Essentials of
Food Science and Nutrition

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Preface

The branch of science, which deals with the study of food as well as the fundamental phenomena that are directly linked to its production, is known as food science. It makes use of biological and physical sciences as well as engineering to study the principles behind food processing, the causes of deterioration and the nature of foods. There are various fields which contribute knowledge to food science such as physiology, biochemistry, chemistry, physics and microbiology. Food chemistry, food engineering, food microbiology and food preservation are some of the sub-disciplines of this field. Food chemistry studies the various chemical activities and interactions which take place between the varied non-biological and biological constituents of food. This book provides comprehensive insights into the field of nutrition and food science. It presents researches and studies performed by experts across the globe. Those in search of information to further their knowledge will be greatly assisted by this book.

A short introduction to every chapter is written below to provide an overview of the content of the book:

Chapter 1 - The branch of science which deals with the study of the chemical, physical and biological makeup of food is known as food science. Food technology is involved in the study of the fundamental phenomena which are connected to the production of food products as well as their properties. This is an introductory chapter which will introduce briefly all the significant aspects of food science and technology.; **Chapter 2** - The science of interpreting the interactions between nutrients and other materials in food with respect to the growth, health and disease in an organism is known as nutrition. Some of the sub-disciplines within nutrition are malnutrition and sports nutrition. The chapter closely examines these key concepts of nutrition to provide an extensive understanding of the subject.; **Chapter 3** - The nutrients are broadly classified into two categories, namely, micronutrients and macronutrients. Some of the micronutrients are vitamins and minerals while carbohydrates, proteins and fats fall under macronutrients. The topics elaborated in this chapter will help in gaining a better perspective about these different types of essential nutrients.; **Chapter 4** - The purpose of food supplements is to provide nutrients which an individual is not able to obtain through normal diet, or is consuming in insufficient quantities. The diverse applications as well as the health benefits of food supplements have been thoroughly discussed in this chapter.; **Chapter 5** - A chemical substance which is added to food in order to produce particular desirable effects is known as a food additive. Anticaking agents, food coloring and sugar substitutes are a few examples of commonly used food additives. The diverse applications of these additives have been thoroughly discussed in this chapter.; **Chapter 6** - Food science and nutrition deal with a lot of processes which are associated with food such as food safety, food preservation and food fortification. It also includes sub-disciplines like food chemistry, food biotechnology and food microbiology. This chapter discusses in detail these processes and disciplines related to food science and nutrition.

Finally, I would like to thank my fellow scholars who gave constructive feedback and my family members who supported me at every step.

Theo Graham

Chapter 1

Food Science and Technology

The branch of science which deals with the study of the chemical, physical and biological makeup of food is known as food science. Food technology is involved in the study of the fundamental phenomena which are connected to the production of food products as well as their properties. This is an introductory chapter which will introduce briefly all the significant aspects of food science and technology.

Food Science

Food science is a discipline which studies chemical, biological, physical and other processes that have an influence on food's safety, flavour, appearance, etc. But it is also interested in other food-related practices and activities including design, packaging, new product development, optimisation of food manufacturing processes and similar.

The main goal of food science is to ensure that food products on store shelves are safe, tasty and nutritious. But in the recent years, food scientists have also started to pay more attention to informing and educating the public about the importance of proper food handling, storage and processing. The aim is to reduce the incidence of food-borne illnesses and help consumers prepare tasty meals that are packed with vitamins, minerals and other essential nutrients.

Food Microbiology

Basically the study of how microorganisms interact with foods, food microbiology focuses on bacteria, molds, yeasts and viruses. Areas of particular importance to product development are food safety and quality. Food safety deals with preventing pathogens in foods. It's nearly impossible to eliminate microorganisms from any food, but many steps can be taken to slow their growth or inactivate them. What food science is doing in the way of food safety is developing very sensitive and rapid techniques to identify pathogenic microorganisms and the toxins they produce in foods, and developing packaging and processes that inhibit their growth and reduce their rate of survival. Not all microorganisms are harmful, but some cause food spoilage.

The key to controlling spoilage is killing as many bacteria as we can without also killing the quality of the food in which they reside. Food microbiology is one of the most important fields of food science when it comes to product development, especially in this age of litigation. When devising a development plan for a new product, it is essential to consider the microbial quality of each component and how that may affect the overall quality of the product—and to determine how to process the product so that it has a sufficient shelf life.

