5. Sesterterpenes

Terpenes having 25 carbons and *five isoprene* units are rare relative to the other sizes (the *sester-* prefix means half to three, i.e. two and a half). An example of a sesterterpenoid is geranylfarnesol isolated from seed oils of *Camellia sasanqua* (sasanqua) and *Camellia japonica* (camellia), family Theaceae [36]. Geranylfarnesol showed cytotoxic activity in mouse leukemic M1 cells [37].

5.6. Triterpenes

They consist of *six isoprene* units and have the molecular formula $C_{30}H_{48}$ (see Figure 13). The linear triterpenesqualene, the major constituent of shark liver oil, is derived from the reductive coupling of two molecules of farnesyl pyrophosphate. Triterpenes constitute a significant portion of the lipid substances of all plants; more than 4000 triterpenoids have been isolated. These compounds are precursors to steroids in both plants and animals. Both triterpenes and steroids occur free, as glycosides or in other combined forms. The structures of triterpenes and steroids

may be subdivided into about 40 major types [1]. β-Boswellic acids (ursane-type triterpene) and α-boswellic acids (oleanane-type triterpene) that are isolated from the oleo-gum-resin of *Boswelliacarterii* are known for their anti-inflammatory and anti-rheumatic activities [38].



Quassin

Figure 13.
Example of triterpene.

One group of compounds showing a range of interesting biological activity is the quassinoids isolated from *Quassiaamara*. These are degradation and rearrangement products of triterpenes. Quassia is used as a bitter tonic, as an insecticide and as an enema for the expulsion of thread worms.

Terpenes also include sesquarterpenes (*seven isoprene* units, $C_{35}H_{56}$), tetraterpenes (*eight isoprene* units, $C_{40}H_{64}$) as well as polyterpenes and norisoprenoids (long chains of *many isoprene* units.

6. Lipids

Lipids comprise a group of naturally occurring molecules that include fixed oils, waxes, essential oils, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), phospholipids and others. Lipids serve various biological actions as major structural components of all biological membranes and as energy reservoirs and fuel for cellular activities in addition to being vitamins and hormones [39, 40]. Although lipids are considered primary plant metabolites, recent studies revealed pharmacological activities to members of this class of phytochemicals.

6.1. Fixed oils

Fixed oils constitute of high molecular aliphatic long-chain fatty acids, such as palmitic, stearic and oleic acids, esterified with glycerol. Fixed oils contain a relatively higher percentage of liquid glycerides (polyunsaturated) such as glycerin oleate, while fats are rich in solid glycerides such as glycerin stearate. [39]. Flax and linseed and its oil are obtained from *Linumusitatissimum*, family Linaceae. Polyunsaturated fatty acids in some fixed oils cause reduced excretion of lipid peroxidation products and hence are potent antioxidants and anti-inflammatory. They are used as prophylactic to decrease the risk of atherosclerosis and cardiovascular disease [41].

6.2. Waxes

Waxes are lipoidal matter constituting mainly from long aliphatic chains that may contain one or more functional groups. They may contain hydroxyl groups as in the case of primary and secondary long-chain alcohols that are frequently present in the form of esters. Others contain unsaturated bonds, aromatic systems, amide, ketonic, aldehydic or carboxylic functional groups. On the other hand, synthetic waxes constitute of long-chain hydrocarbons (alkanes or paraffins) that lack functional groups. They are similar to the fixed oils and fats since they are esters of fatty acids, but with the difference that the alcohol is not glycerin. The seeds of *Simmondsiachinensis* yield the liquid wax, jojoba wax, which consists of straight chain esters of fatty acids and alcohols [42]. Jojoba wax has anti-inflammatory, anti-aging and wound healing activities, and hence it can be utilized in several skin conditions. Jojoba wax has also been used in topical medications to enhance drug absorption. In addition, it is used in skin care products and in cosmetics such as sunscreens and moisturizers [43].

6.3. Essential oils

Essential oils are volatile aromatic complex mixtures of relatively low molecular weight compounds. Although they may contain up to 60 components, yet they are characterized by the presence of two or three major components at fairly high concentrations (20–70%) compared to other components present in trace amounts. For example, *Origanumcompactum* essential oil contains carvacrol (30%) and thymol (27%) as major components. Linalol is the major component of *Coriandrumsativum* essential oil reaching up to 68%. Other examples are *Artemisia herba-alba* essential oil which contains α- and β-thuyone (57%) and camphor (24%) as major constituent, *Cinnamomumcamphora* essential oil with 1,8-cineole (50%) as major constituent and finally *Menthapiperita* essential oil with menthol (59%) and menthone (19%) being the major constituent. Generally, these major components determine the biological properties of the essential oils [44]. They have many and important medical uses such as antiseptic, antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthesic remedies. They are also used as fragrances in embalmment and in food preservation [45].

7. Carbohydrates

Carbohydrates are universally present in living beings on our planet. As the first product of photosynthesis, carbohydrates are the starting point for all phytochemicals and also, by extension, for all animal biochemicals. More carbohydrates occur in nature than any other type of natural compound. The most abundant single organic substance on Earth is cellulose, a polymer of glucose, which is the main structural material of plants. Although carbohydrates are primary metabolites, they are incorporated in plenty of secondary metabolites through glycosidation linkages. Polymers of simple sugars and uronic acids produce mucilages and gums [46].

Carbohydrates consist of carbon, hydrogen and oxygen with the last two elements usually present in the same proportions as in water. They are classified into four chemical groups: monosaccharides, disaccharides, oligosaccharides and polysaccharides. Monosaccharides contain from three to nine carbon atoms, although those with five and six carbon atoms (pentoses, $C_5H_{10}O_5$, and hexoses, $C_6H_{12}O_6$) are accumulated in plants in greatest quantity. Condensation of monosaccharides results in the other types according to the number of saccharide units involved. In addition to the important biological and structural function of carbohydrates in plants, some

members show medicinal effects such as mucilage. Mucilage, viscous sticky material produced by almost all plants and some microorganisms, plays a protective role in thickening membranes in plants. It also serves in storage of water and food and in seed germination. Chemically it constitutes of a polar glycoprotein and an exopolysaccharide. Mucilage is used medicinally as demulcent. Cactus (and other succulents) and *Linumusitatissimum* (flax seeds) are the major sources of mucilage. The extract of the mucilaginous root of the marshmallow plant (*Althaeaofficinalis*); used traditionally to make marshmallows, were used as cough suppressant due to its demulcent effect. *Ulmusrubra* (the slippery elm) inner bark, is also used as a demulcent due to its mucilaginous content. Mucilage acts primarily as a local demulcent or emollient when it comes in direct contact with mucous membrane surfaces or skin. Here, they produce a coating of "slime" that soothes and protects exposed or irritated surfaces of the gastrointestinal tract. They are used extensively in the management of inflammatory digestive disorders, especially when there is ulceration. Their relative indigestibility and hydrophilic properties have important influences on bowel behavior

Cosmeceuticals

The term "cosmeceutical" was introduced by dermatologist Dr Albert Kligman in 1984 and is derived from a combination of the words **cosme**tic and pharma**ceutical**.

What are cosmeceuticals?

Cosmeceuticals are products that have both cosmetic and therapeutic (medical or drug-like) effects, and are intended to have a beneficial effect on skin health and beauty. Like cosmetics, they are applied topically as creams or lotions but contain active ingredients that have an effect on skin cell function. In some cases, their action is limited to the skin surface (such as exfoliants), while others can penetrate to deeper levels, either enhancing or limiting normal skin functions. Cosmeceuticals are available "over-the-counter" (without prescription) and are generally used as part of a regular skin care regime to help improve skin tone and texture, pigmentation and fine lines.

Most moisturisers restore barrier function and water content to the skin, improving the appearance of aged or dry skin. Cosmeceuticals should ideally deliver the active ingredient in a biologically effective form to the skin and reach the target site in sufficient quantity to have an effect.

What are some common ingredients in cosmeceuticals?

- Sunscreens

  These are probably the most important ingredient in cosmeceuticals because they protect against sun damage, photo-ageing and skin cancers.

- Antioxidants

  External factors such as ultraviolet (UV) radiation, pollution and smoking, as well as internal factors including normal cellular metabolism, can generate molecules called free radicals which are damaging to the skin. Antioxidants “mop up” these free radicals thereby reducing inflammation and protecting the skin against sun damage and skin cancers. Some studies suggest that combinations of antioxidants can be more effective than single ingredient formulations.Examples of antioxidants include:

  - Alpha-lipoic acid: Has anti-inflammatory and exfoliating effects.
  - Vitamin C (L-ascorbic acid): Stimulates collagen repair and can improve fine lines, reduce inflammation and pigmentation. Although it is found in a number of cosmeceutical products, many are not effective because the vitamin C is unstable when exposed to air, heat or light, is in too low a concentration or in a form that cannot be absorbed or metabolised by the skin.
  - Nicotinamide (vitamin B3): An antioxidant that improves skin barrier function. It can reduce fine lines, wrinkles and hyperpigmentation and improve skin texture. It may also play a role in skin cancer prevention.
  - Vitamin E (alpha tocopherol): Another antioxidant that reduces UV damage and skin cancer. It also works synergistically with vitamin C in reducing collagen break down.
  - N-Acetyl-Glucosamine (NAG): May help fade pigmentation and prevent sun damage (photo damage).
  - Ubiquinone (CoQ10): A naturally occurring antioxidant that reduces collagen breakdown due to sun exposure.
- Hydroxy acids

  These can be classified according to their molecular structure into alpha hydroxy acids (AHAs), poly hydroxy acids (PHAs) and beta hydroxy acids (BHAs).Hydroxy acids improve skin texture and reduce the skin signs of ageing by hydrating the skin and promoting the shedding of dead skin cells from the outer layer of the skin (epidermis).

  - AHAs are often called “fruit acids” as many are derived from natural fruit sources. AHAs include glycolic acid, lactic acid, citric acid, mandelic acid, malic acid, tartaric acid and lactobionic acid.
  - PHAs include gluconolactone and lactobionic acid.
  - The main BHA used is salicylic acid which is particularly useful in people with oily or acne prone skin because of its fat solubility and ability to penetrate pores.
- Retinoids (vitamin A)

  These are natural or synthetic forms of vitamin A that can partially reverse skin changes induced by sun exposure. Common retinoids include tretinoin or retinoic acid, retinol and retinaldehyde. They act as antioxidants, protecting cells from free radicals, as well as activating specific genes and proteins. Topical tretinoin has been shown to improve the appearance of photo damaged skin by reducing fine lines and wrinkles, skin looseness

(laxity) and excess pigmentation, as well as improving skin texture. Tretinoin can cause side effects such as burning, stinging, redness and flaking.

- Skin lightening agents

  These help inhibit the production of melanin (the main skin pigment) to reduce skin discolouration and pigmentation. Examples include:

  - Hydroquinone. This has been the agent of choice for skin lightening for many years. Concerns regarding skin darkening, loss of pigmentation and possible carcinogenicity have resulted in it being banned from over the counter products in some countries. However, these concerns have mainly arisen from animal studies using long term, high doses and are probably not relevant to topical application in humans.
  - Ascorbic acid (vitamin C)
  - Kojic acid
  - Azelaic acid
  - Licorice extract (glabridin).
- Botanicals

  These include plant extracts from leaves, roots, fruits, berries, stems, bark and flowers. Botanicals may have antioxidant, anti-inflammatory and/or skin soothing properties, however, their effects remain largely untested or unproven. Examples include soy, curcumin, silymarin, pycnogenol, ginkgo biloba, green tea extract, grape seed extract, aloe vera, witch hazel, allantoin and ferulic acid.

- Sunscreens

  (see also A-Z of Skin Sun protection and sunscreens)

- Peptides and proteins

  Peptides are short chains of amino acid sequences that are the building blocks of larger proteins. Cellular “messengers” formed from amino acids can imitate normal biological signals that either stimulate repair or inhibit processes that accelerate skin ageing. Examples include the pentapeptide Pal-KTTKS.

- Growth factors

  These proteins help control chemical signals between and within cells. They are important in wound healing and repair of damaged tissue, and may help to repair skin damage from sun exposure. Studies suggest that the use of multiple growth factors can stimulate collagen and elastin production and improve the appearance of photo-damaged skin.

Do cosmeceuticals really work?

Ideally cosmeceuticals should be clinically tested to ensure they have a proven benefit and can substantiate their claims, however, the cosmeceutical industry is largely unregulated. Unlike medicines, cosmeceuticals are not subject to review by the Food and Drug Administration (FDA) in the United States or the Therapeutic Goods Administration (TGA) in Australia. Although they are usually tested for safety, they do not have to undergo testing to ensure the claims they make regarding efficacy (effectiveness) are accurate. Unfortunately, many creams do not live up to their advertised hype.

What is the future for cosmeceuticals?

Research is continuing into new delivery systems (such as liposomes) and new active ingredients.

Aquaceuticals

A smart dealer wanting to learn about aquaceuticals needs to quickly develop a broad understanding of the key concepts in this fascinating and rapidly growing segment of the water quality improvement industry. Our customers turn to aquaceuticals because they believe in the value of liquid nutrition. Solid pills and powder-filled capsules are generally less effectively absorbed through the human gastro-intestinal (GI) tract than liquid formulations. Aquaceuticals generally come in two varieties: premixed and self-mix. Intelligent customers inevitably gravitate towards the self-mix, since it costs less and they can use their own purified water to mix the supplement into a usable form instead. Aquaceutical concentrates will mix best with purified water since there are no contaminants like metals, minerals, or disinfectants and their byproducts to interfere with the mixing process. Clean, purified water is the building block upon which all good aquaceutical formulations are built.

Metals and minerals

Trace minerals serve as catalysts to vitamin uptake within the cells of the human body. They are essential to our daily health, and have specific minimum daily requirements depending on our body size and level of stress. Unfortunately, this requirement usually goes unmet when living the hectic modern lifestyle. The following are just a few of the trace minerals that should be included in a healthful daily diet, and can be obtained through organic foods and nutritional supplements like aquaceuticals.

Boron is a trace mineral that provides metabolic benefits in the human body. Though its exact role in the body is relatively unknown, recent experimental studies indicate that boron may be essential for energy utilization and the creation and preservation of bone.

Iodine is an important trace mineral, though it is often misunderstood. A deficiency of iodine results in goiters, lower vitality, lower metabolism and the inability to think logically. Iodine is essential for proper thyroid function.

Lithium is a trace mineral that interacts only with sodium. It can be obtained from drinking water and is essential to balancing the part of the brain that dictates behavioral and emotional behaviors.

Magnesium is an abundant element in the body and it is closely related to calcium and phosphorus as far as functions go. About 70 percent of all magnesium is contained in the bones and teeth and the rest is found in the cells of the soft tissue of the body.

Manganese is a natural muscle builder, and it is also known to strengthen bones and ligaments. It is usually found in the bone, liver, kidneys, heart, pituitary gland, pancreas, spleen and intestines.

Molybdenum is an essential trace mineral that is found concentrated in the liver, adrenal glands, kidneys, bones and skin. Most people obtain molybdenum from milk and other dairy products, dried legumes, organ meats and whole grains.

Phosphorus is a vital essential mineral because it provides a wide range of functions throughout the body. It is not only involved in bone and teeth formation, but also the metabolism of every cell in the human body.

Selenium is a trace mineral that helps to prevent oxygen damage to the cell membrane. Cell membranes are critical for the proper absorption of nutrients and the elimination of toxic wastes. It's also known to be a powerful antioxidant.

Silicon is a trace mineral that is found in the hair and skin. It is important in the formation of the collagen found in bone, cartilage and other connective tissues. It is also necessary for the formation of other connective tissues like elastin, which help maintain the integrity of the elastic quality of blood vessels – an important feature of blood pressure control – and other tissues.

Sodium is a major component of positively charged ions found in cellular tissue. An adequate amount of sodium is important to the overall function of the human body.

Organics, inorganics and chelation

It is critical to grasp the difference between organic and inorganic metal and mineralcompounds. Being mammalian organisms, we require specialized types of nutrients and other co-factors to survive and thrive. For example, our bodies require iron to live; we can get iron from nails or from liver…which one is better for your body? Inorganicminerals are not readily metabolized by humans; we require them to be preprocessed by another organism, making them biogenic. When we consume inorganic minerals, our body attempts to convert them to a biogenic compound through a process known as chelation. This chelation process is often disturbed or incomplete, meaning the minerals will exit your body without ever being absorbed and put to good use. Biomolecules can be produced through plants and animals, as well synthetically, by reacting minerals with ligands such as glycine. The food chain exists for a very important reason; vendors selling ground up dirt are not helping their customers' health.

Acidity, alkalinity and pH

pH describes the acidity or basicity of an aqueous solution along a logarithmic scalefrom 0 to 14. 'Pure' water has a neutral pH, close to 7.0 at 77 °F (25 °C). Solutions with a pH less than 7 are described as acidic and solutions with a pH greater than 7 are basic or alkaline. Since alkalinity is the sum of the bicarbonate (HCO_3^-), carbonate (CO_3^{-2}) and hydroxide (OH^-) buffers, it measures the ability of a solution to neutralize acids. Evidently pH and alkalinity are not the

same thing at all. Most of the medical literature indicates that human bodies function well with the appropriate reserves of alkalinity to buffer acidic byproducts of metabolism. Healthy blood is slightly alkaline, and some studies suggest that acidity in the human body can be correlated to a broad spectrum of health maladies. Many enterprising individuals sell ionizing machines and supplements that raise pH, playing in a lack of consumer education. Purchasing an ionizing machine or supplement that only raises pH and not total alkalinity is like trying to cool a room with an ice-cube. At the 2012 WQAAquatech Convention in Las Vegas, NV, Robert Slovak presented an excellent lecture on the differences between pH and alkalinity specifically as pertaining to human health and nutrition. You'd be wise to review the presentation notes to further assist in your understanding of this subject.

Antioxidants, oxidants and ORP

Experienced water quality improvement professionals will be familiar with ORP. We use ORP measurements (oxidation reduction potential) as an indicator of the effectiveness of oxidizing disinfectants in water, like chlorine and bromine. A positive ORP voltage indicates oxidizing potential, with higher numbers indicating greater disinfection capacity. The alternative health industry has begun promoting the value of 'negative ORP' in describing solutions that are anti-oxidants. Negative ORP water has been attributed with numerous health benefits such as increasing cellular hydration, raising physical and mental energy levels, contributing to mitochondrial ATP production, neutralizing free radicals, restoring beneficial intestinal flora, and even curing certain cancers.

Vitamins and electrolytes

An electrolyte is any substance containing free ions to make the substance electrically conductive. Electrolytes are generally solutions of acids, bases or salts. The human body requires specific electrolytes to function properly, especially during periods of exertion or stress. Sodium and potassium salts are the most common forms of electrolytes supplied in aquaceutical concentrates. Vitamins are organic compoundsrequired as vital nutrients in tiny amounts that cannot be synthesized in our bodies and must be supplied from an external source. Vitamins perform a number of biochemical functions. Some vitamins regulate mineral metabolism and others regulate growth and differentiation of cells and tissue. Others function as antioxidants like vitamin E and vitamin C. Most vitamins function as precursors for enzyme cofactors that help enzymes in their work as catalysts in metabolism. Aquaceutical concentrates will generally include vitamins that are not lipid-bound. It is important that a vitamin formulation be manufactured in a way that the ingredients are biochemically balanced and that interfering factors are minimized.

Liquids, powders and effervescence

Aquaceutical concentrates come in a number of forms, with liquids generally being the least shelf-stable. Most manufacturers elect to supply powders and compressed effervescent tablets that quickly dissolve in purified water. Aquaceutical concentrates should always be stored in a temperature-controlled environment to maximize longevity and potency.

It is clear that there are a myriad of aquaceutical options available for the progressive water dealer. There is also a lot of good and bad information out there. It is easy to get confused and

discouraged without proper guidance. Research carefully, find good vendors that you can trust, and make sure you understand what you're selling.

Animal metabolites - Sources and extraction of nutraceuticals of animal origin.

Chitin and chitosan production

Chitin or poly (β-(1→4)-*N*-acetyl-D-glucosamine) is a natural polysaccharide of major importance, first identified in 1884 (Figure 1). This biopolymer is synthesized by enormous number of living organisms [1] and it belongs to the most abundant natural polymers, after cellulose. In the native state, chitin occurs as ordered crystalline microfibrils which form structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. So far, the main commercial sources of chitin are crab and shrimp shells. In industrial processing, chitin is extracted by acid treatment to dissolve the calcium carbonate followed by alkaline solution to dissolve proteins. In addition, a decolorization step is often added in order to remove pigments and obtain a colorless pure chitin. All those treatments must be adapted to chitin source, owing to differences in the ultrastructure of the initial material (the extraction and pre-treatments of chitin will be described later), to produce first a high quality chitin, and then chitosan (after partial deacetylation). Chitin is infusible and sparingly soluble during transformation into different conformations. The question of its solubility is a major problem in the development of both processing and use of chitin as well as its characterization.

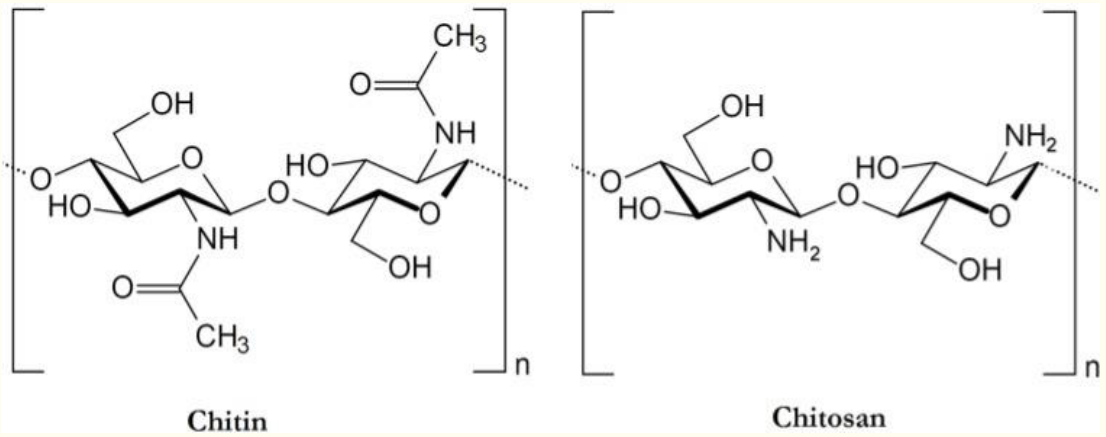


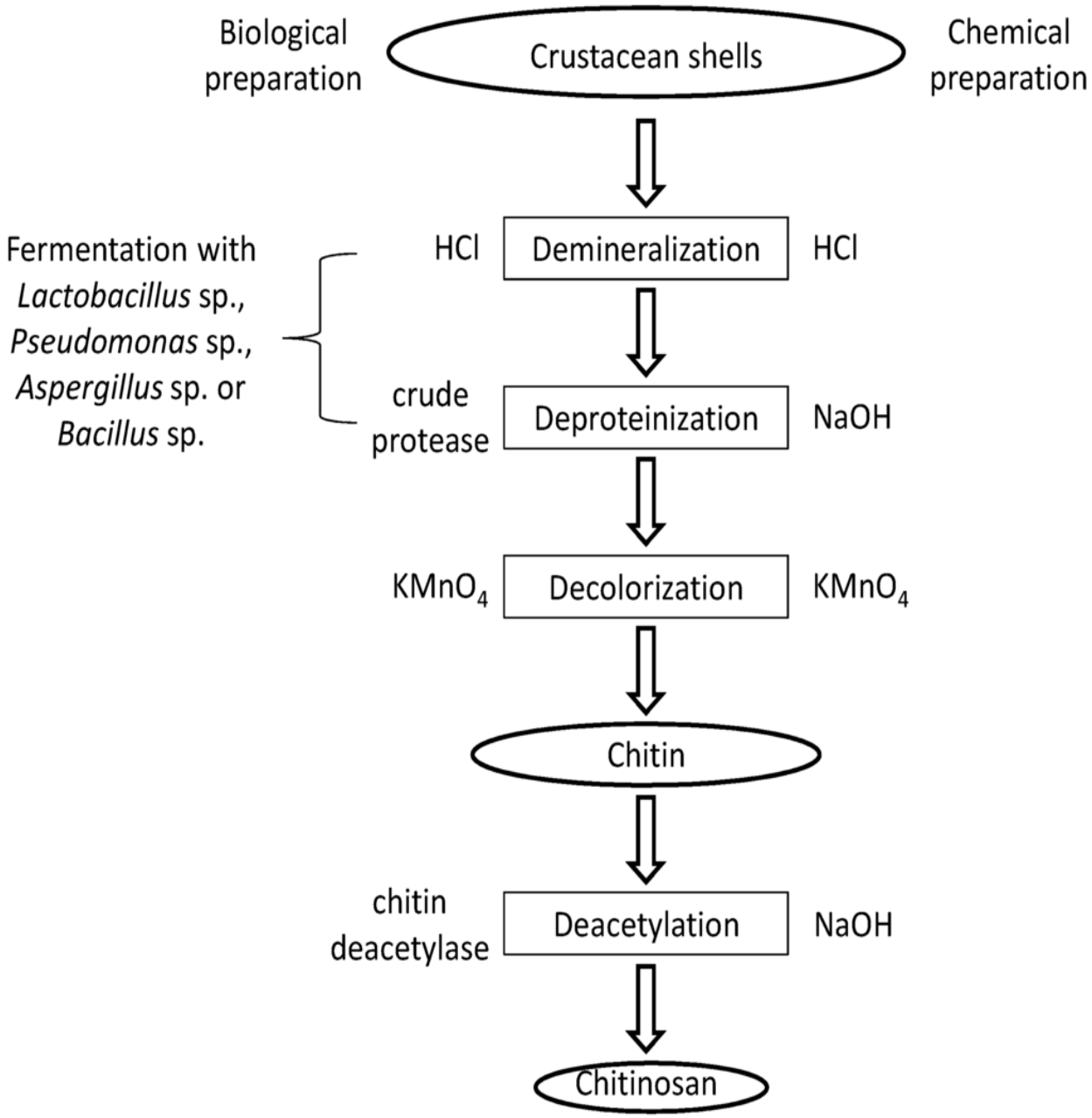
Figure 1

Chemical structure of chitin and chitosan

Chitin has more applications while transforming to chitosan (by partial deacetylation under alkaline conditions) [2,3,4]. Chitosan is a random copolymer with a molar fraction DA (degree of acetylation) of β-(1→4)-*N*-acetyl-D-glucosamine (Figure 1) and a fraction (1-DA) of β-(1→4)-D-glucosamine (Figure 1). The degree of acetylation of chitosan is characterized by the molar fraction of *N*-acetylated units (DA) or as a percentage of acetylation (DA%).

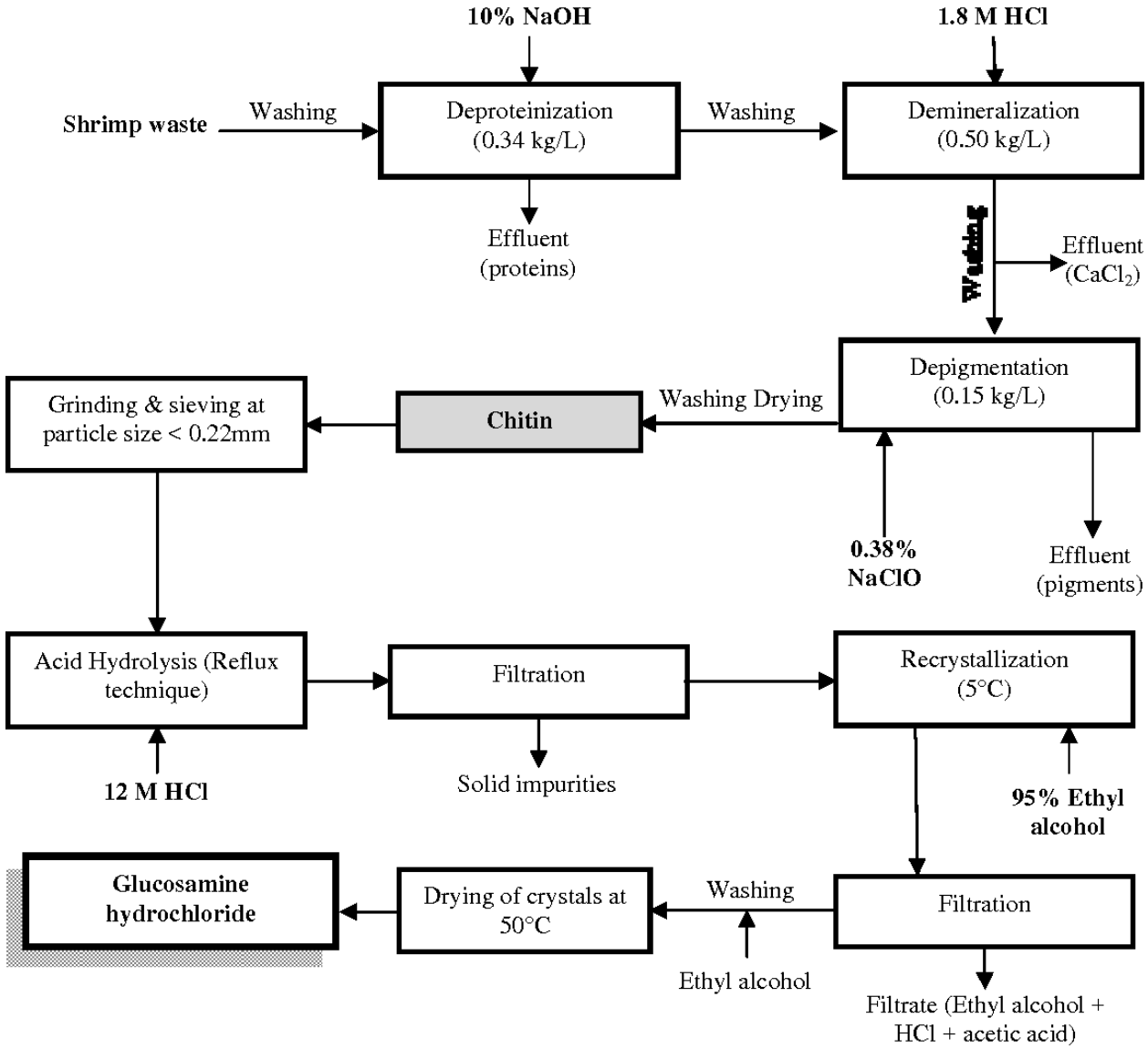
This review aims to present the state-of-the-art knowledge on the morphology of chitin and chitosan, the main techniques applied to chitin isolation and chitosan production. Then, the best methods for characterization in solution or solid state are also indicated. It is pointed out that for

biomedical products, chitin and chitosan need to be highly purified, since residual proteins and pigments can cause side effects. Finally, the main biological properties will be analyzed in relation with the chemical structure (degree of acetylation and molecular weight of chitosan).



Production of Glucosamine

Chitin was prepared from Persian Gulf shrimp (Metapenaeus monoceros), and then, the obtained chitin was hydrolyzed by hydrochloric acid solutions. The production yield of glucosamine hydrochloride from chitin was optimized, and the effect of three factors (acid concentration, acid to chitin ratio, and reaction time) was investigated. A Box-Behnken design by Minitab software created 12 reactions with different conditions. Each reaction was performed in two replicates. Response surface methodology was used for predicting the glucosamine preparation. The optimum conditions for glucosamine hydrochloride preparation were 30 and 37% hydrochloric acid, 9:1 (v/w) acid solution to solid ratio, and 4 h of reaction time. Time ratio and time acid concentrations were the effective factors on the yield.



Production of Chondroitin sulphate

Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). It is usually found attached to proteins as part of a proteoglycan. A chondroitin chain can have over 100 individual sugars, each of which can be sulfated in variable positions and quantities. Chondroitin sulfate is an important structural component of cartilage and provides much of its resistance to compression.[3] Along with glucosamine, chondroitin sulfate has become a widely used dietary supplement for treatment of osteoarthritis.

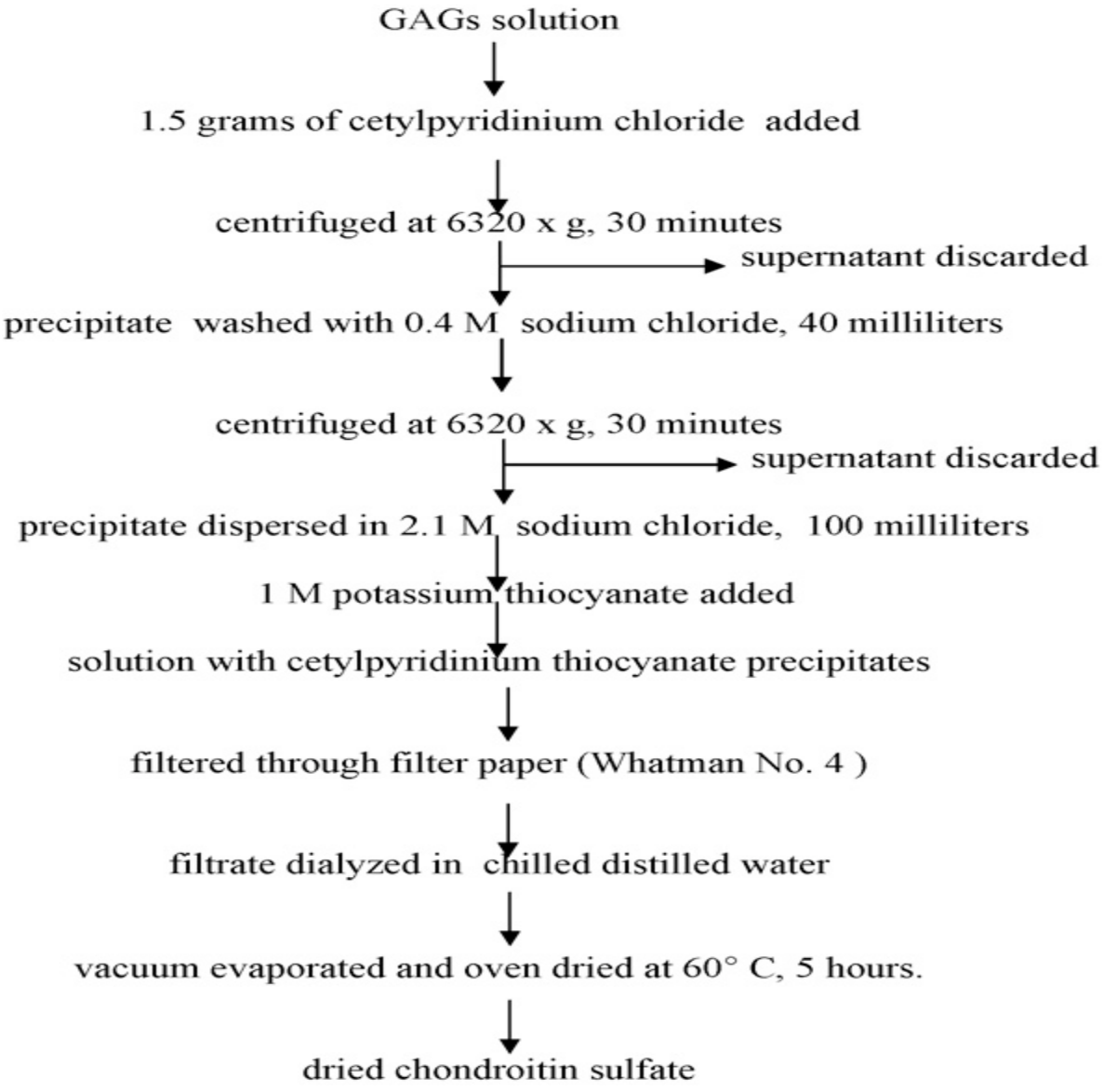


Fig. 1. Chondroitin sulfate extraction procedure.

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMEDTOBEUNIVERSITY)
Accredited'A'GradebyNAAC/12BStatusbyUGC/ApprovedbyAICTE

www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENTOFBIOTECHNOLOGY

M.TECH - BIOTECHNOLOGY

UNIT–III– FOOD & NUTRACEUTICALS–SBTA7013

Yogurt Production

Yogurt Definitions

Yogurt is a fermented milk product that contains the characteristic bacterial cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. All yogurt must contain at least 8.25% solids not fat. Full fat yogurt must contain not less than 3.25% milk fat, lowfat yogurt not more than 2% milk fat, and nonfat yogurt less than 0.5% milk. The full legal definitions for yogurt, lowfat yogurt and nonfat yogurt are specified in the Standards of Identity listed in the U.S. Code of Federal Regulations (CFR), in sections 21 CFR 131.200, 21 CFR 131.203, and 21 CFR 131.206, respectively.

The two styles of yogurt commonly found in the grocery store are set type yogurt and swiss style yogurt. Set type yogurt is when the yogurt is packaged with the fruit on the bottom of the cup and the yogurt on top. Swiss style yogurt is when the fruit is blended into the yogurt prior to packaging.

Ingredients

The main ingredient in yogurt is milk. The type of milk used depends on the type of yogurt – whole milk for full fat yogurt, lowfat milk for lowfat yogurt, and skim milk for nonfat yogurt. Other dairy ingredients are allowed in yogurt to adjust the composition, such as cream to adjust the fat content, and nonfat dry milk to adjust the solids content. The solids content of yogurt is often adjusted above the 8.25% minimum to provide a better body and texture to the finished yogurt. The CFR contains a list of the permissible dairy ingredients for yogurt.

Stabilizers may also be used in yogurt to improve the body and texture by increasing firmness, preventing separation of the whey (syneresis), and helping to keep the fruit uniformly mixed in the yogurt. Stabilizers used in yogurt are alginates (carageenan), gelatins, gums (locust bean, guar), pectins, and starch.

Sweeteners, flavors and fruit preparations are used in yogurt to provide variety to the consumer. A list of permissible sweeteners for yogurt is found in the CFR.

Bacterial Cultures

The main (starter) cultures in yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The function of the starter cultures is to ferment lactose (milk sugar) to produce lactic acid. The increase in lactic acid decreases pH and causes the milk to clot, or form the soft gel that is characteristic of yogurt. The fermentation of lactose also produces the flavor compounds that are characteristic of yogurt. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the only 2 cultures required by law (CFR) to be present in yogurt.

Other bacterial cultures, such as *Lactobacillus acidophilus*, *Lactobacillus subsp. casei*, and Bifido-bacteria may be added to yogurt as probiotic cultures. Probiotic cultures benefit human health by improving lactose digestion, gastrointestinal function, and stimulating the immune system.

General Manufacturing Procedure

The following flow chart and discussion provide a general outline of the steps required for making yogurt. For a more detailed explanation see the literature references by Staff (1998), Tamime and Robinson (1999), Walstra et al. (1999) and the website by Goff, www.foodsci.uoguelph.ca/dairyedu/yogurt.html.

General Yogurt Processing Steps

- Adjust Milk Composition & Blend Ingredients
- Pasteurize Milk
- Homogenize
- Cool Milk
- Inoculate with Starter Cultures
- Hold
- Cool
- Add Flavors & Fruit
- Package

1. Adjust Milk Composition & Blend Ingredients

Milk composition may be adjusted to achieve the desired fat and solids content. Often dry milk is added to increase the amount of whey protein to provide a desirable texture. Ingredients such as stabilizers are added at this time.

2. Pasteurize Milk

The milk mixture is pasteurized at 185°F (85°C) for 30 minutes or at 203°F (95°C) for 10 minutes. A high heat treatment is used to denature the whey (serum) proteins. This allows the proteins to form a more stable gel, which prevents separation of the water during storage. The high heat treatment also further reduces the number of spoilage organisms in the milk to provide a better environment for the starter cultures to grow. Yogurt is pasteurized before the starter cultures are added to ensure that the cultures remain active in the yogurt after fermentation to act as probiotics; if the yogurt is pasteurized after fermentation the cultures will be inactivated.

3. Homogenize

The blend is homogenized (2000 to 2500 psi) to mix all ingredients thoroughly and improve yogurt consistency.

4. Cool Milk

The milk is cooled to 108°F (42°C) to bring the yogurt to the ideal growth temperature for the starter culture.

5. Inoculate with Starter Cultures

The starter cultures are mixed into the cooled milk.

6. Hold

The milk is held at 108°F (42°C) until a pH 4.5 is reached. This allows the fermentation to progress to form a soft gel and the characteristic flavor of yogurt. This process can take several hours.

7. Cool

The yogurt is cooled to 7°C to stop the fermentation process.

8. Add Fruit & Flavors

Fruit and flavors are added at different steps depending on the type of yogurt. For set style yogurt the fruit is added in the bottom of the cup and then the inoculated yogurt is poured on top and the yogurt is fermented in the cup. For swiss style yogurt the fruit is blended with the fermented, cooled yogurt prior to packaging.

9. Package

The yogurt is pumped from the fermentation vat and packaged as desired.

The Preparation of Acidophilus Milk

INTESTINAL toxemia and sour milk therapy are subjects that are far from being on a scientific basis. But it has been demonstrated, at any rate, that milk containing lactic acid is more readily digested than "sweet" milk, whether the acid is a result of bacterial activity or a result of the addition of U. S. P. lactic acid, as is the custom in preparing infant feeding formulas. Furthermore, it has been demonstrated that it is possible under certain conditions to implant the acidophilus type of bacillus in the lower intestinal tract of both man and animal to such an extent that it predominates over all other types. No doubt, too much has been claimed for sour milk therapy; yet there is a preponderance of evidence to support the view that the B. acidophilus culture may be employed in such a way as to have therapeutic value.' In order to have therapeutic value, the culture must contain enormous numbers of bacteria. James2 has shown how the average commercial culture falls down in this respect. A large amount of the culture should be taken daily; and lactose or dextrin or some other carbohydrate that is rather slowly absorbed should be fed at the same time. The kind of culture most satisfactory for supplying the acidophilus organisms in large numbers is a freshly prepared milk culture. Tablets, candies and emulsions have not received the unqualified approval of the Council on Pharmacy and Chemistry,' and any broth culture which has been kept at a refrigerator temperature cannot contain many virulent organisms on account of the fact that they die out rapidly at low temperatures. In a few cities acidophilus milk may be purchased for from $.50 to $1.00 a quart, but it is generally not available at all even at such a price. However, by observing a little care it may be prepared in the home at a very low cost.

The Milk Supply-To start with, the milk must be free from those bacteria which naturally occur in fresh milk. This can be attained by boiling ordinary market milk for a long time, but it has been found that unsweetened evaporated milk is particularly suitable for this purpose. It can be obtained from the grocer at a fair price, can be kept on hand [1105] AMERICAN JOURNAL OF PUBLIC HEALTH always ready for use, and it is certainly sterile. Evaporated milk has been sterilized under rather high steam pressure (about 11 pounds): this is sufficient just to begin to caramelize the milk. Kopeloff has shown that when milk is autoclaved to a "light caramel color" the acidophilus organism grows more rapidly in it than in milk heated to a lesser degree.

The Starter Culture-It is now possible to obtain a pure broth culture of B. acidophilus at, or through, all drug stores. The product of a reliable manufacturer should be specified. One culture which we obtained proved to be B. bulgaricus though it was labeled "B. acidophilus.

" The Control of Temperature-The acidophilus organism grows best at 90° to 105° F. It exhibits less growth at lower temperatures, though it has been found that the organism produces acid at a considerable rate even at room temperature. (Four observations showed an average increase of 0.65 per cent lactic acid in 24 hours at 680 F.)

Milk that has been inoculated can be warmed to 1000 to 1050 F. and transferred to a thermos bottle where it will maintain a temperature high enough to permit very satisfactory growth in 15 to 24 hours. The rate at which the temperature falls off depends upon the quality of the bottle and also, of course, on the temperature of the surrounding air. But it has been found that beginning with a temperature of 1000 F., a quart thermos bottle of average quality will permit the

temperature to drop no more than 100 to 150 in 24 hours. Such temperatures are found quite satisfactory for the rapid production of acidophilus milk.

The Procedure-Carefully clean a quart thermos bottle by allowing it to stand over night full of water containing some washing powder or a little household ammonia; then scald it. Place the cork, a can opener and thermometer in a pan and pour boiling water over them. Wipe the top of a one-pound can of evaporated milk free from dust and pour boiling water over it. Open the can and pour contents into the pan that has been scalded. Fill the can with boiling water and pour into the evaporated milk. Immerse the pan in cool water and stir the mixture with the thermometer until the temperature comes down to about 105° F. Add 2 or 3 ounces of commercial B. acidophilus culture, mix, and transfer to the thermos bottle. (The temperature should now be between 1000 and 1020 F.) Cork and let stand for 24 hours, or until the milk has acquired a pleasantly sour taste. When this is attained, transfer to a clean milk bottle and place in the refrigerator. Succeeding cultures of acidophilus are prepared by using about a teacupful (6 ounces) of milk culture previous made to inoculate the 106 PREPARATION OF AcIDOPHILUS MILK diluted evaporated milk for the next run. Proceeding in such a manner, it will be found that acid is produced at a more rapid rate than when the first quart was prepared using the commercial broth culture as a starter. Thirteen to 17 hours is now quite sufficient. If fermentation is allowed to proceed for a longer time so much acid is developed that the taste becomes unpleasantly sour. After a little experience one may stop the action of the bacteria at any desired degree of sourness. This is effected by merely transferring the milk to a clean glass bottle and placing in the refrigerator. It is perfectly safe to keep the culture at room temperature, but, as has already been mentioned, a considerable increase in acidity may be expected. This is of little consequence, however, if the product is consumed within 24 hours. On the other hand, if the milk is kept in a refrigerator it should be consumed within 48 hours for the reason that the organisms rapidly die out at such low temperatures. One or two tablespoonfuls of lactose or dextrin may be added to each glass of milk consumed, though the pleasant tart taste of the sour milk is to some extent injured by this addition. Most people prefer to take the carbohydrate separately. Typical acidophilus milk has a fine grained curd. It is rather viscous, especially at low temperatures, but not "stringy." It does not develop so high an acidity as is produced by the B. bulgaricus. When a culture becomes rapidly very sour or bitter and is extremely viscous so that when poured, from a bottle it runs out in strings, it is an indication that the culture used was B. bulgaricus. This should be discarded and a new commercial culture obtained from a different source. At any time when souring does not seem to be taking place normally a new start should be made. The nutritive value of this product is exactly the same as that of ordinary cow's milk. Evaporated milk is whole cow's milk evaporated down to one-half its bulk. Therefore, by diluting a can of it with an equal amount of water the composition will be the same as that of normal cow's milk. The evaporated milk flavor, which is objectionable to some people, is scarcely noticeable in acidophilus milk prepared from that product.

Some Experimental Results-A large number of milk cultures have been prepared in thermos bottles on which observations have been made of the influence of (1) the amount of old culture used for inoculation, (2) temperature at beginning of fermentation, and (3) length of time during which fermentation is allowed to proceed.

It has been found that acidophilus milk has its best flavor at 0.8 to 1.0 per cent lactic acid. The result of these observations led to the conclusion that the best results are obtained by the use of about one teacupful of old culture to inoculate one quart of diluted evaporated milk, starting the fermentation in a thermos bottle at 100° to 1020 F. and allowing the fermentation to proceed for 13 to 17 hours. For the first inoculation, however, where the commercial broth culture is used, fermentation should be allowed to proceed for 24 to 27 hours. The fact that the commercial culture is much less active than the prepared milk culture when used for inoculation shows how much greater must be the therapeutic value of the latter.

Method of producing koumiss

The method relates to the dairy industry and can be used in the production of mare's milk products with accelerated (with a single fermentation of mare's milk) and long-term maturation of the koumiss mixture with 2 to 3-fold "rejuvenation" of it with fresh mare's milk.

A known method of producing koumiss (1), including fermentation of mare's milk, making koumiss sourdough, stirring, bottling, cooling, maturation at 16-18 ° C for 1-2 hours and the introduction of a stabilizing additive (apple pectin or agar agar mixed with powdered sugar in a ratio of 1: 5 based on 0.5% of the mass of the milk mixture), followed by spill and high-temperature compensation at 9 ° C for 5 minutes.

A known method of producing koumiss from mare's milk (2), including adding koumiss sourdough milk to the mare, fermenting, kneading the mixture, self-gasing for 2-3 hours without air. Mare's milk is used in dry form, which is pre-mixed with water in a ratio of 1:10, fermentation is carried out until acidity reaches 60-70 ° T, and kneading is carried out for 25-60 minutes depending on the speed of the mixer, followed by exposure for 2 hours moreover, after self-gasification, the mixture is ripened and cooled at a temperature of from 2 to 8 ° C.

The disadvantages of the described methods are the short shelf life due to the lack of environmentally friendly equipment and the loss of organoleptic characteristics of koumiss, the irrational use of raw materials in the summer, low consumer properties, taste and appearance.

The objective of the proposed method is the manufacture of the target product according to the traditional technology of koumiss making-producing natural koumiss (from mare's milk) due to strict adherence to the technological regime, hygiene and high-quality koumiss sourdough.

The cooking method is as follows:

- Fresh mare's milk, with constant stirring, is poured into the sourdough with an acidity of 120 ° T in a ratio of 1: 3. The mixture is stirred at a temperature of 28-30 ° C and left alone for ripening until the acidity of the mixture reaches 60-70 ° T. Then milk of the next milk yield is added, and after repeated kneading for 1 hour and rest for 2 hours, koumiss fermentation is activated, a large amount of carbon dioxide is released, the surface of the mixture is covered with an even layer of the smallest foam (which is not observed when making the initial koumiss mixture) , a specific koumiss flavor and aroma appears. The number (rejuvenation) of koumiss is carried out depending on the number of milks. With each subsequent addition of milk, koumiss wanders harder - its quality improves. After the last "rejuvenation", koumiss is poured into 0.5 liter bottles, corked with a crown plug. For further ripening, koumiss is placed in a refrigerator with a temperature of 4-6 ° C.

For the production of koumiss, mare's milk must meet the following characteristics:

- acidity 5-7 ° T,

- density 1.029 ° -1.033 ° A
- temperature - 28-30 ° C,
- mechanical pollution - not lower than the first group.

The best production sourdough is the daily left part of koumiss in the active phase of fermentation. Koumiss starter culture, subject to the correct technological regime, maintains its activity and stability for months and even years without requiring replacement, however, if the starter culture acquires undesirable properties (sediment, flakes, excess acidity, is not sufficiently carbonated), it must be replaced. In case of spoilage, you should have insurance leaven selected from a good batch of koumiss, which is stored in the refrigerator. Before use, it must be diluted with mare's milk (rejuvenate) in a 1: 1 ratio and kneaded for 60 minutes, in order to further activate microflora and restore normal koumiss fermentation, the mixture needs to be rejuvenated 3-4 times and after each fresh fresh mare's milk is added, knead 60 minutes. Periodically, every 4-5 weeks, the insurance starter should be replaced with a new, fresh koumiss.

Koumiss sourdough must be introduced in such an amount that the acidity of the mixture is 40-45 ° T.

The amount of yeast introduced is determined by the formula:

$$A_З = \frac{(K_С - K_М) \cdot A_М}{K_З - K_С}$$

where

And $_h$ is the amount of ferment required

And $_m$ is the amount of mare's milk,

K $_{With\ the}$ acidity of the mixture

To $_Z$ - the acidity of the starter culture,

K $_M$ - the acidity of milk.

An example of determining the consumed leaven.

There is 100 l of milk with an acidity of 6 ° T, the acidity of the initial mixture (sourdough + mare's milk) is 45 ° T, and the acidity of the starter is 120 ° T.

The required amount of starter culture is determined by the formula:

$$A_{З=} \frac{(45 - 6) \cdot 100}{120 - 45} = 52\ л$$

KEFIR-MANUFACTURE, COMPOSITION, NUTRITIONAL AND THERAPEUTIC PROPERTIES

Introduction

Kefir is a viscous, slightly carbonated dairy beverage that contains small quantities of alcohol and, like yoghurt, is believed to have its origins in the Caucasian mountains of the former USSR. It is also manufactured under a variety of names including kephir, kiaphur, kefer, knapon, kepi and kippi with artisanal production of kefir occurring in countries as widespread as Argentina, Taiwan, Portugal, Turkey and France. It is not clear whether all kefirs originate from a single

original starter culture, since microbial analyses of kefir samples taken from different locations indicate microflora population differences.

Definition

Although no clear definition of kefir exists, it is a viscous, acidic, and mildly alcoholic milk beverage produced by fermentation of milk with a kefir grain as the starter culture (FAO/WHO 2003). The Codex Alimentarius description of kefir state it as Starter culture prepared from kefir grains, Lactobacillus kefir, and species of the genera Leuconostoc, Lactococcus and Acetobacter growing in a strong specific relationship. Kefir grains constitute both lactose-fermenting yeasts (Kluyveromyces marxianus) and non-lactose-fermenting yeasts (Saccharomyces unisporus, Saccharomyces cerevisiae and Saccharomyces exiguus).

Composition

The composition of kefir will be essentially dependant on the type of milk that was used. The major change caused by fermentation measured in term of acid production and alcohol production may also reflect in the composition.Table-30.1 shows the composition standards prescribed by the Codex

Table 30.1 Codex standard for kefir

Milk protein (% w/w)	min. 2.8
Milk fat (% m/m)	<10
Titratable acidity, expressed as % of lactic acid	min. 0.6
Ethanol (% vol. /w)	not stated
Sum of specific microorganisms constituting the starter culture (cfu/g, in total)	10^7 (minimum)
Yeasts (cfu /g)	10^4 (minimum)

(From Codex Standard for Fermented Milks CODEX STAN 243 – 2003)

Kefir Manufacture

Although commercial kefir is traditionally manufactured from cow's milk, it has also been made from the milk of ewes, goats and buffalos. Moreover, kefir produced using soy milk has also been recently reported. The various steps of kefir manufacture are depicted in Figure 30.1.

Traditionally, kefir is produced by adding kefir grains (a mass of proteins, polysaccharides, mesophilic, homofermentative and heterofermentative lactic acid streptocci, thermophilic and mesophilic lactobacilli, acetic acid bacteria, and yeast) to a quantity of milk. The size of the initial kefir grain inoculum affects the pH, viscosity and microbiological profile of the final product. A grain to milk ratios of 1:30 to 1:50 were found optimum. In some manufacturing procedures, a percolate of the grains from a coarse sieve is used as the mother culture to

inoculate fresh milk. Fermentation of the milk by the inoculum proceeds for approximately 24 hours, during which time homofermentative lactic acid streptococci grow rapidly, initially causing a drop in pH. This low pH favours the growth of lactobacilli, but causes the streptococci numbers to decline. The presence of yeasts in the mixture, together with fermentation temperature (21-23°C), encourages the growth of aroma producing heterofermentative streptococci. As fermentation proceeds, growth of lactic acid bacteria is favoured over growth of yeasts and acetic acid bacteria.

Method of manufacture of kefir

Kefir grains are key to kefir production, and it has been found that the finished product has a different microbiological profile from the grains and therefore cannot be used to inoculate a new batch of milk. Grains have been shown to possess a dynamic and complex flora which is not conducive to commercial production of a uniform, stable product; this has prompted researchers to try to produce kefir from a mixture of pure cultures. Some researchers produced a starter consisting of two bacteria (Lactobacillus helveticus and Lactococcus lactis subsp lactis) and one yeast (S. cerevisiae) isolated from kefir grain and combined with two yoghurt strains (Lactobacillus delbrueckii subsp bulgaricus, and Streptococcus thermophilus). Yeast was added to the starter with sucrose either at the beginning, or after lactic acid fermentation. The two resulting kefirs produced were found to have high numbers of viable cocci and lactobacilli and had chemical and organoleptic properties that were similar to traditional kefir. A commercial kefir is being produced in the United States using a mixture of defined microorganisms rather than kefir grains. This starter culture mixture has been reported to contain Streptococcus lactis, Lb. plantarum, Streptococcus cremoris, Lb. casei, Lactococcus lactis subsp Lactis biovar diacetylactis, Leuconostoc cremoris and Saccharomyces florentinus.

Characteristics of Kefir

The flavour, viscosity and microbial/chemical composition of the final kefir product can be affected by the size of the inoculum added to the milk, the occurrence of any agitation during fermentation, and the rate, temperature and duration of the cooling and ripening stages following fermentation. Natural kefir has a refreshing, yeasty taste and a ‘sparkling’ mouth feel. Modern manufacturing procedures for kefir result in ethanol levels in the finished product of 0.01– 0.1% although kefir with ethanol concentrations as high as 0.25% have been produced from grains in the laboratory. The amounts of ethanol and CO2 produced during fermentation of kefir depend on the production conditions used. CO2 content of kefir has been said to be ‘comparatively low’ in relation to other fermented drinks. The distinctive taste of kefir results from the presence of several flavour compounds which are produced during fermentation. Kefir produced from pure cultures did not receive high sensory evaluation scores. Acetaldehyde and acetoin have received particular attention with regard to their roles during kefir manufacture because of their contribution in the taste; both have been found to increase in concentration during kefir fermentation. During storage, acetaldehyde increases in concentration and acetoin decreases.

Kefir Grains

Kefir grains (Figure-30.2) resemble small cauliflower florets: they measure 1-3 cm in length, are lobed, irregularly shaped, white to yellow-white in colour, and have a slimy but firm texture. Grains are kept viable by transferring them daily into fresh milk and allowing them to grow for approximately 20 hours; during this time, the grains will have increased their mass by 25%. Grains must be replicated in this way to retain their viability, since old and dried kefir grains have little or no ability to replicate. In addition, washing grains in water also reduced viability. It has been recommended that in a commercial operation using grains to produce kefir, grains should be kept viable through daily transfers and should only be replaced if their ability to ferment milk becomes impaired. Low temperature storage appears to be the best way to maintain kefir grains for long periods. Storage of kefir grains at 80° or 20°C for 120 days did not change their fermentation properties compared to grains that had not been stored; however, grains stored at 4°C did not produce acceptable kefir after thawing.

Kefir grains

Microbiology of kefir grains

Bacteria found in kefir grains and kefir

The microbial population (Figure 30.3) found in kefir grains have been used as an example of a symbiotic community. This symbiotic nature has made identification and study of the constituent microorganisms within kefir grains difficult.

Bacteria found in kefir grains and kefir

Lactobacilli *Lactobacillus kefir*	*Lactobacillus delbrueckii*
Lactobacillus kefiranofaciens	*Lactobacillus rhamnosus*
Lactobacillus kefirgranum	*Lactobacillus casei*
Lactobacillus parakefir	*Lactobacillus paracasei*
Lactobacillus brevis	*Lactobacillus fructivorans*
Lactobacillus plantarum	*Lactobacillus hilgardii*
Lactobacillus helveticus	*Lactobacillus fermentum*
Lactobacillus acidophilus	*Lactobacillus viridescens*
Lactococci *Lactococcus lactis* subsp *lactis*	**Enterococci** *Enterococcus durans*

Lactococcus lactis subsp *cremoris*	
Streptococci *Streptococcus thermophilus*	**Leuconostocs** *Leuconostoc mesenteroides*
Acetic acid bacteria *Acetobacter pasteurianus* *Acetobacter aceti*	**Other Bacteria** Bacillus spp, Micrococcus spp. *Bacillus subtilis, Escherichia coli*

Yeasts found in kefir grains and kefir

The yeasts in kefir (Table-30.3) have been less well studied than kefir bacteria, although it is obvious that the yeasts in kefir grains provide an environment for the growth of kefir bacteria, producing metabolites that contribute to the flavour and mouthfeel of kefir. To prevent excessive CO2 production (particularly after fermentation), a two stage fermentation process starting with a non-lactose fermenting yeast such as Saccharomyces cerevisiae can be done. The properties of yeasts found in kefir grains vary. For example, some of the yeast found in kefir grains are capable of fermenting lactose, while some are not. Also, it has been observed that some type of yeasts are located at the surface of the grain, while others inhabit the interior. It may be that yeasts located at different locations in the kefir grains play different roles in the fermentation process. Like kefir bacteria, the profile of yeasts is different in kefir grains when compared to the final kefir product.