Food Engineering and Processing

As far as product developers go, food engineers develop the concepts that processors use to turn raw ingredients into safe, long-lasting foods. In menu development, engineers may not play a direct role, but processors will. How an ingredient is processed can have a dramatic effect on the flavor, color, safety and shelf life of food. Consider high-pressure processing (HPP) and its effect on the fresh fruit and vegetable juice industry. HPP is a non-thermal or "cold pasteurization" process developed to allow fruit and vegetable juices to be produced without any of the deleterious effects of thermal treatments, such as changes in flavor, texture or color. The nutritional profile of the juices is unaltered by the process, so fortification is not necessary.

An all-natural, healthy and flavorful product can be produced thanks to food processing engineers. HPP can also be used to process raw clams, destroying potentially harmful bacteria. Think of the potential in a seafood restaurant chain. Clams, even oysters could be kept alive until the customer orders them; place the clams/oysters in the HPP and in a minute they're shucked and cleared of potentially harmful microorganisms.

Food Chemistry and Biochemistry

All foods are biochemicals because in most cases foods or their raw ingredients are or were living entities. Understanding biochemistry can be of vital importance to a product developer. Take the activity of enzymes. Adding the enzyme lactase to milk cuts the disaccharide lactose into its individual components galactose and glucose, making it more digestible. This opens up a whole spectrum of products for the lactose-intolerant. But the enzyme has an optimal activity at a specific pH and temperature—it won't survive certain types of processing. A grounding in biochemistry provides the knowledge to develop a product that takes advantage of such compounds.

Sensory Analysis

Sensory science uses people to describe and evaluate flavors, textures, appearance and other attributes of foods. Sensory evaluation practices are integral to product developers. Sensory techniques can be used to narrow down concepts and choose protocepts, optimize a developing product and monitor and control quality of the finished product. Consumer tests can be used to evaluate preference and acceptance of a product. Products can be evaluated and optimized using quantitative descriptive analysis (QDA), in which a focus group defines the sensory attributes for a product and establishes numerical scores for these attributes. Establishing the appropriate measurement is of major importance, as it allows sensory results to be quantified. This is the essence of sensory science: converting human perception of a food into quantifiable results.

Food Technology

Food Technology is a science which deals with the techniques and principles involved in processing and preserving the food substances. The application of food science helps in manufacturing safe, wholesome and nutritious food products. The study of food technology is to develop new methods and systems for keeping food products safe and resistant from natural harms such as bacteria and other micro-organisms. Food processing helps in preservation enhances the flavor and reduces the toxins in the food product which results in better distributional efficiency and easy marketing of the food product.

The modern food processing techniques is the key to flourishing supermarkets we have today. Extra nutrients can be added while processing the food and processed food is less susceptible to spoilage. Some of the techniques used are spray drying, juice concentrates, freeze drying and the introduction of artificial sweeteners, colorants, and preservatives. Of late, many products such as dried instant soups, reconstituted fruits and juices, and self-cooking meals were developed for the convenience of working people.

The food processing industries are involved in processes such as primary and secondary processing, preservation, quality management, packaging and labeling of a variety of products such as dairy products, fish products, fruit & vegetable products, meat & poultry products, confectionery products and food grains.

Few specializations in this field are:

- Dairy
- Sugar
- Alcohol

- Bakery and confectionery items
- Oil and oil seed processing
- Fruits and vegetables
- Meat- Fish
- Cereals

Food Processing

Food processing is the set of methods and techniques used to transform raw ingredients into food or food into other forms for consumption by humans or animals either in the home or by the food processing industry. Food processing typically takes clean, harvested crops or slaughtered and butchered animal products and uses these to produce attractive, marketable, and often long-life food products. Similar processes are used to produce animal feed. Extreme examples of food processing include the expert removal of toxic portions of the fugu fish or preparing space food for consumption under zero gravity.

Examples of some processed foods

The benefits of food processing include the preservation, distribution, and marketing of food, protection from pathogenic microbes and toxic substances, year-round availability of many food items, and ease of preparation by the consumer. On the other hand, food processing can lower the nutritional value of foods, and processed foods may include additives (such as colorings, flavorings, and preservatives) that may have adverse health effects.

Food Processing Methods

Common food processing techniques include:

- Removal of unwanted outer layers, such as potato peeling or the skinning of peaches,

- Chopping or slicing, such as to produce diced carrots,
- Mincing and macerating,
- Liquefaction, such as to produce fruit juice,
- Fermentation, as in beer breweries,
- Emulsification,
- Cooking, by methods such as baking, boiling, broiling, frying, steaming, or grilling,
- Mixing,
- Addition of gas such as air entrainment for bread or gasification of soft drinks,
- Proofing,
- Spray drying,
- Pasteurization and
- Packaging.