Nutritional Significance of Kefir

The composition of kefir depends greatly on the type of milk that was fermented. However, during the fermentation, changes in composition of nutrients and other ingredients have also been shown to occur. L(+) lactic acid is the organic acid in highest concentrations after fermentation and is derived from approximately 25% of the original lactose in the starter milk. The amino acids valine, leucine, lysine and serine are formed during fermentation, while the quantities of alanine and aspartic acid increase as compared to raw milk. Appreciable amounts of pyridoxine, vitamin B12, folic acid and biotin were synthesized during kefir production, depending on the source of kefir grains used, while thiamine and riboflavin levels were reduced. Some workers reported decreases in biotin, vitamin B12 and pyridoxine, and significant increases in folic acid, as compared to non-fermented milk.

Bio Active Ingredients in kefir

Kefir has a long tradition of offering heath benefits. There are several compounds in kefir that may have bioactive properties.

(i) Exopolysaccharides

Exopolysaccharides of differing structures and compositions are produced by a variety of lactic acid bacteria including Lactobacillus, Streptococcus, Lactococcus and Leuconostoc. These cell-surface carbohydrates confer protective and adaptive properties on their bacterial producers; since they are often loosely bound to the cell membrane, they are therefore, easily lost to their environment. In food products, exopolysaccharides often contribute to organoleptic and stability characteristics. A unique polysaccharide called kefiran has been found in kefir grains. Grains may also contain other exopolysaccharides. Kefiran contains D-glucose and D-galactose only in a ratio of 1:1. Kefiran dissolves slowly in cold water and quickly in hot water, and forms a viscous solution at 2% concentration.

(ii) Bioactive peptides

Many organisms possess enzymes (e.g. proteinases and peptidases) that are able to hydrolyse the protein in a medium, thereby supporting growth of the organism by liberating peptides and amino acids. The action of proteinase and peptidase enzymes on milk proteins can theoretically result in a very large number of possible peptides. An analysis of the proteinase activity of kefir grain bacterial isolates has shown that several isolates have high proteinase activities which increases the possibility that bioactive peptides may be present in kefir. Studies on the peptide content of kefir drink have shown that kefir contains a large number of peptides.

Therapeutic Significance of kefir

Kefir has had a long history of being beneficial to health in Eastern European countries, where it is associated with general wellbeing. It is easily digested and is often the first weaning food received by babies.

- It has been proposed that stimulation of the immune system may be one mechanism whereby probiotic bacteria may exert many of their beneficial effects. Peptides formed during the fermentation process or during digestion have also been shown to be bioactive, and demonstrate a variety of physiological activities, including stimulation of the immune system in animal models. Stimulation of the immune system may also occur due to the action of exopolysaccharides found in kefir grains.
- Anti tumour effects of a water-soluble polysaccharide (approximate molecular weight 10,000,00 Da) isolated from kefir grains is reported.
- A water soluble polysaccharide fraction from kefir grains was shown to inhibit pulmonary metastasis of Lewis lung carcinoma, whether the kefir-derived polysaccharide was given orally before or after tumour transplantation.
- Some kefir grains have been shown to possess b-galactosidase activity which remains active when consumed and thus can be beneficial for lactose intolerant people.
- Many lactobacilli are capable of producing a wide range of antimicrobial compounds, including organic acids (lactic and acetic acids), carbon dioxide, hydrogen peroxide, ethanol, diacetyl and peptides (bacteriocins) that may be beneficial not only in the reduction of food borne pathogens and spoilage bacteria during food production and storage, but also in the treatment and prevention of gastrointestinal disorders and vaginal

infections. Fresh kefir grains were found to inhibit the growth of the pathogens Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli. Leuconostoc mesenteroides and Lactobacillus plantarum, isolated from kefir grains, have been shown to produce antimicrobial compounds which can inhibit Gram-positive and Gram-negative bacteria. These antimicrobial compounds are found to be heat stable. But their antimicrobial properties are reduced after exposure to proteolytic enzymes. Lactobacilli isolated from kefir grains had antimicrobial activities against E. coli, Listeria monocytogenes, Salmonella Typhimurium, S. Enteritidis, S. flexneri and Yersinia enterocolitica. Bacteriocins were thought to be responsible for the antimicrobial activities
.

Kefir is a microbiologically complex product with a large number of different bacteria and yeast involved in its making . Many of these microorganisms are only now being identified by using advanced molecular biological techniques. The study of kefir is made more difficult, because it appears that many different sources of kefir grains exist that are being used to produce kefir. The production of kefir depends on the synergistic interaction of the microflora in kefir grains. During the fermentation process, the yeasts and bacteria in kefir grains produce a variety of ingredients that give kefir its unique taste and texture. After fermentation, the finished kefir product contains many ingredients that are proving to be bioactive and may be used as functional ingredients.

DAHI PREPARATION METHODS, QUALITY OF DAHI, PACKAGING, SHELF LIFE AND DEFECTS

Introduction

Fermentation is one of the simplest ways of preserving milk constituents for human consumption. Fermentation gives an acid taste to milk which is particularly refreshing in worm climate and also imparts certain therapeutic benefits originally absent in milk. Fermented dairy products have assumed prominent position in human diet in many regions of the world. Fermentation leads to partial breakdown of milk constituents and increases the digestibility of cultured milk products.

In Vedic literature also, we could find many references about fermented milk products some are listed below.

- Prasadjya Dahi after dilution and churning, carrying butter grains in the liquid mass
- Payasya Strained curd, when mixed with boiled milk, crystal sugar and fermented herbs
- Shrikarini Consists of strained dahi, crystal sugar and spices
- Rasala -- Sugar and spiced curd

Some of the popular Indian fermented milk products are Dahi, Lassi, Chakka, Shrikhand, Mishti Dahi and Raita.

Product Description

Dahi is produced from heat treated milks after inoculation with certain species of lactic acid bacteria added to milk in the form of starter culture. Lactic acid bacteria added multiply, grow and produce lactic acid, acetic acid and carbon dioxide by utilizing lactose present in milk. Some bacteria uses citric acid of milk to produce certain volatile organic compounds mainly **diacetyl,** which is mainly responsible for flavor of dahi. Judicious combination of acid producing and flavour producing microorganisms in the starter helps in the production of Dahi with a firm body and good flavour.

Definition of *dahi*

Dahi or curd is a semi solid product, obtained from pasteurized or boiled milk by souring, using harmless lactic acid or other bacterial cultures. Dahi may contain additional cane sugar. It should have the same minimum percentage of fat and solids-not-fat as the milk from which it is prepared. Where Dahi or curd, other than skimmed milk Dahi, is sold or offered for sale without any indication of the class of milk, the standards prescribed for Dahi prepared from buffalo milk shall apply.

Table 23.1 FSSR(2011) and BIS standards of *dahi*

Characteristics	FSSR(2011)	BIS
Acidity % lactic acid	-	0.6 - 0.8
Total Plate count	Not more than 1000000/g	
Coliform count	10 per g max	10 per g max
Escherechia coli	Absent in 1g	
Salmonella	Absent in 25g	
Shigella	Absent in 25g	
Stephylococcus aurius	Not more than 100/g	
Yeast and Mould	100 per g max	100 per g max
Anaerobic spore	Absent in 1g	
Listeria monocytogenes	Absent in 1g	
Phosphatase test		Negative
Other requirements	It should have the same minimum percentage of fat and SNF as the milk from which it is prepared. If no standards declared then standards prescribed for dahi from buffalo milk	Dahi shall conform to the requirements of milk fat and MSNF, as laid down in FSSR, 2011

	shall apply	

Table 23.2 Chemical composition of *dahi*

Components	Whole milk Dahi	Skim milk Dahi
	%	%
Water	85-88	90-91
Fat	5 – 8	0.05 - 0.1
protein	3.2-3.4	3.3-3.5
Lactose	4.6-5.2	4.7-5.3
Lactic acid	0.5-1.1	0.5-1.1
Ash	0.7-0.75	0.7-0.75

Method of Preparation

Traditional method

In traditional method of dahi preparation, milk is heated intensively to boil for 5 to 10 min and then it is cooled to room temperature. cooled milk is added with previous day's curd or buttermilk, stirred and allowed to set undisturbed usually for overnight.

At halwai's shop milk is considerably concentrated before being inoculated with starter culture. So that the total solid content of milk gets increased, particularly increase in the protein content of milk. Concentration of milk results in custard like consistency of dahi and keeps the product from wheying off.

Industrial method of making *dahi*

Selection of raw material

Production of cultured/fermented milk demands high quality raw materials with respect to physical, chemical and microbial standards.

Filtration/clarification

Fresh raw milk is heated to 35 to 40°C to aid clarification or filtration process then it is filtered to ensure that, milk is free from extraneous matter.

Standardization: Fat: 0 – 5%, SNF: 11 – 13%

Fat is standardized based on type of product ranging from fat free to full fat and SNF level is increased by min. 2% than that of milk. It is common to boost the SNF content of the milk to about 12% with the addition of skim milk powder or condensed skim milk.

Increased SNF inturn increases the protein, calcium and other nutrients and resulted with improved body and texture, custard like consistency. Higher milk solids prevent wheying off of the product during storage.

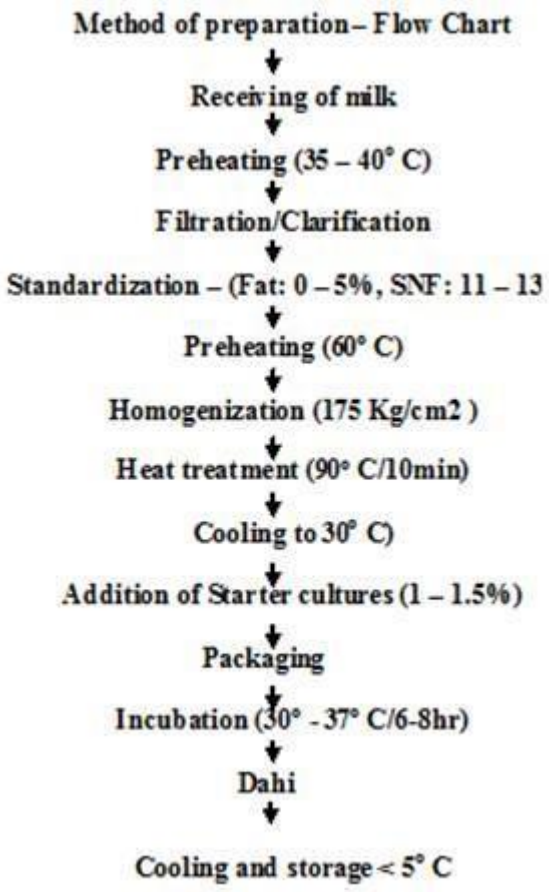


Fig. 23.1 Method of preparation of *dahi*

Homogenization: 175 Kg/cm^2

The standardized milk is subjected to homogenization after heating to 60□C to increase the efficiency. Homogenization reduces the cream layer formation during incubation, Single stage homogenization with 175kg/cm^2 pressure would be sufficient to improve texture of dahi.

Heat treatment: 9□ C/23min

Milk intended for dahi or any other fermented milk product is given severe heat treatment i.e. 90□C for 10min.

Following are the benefits of high heat treatment

- □ Denatures and coagulates milk albumin and globulins which enhance the viscosity and produce custard like consistency

- Kills contaminating and competitive microbes
- Development of relatively sterile medium
- Removal of air form the medium more conducive for the growth of culture bacteria
- Effective thermal breakdown of protein releasing peptones and sulfhydryl groups, this inturn provide nutrients to starter bacteria

Packaging and fermentation

The heat treated product mix is cooled to 37C and it is inoculated with specific dahi culture at the rate of 1 to 1.5%. Starter culture is the most crucial component in the production of high quality fermented milks. Proper selection of culture strains decides the good quality of product. Dairy cultures are available in various forms like freeze dried, liquid and frozen forms. After the product mix is inoculated with dahi culture it is thoroughly mixed and filled into plastic cups, sealed properly to avoid any contamination and spillage of the product. Dahi is packed in food grade polystyrene and polypropylene cups in 100g, 200g and 400g pack sizes. Various packaging machines of upto 400cups/min speed are available to package cultural dairy products in different sizes. The packaged product should be stored at < 5C for extended shelf lifeThus packed product is arranged in cases or crates and transferred to incubation room maintained at 37to 42C. The product mix is incubated till its pH reaches 4.4 to 4.5 and then it is cooled rapidly to less than 5C by exposing the cups to high velocity cold air.

Storage

Dahi is normally stored at 4 5C. Storage area should be maintained clean and tidy to avoid any cross contamination.

Table 23.3 Common defects in dahi

Sl No.	Defect	Probable Cause	Remedy
Flavor defects			
1	Insufficient Flavor	Low citrate level in milk, Low diacetyl content	Add 0.02 0.05% Sodium citrate prior to mixing the starter culture. Cool rapidly after culturing
2	Oxidized flavor	Copper contamination Exposure to fluorescent light Exposure to sunlight	Avoid usage of copper utensils Protect product from direct exposure to Sunlight/ UV light

3	Yeast/cheesy	Contaminating yeast growth	Sanitation check
4	Rancid flavor	Lipolytic activity	Do not mix pasteurized and raw dairy ingredients prior to homogenization
5	High acid	Addition of more culture, Increased incubation time Use of sour milk	Optimum culture addition Blast cool the product immediately after optimum pH is reached Use good quality fresh milk
Body and textural defects			
1	Weak body	Insufficient heat treatment to the mix Too low milk SNF Severe agitation after fermentation	Heat treatment should not be less than 85ºC/30min Homogenize the dahi mix prior to homogenization Increase the MSNF content to 11% by adding Skim milk powder
2	Grainy texture	High acidity Improper dispersion of Skim milk powder	Rapidly cool the product to <5ºC after attaining optimum acidity Use in line screen/filter
3	Syneresis	Insufficient heat treatment to the mix Improper standardization and too low milk SNF Agitation/disturbances during fermentation	Heat treatment should not be less than 85ºC/30min Increase the MSNF content to min. of 11% by adding Skim milk powder Do not disturb the cups during fermentation
4	Ropiness	Contamination of milk with psychotropic microorganisms Culture contamination/impure culture	Proper heat treatment of milk, Avoid cold storage of milk before pasteurization/thermization Use of pure culture

Cheese Production

Cheese Definitions

Cheese comes in many varieties. The variety determines the ingredients, processing, and characteristics of the cheese. The composition of many cheeses is defined by Standards of Identity in the U.S. Code of Federal Regulations (CFR).

Cheese can be made using pasteurized or raw milk. Cheese made from raw milk imparts different flavors and texture characteristics to the finished cheese. For some cheese varieties, raw milk is given a mild heat treatment (below pasteurization) prior to cheese making to destroy some of the spoilage organisms and provide better conditions for the cheese cultures. Cheese made from raw milk must be aged for at least 60 days, as defined in the CFR, section 7 CFR 58.439, to reduce the possibility of exposure to disease causing microorganisms (pathogens) that may be present in the milk. For some varieties cheese must be aged longer than 60 days.

Cheese can be broadly categorized as acid or rennet cheese, and natural or process cheeses. Acid cheeses are made by adding acid to the milk to cause the proteins to coagulate. Fresh cheeses, such as cream cheese or queso fresco, are made by direct acidification. Most types of cheese, such as cheddar or Swiss, use rennet (an enzyme) in addition to the starter cultures to coagulate the milk. The term "natural cheese" is an industry term referring to cheese that is made directly from milk. Process cheese is made using natural cheese plus other ingredients that are cooked together to change the textural and/or melting properties and increase shelf life.

Ingredients

The main ingredient in cheese is milk. Cheese is made using cow, goat, sheep, water buffalo or a blend of these milks.

The type of coagulant used depends on the type of cheese desired. For acid cheeses, an acid source such as acetic acid (the acid in vinegar) or gluconodelta-lactone (a mild food acid) is used. For rennet cheeses, calf rennet or, more commonly, a rennet produced through microbial bioprocessing is used. Calcium chloride is sometimes added to the cheese to improve the coagulation properties of the milk.

Flavorings may be added depending on the cheese. Some common ingredients include herbs, spices, hot and sweet peppers, horseradish, and port wine.

Bacterial Cultures

Cultures for cheese making are called lactic acid bacteria (LAB) because their primary source of energy is the lactose in milk and their primary metabolic product is lactic acid. There is a wide variety of bacterial cultures available that provide distinct flavor and textural characteristics to cheeses. For a more detailed description of cheese cultures and microbiology, see Fox (2004), Kosikowski and Mistry (1997), and Law (1997).

Starter cultures are used early in the cheese making process to assist with coagulation by lowering the pH prior to rennet addition. The metabolism of the starter cultures contribute desirable flavor compounds, and help prevent the growth of spoilage organisms and pathogens. Typical starter bacteria include *Lactococcus lactis* subsp. *lactis* or *cremoris*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbruckii* subsp. *bulgaricus*, and *Lactobacillus helveticus*.

Adjunct cultures are used to provide or enhance the characteristic flavors and textures of cheese. Common adjunct cultures added during manufacture include *Lactobacillus casei* and *Lactobacillus plantarum* for flavor in Cheddar cheese, or the use of *Propionibacterium freudenreichii* for eye formation in Swiss. Adjunct cultures can also be used as a smear for washing the outside of the formed cheese, such as the use of *Brevibacterium linens* of gruyere, brick and limburger cheeses.

Yeasts and molds are used in some cheeses to provide the characteristic colors and flavors of some cheese varieties. Torula yeast is used in the smear for the ripening of brick and limburger cheese. Examples of molds include *Penicillium camemberti* in camembert and brie, and *Penicillium roqueforti* in blue cheeses.

General Manufacturing Procedure

The temperatures, times, and target pH for different steps, the sequence of processing steps, the use of salting or brining, block formation, and aging vary considerably between cheese types. The following flow chart provides a very general outline of cheese making steps. The general processing steps for Cheddar cheese are used for illustration. For a more detailed explanation see the literature references by Fox (2004), Kosikowski and Mistry (1997), Law (1997), Walstra et al. (1999), and the website by Goff, www.foodsci.uoguelph.ca/dairyedu/cheese.html.

General Cheese Processing Steps

- Standardize Milk
- Pasteurize/Heat Treat Milk
- Cool Milk
- Inoculate with Starter & Non-Starter Bacteria and Ripen
- Add Rennet and Form Curd
- Cut Curd and Heat
- Drain Whey
- Texture Curd
- Dry Salt or Brine
- Form Cheese into Blocks
- Store and Age
- Package

The times, temperatures, and target pH values used for cheddar cheese will depend on individual formulations and the intended end use of the cheese. These conditions can be adjusted to optimize the properties of Cheddar cheese for shredding, melting, or for cheese that is meant to be aged for several years.

1. Standardize Milk

Milk is often standardized before cheese making to optimize the protein to fat ratio to make a good quality cheese with a high yield

2. Pasteurize/Heat Treat Milk

Depending on the desired cheese, the milk may be pasteurized or mildly heat-treated to reduce the number of spoilage organisms and improve the environment for the starter cultures to grow. Some varieties of milk are made from raw milk so they are not pasteurized or heat-treated. Raw milk cheeses must be aged for at least 60 days to reduce the possibility of exposure to disease causing microorganisms (pathogens) that may be present in the milk.

3. Cool Milk

Milk is cooled after pasteurization or heat treatment to 90°F (32°C) to bring it to the temperature needed for the starter bacteria to grow. If raw milk is used the milk must be heated to 90°F (32°C).

4. Inoculate with Starter & Non-Starter Bacteria and Ripen

The starter cultures and any non-starter adjunct bacteria are added to the milk and held at 90°F (32°C) for 30 minutes to ripen. The ripening step allows the bacteria to grow and begin fermentation, which lowers the pH and develops the flavor of the cheese.

5. Add Rennet and Form Curd

The rennet is the enzyme that acts on the milk proteins to form the curd. After the rennet is added, the curd is not disturbed for approximately 30 minutes so a firm coagulum forms.

6. Cut Curd and Heat

The curd is allowed to ferment until it reaches pH 6.4. The curd is then cut with cheese knives into small pieces and heated to 100°F (38°C). The heating step helps to separate the whey from the curd.

7. Drain whey

The whey is drained from the vat and the curd forms a mat.

8. Texture curd

The curd mats are cut into sections and piled on top of each other and flipped periodically. This step is called **cheddarin**g. Cheddaring helps to expel more whey, allows the fermentation to continue until a pH of 5.1 to 5.5 is reached, and allows the mats to "knit" together and form a tighter matted structure. The curd mats are then milled (cut) into smaller pieces.

9. Dry Salt or Brine

For cheddar cheese, the smaller, milled curd pieces are put back in the vat and salted by sprinkling dry salt on the curd and mixing in the salt. In some cheese varieties, such as mozzarella, the curd is formed into loaves and then the loaves are placed in a brine (salt water solution).

10. Form Cheese into Blocks

The salted curd pieces are placed in cheese hoops and pressed into blocks to form the cheese.

11. Store and Age

The cheese is stored in coolers until the desired age is reached. Depending on the variety, cheese can be aged from several months to several years.

12. Package

Cheese may be cut and packaged into blocks or it may be waxed.

Dosa

Dosa or Dose is arguably one of the most popular dishes in India; it is a typical part of the South Indian diet and is gaining popularity all over the world. It is a type of pancake made from fermented batter of rice and blackgram. The literary references to dosa date back to the 1st century AD. The place of origin of dosa is debatable but traditionally accepted to be Udupi, a town in the state of Karnataka. However a popular variant, the thin crust-crispy dosa, was believed to be originated from the Indian state of Tamil Nadu.

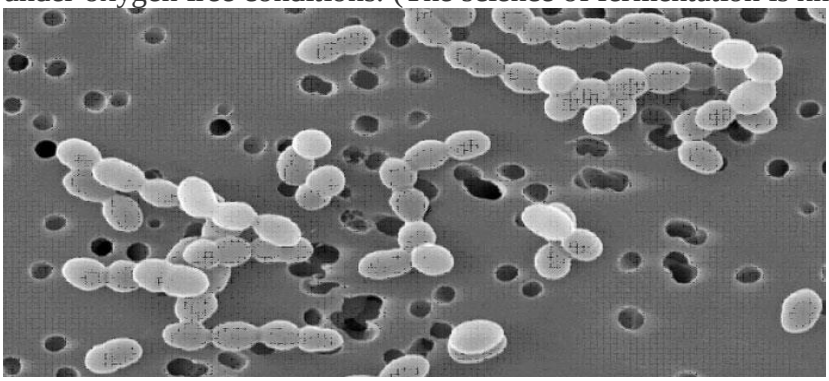
Preparation and serving: Mixture of rice and black grams (usually 2:1) soaked in water overnight is finely ground to form a batter and a pinch of salt is added. The batter is allowed to ferment overnight and then mixed with water to get the desired consistency. The batter is then ladled onto a hot griddle greased with oil or clarified butter. It is spread out evenly with the base of a ladle to form a pancake. Typical dosa is served hot along with vegetable soup (sambar), potato curry and coconut-chilly sauce (chutney) (Fig. 1), but now a day, one can find hundreds of varieties of dosa depending upon their taste and preferences.

Leaving of the batter due to Fermentation

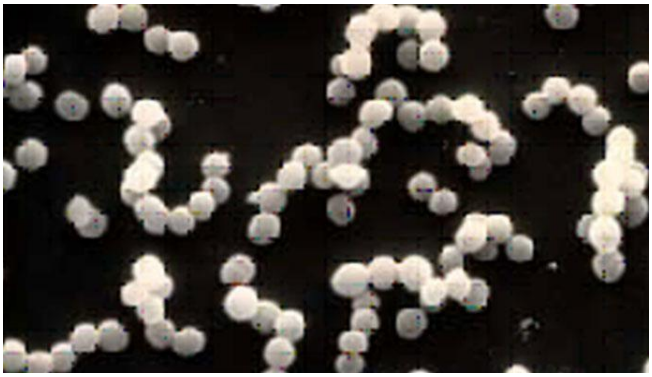
Nutrition: The main ingredients of dosa are rice (Oryza sativa) and blackgram (Phaseolus mungo). White rice, which is normally used for dosa, contains about 90% carbohydrates, 8 percent proteins and 2% fat. It is also a good source of calcium, magnesium, phosphorus, manganese, selenium, iron and vitamins, folic acid, thiamine and niacin.

It has low fiber content and contains pro-inflammatory omega-6 fatty acids. Black gram or Mungo bean is rich in carbohydrates (about 60%) and proteins (about 25%), It also contains about 18% of dietary fiber and is a good source of minerals, potassium, calcium, iron and vitamins, niacin, thiamine, and riboflavin. Black gram has been found to be very useful in controlling cholesterol levels.

Fermentation: Fermentation gives the characteristic texture (leavening), aroma and taste to the dosa batter along with improved digestibility and nutritional value (Fig. 2). Fermentation is the process of converting carbohydrates to alcohol or organic acids with the help of microorganisms, under oxygen free conditions. (The science of fermentation is known as zymology or zymurgy.)



Leuconostoc mesenteroides



Lactococcus lactis

The microorganisms responsible for the fermentation are naturally present in the ingredients of dosa batter, black gram and rice. Some of the fermentation bacteria/microbes are also provided by water and air. A temperature of 25°-30° C is found to be highly favorable for the microorganisms to boost the fermentation process.

Fermentation of dosa batter is carried out mainly by Lactobacillales or lactic acid bacteria (bacteria that convert milk to yogurt), recognized as lactobacillus delbrueckii, L. lactis, Strptociccus lactis, S. faecalis, Leuconostoc mesenteroides and Pedicococcuscerevisiae. Wild yeasts, recognized as Saccharomyces cerevisiae, Debaryomyces hansenii and Trichosporon beigelli, on the other hand, are found to produce flavor compounds and help in the saccharification (hydrolysis) of starch. In the early stages of fermentation, the 'heterofermentative' type bacteria like Leuconostoc mesenteroides (Fig. 3) are found to predominate, producing carbon dioxide and alcohol along with the lactic acid (the mucilaginous property of dosa batter helps to trap the carbon-dioxide evolved during fermentation which results into leavening of the batter).

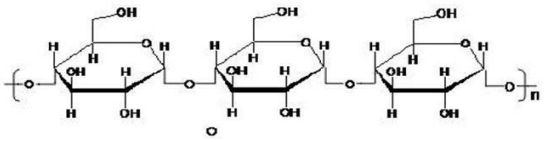


Fig 5 : Amylose (n=5 to 600)

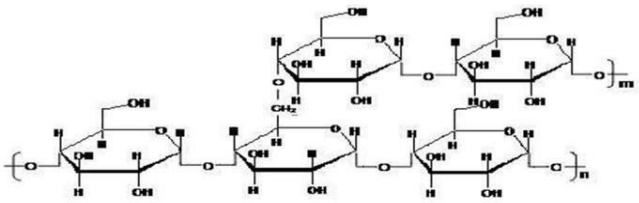


Fig 5 : Amylopectin (m=200 to 2000 ; n=20 to 30)

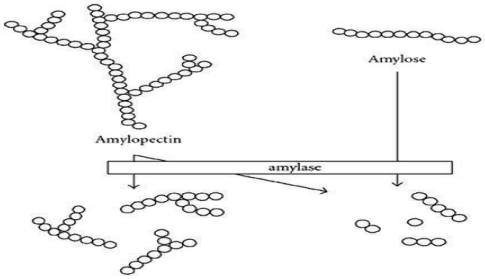


Fig 7 : Hydrolysis of starch by amylase enzymes

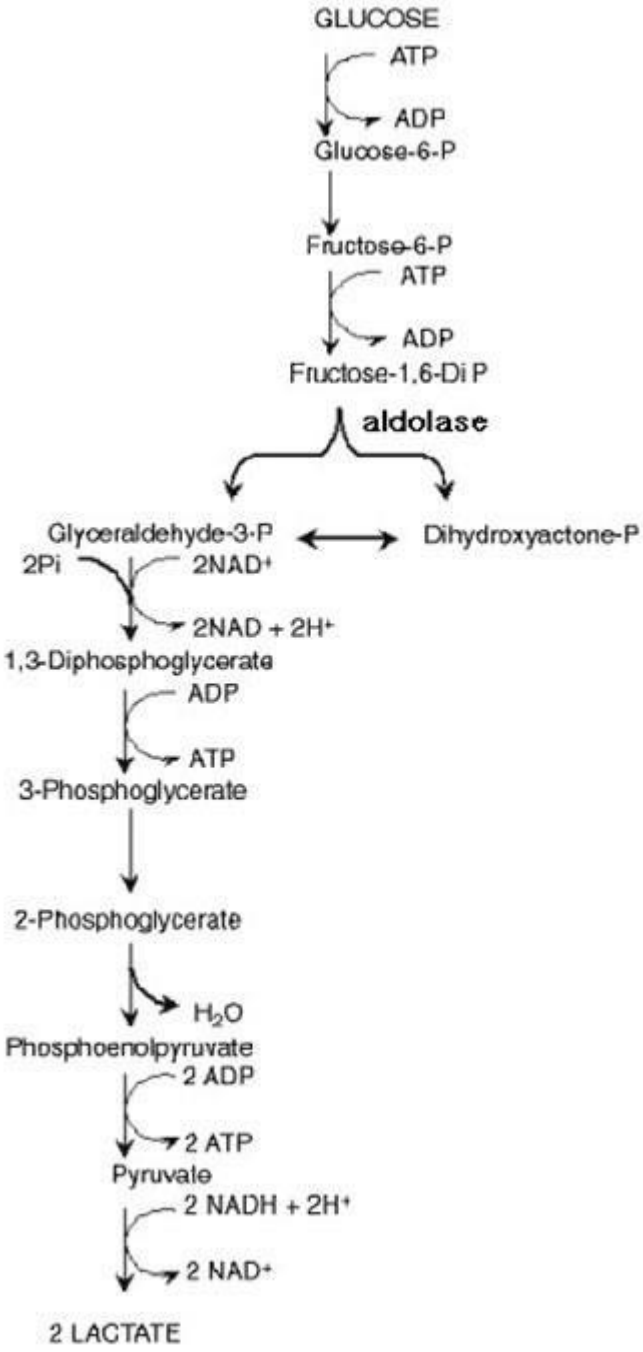
During the later stages of the fermentation the homofermentative lactic acid bacteria like Lactococcus lactis (Fig. 4) dominate and produce only lactic acid. Due to this batter starts turning sour over the time. Starch in the rice and black gram (or in general) contains two types of homopolysaccharides, amylose and amylopectin. Amylose is an unbranched homopolysaccharide consisting of about 5-600 glucose units, linked by α-(1→4) glycosidic bonds.

It forms a helix structure with six glucose units in each helix (Fig. 5). Amylopectin is a branched molecule formed by several glucose units ranging from several hundreds to fifty thousand in a main chain, which are joined by α-(1→4) glycosidic bonds.

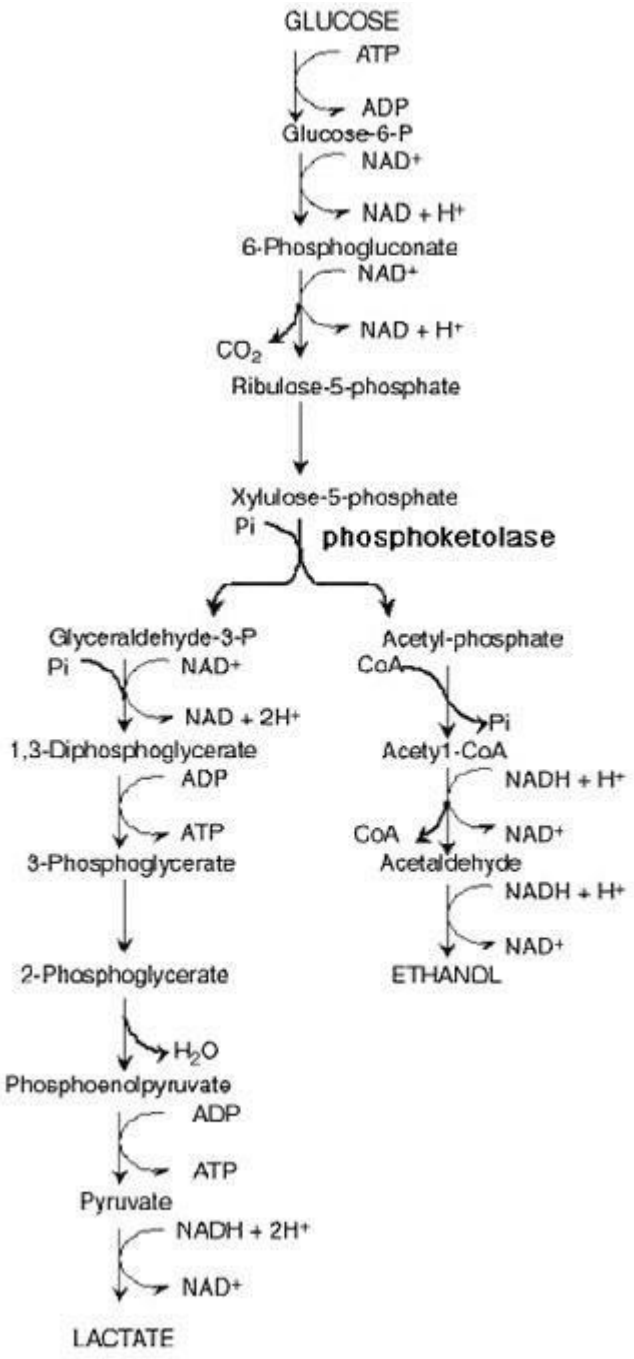
Lateral chains of about 20-30 glucose units are linked to the main chain by a α-(1→6) glycosidic bond. Glucose units on the lateral chain are linked again, joined with themselves by α-(1→4) glycosidic bonds. These branches provide a spongy fiber-like structure to the amylopectin and prevent the formation of a helical structure (Fig. 6). During the fermentation process the starch content of the dosa batter is hydrolysed (broken down) yielding maltotriose and maltose from amylose, or maltose, glucose and limit dextrin from amylopectin, with the help of amylases, the calcium metalloenzymes (Fig. 7). These amylase enzymes (mainly α-amylase, β-amylase and γ-amylase) are provided by the microorganisms (yeasts and bacteria) naturally present in the ingredients of dosa batter and the atmosphere.

The glucose, and other sugar molecules, then undergo a splitting process called glycolysis, a multistep metabolic pathway which involves a sequence of about ten enzyme-catalyzed reactions. Glycolysis can occur either in presence or absence of oxygen. Glycolysis is carried on in two slightly different ways, depending on the microorganisms (enzymes) and conditions

involved in the process. In Embden–Meyerhof–Parnas pathway (homolactic process), glucose is gradually split into two molecules of pyruvate (3 carbon sugar) and yields two molecules of ATP (free energy containing molecule) and two "high energy" electron carrying molecules of NADH.



Scheme 1 : Homolactic fermentation of glucose



Scheme 2 : Homolactic fermentation of glucose

The phosphorylation and transport of glucose usually occurs by an ATP-dependent hexose kinase or a phosphoenolypyruvate (PEP) sugar phosphotransferase system (PTS). In a concomitant step, the 2 electrons that were added to NAD+ in the glycolysis are once again removed from NADH and added back to the pyruvate molecule, regenerating NAD+ and producing lactic acid (or lactate) (Scheme 1). In the phosphoketolase pathway (hetero lactic process), Glucose molecule is converted into glucose-6-phosphate which then dehydrogenates to

give 6-phosphogluconate, upon subsequent decarboxylation it yields pentose-5-phosphate and one molecule of CO2. Pentose-5-phosphate is cleaved into glyceraldehyde phosphate (GAP) and acetyl phosphate. GAP is further metabolized to lactate as in homofermentation, with the acetyl phosphate reduced to ethanol via acetyl-CoA and acetaldehyde intermediates. End-products (CO2, lactate and ethanol) are produced in equimolar quantities (Scheme 2).

Benefits of fermentation: Fermentation process helps to break down the anti-nutrients like phytic acid present in rice and blackgram (phytic acid is known to block the mineral absorption and cause deficiencies). The mocroorganisms involved in the fermentation also produce useful substances like vitamins, folic acid, riboflavin, niacin, thiamin, biotin, vitamin K and some free amino acids as well as some antibiotic and anti-carcinogenic substances, hence increasing the total nutritional value of the dosa. Since dosa batter is predigested by bacteria, it is easier to digest. The lactic acid formed during the fermentation process, along with the various enzymes, aid the digestion of food, especially protein digestion. Lactic acid formed during fermentation not only preserves the food but also promotes the growth of a healthy intestinal flora. Lactobacilli are known to promote digestive health by inhibiting bacteria like Shigella, Salmonella and E.coli.

Role of water: Water is important to maintain the consistency (swelling and gelatinization) of the dosa batter. It acts as a solvent medium for sugars and other substrtates of fermentation process and also for enzymes. Water also acts as a source for the various microorganisms and minerals needed for the fermentation process.

Pinch of salt: Presence of salt helps to controll the fermentation process. It slows down the yeast action and helps the liberation of yeasts enzymes and other useful substances into the batter via osmosis. Since the lactic acid bacteria can tolerate high salt concentrations, the presence of salt gives them an advantage over other less tolerant species and allows the lactic acid fermenters to start the metabolism, which produces lactic acid, which further inhibits the growth of non-desirable organisms. Further addition of salt increases the mineral value of the dosa and the taste.

Introduction to Sauerkraut:

The use of cabbage (Brassica oleracea) as a food antedates known recorded history. Sauerkraut, a product resulting from the lactic acid fermentation of shredded cabbage, is literally acid (sour) cabbage. The antecedents of sauerkraut differed considerably from that prepared at present. At first the cabbage leaves were dressed with sour wine or vinegar.

Later the cabbage was broken or cut into pieces, packed into containers, and covered with verjuice (the juice expressed from immature apples or grapes), sour wine, or vinegar. Gradually the acid liquids were replaced by salt and a spontaneous fermentation resulted.

One may speculate that sauerkraut manufacture comparable to the method used today developed during the period of 1550 to 1750 A.D. although cabbage has been known and used commonly for about 4000 years. Those readers particularly interested in the historical evolution of the sauerkraut fermentation should consult Pederson (1960, 1979) and Pederson and Albury (1969).

Originally sauerkraut was made only in the home because it provided a means for utilizing fresh cabbage which otherwise would spoil before it could be used Now the commercial production of sauerkraut has become an important food industry. Even so, a significant quantity is still produced in the home, particularly in rural and suburban areas where home vegetable gardens still exist.

Cabbage varieties best suited for growth in the major production areas are used early, midseason, and late types are grown. Varieties formerly used such as Early Flat Dutch, Late Flat Dutch, Early Jersey Wakefield, and others have been replaced in part by new cultivars which have been bred to be well-adapted to mechanical harvesting and at the same time inherently contain less water, thus reducing the generation of in-plant liquid wastes. Mild-flavored, sweet, solid, white-headed cabbage is the choice as it makes a superior kraut.

Process for Sauerkraut Fermentation:

Properly matured sound heads of cabbage are first trimmed to remove the outer green broken or dirty leaves. The cores are cut mechanically by a reversing corer that leaves the core in the head. Then the cabbage is sliced by power-driven, rotary, adjustable knives into long shreds as fine as 0.16 to 0.08 cm (1/16 to 1/32 inches) in thickness.

In general, long, finely cut shreds are preferred, but the thickness is determined by the judgment of the manufacturer. The shredded cabbage (known also as slaw) is then conveyed by belts or by carts to the vats or tanks for salting and fermentation.

Salt plays a primary role in the making of sauerkraut and the concentrations used are carefully controlled. According to the legal standard of identity the concentration of salt must not be less than 2%, nor more than 3%. As a result most producers use a concentration in the range of 2.25 to 2.5% of salt. Salt is required for several reasons.

It extracts water from the shredded cabbage by osmosis, thus forming the fermentation brine It suppresses the growth of some undesirable bacteria which might cause deterioration of the product and, at the same time, makes conditions favorable for the desirable lactic acid bacteria. Salt also contributes to the flavor of the finished sauerkraut by yielding a proper salt-acid ratio (balance) if the cabbage is properly salted.

The use of too little salt causes softening of the tissue and produces a product lacking m flavor. Too much salt interferes with the natural sequence of lactic acid bacteria, delays fermentation and, depending on the amount of over-salting, may produce a product with a sharp, bitter taste, cause darkening of color, or favor growth of pink yeasts.

Uniform distribution of salt throughout the mass of shredded cabbage cannot be overemphasized. In some factories the slaw is weighed on conveyor belt lines and the desired amount of salt is sprinkled on the shreds by means of a suitable proportioner as it moves along the conveyor to the vat.

In other plants hand-carts are used to carry the shredded cabbage to the vat. Some prefer to salt the weighed cabbage in each cart. Others transport the slaw in carts which are weighed occasionally to check the capacity. The shreds are then dumped into the vat, distributed by forks, and then salted with a specific weight of salt.

The variations of salt concentrations in the brines covering kraut have been thoroughly investigated by Pederson and Albury (1969) and discussed by Pederson (1975, 1979). No mention of recirculation of the brines to gain uniformity in concentration of salt was noted.

It would seem that this method of ensuring uniform salt distribution in sauerkraut brines would be as effective as it is in the olive industry. Only small alterations in tank or vat design would be required to make it possible to completely recirculate the brine, pumping from the bottom and discharging at the surface.

Brine begins to form once the shreds are salted, and the tank is closed once it has been filled to the proper level. Formerly, the slaw was covered with a thick layer of outer leaves and then fitted with a wood cover (head) which was heavily weighted. Within a few hours the brine had formed and the fermentation had started. The head then was fixed in position in much the same manner as with pickle or olive tanks.

Now, however, a sheet plastic cover is used. This cover is much larger in area than the top of the vat or tank itself. The plastic sheeting is placed firmly against the top of the shredded cabbage with the edges draped over the sides of the container to form an open bag. Then enough water or preferably salt brine is placed in this bag so that the weight of the liquid added forces the cabbage shreds down into the brine until the brine covers the surface of the uppermost shreds. Unless the shreds are completely covered with brine, undesirable discoloration together with

undesirable flavor changes will occur. This newer method of covering and weighting provides nearly anaerobic conditions, particularly after fermentation becomes acid and quantities of carbon dioxide are produced. Precautions to avoid pin holes or tears in the plastic are mandatory if aerobic yeast growth is to be avoided.

With the old method of closure film forming yeasts always were a problem and if the scum was not removed at intervals a yeasty flavor was imparted to the kraut. Pichia membranaefaciens yeast strains, in particular, voraciously oxidize lactic acid contained in salt brines. Other genera also may be involved and besides destroying acid also contribute to yeasty flavor.

By the time the tank or vat is filled with the salted shreds and weighted, brine has formed and fermentation has started in a sequence of bacterial species responsible for the lactic acid fermentation.

Microbiology of the Sauerkraut Fermentation:

Although the lactic acid fermentation was described by Pasteur in 1858 and much work had been done in the intervening years with various lactic bacteria from cabbage and cucumber fermentations, it was not established that a definite sequence of bacterial species of lactic acid bacteria were responsible for the fermentation of either vegetable until 1930 when Pederson first described the lactic acid bacteria he observed in fermenting sauerkraut.

Pederson found that the fermentation was initiated by the species Leuconostoc mesenteroides. This species was followed by gas-forming rods and finally by non-gas-forming rods and cocci. Since 1930 additional studies by Pederson and Albury (1954, 1969) have firmly established the importance of Leuconostoc mesenteroides in initiating the lactic fermentation of sauerkraut.

Also they more closely identified the species and sequence of the other lactic acid bacteria involved. Now it is accepted that the kraut, fermentation is initiated by Leuconostoc mesenteroides, a heterofermentative species, whose early growth is more rapid than other lactic acid bacteria and is active over a wide range of temperatures and salt concentrations.

It produces acids and carbon dioxide that rapidly lower the pH, thus inhibiting the activity of undesirable microorganisms and enzymes that may soften the shredded cabbage. The carbon dioxide replaces air and creates an anaerobic condition favorable to prevention of oxidation of ascorbic acid and the natural color of the cabbage. Also carbon dioxide stimulates the growth of many lactic acid bacteria. It also may be that this species provides growth factors needed by the more fastidious types found in the fermentation.

While this initial fermentation is developing, the heterofermentative species Lactobacillus brevis and the homofermentative species Lactobacillus plantarum and sometimes Pediococcus cerevisiae begin to grow rapidly and contribute to the major end products including lactic acid, carbon dioxide, ethanol, and acetic acid. Minor end products also appear.

These are a variety of additional volatile compounds produced by the various bacteria responsible for the fermentation, by auto-chemical reactions, or the intrinsic enzymes of the fermenting cabbage itself. Hrdlicka et al (1967) reported the formation of diacetyl and acetaldehyde, the primary carbonyls formed during cabbage fermentation.

Volatile sulfur compounds are major flavor components of fresh cabbage according to Bailey et al. (1961) and Clapp et al. (1959) and also of sauerkraut. However, according to Lee et al. (1976), the major portion of the volatiles of sauerkraut is accounted for by acetal, isoamyl alcohol, n-hexanol, ethyl lactate, cis-hex-3-ene-l-ol, and allyl isothiocyanate. Of these, only the latter two have been identified as major constituents of fresh cabbage.

These latter authors concluded that although these two compounds define the character of cabbage products (kraut) they do not contribute significantly to the determination of its quality. They further believe that the fresh and fruity odor of such compounds as ethyl butyrate, isoamyl acetate, n-hexyl acetate, and mesityl oxide are probably more important in determining the acceptability of sauerkraut.

Temperature is a controlling factor in the sequence of desirable bacteria in the sauerkraut fermentation at a salt concentration of 2.25%. At the optimum of 18.3°C (65°F) or lower the quality of the sauerkraut is generally superior in flavor, color and ascorbic acid content because the heterofermentative lactic acid bacteria exert a greater effect.

According to Pederson and Albury (1969) an average temperature of about 18°C (65°F) with a salt concentration of 2.25% may be considered normal in the kraut-producing areas of the United States. At (or near) this temperature, fermentation is initiated by Leuconostoc mesenteroides and continued by Lactobacillus brevis and Lactobacillus plantarum, the latter species being most active in the final stages of fermentation.

Under these conditions a final total acidity of 1.7 to 2.3% acid (calculated as lactic acid) is formed, and the ratio of volatile to nonvolatile acid (acetic/lactic) is about 1 to 4. The fermentation is completed in 1 to 2 months, more or less, depending upon the quantity of fermentable materials, concentration of salt, and fluctuations in temperature.

At higher temperatures, as would be expected, they found that the rate of acid production was faster. For example, at 23°C (73.4°F) a brine acidity of 1.0 to 1.5% (calculated as lactic acid) may be observed in 8 to 10 days and the sauerkraut may be completely fermented in about 1 month.

At a still higher temperature of 32°C (89.6°F), the production of acid generally is very rapid with acid production of 1.8 to 2.0% being obtained in 8 to 10 days. As the temperature increased, they observed a change in the sequence of lactic acid bacteria. First, the growth of Leuconostoc

mesenteroides was retarded and Lactobacillus brevis and Lactobacillus plantarum dominated the fermentation. At higher temperatures the kraut fermentation became essentially a homofermentation dominated by Lactobacillus plantarum and Pediococcus cerevisiae.
As a result, the quality attributes of flavor and aroma deteriorated and the kraut was reminiscent of acidified cabbage because of the large quantity of lactic acid and little acetic acid produced by the homo-fermentative species. They also observed that sauerkraut fermented at higher temperatures would darken readily and, therefore, should be canned as quickly as possible after the fermentation was completed.

An extremely important observation they made was that kraut could be successfully fermented even when started at the low temperature of 7.5°C (45.5°F). Leuconostoc mesenteroides can grow at lower temperatures than the other lactic acid bacteria involved in the fermentation. At this low temperature (7.5°C or 45.5°F) an acidity of 0.4% (as lactic acid) is produced in about 10 days and 0.8 to 0.9% in less than a month.
This amount of acidity coupled with saturation of the mass of kraut and brine with carbon dioxide is sufficient to provide the conditions necessary for preservation and later completion of the fermentation provided that anaerobiosis is maintained throughout the period of latency. When the kraut mass warms enough, the fermentation then is completed by the lactic acid bacteria of the genera Lactobacillus and Pediococcus, known to grow poorly if at all at 7.5°C (45.5°F).
Thus, it may require 6 months or more before the fermentation is completed. Such kraut is generally of superior quality because it remains cool and is not subjected to high temperature during-fermentation. In good commercial practice this variation in temperature permits the processor to maintain a supply of new, completely fermented sauerkraut throughout most of the year.
Precedent for the recommendation by Pederson and Albury that sauerkraut be fermented at not over 18.3°C (65°F) had already been recorded by Parmele et al. (1927), Marten et al. (1929), and others.

Defects and Spoilage of Sauerkraut:

Abnormalities of sauerkraut, although varied, with few exceptions can be and generally have been avoided by application of scientific knowledge already available to the industry. For example, the simple expedient of providing anaerobiosis has eliminated most of the problems involving discoloration (auto-chemical oxidation), loss of acidity caused by growth of, molds and yeasts, off-flavors and odors (yeasty and rancid) caused by excessive aerobic growth of molds and yeasts, slimy, softened kraut caused by pectolytic activity of these same molds and

yeasts, and pink kraut caused by aerobic growth of asporogenous yeasts, presumably members of the genus Rhodotorula.

Stamer et al. (1973) described the induction of red color in white cabbage juice by L. brevis while studying the effects of pH on the growth rates of the 5 species of lactic acid bacteria commonly associated with the kraut fermentation. L. brevis was the only species which produced such color formation in white cabbage juice and did so only when the juice was buffered with either calcium carbonate or sodium hydroxide.

No color development occurred when the pH of the juice (3.9) was not adjusted or when the pH of the juice was raised to 5.5 and the juice sterilized by filtration before it was re-incubated. Therefore, red color formation was caused by L. brevis and did not arise as the result of chemical or inherent enzymatic reactions of the juice.

It remains to be seen whether this interesting phenomenon will be observed in industrial kraut fermentations. Since color induction by L. brevis was found to be pH dependent it seems unlikely to be found in normal kraut fermentations but could easily result from accidental addition of alkali to the shredded cabbage during salting.

Slimy or ropy kraut has been observed for many years. It is generally caused by dextran formation induced by Leuconostoc mesenteroides and is transitory in nature. This species prefers to ferment fructose rather than glucose. Therefore, in the fermentation of sucrose, the fructose is fermented leaving the glucose which interacts to form the slimy, ropy, water-insoluble dextrans. These vary from an almost solid, gelatinous mass to a ropy slime surrounding the bacterial cells. These variations are easily demonstrated by growing L. mesenteroides in a 10% sucrose solution containing adequate accessory nutrients. The fermenting kraut may become very slimy during the intermediate stage of fermentation but with additional time the dextrans are utilized by other lactic acid bacteria. Thus, it is imperative to distinguish between dextran induced slimy kraut and permanently slimy kraut caused by pectolytic activity. The former condition certainly is not a defect but should be considered a normal step in a natural progression.

Raw Materials

Soybeans

Soybeans *(Glycine max)* are also called soya beans, soja beans, Chinese peas, soy peas, and Manchurian beans. They have been referred to as the "King of Legumes" because of their valuable nutritive properties. Of all beans, soybeans are lowest in starch and have the most complete and best protein mix. They are also high in minerals, particularly calcium and magnesium, and in Vitamin B. They have been cultivated since the dawn of civilization in China and Japan and were introduced into the United States in the nineteenth century. In the 1920s and 1930s, soybeans gained popularity in the U.S. as a food crop.

Soybeans are short, hairy pods containing two or three seeds which may be small and round or larger and more elongated. Their color varies from yellow to brown, green, and black. The variety designated yellow #2 are most commonly used for food products. These soybeans get their name from the yellow hilum or seed scar which runs down the side of the pod. The grades of grain allowed for trading are established by the United States Grain Standards which are administered by the U.S. Department of Agriculture. Soybeans are unusual in that, unlike other grains, most are used in processing or exporting, and not much as direct animal feed. This is because soybeans contain "anti-nutritional" factors that must be removed from the beans before they can be of nutritional value to animals. The soybeans used in soy sauce are mashed prior to mixing them with other ingredients.

Wheat

In many traditional brewed recipes, wheat is blended in equal parts with the soybeans. Pulverized wheat is made part of the mash along with crushed soy beans. The nonbrewed variety does not generally use wheat.

Salt

Salt, or sodium chloride, is added at the beginning of fermentation at approximately 12-18% of the finished product weight. The salt is not just added for flavor; it also helps establish the proper chemical environment for the lactic acid bacteria and yeast to ferment properly. The high salt concentration is also necessary to help protect the finished product from spoilage.

American farmers produced surpluses of many agricultural commodities in 1930, but soybeans were not one of them. During the early years of the Great Depression, few farmers raised soybeans, but this changed in just 10 years. In 1929, American farmers produced less than 10 million bushels (352 million L) of soybeans. By 1939 production approached 100 million bushels (3.5 billion L), and in 1995, American farmers raised more than 2.1 billion (74 billion L) bushels of soybeans. No one surpassed Henry Ford as a promoter of soybean production in the 1930s.

In 1929, Henry Ford constructed a research laboratory in Greenfield Village and hired Robert Boyer to oversee experimentation related to farm crops. Ford hired additional scientists to investigate the industrial uses of many agricultural commodities, including vegetables such as

carrots. The greatest success was in soybean experimentation. The researchers developed soy-based plastics and made parts for automobiles out of the products. The scientists manufactured ink made from soy oil, and produced soy-based whipped topping. Many of these processes and products remain in use.

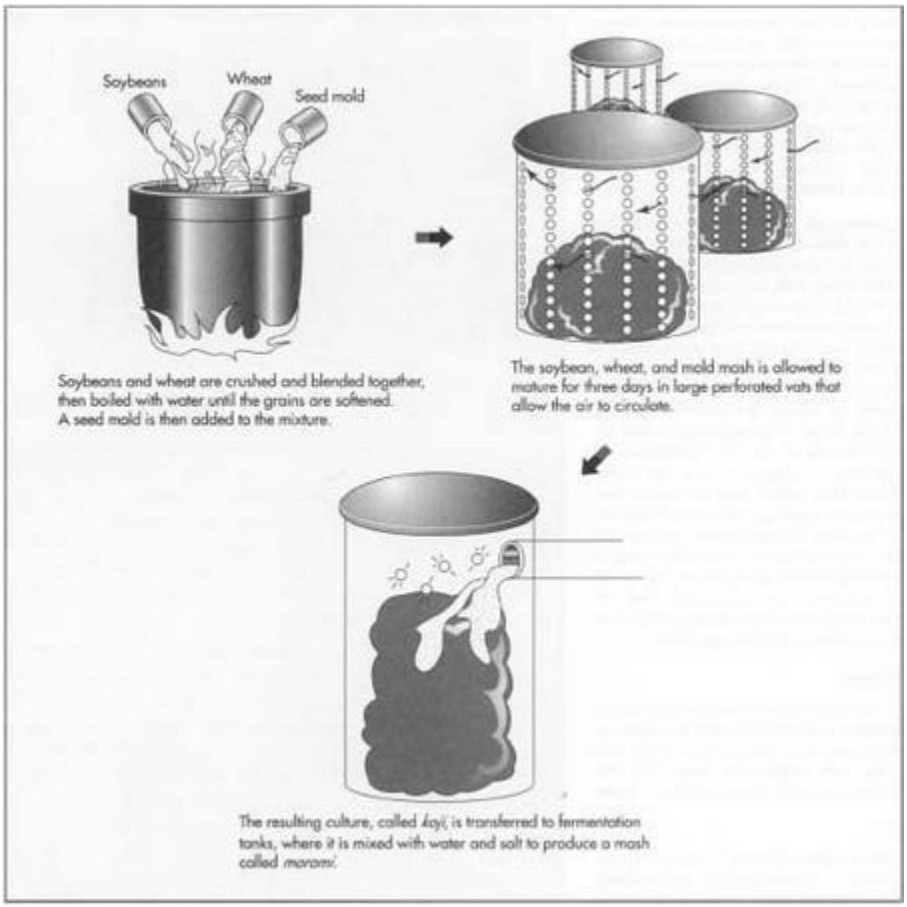
Ford believed that farmers should have one foot on the soil and the other in industry. Ford promoted agricultural production of soybeans through an exhibit in a barn at the Chicago "Century of Progress" World Exposition in 1933. He hosted a meal which included a variety of soybean items and supported the publication of recipe booklets full of soybean-based recipes.

Henry Ford wished to see farmers to produce soybeans on their farms and process them for industrial purposes. Though his vision was not realized, the importance of soybeans in American agriculture came to fruition. Soybeans are one of most important crops raised in America, and provide American farmers millions of dollars in income.

Leo Landis

Fermenting agents

The wheat-soy mixture is exposed to specific strains of mold called *Aspergillus oryzae* or *Aspergillus soyae*, which break down the proteins in the mash. Further fermentation occurs through addition of specific



bacteria *(lactobaccillus)* and yeasts which enzymatically react with the protein residues to

produce a number of amino acids and peptides, including glutamic and aspartic acid, lysine, alanine, glycine, and tryptophane. These protein derivatives all contribute flavor to the end product.

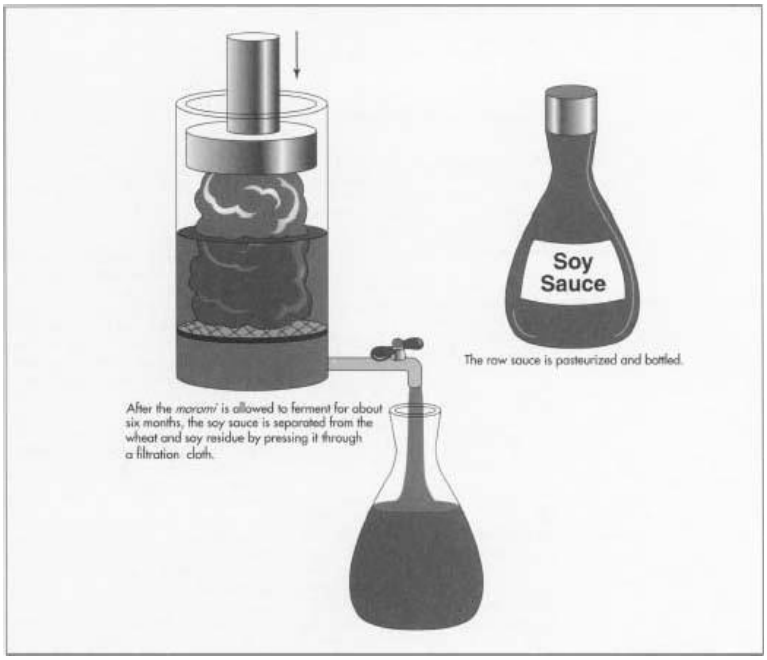
Preservatives and other additives

Sodium benzoate or benzoic acid is added to help inhibit microbial growth in finished soy sauce. The non-brewed process requires addition of extra color and flavor agents.

The Manufacturing Process

Traditional brewed method

Brewing, the traditional method of making soy sauce, consists of three steps: *koji* -making, brine fermentation, and refinement.



Koji-making

- 1 Carefully selected soybeans and wheat are crushed and blended together under controlled conditions. Water is added to the mixture, which is boiled until the grains are thoroughly cooked and softened. The mash, as it is known, is allowed to cool to about 80°F (27°C) before a proprietary seed mold *(Aspergillus)* is added. The mixture is allowed to mature for three days in large perforated vats through which air is circulated. This resulting culture of soy, wheat, and mold is known as *koji*.

Brine fermentation

- 2 The *koji* is transferred to fermentation tanks, where it is mixed with water and salt to produce a mash called *moromi*. Lactic acid bacteria and yeasts are then added to promote further fermentation. The *moromi* must ferment for several months, during which time the soy and wheat paste turns into a semi-liquid, reddish-brown "mature mash." This fermentation process creates over 200 different flavor compounds.

Refinement

- 3 After approximately six months of *moromi* fermentation, the raw soy sauce is separated from the cake of wheat and soy residue by pressing it through layers of filtration cloth. The liquid that emerges is then pasteurized. The pasteurization process serves two purposes. It helps prolong the shelf life of the finished product, and it forms additional aromatic and flavor compounds. Finally, the liquid is bottled as soy sauce.

Non-brewed method (chemical hydrolysis)

Instead of fermenting, many modern manufactures artificially break down the soy proteins by a chemical process known as hydrolysis because it is much faster. (Hydrolysis takes a few days as compared to several months for brewing.)

1. In this method, soybeans are boiled in hydrochloric acid for 15-20 hours to remove the amino acids. When the maximum amount has been removed, the mixture is cooled to stop the hydrolytic reaction.
2. The amino acid liquid is neutralized with sodium carbonate, pressed through a filter, mixed with active carbon, and purified through filtration. This solution is known as hydrolyzed vegetable protein.
3. Caramel color, corn syrup, and salt are added to this protein mixture to obtain the appropriate color and flavor. The mixture is then refined and packaged.

Sauces produced by the chemical method are harsher and do not have as desirable a taste profile as those produced in the traditional brewed manner. The difference in taste occurs because the acid hydrolysis used in the non-brewed method tends to be more complete than its fermentation counterpart. This means that almost all the proteins in the non-brewed soy sauce are converted into amino acids, while in the brewed product more of the amino acids stay together as peptides, providing a different flavor. The brewed product also has alcohols, esters, and other compounds which contribute a different aroma and feel in the mouth.

In addition to the brewed method and the non-brewed method, there is also a semi-brewed method, in which hydrolyzed soy proteins are partially fermented with a wheat mixture. This method is said to produce higher quality sauces than can be produced from straight hydrolysis.

Quality Control

Numerous analytical tests are conducted to ensure the finished sauce meets minimum quality requirements. For example, in brewed sauces, there are several recommended specifications. Total salt should be 13-16% of the final product; the pH level should be 4.6-5.2; and the total

sugar content should be 6%. For the non-brewed type, there is 42% minimum of hydrolyzed protein; corn syrup should be less than 10%; and carmel color 1-3%.

In the United States, the quality of the finished sauce is protected under federal specification EE-S-610G (established in 1978) which requires that fermented sauce must be made from fermented mash, salt brine, and preservatives (either sodium benzoate or benzoic acid). This specification also states that the final product should be a clear, reddish brown liquid which is essentially free from sediment. The non-fermented sauce is defined as a formulated product consisting of hydrolyzed vegetable protein, corn syrup, salt, caramel color, water, and a preservative. It should be a dark brown, clear liquid.

The Japanese, on the other hand, are more specific in grading the quality of their soy sauces. They have five types of soy sauce: *koikuchi-shoyu* (regular soy sauce), *usukuchi-shoyu* (light colored soy sauce), *tamari-shoyu, saishikomi-shoyu,* and *shiro-shoyu.* These types are classified into three grades, Special, Upper, and Standard, depending upon sensory characteristics such as taste, odor, and feel in the mouth, as well as analytical values for nitrogen content, alcohol level, and soluble solids.

Byproducts/Waste

The fermentation process produces many "byproducts" that are actually useful flavor compounds. For example, the various sugars are derived from the vegetable starches by action of the *moromi* enzymes. These help subdue the saltiness of the finished product. Also, alcohols are formed by yeast acting on sugars. Ethanol is the most common of these alcohols, and it imparts both flavor and odor. Acids are generated from the alcohols and sugars, which round out the flavor and provide tartness. Finally, aromatic esters (chemicals that contribute flavor and aroma) are formed when ethanol combines with organic acids.

Chemical hydrolyzation also leads to byproducts, but these are generally considered undesirable. The byproducts are a result of secondary reactions that create objectionable flavoring components such as furfural, dimethyl sulfide, hydrogen sulfide, levulinic acid, and formic acid. Some of these chemicals contribute off odors and flavors to the finished product.

The Future

The future of soy sauce is constantly evolving as advances are made in food technology. Improved processing techniques have already allowed development of specialized types of soy sauces, such as low-sodium and preservative-free varieties. In addition, dehydrated soy flavors have been prepared by spray drying liquid sauces. These powdered materials are used in coating mixes, soup bases, seasoning rubs, and other dry flavorant applications. In the future, it is conceivable that advances in biotechnology will lead to improved understanding of enzymatic reactions and lead to better fermentation methods. Technology may someday allow true brewed flavor to be reproduced through synthetic chemical processes.

Tempeh Fermentation Process

What happens during the fermentation of soybeans into tempeh? Generally it is agreed that not only is the flavor, aroma, and texture of plain cooked soybeans enhanced during the fermentation process, but there are several nutritional benefits derived in the process as well to make the beans more easily assimilated by the human body. This is done by the main mold Rhizopus oligosporus, and other minor organisms or their enzymes hydrolizing proteins, carbohydrates, and fats to create smaller and more digestible units. This process makes the protein in tempeh more digestible and usable.

A. Changes in Lipids

Free fatty acids increased from 0.5% in the unfermented control to 21.0% in the dehydrated tempeh (with the same moisture content). During fatty acid synthesis, Rhizopus sp. produced only gamma-linolenic acid (GLA) instead of alpha-linoleic acid. GLA is a prostaglandin and leukotriene precursor. It is used therapeutically to decrease the cholesterol and triglyceride content in blood. It is not found in soybean (3).

B. Changes in Carbohydrates

During fermentation, the principal changes in carbohydrates are the rapid decrease of hex-oses and the slow hydrolysis of stachyose, the flatulence factor in beans (4). This makes tempeh a more socially acceptable soybean product.

C. Changes in Proteins and Amino Acids

Steinkraus (5) summarized the biochemical analysis of tempeh. The most significant changes are in the proteins and vitamins. Ammonia (% of total nitrogen) increased from 0.1 to 1.7. Percent nitrogen soluble in water increased from 6.5 to 39.0. Percent nitrogen soluble in trichloroacetic acid increased from 5.9 to 28. There were no significant changes in the amino acid patterns between soybeans and tempeh (6). It is likely that there is no de novo synthesis of amino acids, but only a degradation and consumption of soy protein by the fungi.

D. Changes in Vitamins

Steinkraus (5) also summarized reported work on changes in vitamins during tempeh fermentation. Riboflavin increased by 2-47 times, niacin increased by 2-7 times, and vitamin B12 by 33 times. Thiamin, unfortunately, decreased. Panthothenic acid has been reported to stay the same or increased by 2-4 times. Pyridoxine increased by 4-14 times. Biotin and total folate

compounds were respectively 2.3 and 4-5 times higher in tempeh than in unfermented soybeans. The variations in reported changes may be due to the way tempeh was made in various laboratories and locations, as well as the beans and microorganisms associated with them. For example, according to this author's experience, the production of vitamin B12 fluctuated considerably even under similar fermentation conditions in the same location.

E. Presence of Antioxidants and Antibiotic in Tempeh

An isoflavone identified as 6,7,4'-trihydroxy isoflavone (called Factor 2) has been reported (6,7). The antioxidative effect of factor 2 on retinol was about the same as DL-alpha-tocopherol, and three times that of genistein. Other isoflavones were later reported (8,9).

In Indonesia, tempeh is widely used to ween babies off mother's milk and to help patients recover strength from dysentery and other ailments of the intestinal tract (1). R. oligosporus NRRL 2710 was reported to produce an antibiotic active compound against a number of gram-positive bacteria including S. aureus and B. subtilis, as well as the gramnegative K. pneumoniae (10). It was demonstrated later that K. pneumoniae and R. oligosporus NRRL 2710 grows well together in tempeh fermentation. There was no evidence of K. pneumoniae inhibition by the mold (11,12). This may help explain why tempeh is provided to patients with dysentery and other ailments of the intestinal tract.

F. Reduction of Phytate

Phytate is considered to exacerbate mineral deficiency in human by hindering absorption in the gut. Reduction of phytate was reported to be 22% during tempeh fermentation due to phytase active in R. oligosporus (13). This again demonstrated the benefits of tempeh.

IV. TEMPEH FERMENTATION IN INDONESIA (1) A. A Small Tempeh Manufacturer in Denpassar, Bali

A home-based tempeh factory can be operated by a small family group of four adults and several small children. Fifty kilos of tempeh are made each day in the house. Cleaned soybean is cooked in the early morning. At mid-morning, the cooked beans are cooled manually in a large bamboo colander placed on the floor. The tempeh from the day before is incubated in small 3" x 3" perforated plastic bags, each of which weigh about 3 ounces. These small cakes are incubated for 2 days on wooden slats in a dark room. A bicycle-powered mill, the colander, and an aluminum cooking pot and heat sealer are the only pieces of equipment. The 3-ounce cakes are sold to restaurants and in the market for 150 rupiah (about 6 US cents).

B. A Medium-Scale Manufacturer in Denpassar, Bali

A substantial medium operation produces 750 kilos of tempeh, 7 days a week, 30 days a month. The manufacturer employs 10 young men in this operation.

The soybeans are cooked in delidded 55-gallon drums placed over propane burners. The hulls are skimmed off the beans manually with plastic colanders by two persons. Two others cool and package cooked beans in perforated plastic bags in another room. The beans are piled not on the floor but on a piece of white canvas. Tempe Murni sells 250 grams of finished tempeh for 400 rupiah (U.S. 160) to their distributors, who sell it in the market for 500 rupiah (U.S. 210).

C. A Large Tempeh Manufacturer in Yogyakarta, Java

Yogyakarta has always been viewed as the cultural capital of Indonesia, it has the highest per capita consumption of tempeh in all of Indonesia. On a weekly basis, the average person in Yogyakarta consumes nearly 200 grams of tempeh versus only 75 grams per Balinese citizen. This shows in the local market which is totally inundated by a large variety of tempeh products—both raw cakes and tempeh prepared in various sauces. These products are supplied mainly by one large tempeh maker. This innovative plant produces 5000 pounds of product each day from a 1500 square foot area. This shop does have a gas-fired boiler and copper kettle for cooking the beans but everything else is done with the same level of technology witnessed elsewhere (bamboo colanders on the floor for mixing and packaging, etc.). What is unique about this shop is the ingenious barter system that exists here. The main operation is run by a paid staff of 24 people but the filling is almost all done manually by local workers, They seal the inoculated beans in plastic bags, sometimes melting the plastic by running it near the open flame of a cloth wick stuck into a coke can filled with kerosene! After the bags are sealed, the workers pack them up into the cloth sacks the beans came in, load them onto bicycles, and pedal the load home. There they incubate the beans for several days, and when the beans are white and ripe, take them to the local market for sale. In Yogyakarta, small, 1 ounce packages of tempeh incubated for an extra long time in banana leaves are available and sold for 25 rupiah (about U.S. 10).

In Indonesia, the same basic processing steps for tempeh making takes place. Figure 3 presents a generalized flow chart on these procedures followed in Indonesia (1,14). The steps are as follows:

1. Beans are soaked overnight in what is known as the prefermentation.

2. Early the next morning the beans are dehulled and split, using anything from mills to hands and feet.

3. Beans are cooked in open kettles where more dehulling takes place.

4. Cooked beans are placed in a large woven colander about 3 feet across and placed in front of fans to dry.

5. Cooled beans are inoculated with the culture. Most culture comes from the same source, a local Indonesian lab, and is a type of Rhizopus that is incubated at around 30 °C for 48 hr.

6. Inoculated beans are now scooped into perforated plastic bags or banana leaves. Whereas the perforated plastic bags were the most common way of incubating tempeh, most cooks preferred tempeh incubated in banana leaves. Observations in the market bore this out as tempeh incubated in the leaves was consistently of higher quality than that in the plastic bags.

Soak beans overnight (Pre-fermentation) Dehull and split beans Cook beans in open kettle

Inoculate beans at room temperature (30°C) for 2 days Pack beans in plastic bags or banana leaf

Marketing final product Figure 3 Flowchart of tempeh manufacturing in Indonesia.

7. Incubation takes place in a room or closet where inoculated beans sit out at room temperature on wooden slats.

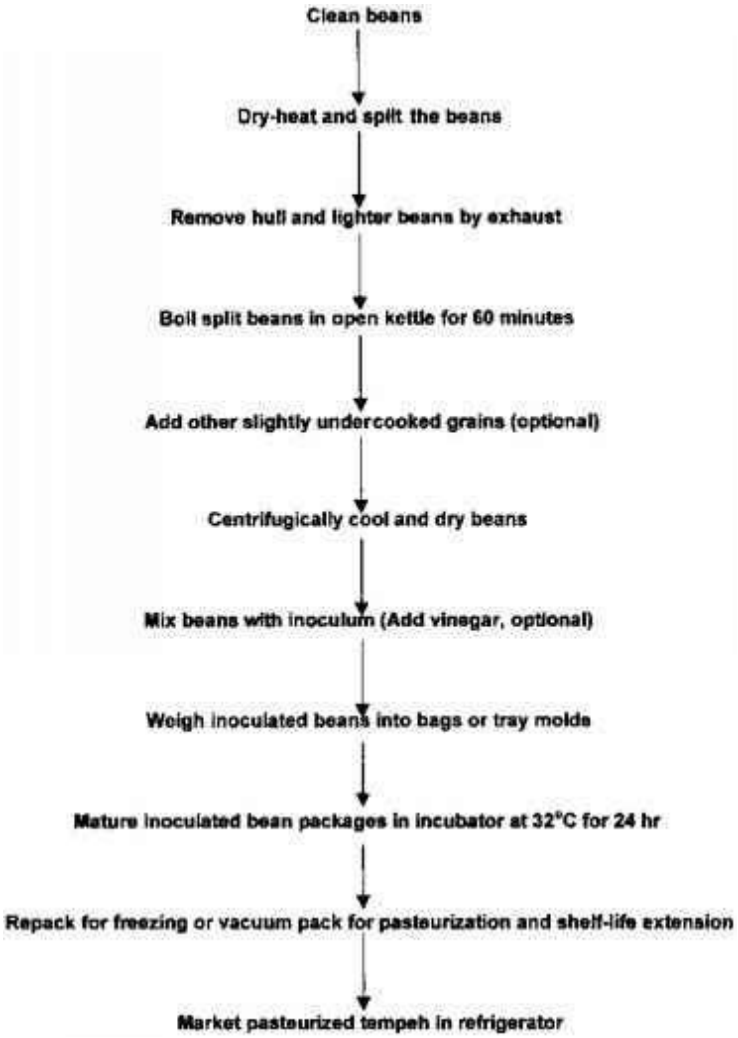
8. After 2 days the tempeh is sold in its perforated incubation bag or banana leaf in the marketplace.

V. TEMPEH MAKING IN NORTH AMERICA, CA 2000

In the year 2000, only about seven shops existed in North America capable of making a thousand pounds of tempeh or more per day. These were Lightlife Foods in Massachusetts, Turtle Island Foods in Oregon, White Wave in Colorado, Northern Soy in New York, Cricklewood Foods in Pennsylvania, Surata Soyfoods in Oregon, and 21st Century Foods in Massachusetts. Even the smallest of these would be viewed as a sanitary modern factory compared to Indonesian methods. Most shops follow a flow chart or processing scheme (Fig. 4) similar to the following:

1. Whole soybeans are heated and split dry in a mill.

2. The split beans and hulls fall into a tube where the lighter hulls are sucked out from the top by connecting it to an exhaust fan. The heavier beans fall to the bottom of the tube into a collection bucket.



Market pasteurized tempeh in refrigerator Figure 4 Flowchart for tempeh manufacturing in the United States.

3. The split beans are cooked at a boil for 60 min in an open steam jacketed stainless steel kettle.

4. Other grains may be added toward the end of the cooking time. These grains are always slightly undercooked. The rule here is to cook the grains for about half as long as one normally would if one were preparing them to eat at our own supper table.

5. Cooked beans are now placed into a centrifuge. These have stainless steel baskets and the beans are spun rapidly for a short period of time to cool and remove excess water.

6. Cooled beans are placed in a horizontal mixer where they are mixed with the innoculant and other grains. Some people acidify the beans with a small amount of vinegar at this point.

7. Beans are now either placed in a mechanical scale of some kind or weighed out by hand into perforated bags or tray molds for burgers.

8. Bags of inoculated beans are now laid flat on perforated trays and placed inside an incubation room where they are heated at 32°C (89.6) for 24 hr.

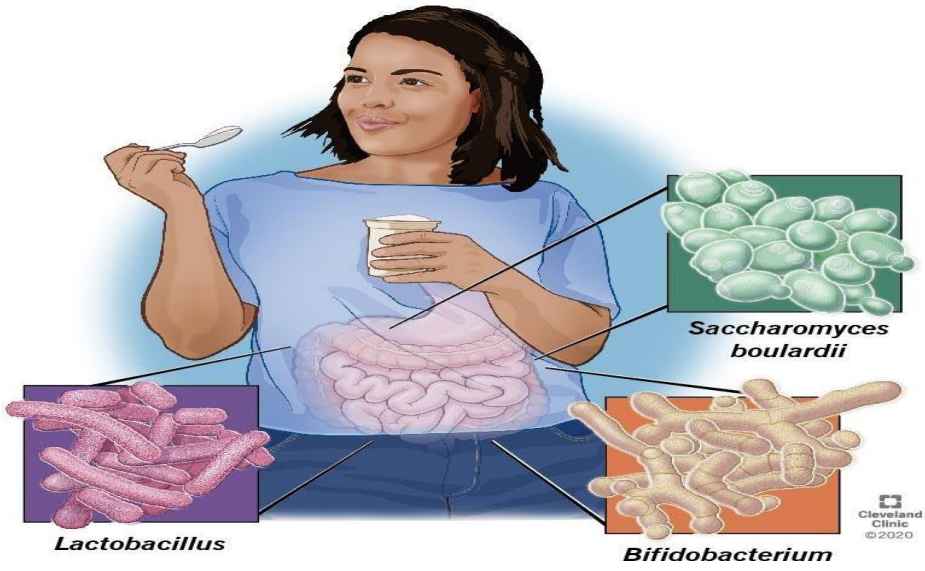
9. At this point tempeh is now bound into a firm, fragrant white cake. Some workers slip an outer bag over the tempeh at this point and freeze the product.

10. Most commonly at this point, finished tempeh is now vacuum packaged and then steam or hot water-pasteurized to extend the shelf life.

11. Tempeh is now cooled down and sold refrigerated it has a shelf life of approximately 3 months.

Probiotics

Probiotics are made of good live bacteria and/or yeasts that naturally live in your body. You constantly have both good and bad bacteria in your body. When you get an infection, there's more bad bacteria, knocking your system out of balance. Good bacteria helps eliminate extra bad bacteria, returning the balance. Probiotic-supplements are a way to add good bacteria to your body.



Probiotics are made of both bacteria and yeast. Common probiotic bacteria can include lactobacillus and bifidobacterium. The most common yeast found in probiotics is saccharomyces boulardii.

What are probiotics?

Probiotics are a combination of live beneficial bacteria and/or yeasts that naturally live in your body. Bacteria is usually viewed in a negative light as something that makes you sick. However, you have two kinds of bacteria constantly in and on your body — good bacteria and bad bacteria. Probiotics are made up of good bacteria that helps keep your body healthy and working well. This good bacteria helps you in many ways, including fighting off bad bacteria when you have too much of it, helping you feel better.

Probiotics are part of a larger picture concerning bacteria and your body — your microbiome. Think of a microbiome as a diverse community of organisms, such as a forest, that work together to keep your body healthy. This community is made up of things called microbes. You have trillions of microbes on and in your body. These microbes are a combination of:

- Bacteria.
- Fungi (including yeasts).
- Viruses.
- Protozoa.

Everyone's microbiome is unique. No two people have the same microbial cells — even twins are different.

For a microbe to be called a probiotic, it must have several characteristics. These include being able to:

- Be isolated from a human.
- Survive in your intestine after ingestion (being eaten).
- Have a proven benefit to you.
- Be safely consumed.

Where do beneficial probiotics (microbes) live in my body?

Though the most common place linked to beneficial microbes is your gut (mostly large intestines), you have several locations in and on your body that host good microbes. These locations are in contact with the “outside world" and include your:

- Gut.
- Mouth.
- Vagina.
- Urinary tract.
- Skin.
- Lungs.

How do probiotics work?

The main job of probiotics, or good bacteria, is to maintain a healthy balance in your body. Think of it as keeping your body in neutral. When you are sick, bad bacteria enters your body and increases in number. This knocks your body out of balance. Good bacteria works to fight off the bad bacteria and restore the balance within your body, making you feel better.

Good bacteria keeps you healthy by supporting your immune function and controlling inflammation. Certain types of good bacteria can also:

- Help your body digest food.
- Keep bad bacteria from getting out of control and making you sick.
- Create vitamins.
- Help support the cells that line your gut to prevent bad bacteria that you may have consumed (through food or drinks) from entering your blood.
- Breakdown and absorb medications.

This balancing act is naturally happening in your body all of the time. You don’t actually need to take probiotic supplements to make it happen. Good bacteria is just a natural part of your body. Eating a well-balanced diet rich in fiber every day helps to keep the number of good bacteria at proper levels.

What are the most common types of probiotic bacteria?

Though there are many types of bacteria that can be considered probiotics, there are two specific types of bacteria that are common probiotics found in stores. These include:

- *Lactobacillus*.
- *Bifidobacterium*.

Probiotics are also made up of good yeast. The most common type of yeast found in probiotics is:

- *Saccharomyces boulardii*.

Can I use probiotics to help with medical conditions?

There is currently a large amount of research happening around the idea of what probiotics can do for your body. Even though there are a lot of possibly positive outcomes, researchers are still working to find definitive answers about how probiotics can help with various conditions.

However, there are some medical conditions where probiotics may help. This can vary between people meaning that what works for one person may not work for another. These can also vary based on the certain probiotic that is taken.

Some of the conditions that might be helped by increasing the amount of probiotics in your body (through food or supplements) include:

- Diarrhea (both diarrhea caused by antibiotics and from *Clostridioides difficile* (C. diff) infection).
- Constipation.
- Inflammatory bowel disease (IBD).
- Irritable bowel syndrome (IBS).
- Yeast infections.
- Urinary tract infections.
- Gum disease.
- Lactose intolerance.
- Eczema (atopic dermatitis).
- Upper respiratory infections (ear infections, common cold, sinusitis).
- Sepsis (specifically in infants).

Can I take or eat something to increase the good probiotics (microbes) in my body?

You can increase the amount of good microbes in your body through foods, drinks and supplements. You may already have certain foods in your daily diet that contain probiotics. Fermented foods in particular (yogurt and pickles, for example) are home to a host of good bacteria that benefit your body. There are also fermented drinks like kombucha (fermented tea) or kefir (fermented dairy drink) that introduce extra probiotics into your diet.

Apart from food, you can add probiotics to your diet through dietary supplements. These aren't drugs, so they do not need to be approved by the Federal Drug Administration (FDA). It's important that you always talk to your healthcare provider before starting any kind of supplement or major change to your diet.

Can I get probiotics from food?

You can absolutely increase beneficial microbes in your body from the foods you eat. Certain foods have probiotics (good bacteria) in them and can benefit the health of your microbiome.

These foods can be introduced into your diet at any point of the day. You may even be regularly eating them now and not realize that they contain probiotics. You will want to check the food label for "live and active cultures." A few suggestions for just some of the probiotic-rich foods you can add to your diet and some times to try them include:

For breakfast, try:

- Yogurt.
- Buttermilk.
- Sourdough bread.

For lunch, try:

- Cottage cheese.
- Kombucha.
- Tempeh.

For a snack, try:

- Fermented pickles.

For dinner, try:

- Fermented sauerkraut.
- Kimchi.
- Miso soup.

Make sure you are still creating a balanced and healthy meal each time you sit down to eat. Though adding probiotic-rich foods into your diet won't hurt you, balance is still key. Adding too much of just one food prevents your body from reaping the benefits of other food groups.

How do I take a probiotic supplement?

There are several ways you can take a probiotic supplement. They come in a variety of forms, including in:

- Foods.
- Drinks.
- Capsules or pills.
- Powders.
- Liquids.

Probiotic supplements may be combined with a prebiotic. Prebiotics are complex carbohydrates that feed the microorganisms in your gut. Basically, prebiotics are the “food source” for the good bacteria. They help feed the good bacteria and keep it healthy. Prebiotics include inulin, pectin and resistant starches.

When you have a supplement that combines a probiotic and prebiotic, it’s called a synbiotic.

How effective are probiotics?

Researchers are currently unsure how effective probiotic supplements are for treating conditions. There’s constant research on the topic. While many research studies have had positive results on the impact of probiotic supplements, more research is still needed.

It’s also important to keep in mind that unlike medications, dietary supplements do not need to be approved by the FDA. This means that manufacturers can sell supplements simply with “claims” of safety and effectiveness.

Always talk with your healthcare provider (or pediatrician) before taking a supplement or giving one to your child. Supplements might interfere with medicines you may be taking. If you are pregnant or breast feeding, check with your provider before taking any supplement.

Are there any storage instructions for probiotics?

Several probiotic strains are very fragile and need to be protected from heat, oxygen, light and humidity. The probiotics might start to break down or die if they are exposed to these elements. Because of this, you may need to refrigerate your probiotics or store it in a particular place. Refrigerating certain probiotic strains ensures that they’re still viable when you go to use them and will still provide the full benefit of the probiotic. Always read the labels on any probiotic product you purchase to make sure you store it correctly and use it within the expiration date.

How safe are probiotics?

Because microbes used as probiotics already exist naturally in your body, probiotic foods and supplements are generally considered safe. They may trigger allergic reactions, and may also cause mild stomach upset, diarrhea, or flatulence (passing gas) and bloating for the first few days after starting to take them.

There are certain people who need to use caution when using probiotic supplements. There is a risk of infection in some people. These people include those who have:

- A weakened immune system (those going through chemotherapy for example).
- A critical illness.
- Recently had surgery.

Caution should also be used when giving probiotics to very sick infants.

Always talk to your healthcare provider before starting a probiotic supplement.

Can probiotics hurt me?

For most healthy people, probiotics don't cause any harm. They are generally considered safe and are often "given a try" to see if they could help with various medical conditions. There's a lot of research around the topic of probiotics. Scientists are trying to determine when and how they should be used, as well as how effective they are. Talk to your healthcare provider before starting a probiotic supplement because there are some cases where you shouldn't be taking them. It's always best to have the conversation first before starting a new supplement.

Are there any risks related to probiotics?

Probiotics are generally considered safe. However, there are some risks linked to the supplements. These risks are increased if you have a medical condition that weakens your immune system, have recently had surgery or have other serious medical conditions.

Unlikely, but possible, risks can include:

- Developing an infection.
- Developing a resistance to antibiotics.
- Developing harmful byproducts from the probiotic supplement.

Should I give probiotics to my kids?

Probiotics can be beneficial for both adults and kids. If your child has an illness that requires an antibiotic medication for treatment, taking a probiotic can help shorten symptoms. Probiotics can also be used to help relieve constipation, acid reflux, diarrhea, gas and eczema in children.

Introducing probiotics into your child's diet through food is typically a safe way to give them probiotics. Foods like yogurt and cottage cheese are often part of a balanced diet and can add in good bacteria without much risk.

There are commercially available probiotic supplements specifically designed for infants and children. However, it is important to talk to your child's pediatrician before giving them any probiotic supplement or changing the child's diet to include probiotic-rich foods.

Do I need to take probiotics after I take antibiotics?

Antibiotic medications are often needed to fight an infection. However, while antibiotics are killing the bad bacteria, they are also knocking out the good bacteria in your body. Some people develop conditions like diarrhea after taking an antibiotic. In other people, this may allow for really bad bacteria to take over and populate the gut, such as with C. diff. Some research has shown a positive connection between taking probiotics after an antibiotic and relief from diarrhea. This hasn't been proven yet and doesn't work for everyone.

The thought behind adding probiotics back into your body after taking an antibiotic is that it can repopulate the good bacteria that was destroyed by the antibiotics and re-boot your system. The extra good bacteria helps repopulate your gut and fight off any remaining bad bacteria. Many people feel that adding in probiotics won't hurt, might help you feel better a little faster and prevent diarrhea.

Should I try probiotics?

If you are interested in adding probiotics to your diet, it's worth a conversation with your healthcare provider. Many providers may suggest giving them a try to see if they help with your general health. It is important to remember that not all probiotics behave the same way and have the same effects. Each has their own individual benefits. They generally don't cause harm. One easy way to start can be by simply introducing probiotic-rich foods into your diet, like yogurt.

Before you start any supplements, make sure you talk to your healthcare provider. Your provider may be able to point you in the right direction, helping you figure out the best probiotic to take, how much to take and when to take it. A conversation is always worth the time when it concerns your health.

Prebiotics

The prebiotics concept was introduced for the first time in 1995 by Glenn Gibson and Marcel Roberfroid [4]. Prebiotic was described as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health". This definition was almost unchanged for more than 15 years. According to this definition, only a few compounds of the carbohydrate group, such as short and long chain β-fructans [FOS and inulin], lactulose, and GOS, can be classified as prebiotics. In 2008, the 6th Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) defined "dietary prebiotics" as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" [19].

The following criteria are used to classify a compound as a prebiotic: (i) it should be resistant to acidic pH of stomach, cannot be hydrolyzed by mammalian enzymes, and also should not be absorbed in the gastrointestinal tract, (ii) it can be fermented by intestinal microbiota, and (iii) the growth and/or activity of the intestinal bacteria can be selectively stimulated by this compound and this process improves host's health [19].

Although not all the prebiotics are carbohydrates, the following two criteria can be exploited to distinguish fiber from carbohydrate-derived prebiotics: (i) fibers are carbohydrates with a degree

of polymerization (DP) equal or higher than 3 and (ii) endogenous enzymes in the small intestine cannot hydrolyze them. It should be taken into account that the fiber solubility or fermentability is not crucial [20,21].

There are also some revised definitions for prebiotics published in the scientific literature [22]. However, the above-mentioned definition, which was given in 2008, has been accepted in recent years. Despite the absence of a consensus definition, the important part of the original and other definitions is that the consumption of prebiotics is associated with human well-being. The word "selectivity", or the potency of a prebiotic to stimulate a specific gut microbiota, was another key element of the original definition; however, this concept has been questioned recently [23]. In 2013, Scott et al. [24] reported that the prebiotic effect was enhanced by cross-feeding, defined as the product of one species which can be consumed by another one. This implication raises doubt for utilizing the "selectivity" term in the prebiotics definition. A review on the evolution of prebiotics concept through history can be found in a previous publication [23], and the debate on their definition is still ongoing [25].

Go to:

3. Types of Prebiotics

There are many types of prebiotics. The majority of them are a subset of carbohydrate groups and are mostly oligosaccharide carbohydrates (OSCs). The relevant articles are mainly on OSCs, but there are also some pieces of evidence proving that prebiotics are not only carbohydrates.

3.1. Fructans

This category consists of inulin and fructo-oligosaccharide or oligofructose. Their structure is a linear chain of fructose with β(2→1) linkage. They usually have terminal glucose units with β(2→1) linkage. Inulin has DP of up to 60, while the DP of FOS is less than 10 [2].

Previously, some studies implicated that fructans can stimulate lactic acid bacteria selectively. However, over recent years, there are some investigations showing that the chain length of fructans is an important criterion to determine which bacteria can ferment them [26]. Therefore, other bacterial species can also be promoted directly or indirectly by fructans.

3.2. Galacto-Oligosaccharides

Galacto-oligosaccharides (GOS), the product of lactose extension, are classified into two subgroups: (i) the GOS with excess galactose at C_3, C_4 or C_6 and (ii) the GOS manufactured from lactose through enzymatic trans-glycosylation. The end product of this reaction is mainly a mixture of tri- to pentasaccharides with galactose in β(1→6), β(1→3), and β(1→4) linkages. This type of GOS is also termed as trans-galacto-oligosaccharides or TOS [19,27].

GOSs can greatly stimulate *Bifidobacteria* and *Lactobacilli*. *Bifidobacteria* in infants have shown high incorporation with GOS. *Enterobacteria*, *Bacteroidetes*, and *Firmicutes* are also stimulated by GOS, but to a lesser extent than *Bifidobacteria* [2].

There are some GOSs derived from lactulose, the isomer of lactose. This lactulose-derived GOSs are also considered as prebiotics [19]. Besides these types of GOS, the other types are based on sucrose extension named raffinose family oligosaccharides (RFO). The effect of RFO on gut microbiota has not been elucidated yet [28,29].

3.3. Starch and Glucose-Derived Oligosaccharides

There is a kind of starch that is resistant to the upper gut digestion known as resistant starch (RS). RS can promote health by producing a high level of butyrate; so it has been suggested to be classified as a prebiotic [30]. Various groups of *Firmicutes* show the highest incorporation with a high amount of RS [3]. An in vitro study demonstrated that RS could also be degraded by *Ruminococcus bromii*, and *Bifidobacterium adolescentis*, and also to a lesser extent by *Eubacterium rectale* and *Bacteroides thetaiotaomicron*. However, in the mixed bacterial and fecal incubations, RS degradation is impossible in the absence of *R. bromii* [31].

Polydextrose is a glucose-derived oligosaccharide. It consists of glucan with a lot of branches and glycosidic linkages. There is some evidence that it can stimulate *Bifidobacteria*, but it has not been confirmed yet [32].

3.4. Other Oligosaccharides

Some oligosaccharides are originated from a polysaccharide known as pectin. This type of oligosaccharide is called pectic oligosaccharide (POS). They are based on the extension of galacturonic acid (homogalacturonan) or rhamnose (rhamnogalacturonan I). The carboxyl groups may be substituted with methyl esterification, and the structure can be acetylated at C_2 or C_3. Various types of sugars (e.g., arabinose, galactose, and xylose) or ferulic acid are linked to the side chains [33]. Their structures vary significantly depending on the sources of POSs [34].

3.5. Non-Carbohydrate Oligosaccharides

Although carbohydrates are more likely to meet the criteria of prebiotics definition, there are some compounds that are not classified as carbohydrates but are recommended to be classified as prebiotics, such as cocoa-derived flavanols. In vivo and in vitro experiments demonstrate that flavanols can stimulate lactic acid bacteria [35].

Go to:

4. Production of Prebiotics

Prebiotics play an important role in human health. They naturally exist in different dietary food products, including asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato, rye, soybean, human's and cow's milk, peas, beans, etc., and recently, seaweeds and microalgae [36]. Because of their low concentration in foods, they are manufactured on industrial large scales. Some of the prebiotics are produced by using lactose, sucrose, and starch as raw material [37,38]. Since most prebiotics are classified as GOS and FOS regarding industrial scale (Figure 1), there are many relevant studies on their production.

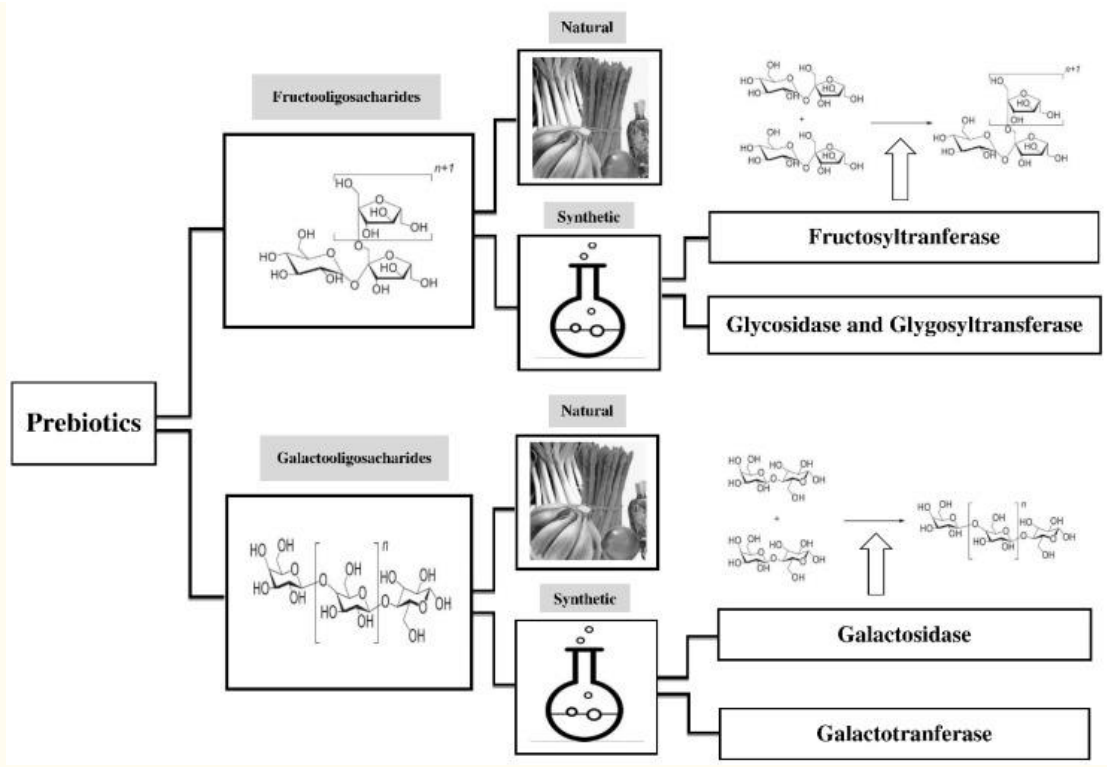
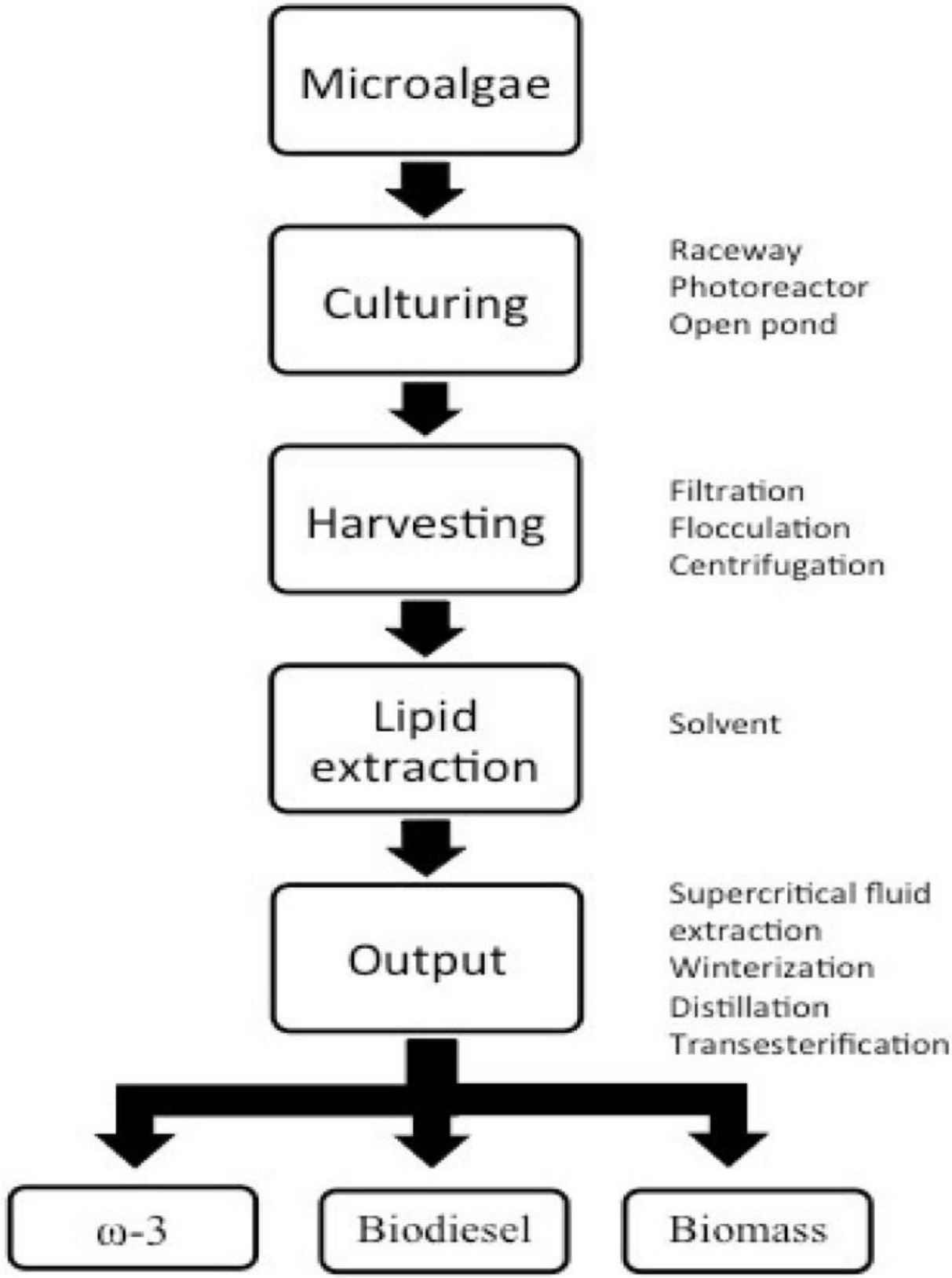


Figure 1

Sources and production of major prebiotics, including fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS). Prebiotics exist in human diets in small concentration. Since they have crucial roles in health maintenance, they are manufactured on industrial large scales.

Extraction and purification of omega-3 fatty acids from microalgal biomass

Figure 4 summarizes an integrated system for the large-scale production of microalgal bio-products. A microalgae strain is cultivated to increase cell density using photobioreactors, open ponds, race ways or hybrid systems. Algal cells are separated from culture media by filtration, flocculation or centrifugation, followed by drying to improve extraction [1]. Lipid extraction is then commonly performed using a non-water miscible organic solvent. A typical extraction protocol in small scale is often based on the method of Bligh and Dyer [103], which uses a solvent mixtures made of methanol/chloroform for the cell disruption and lipid extraction. Larger scale extraction is typically carried out with hexane as a solvent. Subsequently, unsaturated fatty acids are separated from the total lipids by fractional (molecular) distillation or winterization, whereby oil temperature is reduced to precipitate the more saturated lipids. Further processing to improve the quality, shelf-life and quantity of PUFA oil can include filtration, bleaching, deodorization, polishing and antioxidant addition [1, 104] (Table 2).



Examples of a bioprocess production chain in a microalgal biorefinery. Apart from omega-3 fatty acids (ω-3), the product portfolio includes biodiesel and protein-rich animal feed from the remaining biomass.

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited 'A' Grade by NAAC / 12B Status by UGC / Approved by AICTE

www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

M.TECH - BIOTECHNOLOGY

UNIT – IV – FOOD & NUTRACEUTICALS – SBTA7013

A plant can actually have properties similar to pharmaceutical drugs, and people have been using them throughout history to either cure an illness or lessen its symptoms – and these plants fall in the category of medicinal plants. Medicinal plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals.

Some common medicinal plants are aloe vera, ginseng, sage, chamomile, tea tree, and pot marigold, to name a few. Used since ancient times, all civilizations and cultures are familiar with the benefits of these plants, having used them for therapeutic, religious, cosmetic, nutritional and beautification purposes since ancient times.

In fact, some of the medicinal plants and their benefits are so simple- they boil down to kitchen remedies, making them both accessible and cost-effective. For example, ginger and garlic are used to boost immune function, reduce inflammation and even to fight cancer. Lavender is another such plant that can improve digestion and treat skin disorders. Many serve as home remedies to treat seasonal cough, cold, stomach ache and other symptoms.

Aromatic plants

Aromatic plants are another group of plants that produce and exude aromatic substances, which are used in making perfumes, in cooking, and in food, pharmaceutical, and liqueur industries. The particular aroma is due to a variety of complex chemical compounds. The term essential oil is similar to fragrance or perfumes because these fragrances are oily in nature and they represent the essence of the active constituents of the plants.

Essential oils and aroma chemicals constitute a major group of industrial products. **Demand and trade for these plant materials initiated globalization that spread new ideas and new settlements.**

Many species of aromatic plants are cultivated for such industrial uses, but most are still wild-collected. The need for renewable sources of industrial products as well as the need to protect plant biodiversity has created a ripe opportunity for farmers to produce such crops. No doubt then, the cultivation of medicinal plants and aromatic herbs has become a profitable business.

Making cultivation profitable

Led by strong and rapidly growing industry demand, here's a little-known story – a happy one, in which small groups of farmers have started earning as much as Rs.30,000 per acre through the cultivation of medicinal and aromatic plants. That's a rather profitable figure when compared to wheat and rice farming, which earns only as much as Rs. 30,000 an acre.

There are companies that have taken on this market on a commercial scale – such as Dabur, Himalaya, and Patanjali. Industry estimates put the market for herbal products at Rs.50,000 Crore, growing at a fast annual rate of 15%. Although the acreage devoted to herbs and aromatic plants in comparison to other crops is still proportionately small, the farmers' returns have been impressive.

A herb called *atheesh*, for example, that grows in the higher reaches of Uttarakhand and Himachal Pradesh can easily fetch a farmer Rs. 2-3 lakh per acre. A lavender farmer may get Rs 1.2 – 1.5 lakh as a return for his 2-acre plot. The farmers say that there is also another huge advantage these crops have for growers – it's not necessary to water these herbs too much or spray fertilizers on it. This has allowed farming in areas where even one crop a year was tough on account of poor rainfall.

The medicinal and aromatic plant industry

There are several companies that are the prime players in the industry that work with farmers to grow medicinal and aromatic plants. For example, Dabur has been able to work with farmers to grow medicinal plants like *shankapushpi*. Companies who buy these herbs and aromatic plants are also expecting a good level of growth and profit.

Amit Agarwal, Director of Natural Remedies, says that some high-value herbs like ateesh, kuth, kutki are currently more profitable because of the supply shortage. Natural Remedies says it is doing contract farming of herbs on 1,043 acres of land.

Patanjali's CEO Acharya Balkrishna says the company is "helping farmers cultivate herbs on 40,000 acres".

Here again, *kutki*, *shatavari* and *chirayata* are on top of his list of best earners. And India has plenty of potentials to grow this business – he says because it is way behind China and there is high global and domestic demand. Taking a cue, the Indian Institute of Integrative Medicine in Jammu has been promoting the cultivation of lavender and other aromatic plants like rosemary, geranium, and sage.

"Demand for oils from these plants is coming from domestic companies dealing in perfumery and cosmetics," says Ram Vishwakarma, director, Indian Institute of Integrative Medicine, Jammu.

Further, in 2017-18, Dabur, under its Bio-Resources Development program, saw an increase of 25% in area under cultivation of medicinal herbs —more than 5,000 acres across 19 states, involving 2,400 farmer families, according to Dabur India CSR head A. Sudhakar. Himalaya

Drug Company works with over 800 farmers, covering over 3,500 acres, says a company spokesperson. Around 62 types of essential oils come to the international market with a large and consistent demand.

India's advantage in making itself a hub for the aromatic oil industry

India is rich in biodiversity and host to many medicinal and aromatic plants which are now being used for various ailments and as nutraceuticals. In India, two major scientific councils are working on medicinal and aromatic plants. One is Central Institute of Medicinal and Aromatic Plants under CSIR which is looking at industry point of view and other is Directorate of Medicinal and Aromatic Plants under ICAR which is looking at the issue from the farmers' point of view.

The demand for aromatic plants is currently increasing in both developed and developing countries for various reasons – accessibility and affordability, and also because there is no worry of side effects. Under the mission by the Central Institute of Medicinal and Aromatic Plants, 'Aroma and Phyto-Pharmaceutical Mission' was launched to boost cultivation of aromatic crops keeping in view the plight of farmers involved in traditional agriculture, and who are trying to shift out of it due to climate change.

Plant tissue culture

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. It is widely used to produce clones of a plant in a method known as micropropagation. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

- The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.
- To quickly produce mature plants.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.
- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e. orchids and *Nepenthes*.
- To clean particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones.

Techniques

Preparation of plant tissue for tissue culture is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet. Thereafter, the tissue is grown in sterile containers, such as Petri dishes or flasks in a growth room with controlled temperature and light intensity. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so their surfaces are sterilized in chemical solutions (usually alcohol and sodium or calcium hypochlorite)[1] before suitable samples (known as explants) are taken. The sterile explants are then usually placed on the surface of a sterile solid culture medium but are sometimes placed directly into a sterile liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar.

In vitro tissue culture of potato explants

The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots. A balance of both auxin and cytokinin will often produce an unorganised growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and subcultured onto new media to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard.

As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants.[2]

Plant tissue cultures being grown at a USDA seed bank, the National Center for Genetic Resources Preservation.

The specific differences in the regeneration potential of different organs and explants have various explanations. The significant factors include differences in the stage of the cells in the cell cycle, the availability of or ability to transport endogenous growth regulators, and the metabolic capabilities of the cells. The most commonly used tissue explants are the meristematic ends of the plants like the stem tip, axillary bud tip and root tip. These tissues have high rates of cell division and either concentrate or produce required growth-regulating substances including auxins and cytokinins.

Shoot regeneration efficiency in tissue culture is usually a quantitative trait that often varies between plant species and within a plant species among subspecies, varieties, cultivars, or ecotypes. Therefore, tissue culture regeneration can become complicated especially when many regeneration procedures have to be developed for different genotypes within the same species.

The three common pathways of plant tissue culture regeneration are propagation from preexisting meristems (shoot culture or nodal culture), organogenesis and non-zygotic embryogenesis.

The propagation of shoots or nodal segments is usually performed in four stages for mass production of plantlets through in vitro vegetative multiplication but organogenesis is a common method of micropropagation that involves tissue regeneration of adventitious organs or axillary buds directly or indirectly from the explants. Non-zygotic embryogenesis is a noteworthy developmental pathway that is highly comparable to that of zygotic embryos and it is an important pathway for producing somaclonal variants, developing artificial seeds, and synthesizing metabolites. Due to the single-cell origin of non-zygotic embryos, they are preferred in several regeneration systems for micropropagation, ploidy manipulation, gene transfer, and synthetic seed production. Nonetheless, tissue regeneration via organogenesis has also proved to be advantageous for studying regulatory mechanisms of plant development.

Choice of explant[edit]

The tissue obtained from a plant to be cultured is called an explant.

Explants can be taken from many different parts of a plant, including portions of shoots, leaves, stems, flowers, roots, single undifferentiated cells and from many types of mature cells provided they still contain living cytoplasm and nuclei and are able to de-differentiate and resume cell division. This has given rise to the concept of totipotency of plant cells.[3][4] However, this is not true for all cells or for all plants.[5] In many species explants of various organs vary in their rates of growth and regeneration, while some do not grow at all. The choice of explant material also determines if the plantlets developed via tissue culture are haploid or diploid. Also, the risk of microbial contamination is increased with inappropriate explants.

The first method involving the meristems and induction of multiple shoots is the preferred method for the micropropagation industry since the risks of somaclonal variation (genetic variation induced in tissue culture) are minimal when compared to the other two methods. Somatic embryogenesis is a method that has the potential to be several times higher in multiplication rates and is amenable to handling in liquid culture systems like bioreactors.

Some explants, like the root tip, are hard to isolate and are contaminated with soil microflora that becomes problematic during the tissue culture process. Certain soil microflora can form tight associations with the root systems, or even grow within the root. Soil particles bound to roots are difficult to remove without injury to the roots that then allows a microbial attack. These associated microflora will generally overgrow the tissue culture medium before there is significant growth of plant tissue.

Some cultured tissues are slow in their growth. For them there would be two options: (i) Optimizing the culture medium; (ii) Culturing highly responsive tissues or varieties.[6] Necrosis can spoil cultured tissues. Generally, plant varieties differ in susceptibility to tissue culture necrosis. Thus, by culturing highly responsive varieties (or tissues) it can be managed.[6]

Aerial (above soil) explants are also rich in undesirable microflora. However, they are more easily removed from the explant by gentle rinsing, and the remainder usually can be killed by surface sterilization. Most of the surface microflora do not form tight associations with the plant tissue. Such associations can usually be found by visual inspection as a mosaic, de-colorization or localized necrosis on the surface of the explant.

An alternative for obtaining uncontaminated explants is to take explants from seedlings which are aseptically grown from surface-sterilized seeds. The hard surface of the seed is less permeable to the penetration of harsh surface sterilizing agents, such as hypochlorite, so the acceptable conditions of sterilization used for seeds can be much more stringent than for vegetative tissues.

Tissue cultured plants are clones. If the original mother plant used to produce the first explants is susceptible to a pathogen or environmental condition, the entire crop would be susceptible to the same problem. Conversely, any positive traits would remain within the line also.

Applications[edit]

Plant tissue culture is used widely in the plant sciences, forestry, and in horticulture. Applications include:

- The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.
- To conserve rare or endangered plant species.[7]
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.[8]
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.

- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants.[9]
- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
- For chromosome doubling and induction of polyploidy,[10] for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as sugarcane,[11] potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.
- Large scale production of artificial seeds through somatic embryogenesis[12]
- Synthetic seeds - A somatic embryo is encapsulated by artificial endosperm and artificial seed coat

CONCEPT OF PLANT TISSUE CULTURE

Gottlieb Haberlandt (1854-1945), a German botanist is considered as the father of plant tissue culture, was the first to separate and culture plant cells on Knop's salt solution in 1898**[7].** Plant tissue culture is the technique of maintaining and growing plant cells, tissues or organs or any plant part on artificial nutrient solid or liquid media in suitable containers under controlled environmental conditions. This practice is used to propagate plant clones under sterile conditions. In vitro culture is one of the key tools of plant biotechnology that exploits the totipotency nature.

of plant cells **[8].** and this was demonstrated for the first time in plants by **[9].** After the discovery of kinetin **[10],** the major work on in vitro regeneration has been done on tobacco (*Nicotiana tabacum L.*) tissue culture, culminating in the first convincing demonstration of the control of differentiation of shoots or roots or both by the kinetin-auxin ratio **[11]** followed by carrot (*Daucus carota L.*) tissue culture and birth of the concept of totipotency of plant cell with the regeneration of complete flowering plants of carrot from its phloem cells **[9].** The cultured cells and tissue can take several pathways to produce a complete plant. Among these are organogenesis and somatic embryogenesis are common. In organogenesis, groups of cells of the apical meristem in the shoot apex, axillary buds, root tips etc are stimulated to differentiate and grow into shoots and ultimately into complete plants. In somatic mbryogenesis, groups of somatic cells/tissues lead to the formation of somatic embryos which resemble the zygotic embryos of intact seeds and can grow into seedlings on suitable medium **II MICROPROPAGATION** The technique was heralded as the universal mass clonal plant propagation system for the future and the term 'micropropagation' was introduced to describe more accurately the processes. In micropropagation rapid proliferation is achieved from tiny plant part such as stem cuttings, auxiliary buds, and to a limited extent from somatic embryos. The process of micropropagation is usually divided into several stages i.e., propagation, initiation of explants, subculture of explants for proliferation, shooting and rooting, and hardening. These stages are universally applicable in large-scale multiplication of plants. The in vitro propagated medicinal plants are genetically pure elite. Micropropagation techniques are must for conservation of an endangered medicinally important species within short period and limited space. Today plant tissue culture applications encompass much more than clonal propagation and micropropagation. The range of routine technologies has expanded to include somatic embryogenesis, somatic hybridization etc. Micropropagation offers several distinct advantages not possible with conventional propagation technique of medicinal plants;

1. Rapid multiplication of genetically uniform plants (clones) that possess desirable traits.
2. The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
3. The regeneration of whole plants from plant cells.
4. Production of secondary metabolites.
5. *In vitro* conservation of germplasm.
6. New and genetically engineered plants can be produced.
7. Conservation of threatened plant species.
8. Plants are obtained throughout the year under controlled conditions, independent of seasons.

2.1 Explants source: Micropropagation start with the selection of plant tissue (explants) from a young, healthy mother plant. For tissue culture, any part of the plant (leaf, root etc.) can be used as explants. Tissue culture

success mainly depends on the age, types and position of explants **[12].** Sometimes Large size explants can increase chances of contamination and small explants (meristems) can show less growth **[13] [14].** To increase the probability of success, the mother plant should be ex-vitro cultivated under optimal condition to minimize contamination in the *in-vitro* culture.

2.2 Sterilization : Microbial contamination is a very common problem in plant tissue culture **[13][15].** For this purpose explants is surface sterilized by different reagents then transferred into nutrient medium generally. Explants are cleaned by distilled water and sterilized using mercuric chloride, ethyl alcohol, and liquid bleach **[16].** Sterilization of laboratory instruments is carried out by autoclaving, alcohol washing, baking, radiations, flaming and fumigation **[17].** The conserved application and bactericide and fungicide products is suggested. The selection of products depends on type of explants to be introduced. Surface sterilization is used to remove contaminants with minimal damage to plant cells.

2.3 Tissue culture Media and Plant Growth Hormones : Media for tissue culture contains vital nutrients and elements for *in vitro* growth of plant tissues. Selection of the right media composition is important for successful tissue culturing. A wide range of media are available for plant tissue culture, but MS **[18]** medium is commonly**[12].** Other media used are Linsmaier-Skoog (LS) **[19]**, Schenk and Hilderbrandt (SH) **[20],** WPM (Woody plant medium **[21]**and the Nitsch and Nitsch (NN) **[22]**. Agar is not essential media component but is used as gelling agent **[23].** The *pH* of culture media is normally between pH 5.7 to 5.8. Optimization of regeneration protocol supporting the action of growth additives as a supplement of growth regulators will be useful in the establish of tissue cultures. Growth hormones regulate various physiological and morphological processes in plants and are also known as plant growth regulators (PGRs) or phytohormones **[23] [1].** PGRs are synthesized by plants; therefore many plant species can grow successfully without external medium supplements [**[24] [25].** Hormones can also be added into cultures to improve plant growth and to enhance metabolite synthesis. Growth and morphogenesis of cultured plants depends on the selection of the initial material, media composition, plant growth regulators, cultivar and environmental factors **[26].** The effects of auxins and cytokinins on shoot multiplication of various medicinal plants have been reported by **[27] [28].** has indicated that the production of multiple shoots is higher in the medium having kinetin along with NAA. *in vitro* growth and shoot formation was not achieved without adequate concentrations exogenous hormones [10] Category of plant growth hormones and their functions.
1) Auxins : Mainly play role in cell division, elongation and root differentiation. Some are - Indole -3-acetic acid (IAA) , indole -3- butyric acid (IBA) , 2,4- dichlorophenoxyacetic acid naphthalene acetic acid (NAA) , 2,4,5- trichl orophenoxy acetic acid naphthoxyacetic acid (NOA**) [23].**

2) Cytokinins : main functions are cell division, shoot induction, development and proliferation. Some are - benzyl amino purine (BAP) , isopentenyl adenine (IPA or 2i -p), kinetin (furfurylamino purine) , 4-hydroxy -3-methyl -trans -2- butenylaminopurine (zeatin) **[23].**

3) Gibberellins: Play role in growth, elongation and flowering such asGA3 **[23].**

2.4 In vitro Plant tissue culture stages and techniques: Any part of plant can be used as explants.The process of tissue culturing, from explants to mature stage plants involves some basic steps:

1) Initiation stage or culture establishment--In this, surface sterilized explants transferred into nutrient medium. The conserved application and bactericide and fungicide products is suggested. The selection of products depends on type of explants to be introduced.

2) Multiplication stage- The aim of this phase is to increase the number of propaganules **[29].** The number of propagranules is multiplied by repeated sub cultures until the desired (or planned) number of plant is attained.

3) Rooting stage- The rooting stage may occur simultaneously in the same culture media used for multiplication of the explants. After changes the nutritional modification and growth regulator composition to induce rooting and the development of stage root growth. For rooting half strength MS medium supplemented with 1.0mg/l auxin was used.

4) Stage of Hardening and Acclimatization of Tissue Culture Plantlets - In this stage in vitro cultured plants hardened and acclimatized. Hardening is done gradually from high to low humidity and from low light intensity to high light intensity. The plantlets were removed from artificial medium and rinsed with sterile water to remove excess of agar on roots, to be then placed into the cup, introducing the roots into the soil sand mixture. The newly transferred plantlets were spray-irrigated while they stilled under transparent plastic cover on the top to prevent quick dehydration. The regenerated plantlets were placed in growth chamber (room) for one week, before they were transferred to green house for several weeks, with gradual release of plastic covers.

2.5 Callus Cultures: Primary callus culture derived from tissues with high contents of parenchyma or meristematic

cells. Callus is an undifferentiated mass of tissue which appears on explants within a few weeks of transfer onto growth medium with suitable hormones**[23]. 2.6 Cell Suspensions culture :** Suspension culture is a type of culture in which single cell or small aggregates of cell multiply while suspended in agitated liquid medium. Suspension cultures are of two types batch and continuous.These cultures are formed *in vitro* when friable calli are grown on liquid media in suitable container and constantly agitated to provide suspension of free cells **[23].** Suspension cultures are widely used in large scale production of secondary metabolites **[30].**

2.7 Protoplast Cultures: Protoplasts are plant cells in which do not possess cell wall and wall has been removed by enzyme digestion or mechanical process **[23].** Plant Cell wall can be removed through enzymatic digestion

(pectinase and cellulose) or by mechanical methods **[31].** The main aim in using this approach in the past however has been interspecies hybridizations. Plant regeneration was carried out successfully in *A. judaica and E. spinosissimus* by protoplast culture **[32]. 2.8 Embryo culture:** Embryo culture or Somatic embryogenesis is process by which a non zygotic embryo is produced in glass vials from plant tissue or cell, which can develop into a new plant **[33] [34].** *In vitro* embryo culture represents a potential tool for improved recovery of hybrid germplasm **[35].** Successful plant regeneration has been studied *in Aloe vera* and *Vitis vinifera* by somatic embryogenesis **[36[37]. 2.9 Anther or Microspore Cultures:** Anther culture is the *in vitro* development of haploid plants originating from potent pollen grains through a series of cell division and differentiation. Andrgenesis has become a powerful tool for the rapid production of haploid and inbred lines used for obtaining hybrid cultivars **[38].** An auxin has been reported to be absolutely required to initiate and promote microspore embryogenesis and a small amount of cytokinin in addition to auxin improves the yield of embryoids in anther culture of oilseed rape **[39] 2.10 Pollen culture:** Pollen culture is the in vitro technique by which the pollen grains (preferably at the microscope stages) are squeezed from the intact anther and then cultured on nutrient medium where the microspores without producing male gametes. **2.11 Shoot tip and Meristem culture:** The shoot tips (shoot apical meristems) can be cultured in vitro producing clumps of shoots from auxiliary and adventitious buds. Shoot meristem multiplication is generally used for producing virus free material and maintaining germplasm via cryopreservation **[40].** Several micropropagation protocols of medicinal plants such as Ashwaganda have been reported from shoot tip explants **[41] . III SECONDARY METABOLITES** Plants produce two types of metabolites -primary and secondary. Primary metabolites are essential for the growth and development of the plants. Plants are attacked by pests and predators. To overcome this problem, during metabolism plants produce enormous number of compounds as part of defense Mechanism**[42] [43]**. These compounds do not play essential role like primary metabolites, they are called secondary metabolites. . Secondary metabolites are used as pharmaceutical, agrochemicals, aromatics and food additives **[42] [44].** I*n vitro* tissue culture offers an effective and potential alternative of production of bioactive compound because the amount of secondary metabolites produced in this technique can be even higher than in parent plants **[44] [45]**. Plant derived compounds include many terpenes, polyphenols, cardenolides, steroids, alkaloids and glycosides **[30] [46].** Figure-1 shows different groups of chemical compounds produced found plants.

Figure -1 : Adapted schematic showing the classification of plant derived compounds **[44]** *In vitro* grown plant cells and tissues have been used extensively for the production of secondary metabolites. Depending on the objectives, biotechnological techniques are used for understanding metabolic pathways and improvement of plants for the production of secondary metabolites. Plants are potential source of various pharmaceutical and industrial products. Nearly 30% of the drugs produced are of plant origin. Tissue culture of medicinal plants provides a continuous and reliable source of these bioactive compounds round the year without the destruction of the entire plant. Hashimoto *et al*. **[47]** reported increased production of tropane alkaloids in genetically engineered root cultures. There are several strategies that can be used to enhance the production of desired pharmaceuticals by genetic engineering **[48]**. Oksman-Caldentey and **[42]** have reviewed the work on the production of designer metabolites in the post-genomic domain. **IV DISADVANTAGES OF MICROPROPAGATION** Micropropagation is not always the perfect means of multiplying plants, conditions that limits its use include:

 It is very expensive, and can have a labour cost of more than 70%.

 A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.

 An infected plant sample can produce infected progeny. This is uncommon if the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.

Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the

Plant name	Common name	Preventive diseases
Aegle marmelos	Beal tree	Diarrhea, dysentery, malaria, fever, jaundice.
Acorus calamus	Sweet flag, Bach	Anti-spasmotic, anti-helminthic properties also used for treatment of epilepsy, mental aliment, diarrhea, dysentery
Celestrus paniculatus	Malkangani	Memory booster, depression, paralysis.
Bacopa moneria	Brahmi	Mental function logativity, disease fatigue and depression, energise the CNS.
Glycerrhiza glabra	Brahmi	Ulcer, anti-spasmotic, asthama, cough.

Genetically modified food

Genetically modified foods (**GM foods**), also known as **genetically engineered foods** (**GE foods**), or **bioengineered foods** are foods produced from organisms that have had changes introduced into their DNA using the methods of genetic engineering. Genetic engineering techniques allow for the introduction of new traits as well as greater control over traits when compared to previous methods, such as selective breeding and mutation breeding.[1]

Commercial sale of genetically modified foods began in 1994, when Calgene first marketed its unsuccessful Flavr Savr delayed-ripening tomato.[2][3] Most food modifications have primarily focused on cash crops in high demand by farmers such as soybean, corn, canola, and cotton. Genetically modified crops have been engineered for resistance to pathogens and herbicides and for better nutrient profiles. GM livestock have been developed, although, as of 2015, none were on the market.[4] As of 2015, the AquAdvantage salmon was the only animal approved for commercial production, sale and consumption by the FDA.[5][6] It is the first genetically modified animal to be approved for human consumption.

There is a scientific consensus[7][8][9][10] that currently available food derived from GM crops poses no greater risk to human health than conventional food,[11][12][13][14][15] but that each GM food needs to be tested on a case-by-case basis before introduction.[16][17][18] Nonetheless, members of the public are much less likely than scientists to perceive GM foods as safe.[19][20][21][22] The legal and regulatory status of GM foods varies by country, with some nations banning or restricting them, and others permitting them with widely differing degrees of regulation.[23][24][25][26]

However, there are ongoing public concerns related to food safety, regulation, labelling, environmental impact, research methods, and the fact that some GM seeds, along with all new plant varieties, are subject to plant breeders' rights owned by corporations.

Definition

Genetically modified foods are foods produced from organisms that have had changes introduced into their DNA using the methods of genetic engineering as opposed to traditional cross breeding.[28][29] In the U.S., the Department of Agriculture (USDA) and the Food and Drug Administration (FDA) favor the use of the term *genetic engineering* over *genetic modification* as being more precise; the USDA defines *genetic modification* to include "genetic engineering or other more traditional methods".[30][31]

According to the World Health Organization, "Foods produced from or using GM organisms are often referred to as GM foods."[28]

History

Human-directed genetic manipulation of food began with the domestication of plants and animals through artificial selection at about 10,500 to 10,100 BC.[32]:1 The process of selective breeding, in which organisms with desired traits (and thus with the desired genes) are used to breed the next generation and organisms lacking the trait are not bred, is a precursor to the modern concept of genetic modification (GM).[32]:1[33]:1 With the discovery of DNA in the early 1900s and various advancements in genetic techniques through the 1970s[34] it became possible to directly alter the DNA and genes within food.

Genetically modified microbial enzymes were the first application of genetically modified organisms in food production and were approved in 1988 by the US Food and Drug Administration.[35] In the early 1990s, recombinant chymosin was approved for use in several countries.[35][36] Cheese had typically been made using the enzyme complex rennet that had been extracted from cows' stomach lining. Scientists modified bacteria to produce chymosin, which was also able to clot milk, resulting in cheese curds.[37]

The first genetically modified food approved for release was the Flavr Savr tomato in 1994.[2] Developed by Calgene, it was engineered to have a longer shelf life by inserting an antisense gene that delayed ripening.[38] China was the first country to commercialize a transgenic crop in 1993 with the introduction of virus-resistant tobacco.[39] In 1995, *Bacillus thuringiensis* (Bt) Potato was approved for cultivation, making it the first pesticide producing crop to be approved in the US.[40] Other genetically modified crops receiving marketing approval in 1995 were: canola with modified oil composition, Bt maize, cotton resistant to the herbicide bromoxynil, Bt cotton, glyphosate-tolerant soybeans, virus-resistant squash, and another delayed ripening tomato.[2]

With the creation of golden rice in 2000, scientists had genetically modified food to increase its nutrient value for the first time.[41]

By 2010, 29 countries had planted commercialized biotech crops and a further 31 countries had granted regulatory approval for transgenic crops to be imported.[42] The US was the leading country in the production of GM foods in 2011, with twenty-five GM crops having received regulatory approval.[43] In 2015, 92% of corn, 94% of soybeans, and 94% of cotton produced in the US were genetically modified strains.[44]

The first genetically modified animal to be approved for food use was AquAdvantage salmon in 2015.[45] The salmon were transformed with a growth hormone-regulating gene from a Pacific Chinook salmon and a promoter from an ocean pout enabling it to grow year-round instead of only during spring and summer.[46]

In April 2016, a white button mushroom (*Agaricus bisporus*) modified using the CRISPR technique received *de facto* approval in the United States, after the USDA said it would not have to go through the agency's regulatory process. The agency considers the mushroom exempt because the editing process did not involve the introduction of foreign DNA.[47]

The most widely planted GMOs are designed to tolerate herbicides. By 2006 some weed populations had evolved to tolerate some of the same herbicides. Palmer amaranth is a weed that competes with cotton. A native of the southwestern US, it traveled east and was first found resistant to glyphosate in 2006, less than 10 years after GM cotton was introduced.[48][49][50]

Process[edit]

Main article: Genetic engineering techniques

Creating genetically modified food is a multi-step process. The first step is to identify a useful gene from another organism that you would like to add. The gene can be taken from a cell[51] or artificially synthesised,[52] and then combined with other genetic elements, including a promoter and terminator region and a selectable marker.[53] Then the genetic elements are inserted into the targets genome. DNA is generally inserted into animal cells using microinjection, where it can be injected through the cell's nuclear envelope directly into the nucleus, or through the use of viral vectors.[54] In plants the DNA is often inserted using *Agrobacterium*-mediated recombination,[55][56] biolistics[57] or electroporation. As only a single cell is transformed with genetic material, the organism must be regenerated from that single cell. In plants this is accomplished through tissue culture.[58][59] In animals it is necessary to ensure that the inserted DNA is present in the embryonic stem cells.[55] Further testing using PCR, Southern hybridization, and DNA sequencing is conducted to confirm that an organism contains the new gene.[60]

Traditionally the new genetic material was inserted randomly within the host genome. Gene targeting techniques, which creates double-stranded breaks and takes advantage on the cells natural homologous recombination repair systems, have been developed to target insertion to exact locations. Genome editing uses artificially engineered nucleases that create breaks at specific points. There are four families of engineered nucleases: meganucleases,[61][62] zinc finger nucleases,[63][64] transcription activator-like effector nucleases (TALENs),[65][66] and the Cas9-guideRNA system (adapted from CRISPR).[67][68] TALEN and CRISPR are the two most commonly used and each has its own advantages.[69] TALENs have greater target specificity, while CRISPR is easier to design and more efficient.[69]

Crops[edit]

Main article: Genetically modified crops

Genetically modified crops (GM crops) are genetically modified plants that are used in agriculture. The first crops developed were used for animal or human food and provide resistance to certain pests, diseases, environmental conditions, spoilage or chemical treatments (e.g. resistance to a herbicide). The second generation of crops aimed to improve the quality, often by altering the nutrient profile. Third generation genetically modified crops could be used for non-food purposes, including the production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation.[70] GM crops have been produced to improve harvests through reducing insect pressure, increase nutrient value and tolerate different abiotic stresses. As of 2018, the commercialised crops are limited mostly to cash crops like cotton, soybean, maize and canola and the vast majority of the introduced traits provide either herbicide tolerance or insect resistance.[70]

The majority of GM crops have been modified to be resistant to selected herbicides, usually a glyphosate or glufosinate based one. Genetically modified crops engineered to resist herbicides are now more available than conventionally bred resistant varieties.[71] Most currently available genes used to engineer insect resistance come from the *Bacillus thuringiensis* (Bt) bacterium and code for delta endotoxins. A few use the genes that encode for vegetative insecticidal proteins.[72] The only gene commercially used to provide insect protection that does not originate

from *B. thuringiensis* is the Cowpea trypsin inhibitor (CpTI). CpTI was first approved for use cotton in 1999 and is currently undergoing trials in rice.[73][74] Less than one percent of GM crops contained other traits, which include providing virus resistance, delaying senescence and altering the plants composition.[75]

Adoption by farmers has been rapid, between 1996 and 2013, the total surface area of land cultivated with GM crops increased by a factor of 100.[76] Geographically though the spread has been uneven, with strong growth in the Americas and parts of Asia and little in Europe and Africa.[70] Its socioeconomic spread has been more even, with approximately 54% of worldwide GM crops grown in developing countries in 2013.[76] Although doubts have been raised,[77] most studies have found growing GM crops to be beneficial to farmers through decreased pesticide use as well as increased crop yield and farm profit.[78][79][80]

Fruits and vegetables[edit]

Three views of a papaya, cultivar "Sunset", which was genetically modified to create the cultivar 'SunUp', which is resistant to Papaya ringspot virus[81]

Papaya was genetically modified to resist the ringspot virus (PSRV). "SunUp" is a transgenic red-fleshed Sunset papaya cultivar that is homozygous for the coat protein gene PRSV; "Rainbow" is a yellow-fleshed F1 hybrid developed by crossing 'SunUp' and nontransgenic yellow-fleshed "Kapoho".[81] The GM cultivar was approved in 1998[82] and by 2010 80% of Hawaiian papaya was genetically engineered.[83] *The New York Times* stated, "without it, the state's papaya industry would have collapsed".[83] In China, a transgenic PRSV-resistant papaya was developed by South China Agricultural University and was first approved for commercial planting in 2006; as of 2012 95% of the papaya grown in Guangdong province and 40% of the papaya grown in Hainan province was genetically modified.[84] In Hong Kong, where there is an exemption on growing and releasing any varieties of GM papaya, more than 80% of grown and imported papayas were transgenic.[85][86]

The New Leaf potato, a GM food developed using *Bacillus thuringiensis* (Bt), was made to provide in-plant protection from the yield-robbing Colorado potato beetle.[87] The New Leaf potato, brought to market by Monsanto in the late 1990s, was developed for the fast food market. It was withdrawn in 2001 after retailers rejected it and food processors ran into export problems. In 2011, BASF requested the European Food Safety Authority's approval for cultivation and marketing of its Fortuna potato as feed and food. The potato was made resistant to late blight by adding resistant genes blb1 and blb2 that originate from the Mexican wild potato Solanum bulbocastanum.[88][89] In February 2013, BASF withdrew its application.[90][91] In 2014, the USDA approved a genetically modified potato developed by J. R. Simplot Company that contained ten genetic modifications that prevent bruising and produce less acrylamide when

fried. The modifications eliminate specific proteins from the potatoes, via RNA interference, rather than introducing novel proteins.[92][93]

As of 2005, about 13% of the Zucchini (a form of squash) grown in the US was genetically modified to resist three viruses; that strain is also grown in Canada.[94][95]

Plums genetically engineered for resistance to plum pox, a disease carried by aphids

In 2013, the USDA approved the import of a GM pineapple that is pink in color and that "overexpresses" a gene derived from tangerines and suppress other genes, increasing production of lycopene. The plant's flowering cycle was changed to provide for more uniform growth and quality. The fruit "does not have the ability to propagate and persist in the environment once they have been harvested", according to USDA APHIS. According to Del Monte's submission, the pineapples are commercially grown in a "monoculture" that prevents seed production, as the plant's flowers aren't exposed to compatible pollen sources. Importation into Hawaii is banned for "plant sanitation" reasons.[96]

In February 2015 Arctic Apples were approved by the USDA,[97] becoming the first genetically modified apple approved for sale in the US.[98] Gene silencing is used to reduce the expression of polyphenol oxidase (PPO), thus preventing the fruit from browning.[99]

Corn[edit]

Corn used for food and ethanol has been genetically modified to tolerate various herbicides and to express a protein from *Bacillus thuringiensis* (Bt) that kills certain insects.[100] About 90% of the corn grown in the US was genetically modified in 2010.[101] In the US in 2015, 81% of corn acreage contained the Bt trait and 89% of corn acreage contained the glyphosate-tolerant trait.[44] Corn can be processed into grits, meal and flour as an ingredient in pancakes, muffins, doughnuts, breadings and batters, as well as baby foods, meat products, cereals and some fermented products. Corn-based masa flour and masa dough are used in the production of taco shells, corn chips and tortillas.[102]

Soy[edit]

Soybeans accounted for half of all genetically modified crops planted in 2014.[75] Genetically modified soybean has been modified to tolerate herbicides and produce healthier oils.[103] In 2015, 94% of soybean acreage in the U.S. was genetically modified to be glyphosate-tolerant.[44]

Rice[edit]

Golden rice is the most well known GM crop that is aimed at increasing nutrient value. It has been engineered with three genes that biosynthesise beta-carotene, a precursor of vitamin A, in the edible parts of rice.[104] It is intended to produce a fortified food to be grown and consumed in

areas with a shortage of dietary vitamin A,[105] a deficiency which each year is estimated to kill 670,000 children under the age of 5[106] and cause an additional 500,000 cases of irreversible childhood blindness.[107] The original golden rice produced 1.6μg/g of the carotenoids, with further development increasing this 23 times.[108] In 2018 it gained its first approvals for use as food.[109]

Wheat[edit]

As of December 2017, genetically modified wheat has been evaluated in field trials, but has not been released commercially.[110][111][112]

Derivative products[edit]

Corn starch and starch sugars, including syrups[edit]

Starch or amylum is a polysaccharide produced by all green plants as an energy store. Pure starch is a white, tasteless and odourless powder. It consists of two types of molecules: the linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight.[113]

Starch can be further modified to create modified starch for specific purposes,[114] including creation of many of the sugars in processed foods. They include:

- Maltodextrin, a lightly hydrolyzed starch product used as a bland-tasting filler and thickener.
- Various glucose syrups, also called corn syrups in the US, viscous solutions used as sweeteners and thickeners in many kinds of processed foods.
- Dextrose, commercial glucose, prepared by the complete hydrolysis of starch.
- High fructose syrup, made by treating dextrose solutions with the enzyme glucose isomerase, until a substantial fraction of the glucose has been converted to fructose.
- Sugar alcohols, such as maltitol, erythritol, sorbitol, mannitol and hydrogenated starch hydrolysate, are sweeteners made by reducing sugars.

Lecithin[edit]

Lecithin is a naturally occurring lipid. It can be found in egg yolks and oil-producing plants. It is an emulsifier and thus is used in many foods. Corn, soy and safflower oil are sources of lecithin, though the majority of lecithin commercially available is derived from soy.[115][116][117][*page needed*] Sufficiently processed lecithin is often undetectable with standard testing practices.[113][*failed verification*] According to the FDA, no evidence shows or suggests hazard to the public when lecithin is used at common levels. Lecithin added to foods amounts to only 2 to 10 percent of the 1 to 5 g of phosphoglycerides consumed daily on average.[115][116] Nonetheless, consumer concerns about GM food extend to such products.[118][*better source needed*] This concern led to policy and regulatory changes in Europe in 2000,[*citation needed*] when Regulation (EC) 50/2000 was passed[119] which required labelling of food containing additives derived from GMOs, including lecithin.[*citation needed*] Because of the difficulty of detecting the origin of derivatives like lecithin with current testing practices, European regulations require those who wish to sell lecithin in Europe to employ a comprehensive system of Identity preservation (IP).[120][*verification needed*][121][*page needed*]

Sugar[edit]

The US imports 10% of its sugar, while the remaining 90% is extracted from sugar beet and sugarcane. After deregulation in 2005, glyphosate-resistant sugar beet was extensively adopted in the United States. 95% of beet acres in the US were planted with glyphosate-resistant seed in 2011.[122] GM sugar beets are approved for cultivation in the US, Canada and Japan; the vast majority are grown in the US. GM beets are approved for import and consumption in Australia, Canada, Colombia, EU, Japan, Korea, Mexico, New Zealand, Philippines, the Russian Federation and Singapore.[123] Pulp from the refining process is used as animal feed. The sugar produced from GM sugar beets contains no DNA or protein – it is just sucrose that is chemically indistinguishable from sugar produced from non-GM sugar beets.[113][124] Independent analyses conducted by internationally recognized laboratories found that sugar from Roundup Ready sugar beets is identical to the sugar from comparably grown conventional (non-Roundup Ready) sugar beets.[125]

Vegetable oil[edit]

Most vegetable oil used in the US is produced from GM crops canola,[126] corn,[127][128] cotton[129] and soybeans.[130] Vegetable oil is sold directly to consumers as cooking oil, shortening and margarine[131] and is used in prepared foods. There is a vanishingly small amount of protein or DNA from the original crop in vegetable oil.[113][132] Vegetable oil is made of triglycerides extracted from plants or seeds and then refined and may be further processed via hydrogenation to turn liquid oils into solids. The refining process removes all, or nearly all non-triglyceride ingredients.[133] Medium-chain triglycerides (MCTs) offer an alternative to conventional fats and oils. The length of a fatty acid influences its fat absorption during the digestive process. Fatty acids in the middle position on the glycerol molecules appear to be absorbed more easily and influence metabolism more than fatty acids on the end positions. Unlike ordinary fats, MCTs are metabolized like carbohydrates. They have exceptional oxidative stability, and prevent foods from turning rancid readily.[134]

Other uses[edit]

Animal feed[edit]

Livestock and poultry are raised on animal feed, much of which is composed of the leftovers from processing crops, including GM crops. For example, approximately 43% of a canola seed is oil. What remains after oil extraction is a meal that becomes an ingredient in animal feed and contains canola protein.[135] Likewise, the bulk of the soybean crop is grown for oil and meal. The high-protein defatted and toasted soy meal becomes livestock feed and dog food. 98% of the US soybean crop goes for livestock feed.[136][137] In 2011, 49% of the US maize harvest was used for livestock feed (including the percentage of waste from distillers grains).[138] "Despite methods that are becoming more and more sensitive, tests have not yet been able to establish a difference in the meat, milk, or eggs of animals depending on the type of feed they are fed. It is impossible to tell if an animal was fed GM soy just by looking at the resulting meat, dairy, or egg products. The only way to verify the presence of GMOs in animal feed is to analyze the origin of the feed itself."[139]

A 2012 literature review of studies evaluating the effect of GM feed on the health of animals did not find evidence that animals were adversely affected, although small biological differences

were occasionally found. The studies included in the review ranged from 90 days to two years, with several of the longer studies considering reproductive and intergenerational effects.[140]

Enzymes produced by genetically modified microorganisms are also integrated into animal feed to enhance availability of nutrients and overall digestion. These enzymes may also provide benefit to the gut microbiome of an animal, as well as hydrolyse antinutritional factors present in the feed.[141]

Proteins[edit]

Rennet is a mixture of enzymes used to coagulate milk into cheese. Originally it was available only from the fourth stomach of calves, and was scarce and expensive, or was available from microbial sources, which often produced unpleasant tastes. Genetic engineering made it possible to extract rennet-producing genes from animal stomachs and insert them into bacteria, fungi or yeasts to make them produce chymosin, the key enzyme.[142][143] The modified microorganism is killed after fermentation. Chymosin is isolated from the fermentation broth, so that the Fermentation-Produced Chymosin (FPC) used by cheese producers has an amino acid sequence that is identical to bovine rennet.[144] The majority of the applied chymosin is retained in the whey. Trace quantities of chymosin may remain in cheese.[144]

FPC was the first artificially produced enzyme to be approved by the US Food and Drug Administration.[35][36] FPC products have been on the market since 1990 and as of 2015 had yet to be surpassed in commercial markets.[145] In 1999, about 60% of US hard cheese was made with FPC.[146] Its global market share approached 80%.[147] By 2008, approximately 80% to 90% of commercially made cheeses in the US and Britain were made using FPC.[144]

In some countries, recombinant (GM) bovine somatotropin (also called rBST, or bovine growth hormone or BGH) is approved for administration to increase milk production. rBST may be present in milk from rBST treated cows, but it is destroyed in the digestive system and even if directly injected into the human bloodstream, has no observable effect on humans.[148][149][150] The FDA, World Health Organization, American Medical Association, American Dietetic Association and the National Institutes of Health have independently stated that dairy products and meat from rBST-treated cows are safe for human consumption.[151] However, on 30 September 2010, the United States Court of Appeals, Sixth Circuit, analyzing submitted evidence, found a "compositional difference" between milk from rBGH-treated cows and milk from untreated cows.[152][153] The court stated that milk from rBGH-treated cows has: increased levels of the hormone Insulin-like growth factor 1 (IGF-1); higher fat content and lower protein content when produced at certain points in the cow's lactation cycle; and more somatic cell counts, which may "make the milk turn sour more quickly".[153]

Livestock[edit]

Main article: Genetically modified livestock

Genetically modified livestock are organisms from the group of cattle, sheep, pigs, goats, birds, horses and fish kept for human consumption, whose genetic material (DNA) has been altered using genetic engineering techniques. In some cases, the aim is to introduce a new trait to the animals which does not occur naturally in the species, i.e. transgenesis.

A 2003 review published on behalf of Food Standards Australia New Zealand examined transgenic experimentation on terrestrial livestock species as well as aquatic species such as fish and shellfish. The review examined the molecular techniques used for experimentation as well as

techniques for tracing the transgenes in animals and products as well as issues regarding transgene stability.[154]

Some mammals typically used for food production have been modified to produce non-food products, a practice sometimes called Pharming.

Salmon*[edit]*

See also: Genetically modified fish § AquAdvantage salmon, and Genetically modified fish § AquAdvantage salmon 2

A GM salmon, awaiting regulatory approval[155][156][5] since 1997,[157] was approved for human consumption by the American FDA in November 2015, to be raised in specific land-based hatcheries in Canada and Panama.[158]

Health and safety[edit]

See also: Genetically modified food controversies § Health

There is a scientific consensus[7][8][9][10] that currently available food derived from GM crops poses no greater risk to human health than conventional food,[11][12][13][14][15] but that each GM food needs to be tested on a case-by-case basis before introduction.[16][17][18] Nonetheless, members of the public are much less likely than scientists to perceive GM foods as safe.[19][20][21][22] The legal and regulatory status of GM foods varies by country, with some nations banning or restricting them, and others permitting them with widely differing degrees of regulation.[23][24][25][26]

Opponents claim that long-term health risks have not been adequately assessed and propose various combinations of additional testing, labeling[159] or removal from the market.[160][161][162][163] The advocacy group European Network of Scientists for Social and Environmental Responsibility (ENSSER), disputes the claim that "science" supports the safety of current GM foods, proposing that each GM food must be judged on case-by-case basis.[164]

Testing[edit]

The legal and regulatory status of GM foods varies by country, with some nations banning or restricting them, and others permitting them with widely differing degrees of regulation.[23][24][25][26] Countries such as the United States, Canada, Lebanon and Egypt use *substantial equivalence* to determine if further testing is required, while many countries such as those in the European Union, Brazil and China only authorize GMO cultivation on a case-by-case basis. In the U.S. the FDA determined that GMO's are "Generally Recognized as Safe" (GRAS) and therefore do not require additional testing if the GMO product is substantially equivalent to the non-modified product.[165] If new substances are found, further testing may be required to satisfy concerns over potential toxicity, allergenicity, possible gene transfer to humans or genetic outcrossing to other organisms.[28]

Regulation[edit]

See also: Regulation of genetic engineering

Green: Mandatory labeling required; Red: Ban on import and cultivation of genetically engineered food.

Government regulation of GMO development and release varies widely between countries. Marked differences separate GMO regulation in the U.S. and GMO regulation in the European Union.[26] Regulation also varies depending on the intended product's use. For example, a crop not intended for food use is generally not reviewed by authorities responsible for food safety.[166]

United States regulations[edit]

Main article: Genetic engineering in the United States § Regulation

In the U.S., three government organizations regulate GMOs. The FDA checks the chemical composition of organisms for potential allergens. The United States Department of Agriculture (USDA) supervises field testing and monitors the distribution of GM seeds. The United States Environmental Protection Agency (EPA) is responsible for monitoring pesticide usage, including plants modified to contain proteins toxic to insects. Like USDA, EPA also oversees field testing and the distribution of crops that have had contact with pesticides to ensure environmental safety.[167][*better source needed*] In 2015 the Obama administration announced that it would update the way the government regulated GM crops.[168]

In 1992 FDA published "Statement of Policy: Foods derived from New Plant Varieties". This statement is a clarification of FDA's interpretation of the Food, Drug, and Cosmetic Act with respect to foods produced from new plant varieties developed using recombinant deoxyribonucleic acid (rDNA) technology. FDA encouraged developers to consult with the FDA regarding any bioengineered foods in development. The FDA says developers routinely do reach out for consultations. In 1996 FDA updated consultation procedures.[169][170]

The StarLink corn recalls occurred in the autumn of 2000, when over 300 food products were found to contain a genetically modified corn that had not been approved for human consumption.[171] It was the first-ever recall of a genetically modified food.

Labeling[edit]

As of 2015, 64 countries require labeling of GMO products in the marketplace.

US and Canadian national policy is to require a label only given significant composition differences or documented health impacts, although some individual US states (Vermont, Connecticut and Maine) enacted laws requiring them.[172][173][174][175] In July 2016, Public Law 114-214 was enacted to regulate labeling of GMO food on a national basis.

In some jurisdictions, the labeling requirement depends on the relative quantity of GMO in the product. A study that investigated voluntary labeling in South Africa found that 31% of products labeled as GMO-free had a GM content above 1.0%.[176]

In the European Union all food (including processed food) or feed that contains greater than 0.9% GMOs must be labelled.[177]

Detection[edit]

Main article: Detection of genetically modified organisms

Testing on GMOs in food and feed is routinely done using molecular techniques such as PCR and bioinformatics.[178]

In a January 2010 paper, the extraction and detection of DNA along a complete industrial soybean oil processing chain was described to monitor the presence of Roundup Ready (RR) soybean: "The amplification of soybean lectin gene by end-point polymerase chain reaction (PCR) was successfully achieved in all the steps of extraction and refining processes, until the fully refined soybean oil. The amplification of RR soybean by PCR assays using event-specific primers was also achieved for all the extraction and refining steps, except for the intermediate steps of refining (neutralisation, washing and bleaching) possibly due to sample instability. The real-time PCR assays using specific probes confirmed all the results and proved that it is possible to detect and quantify genetically modified organisms in the fully refined soybean oil. To our knowledge, this has never been reported before and represents an important accomplishment regarding the traceability of genetically modified organisms in refined oils."[179]

According to Thomas Redick, detection and prevention of cross-pollination is possible through the suggestions offered by the Farm Service Agency (FSA) and Natural Resources Conservation Service (NRCS). Suggestions include educating farmers on the importance of coexistence, providing farmers with tools and incentives to promote coexistence, conduct research to understand and monitor gene flow, provide assurance of quality and diversity in crops, provide compensation for actual economic losses for farmers.[180]

Controversies[edit]

Main article: Genetically modified food controversies

The genetically modified foods controversy consists of a set of disputes over the use of food made from genetically modified crops. The disputes involve consumers, farmers, biotechnology companies, governmental regulators, non-governmental organizations, environmental and political activists and scientists. The major disagreements include whether GM foods can be safely consumed, harm the environment and/or are adequately tested and regulated.[161][181] The objectivity of scientific research and publications has been challenged.[160] Farming-related disputes include the use and impact of pesticides, seed production and use, side effects on non-GMO crops/farms,[182] and potential control of the GM food supply by seed companies.[160]

The conflicts have continued since GM foods were invented. They have occupied the media, the courts,[183] local, regional, national governments, and international organizations.[*citation needed*]

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited 'A' Grade by NAAC / 12B Status by UGC / Approved by AICTE

www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

M.TECH - BIOTECHNOLOGY

UNIT – V – FOOD & NUTRACEUTICALS – SBTA7013

An **energy drink** is a type of drink containing stimulant compounds, usually caffeine, which is marketed as providing mental and physical stimulation (marketed as "energy", but distinct from food energy). They may or may not be carbonated and may also contain sugar, other sweeteners, herbal extracts, taurine, and amino acids. They are a subset of the larger group of energy products, which includes bars and gels, and distinct from sports drinks, which are advertised to enhance sports performance. There are many brands and varieties in this drink category.

Coffee, tea and other naturally caffeinated drinks are usually not considered energy drinks. Other soft drinks such as cola may contain caffeine, but are not considered energy drinks either. Some alcoholic drinks, such as Buckfast Tonic Wine, contain caffeine and other stimulants. According to the Mayo Clinic, it is safe for the typical healthy adult to consume a total of 400 mg of caffeine a day. This has been confirmed by a panel of the European Food Safety Authority, which also concludes that a caffeine intake of up to 400 mg per day does not raise safety concerns for adults. According to the ESFA this is equivalent to 4 cups of coffee (90 mg each) or 2 1/2 standard cans (250 ml) of energy drink (160 mg each/80 mg per serving).[1][2]

Energy drinks have the effects of caffeine and sugar, but there is little or no evidence that the wide variety of other ingredients have any effect.[3] Most effects of energy drinks on cognitive performance, such as increased attention and reaction speed, are primarily due to the presence of caffeine.[4] Other studies ascribe those performance improvements to the effects of the combined ingredients.[5] Advertising for energy drinks usually features increased muscle strength and endurance, but there is no scientific consensus to support these claims.[6] Energy drinks have been associated with many health risks, such as an increased rate of injury when usage is combined with alcohol,[7] and excessive or repeated consumption can lead to cardiac and psychiatric conditions.[8][9] Populations at risk for complications from energy drink consumption include youth, caffeine-naïve or caffeine-sensitive, pregnant, competitive athletes and people with underlying cardiovascular disease.

Uses[edit]

Energy drinks are marketed to provide the benefits among health effects of caffeine along with benefits from the other ingredients they contain.[11] Health experts agree that energy drinks which contain caffeine do improve alertness.[11] The consumption of alcoholic drinks combined with energy drinks is a common occurrence on many high school and college campuses.[12] The alcohol industry has recently been criticized for marketing cohesiveness of alcohol and energy drinks. The combination of the two in college students is correlated to students experiencing alcohol-related consequences, and several health risks.[12]

There is no reliable evidence that other ingredients in energy drinks provide further benefits, even though the drinks are frequently advertised in a way that suggests they have unique benefits.[11][13] The dietary supplements in energy drinks may be purported to provide produce benefits, such as for vitamin B12,[11][14] but no claims of using supplements to enhance health in otherwise normal people have been verified scientifically. Various marketing organizations such as Red Bull and Monster have described energy drinks by saying their product "gives you wings",[15] is "scientifically formulated", or is a "killer energy brew".[11] Marketing of energy drinks has been particularly directed towards teenagers, with manufacturers sponsoring or

advertising at extreme sports events and music concerts, and targeting a youthful audience through social media channels.[16]

When mixed with alcohol, either as a prepackaged caffeinated alcoholic drink, a mixed drink, or just a drink consumed alongside alcohol, energy drinks are often consumed in social settings.

Effects[edit]

A health warning on a can of the Austrian Power Horse energy drink

Energy drinks have the effects caffeine and sugar provide, but there is little or no evidence that the wide variety of other ingredients have any effect.[3] Most of the effects of energy drinks on cognitive performance, such as increased attention and reaction speed, are primarily due to the presence of caffeine.[4] Advertising for energy drinks usually features increased muscle strength and endurance, but there is little evidence to support this in the scientific literature.[6]

A caffeine intake of 400 mg per day (for an adult) is considered as safe from the European Food Safety Authority (EFSA).[2] Adverse effects associated with caffeine consumption in amounts greater than 400 mg include nervousness, irritability, sleeplessness, increased urination, abnormal heart rhythms (arrhythmia), and dyspepsia. Consumption also has been known to cause pupil dilation.[17][*medical citation needed*] Caffeine dosage is not required to be on the product label for food in the United States, unlike drugs, but most (although not all) place the caffeine content of their drinks on the label anyway, and some advocates are urging the FDA to change this practice.[18]

With alcohol[edit]

Combined use of caffeine and alcohol may increase the rate of alcohol-related injury.[7] Energy drinks can mask the influence of alcohol, and a person may misinterpret their actual level of intoxication.[19] Since caffeine and alcohol are both diuretics, combined use increases the risk of dehydration, and the mixture of a stimulant (caffeine) and depressant (alcohol) sends contradictory messages to the nervous system and can lead to increased heart rate and palpitations.[19] Although people decide to drink energy drinks with alcohol with the intent of counteracting alcohol intoxication, many others do so to hide the taste of alcohol.[20] However, in the 2015, the EFSA concluded, that "Consumption of other constituents of energy drinks at concentrations commonly present in such beverages would not affect the safety of single doses of caffeine up to 200 mg." Also the consumption of alcohol, leading to a blood alcohol content of about 0.08%, would, according to the EFSA, not affect the safety of single doses of caffeine up to 200 mg. Up to these levels of intake, caffeine is unlikely to mask the subjective perception of alcohol intoxication.[2]

Health problems

Excessive consumption of energy drinks can have serious health effects resulting from high caffeine and sugar intakes, particularly in children, teens, and young adults.[21][22] Excessive energy drink consumption may disrupt teens' sleep patterns and may be associated with increased risk-taking behavior.[21] Excessive or repeated consumption of energy drinks can lead to cardiac problems, such as arrhythmias and heart attacks, and psychiatric conditions such as anxiety and phobias.[8][9][21] In Europe, energy drinks containing sugar and caffeine have been associated with the deaths of athletes.[23] Reviews have noted that caffeine content was not the only factor, and that the cocktail of other ingredients in energy drinks made them more dangerous than drinks whose only stimulant was caffeine; the studies noted that more research and government regulation were needed.[21][24]

Research suggests that emergency department (ED) visits are on the increase. In 2005, there were 1,494 emergency department visits related to energy drink consumption in the United States; whereas, in 2011, energy drinks were linked to 20,783 emergency department visits.[25] During this period of increase, male consumers consistently had a higher likelihood of visiting the emergency department over their female counterparts.[25] Research trends also show that emergency department visits are caused mainly by adverse reactions to the drinks. In 2011, there were 14,042 energy drink-related hospital visits.[25] Misuse and abuse of these caffeinated drinks also cause a significant amount of emergency department visits. By 2011, there were 6,090 visits to the ED due to misuse/abuse of the drinks.[25] In many cases 42% of patients had mixed energy drinks with another stimulant, and in the other 58% of cases the energy drink was the only thing that had been consumed.[26] Several studies suggest that energy drinks may be a gateway drug.[7]

The American Academy of Pediatrics recommends that children not consume caffeinated energy drinks.

Immune Booster Foods

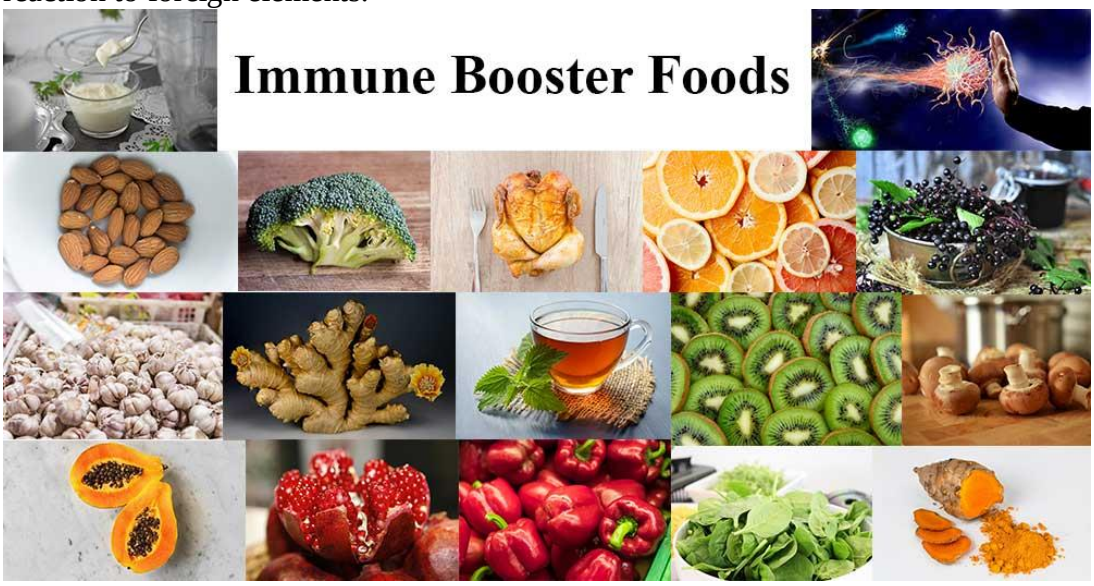
Immunity definition

Immunity is the main process that defends the body from infections and sometimes, the body may be unable to elicit enough responses that can eliminate them. Therefore, it is important to consume immune-boosting foods to strengthen the body against seasonal infections such as cases of flu and viral infections, cancer, arthritis, allergies.

Sometime, during a cold or flu season, one will wonder why some people do not get infected despite being in contact with those infected, considering the flu and cold are spread by air droplets. Yes, its because some people have a stronger immune system as compared to others. How does this happen? Remember, the immune system is the first line of defense against antigens that invade the body. so, with a strong immune system, there is less likelihood of contracting some illnesses but not totally.

A change in diet can, however, boost and strengthen the immunity of a person with weakened immune systems such as persons with lifestyle disorders like diabetes hypertension, some immune deficiency diseases like HIV/AIDs, immune-suppressed patients like those under

medication, etc. An immunity that is suppressed or compromised, it is important that such an individual is encouraged and administered with immune-boosting foods to strengthen them in reaction to foreign elements.



How the Immune System Works

The immune system is a natural defense system made up of a wide range of cells, tissues, and organs that work together to defend the body from foreign elements, known as antigens. These foreign elements include bacteria, viruses, fungi, and parasites, which confer infections to the body making one feel or become sick.

These foreign bodies are found in a wide range of environments so they can be acquired and transmitted in several other ways. A healthy body means a healthy immune system that has the ability to protect itself against these antigens. The body naturally produces white blood cells which play a key role in producing immune cells for body defense. The body also produces chemicals and proteins that can also attack and eliminate the antigens. The body's defense mechanism finds the antigens and eliminates it before it can be able to replicate and spread.

Depending on the load of the antigen, the immune system responds rapidly by producing the specific responders against the antigen in large quantities. The effectiveness of the immune system is favored by its ability to recognize millions of antigens and producing specific immune molecules to combat the antigens.

Besides ita ability to attack the millions of antigens, it is essential to boost the immunity to act viciously against the antigens. To boost immunity means intake or consumption of certain food that provides additional benefits to the body. To boost immunity its important to take the right kind of foods in the right quantities.

Research has indicated that foods that have vitamin C are huge immune boosters especially for flu and the common cold.

Some of these foods that boost immunity and offer many benefits to the body include:

(Alphabetical Order)

1. Almonds as Immune Booster Foods

Almonds are a type of dry fruits that are majorly consumed to prevent and fight off colds. They are small in size but they contain a lot of nutrients beneficial to the body, whole body pulp made up of vitamins and minerals and healthy fats.

The benefits of almonds include:

- Lowering blood pressure
- Control blood sugar
- Regulate cholesterol level
- Ellevate constipation, respiratory disorders, and anemia.
- They also help in hair repair and growth, nail strengthening, and dental strength and care.
- They have Vitamin E and C which are great immune boosters
- A nut contains 5 grams of carbohydrates which maintain a low-carb level in the diet.
- It is rich in antioxidants that regulate free radicals preventing infections, with an anti-inflammatory effect,immune-boosting properties, and anti-hepatotoxicity effect.
- Almonds improve the movement of food through the colon, thereby preventing build-up and possibly the subsequent colon cancer.
- The presence of Vitamin E is an effective antioxidant that reduces the risks of heart diseases and coupled with magnesium they help prevent heart attacks.
- Almond oil is a nutritive element providing good health and functioning of the nervous systems. As a dry fruit, it contains riboflavin and L-carnitine which have been associated with increased brain development and activity. They are recommended for children to help brain development. They also reduce the risks of Alzheimer's disease.
- The vitamins, minerals, and phosphorus have been linked to bone development and strengthening.
- Almond oil is used for massaging and improves skin appearance and reverses signs of aging.

Almonds can be consumed in different ways including, making almond milk a nutritive beverage better than milk; can be used as a top-up in yogurt or oatmeal; grinding the nuts to make powder which can be used to prepare almond butter by adding salt; sprinkling almond powder in vegetables and salads; used as a garnish in dishes and pizzas.

2. Broccoli as Immune Booster Foods

- This is a vegetable that is rich in vitamins and minerals and fiber.
- They have Vitamin A, C, and E, with several antioxidants, qualifying then as one of the healthiest vegetables.
- To ensure that you get all the nutrients from broccoli, cook it for a short time or eat it raw.

3. Citrus fruit as Immune Booster Foods

- This is a vitamin C food that mainly helped in treating flu and colds.
- They boost the immune system by increasing white blood cell production, which are the major elements involved in protecting and fighting infections in the body.
- These fruits include lemons, oranges, limes, grapefruits, tangerine, and clementines.
- However, it is important to consume citrus fruits daily to boost immunity continuously because the body does not produce vitamin C naturally.
- They can be consumed whole or they can be squeezed into the meal while eating.

4. Elderberry as Immune Booster Foods

- This is a type of bush tree whose berries and flowers have been studied and known for their health benefits for centuries now. It is scientifically known as *Sambucus nigra.*
- It has been used to make syrups, jams, food colors, wine, and lozenges.

- Elderberry extracts have proved to have antibacterial, antiviral, anticancer and anti-inflammatory properties.
- It has several flavonoids.
- Elderberries are added in syrups which are used as remedies for colds, flu, and bacterial sinus infections. It reduces the swelling of the mucus membrane.
- Being a plant that helps in the management of flu, it may also be helpful in providing a stronger immune response against COVID-19.
- They have a laxative effect.
- They also lower sugar levels for diabetic patients.
- Generally, elderberries stimulate the immune system to produce immune elements that protect the body from foreign agents.

- Elderberries have been used to manage conditions such as;
- Headache
- Fever
- Constipation
- Upper and lower respiratory infections
- Joint and muscle pain
- kidney conditions
- epilepsy
- Minor skin conditions
- Stress

NOTE: Elderberry can counteract with certain medications such as diuretics, laxatives, steroids, chemotherapy, diabetes medications, and theophylline/ therefore it is important to check with a doctor or/and pharmacist if you are under medication before taking the elderberries to prevent interactions that may harm the body.

In addition to counterreaction with certain drugs, pregnant women should also consult with the doctor before taking them. In case of allergic reactions, terminate the consumption of the berries.

5. Garlic as Immune Booster Foods

- Garlic is commonly used to cook food to add a little zing to a meal.
- It is the most popular supplement that is well known for its ability to boost immunity.
- For centuries now, garlic has been used to manage superficial and systemic infections. It has antibacterial, antiviral and antifungal properties.
- The garlic bulbs are also rich in antioxidants that contain free radicals that can boost the treatment of Alzheimer's, cancers and heart conditions among others. it is commonly known for its role in managing and reduction of cold and flu symptoms.
- Especially during this season of COVID-19 outbreak, garlic consumption has become an essential part of most people's diets.
- Research has shown that people who consume garlic in their diet have fewer occurrences of cold and flu as compared to those who do not take it at all or those who use over the counter medications.
- Even so, the consumption of garlic helps shorten the period of flu and cold infections.

- It has also been documented that garlic helps reduce rates of different kinds of cancer for example, people that consume garlic have reduced cases of colorectal cancers, pancreatic and liver cancer.
- It also plays a major role in reducing blood pressure and the reduction of the hardening of arteries.
- It is also a good relief remedy for the chronic pain caused by arthritis.
- The immune-boosting properties of garlic are due to the presence of high concentrations of sulfur-containing compounds such as allicin.

6. Ginger as Immune Booster Foods

- This is a root tuber that's known for its anti-inflammatory effect against flu and cold, especially when an individual has a sore throat and/or inflammatory illnesses.
- It also reduces nausea.
- Ginger contains gingerol which helps reduce chronic pain and also reduces cholesterol levels.
- It has several antioxidants which are very effective anti-inflammatory and immune-boosting elements. These effects are enabled by the mechanisms elicited by the body during an infection whereby, free radicals are the body causing oxidative stress. These free radicals combine with the antioxidants produced by ginger causing the anti-inflammatory effect and the immune-boosting properties.
- The anti-inflammatory effects help protect the body against arthritis, cancers, neurodegenerative disorders, and hypersensitivity.
- It has also been proven that ginger has antibacterial and antiviral effects.

- Ginger can be eaten in various ways:
- Adding grated ginger in hot tea or chocolate
- Grated ginger can also be added to pastries when cooking such as muffins and cupcakes, cookies, etc
- It can be used to cook and marinate meats and chicken and vegetables as well.
- It can be added to honey and hot/warm water.

7. Green Tea as Immune Booster Foods

- This is a herbal type of beverage packed with flavonoids.
- They are natural anti-oxidants, which has several polyphenols with anti-inflammatory properties which prevents premature aging.
- The antioxidants protect against cell damage which may cause chronic illnesses.
- They also have a common antioxidant known as epigallocatechin gallate, EGCG which has immune-boosting properties.
- Green tea has caffeine and L-theanine an amino acid that provides the calming effect, by optimizing brain function and enhance memory, elevate mood and cognitive performance.
- L-theanine also helps in the production of T-cells that fight germs.
- Green tea's ability to counter oxidative stress also makes it a potent protector against neurodegenerative disease, including Alzheimer's disease and Parkinson's disease.
- It also helps manage and maintain body weight but reducing the cholesterol content

- They are great immune boosters because they have antioxidants
- They have an anti-angiogenesis activity, therefore, reducing the risks of cancer and also blocking the spread of cancer cells.
- They help strengthen bone density and bone development preventing occurrences of osteoarthritis and bone degenerative defects.
- They help in balancing sugar levels and reduce blood sugar in diabetics.
- They also maintain blood pressure and prevent heart attacks and strokes.
- They protect the skin from ultraviolet rays thus reducing skin aging.

Toxicology is a scientific discipline, overlapping with biology, chemistry, pharmacology, and medicine, that involves the study of the adverse effects of chemical substances on living organisms[1] and the practice of diagnosing and treating exposures to toxins and toxicants. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. Factors that influence chemical toxicity include the dosage, duration of exposure (whether it is acute or chronic), route of exposure, species, age, sex, and environment. **Toxicologists** are experts on poisons and poisoning. There is a movement for evidence-based toxicology as part of the larger movement towards evidence-based practices. Toxicology is currently contributing to the field of Cancer research, since some toxins can be used as drugs for killing tumor cells. One prime example of this is Ribosome Inactivating Proteins, tested in the treatment of Leukemia.[2]

The word *toxicology* (/ˌtɒksɪˈkɒlədʒi/) is a neoclassical compound from New Latin, first attested circa 1799,[3] from the combining forms *toxico-* + *-logy*, which in turn come from the Ancient Greek words τοξικός *toxikos*, "poisonous", and λόγος *logos*, "subject matter").

Basic principles[edit]

The goal of toxicity assessment is to identify adverse effects of a substance.[11] Adverse effects depend on two main factors: i) routes of exposure (oral, inhalation, or dermal) and ii) dose (duration and concentration of exposure). To explore dose, substances are tested in both acute and chronic models.[12] Generally, different sets of experiments are conducted to determine whether a substance causes cancer and to examine other forms of toxicity.[12]

Factors that influence chemical toxicity:[8]

- Dosage
  - Both large single exposures (acute) and continuous small exposures (chronic) are studied.
- Route of exposure
  - Ingestion, inhalation or skin absorption
- Other factors
  - Species
  - Age

- Sex
- Health
- Environment
- Individual characteristics

The discipline of evidence-based toxicology strives to transparently, consistently, and objectively assess available scientific evidence in order to answer questions in toxicology,[13] the study of the adverse effects of chemical, physical, or biological agents on living organisms and the environment, including the prevention and amelioration of such effects.[14] Evidence-based toxicology has the potential to address concerns in the toxicological community about the limitations of current approaches to assessing the state of the science.[15][16] These include concerns related to transparency in decision making, synthesis of different types of evidence, and the assessment of bias and credibility.[17][18][19] Evidence-based toxicology has its roots in the larger movement towards evidence-based practices.

Testing methods[edit]

Toxicity experiments may be conducted *in vivo* (using the whole animal) or *in vitro* (testing on isolated cells or tissues), or *in silico* (in a computer simulation).[20]

Non-human animals[edit]

The classic experimental tool of toxicology is testing on non-human animals.[8] Example of model organisms are *Galleria mellonella*, [21] which can replace small mammals, and Zebrafish, which allow for the study of toxicology in a lower order vertebrate in vivo.[22][23] As of 2014, such animal testing provides information that is not available by other means about how substances function in a living organism.[24] The use of non-human animals for toxicology testing is opposed by some organisations for reasons of animal welfare, and it has been restricted or banned under some circumstances in certain regions, such as the testing of cosmetics in the European Union.[25]

Alternative testing methods[edit]

While testing in animal models remains as a method of estimating human effects, there are both ethical and technical concerns with animal testing.[26]

Since the late 1950s, the field of toxicology has sought to reduce or eliminate animal testing under the rubric of "Three Rs" - reduce the number of experiments with animals to the minimum necessary; refine experiments to cause less suffering, and replace *in vivo* experiments with other types, or use more simple forms of life when possible.[27][28]

Computer modeling is an example of alternative testing methods; using computer models of chemicals and proteins, structure-activity relationships can be determined, and chemical structures that are likely to bind to, and interfere with, proteins with essential functions, can be identified.[29] This work requires expert knowledge in molecular modeling and statistics together with expert judgment in chemistry, biology and toxicology.[29]

In 2007 the American NGO National Academy of Sciences published a report called "Toxicity Testing in the 21st Century: A Vision and a Strategy" which opened with a statement: "Change often involves a pivotal event that builds on previous history and opens the door to a new era. Pivotal events in science include the discovery of penicillin, the elucidation of the DNA double

helix, and the development of computers. ...Toxicity testing is approaching such a scientific pivot point. It is poised to take advantage of the revolutions in biology and biotechnology. Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."[30] As of 2014 that vision was still unrealized.[24][31]

The United States Environmental Protection Agency studied 1,065 chemical and drug substances in their ToxCast program (part of the CompTox Chemicals Dashboard) using *in silica* modelling and a human pluripotent stem cell-based assay to predict *in vivo* developmental intoxicants based on changes in cellular metabolism following chemical exposure. Major findings from the analysis of this ToxCast_STM dataset published in 2020 include: (1) 19% of 1065 chemicals yielded a prediction of developmental toxicity, (2) assay performance reached 79%–82% accuracy with high specificity (> 84%) but modest sensitivity (< 67%) when compared with *in vivo* animal models of human prenatal developmental toxicity, (3) sensitivity improved as more stringent weights of evidence requirements were applied to the animal studies, and (4) statistical analysis of the most potent chemical hits on specific biochemical targets in ToxCast revealed positive and negative associations with the STM response, providing insights into the mechanistic underpinnings of the targeted endpoint and its biological domain.[32]

In some cases shifts away from animal studies have been mandated by law or regulation; the European Union (EU) prohibited use of animal testing for cosmetics in 2013.[33]

Dose response complexities[edit]

Most chemicals display a classic dose response curve – at a low dose (below a threshold), no effect is observed.[8]:80 Some show a phenomenon known as sufficient challenge – a small exposure produces animals that "grow more rapidly, have better general appearance and coat quality, have fewer tumors, and live longer than the control animals".[34] A few chemicals have no well-defined safe level of exposure. These are treated with special care. Some chemicals are subject to bioaccumulation as they are stored in rather than being excreted from the body;[8]:85–90 these also receive special consideration.

Several measures are commonly used to describe toxic dosages according to the degree of effect on an organism or a population, and some are specifically defined by various laws or organizational usage. These include:

- LD50 = Median lethal dose, a dose that will kill 50% of an exposed population
- NOEL = No-Observed-Effect-Level, the highest dose known to show no effect
- NOAEL = No-Observed-Adverse-Effect-Level, the highest dose known to show no adverse effects
- PEL = Permissible Exposure Limit, the highest concentration permitted under US OSHA regulations
- STEL = Short-Term Exposure Limit, the highest concentration permitted for short periods of time, in general 15–30 minutes
- TWA = Time-Weighted Average, the average amount of an agent's concentration over a specified period of time, usually 8 hours.

- TTC = Threshold of Toxicological Concern have been established for the constituents of tobacco smoke[35]

Types[edit]

"Clinical toxicology" redirects here. For the journal, see Clinical Toxicology.

Medical toxicology is the discipline that requires physician status (MD or DO degree plus specialty education and experience).

Clinical toxicology is the discipline that can be practiced not only by physicians but also other health professionals with a master's degree in clinical toxicology: physician extenders (physician assistants, nurse practitioners), nurses, pharmacists, and allied health professionals.

Forensic toxicology is the discipline that makes use of toxicology and other disciplines such as analytical chemistry, pharmacology and clinical chemistry to aid medical or legal investigation of death, poisoning, and drug use. The primary concern for forensic toxicology is not the legal outcome of the toxicological investigation or the technology utilized, but rather the obtainment and interpretation of results.[36]

Computational toxicology is a discipline that develops mathematical and computer-based models to better understand and predict adverse health effects caused by chemicals, such as environmental pollutants and pharmaceuticals.[37] Within the *Toxicology in the 21st Century* project,[38][39] the best predictive models were identified to be Deep Neural Networks, Random Forest, and Support Vector Machines, which can reach the performance of in vitro experiments.[40][41][42][43]

Occupational toxicology is the application of toxicology to chemical hazards in the workplace.[44]

Toxicology as a profession

A toxicologist **is a scientist or medical personnel who specializes in the study of symptoms, mechanisms, treatments and detection of venoms and toxins; especially the poisoning of people.**

Requirements[edit]

To work as a toxicologist one should obtain a degree in toxicology or a related degree like biology, chemistry, pharmacology or biochemistry.[45][*citation needed*]Bachelor's degree programs in toxicology cover the chemical makeup of toxins and their effects on biochemistry, physiology and ecology. After introductory life science courses are complete, students typically enroll in labs and apply toxicology principles to research and other studies. Advanced students delve into specific sectors, like the pharmaceutical industry or law enforcement, which apply methods of toxicology in their work. The Society of Toxicology (SOT) recommends that undergraduates in postsecondary schools that don't offer a bachelor's degree in toxicology consider attaining a degree in biology or chemistry. Additionally, the SOT advises aspiring toxicologists to take statistics and mathematics courses, as well as gain laboratory experience through lab courses, student research projects and internships.

Duties[edit]

Toxicologists perform many different duties including research in the academic, nonprofit and industrial fields, product safety evaluation, consulting, public service and legal regulation. In order to research and assess the effects of chemicals, toxicologists perform carefully designed studies and experiments. These experiments help identify the specific amount of a chemical that may cause harm and potential risks of being near or using products that contain certain chemicals. Research projects may range from assessing the effects of toxic pollutants on the environment to evaluating how the human immune system responds to chemical compounds within pharmaceutical drugs. While the basic duties of toxicologists are to determine the effects of chemicals on organisms and their surroundings, specific job duties may vary based on industry and employment. For example, forensic toxicologists may look for toxic substances in a crime scene, whereas aquatic toxicologists may analyze the toxicity level of water bodies.

Compensation[edit]

The salary for jobs in toxicology is dependent on several factors, including level of schooling, specialization, experience. The U.S. Bureau of Labor Statistics (BLS) notes that jobs for biological scientists, which generally include toxicologists, were expected to increase by 21% between 2008 and 2018. The BLS notes that this increase could be due to research and development growth in biotechnology, as well as budget increases for basic and medical research in biological science