Beer fermenting at a brewery

Performance Parameters for Food Processing

When designing processes for the food industry, the following performance parameters may be taken into account:

- Hygiene, measured, for instance, by the number of microorganisms per ml of finished product.
- Energy consumption, measured, for instance, by “ton of steam per ton of sugar produced”.
- Minimization of waste, measured, for instance, by the “percentage of peeling loss during the peeling of potatoes”.

- Labor used, measured, for instance, by the ”number of working hours per ton of finished product”.
- Minimization of cleaning stops, measured, for instance, by the “number of hours between cleaning stops”.

Benefits

More and more people live in the cities far away from where food is grown and produced. In many families, the adults are work from home and therefore there is little time for the preparation of food based on fresh ingredients. The food industry offers products that fulfill many different needs: from peeled potatoes that simply need to be boiled at home to fully prepared ready meals that can be heated up in the microwave oven in a few minutes.

Microwave oven

Benefits of food processing include toxin removal, preservation, easing marketing and distribution tasks, and increasing food consistency. In addition, it increases seasonal availability of many foods, enables transportation of delicate perishable foods across long distances, and makes many kinds of foods safe to eat by de-activating spoilage and pathogenic micro-organisms. Modern supermarkets would not be feasible without modern food processing techniques, long voyages would not be possible, and military campaigns would be significantly more difficult and costly to execute.

Modern food processing also improves the quality of life for allergy sufferers, diabetics, and other people who cannot consume some common food elements. Food processing can also add extra nutrients such as vitamins.

Processed foods are often less susceptible to early spoilage than fresh foods, and are better suited for long distance transportation from the source to the consumer. Fresh materials, such as fresh produce and raw meats, are more likely to harbor pathogenic microorganisms (for example, Salmonella) capable of causing serious illnesses.

Drawbacks

In general, fresh food that has not been processed other than by washing and simple kitchen preparation, may be expected to contain a higher proportion of naturally occurring vitamins, fiber and minerals than the equivalent product processed by the food industry. Vitamin C, for example, is destroyed by heat and therefore canned fruits have a lower content of vitamin C than fresh ones.

Food processing can lower the nutritional value of foods. Processed foods tend to include food additives, such as flavorings and texture enhancing agents, which may have little or no nutritive value, and some may be unhealthy. Some preservatives added or created during processing, such as nitrites or sulfites, may cause adverse health effects.

Processed foods often have a higher ratio of calories to other essential nutrients than unprocessed foods, a phenomenon referred to as “empty calories.” Most junk foods are processed, and fit this category.

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High quality and hygiene standards must be maintained to ensure consumer safety, and failure to maintain adequate standards can have serious health consequences.

Processing food is a very costly process, thus increasing the prices of foods products.

Trends in Modern Food Processing

Health

- Reduction of fat content in final product, for example, by using baking instead of deep-frying in the production of potato chips.
- Maintaining the natural taste of the product, for example, by using less artificial sweetener.

Hygiene

The rigorous application of industry and government endorsed standards to minimize possible risk and hazards. In the U.S., the standard adopted is HACCP.

Efficiency

- Rising energy costs lead to increasing usage of energy-saving technologies, for example, frequency converters on electrical drives, heat insulation of factory buildings, and heated vessels, energy recovery systems.
- Factory automation systems (often Distributed control systems) reduce personnel costs and may lead to more stable production results.

Industries

Food processing industries and practices include the following:

- Cannery
- Industrial rendering
- Meat packing plant
- Slaughterhouse
- Sugar industry
- Vegetable packing plant.

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Chapter 2

Understanding Nutrition

The science of interpreting the interactions between nutrients and other materials in food with respect to the growth, health and disease in an organism is known as nutrition. Some of the sub-disciplines within nutrition are malnutrition and sports nutrition. The chapter closely examines these key concepts of nutrition to provide an extensive understanding of the subject.

Nutrition is defined as the processes by which an animal or plant takes in and utilises food substances. Essential nutrients include protein, carbohydrate, fat, vitamins, minerals and electrolytes. Normally, 85% of daily energy use is from fat and carbohydrates and 15% from protein. In humans, nutrition is mainly achieved through the process of putting foods into our mouths, chewing and swallowing it. The required amounts of the essential nutrients differ by age and the state of the body.

Nutrition is essential for growth and development, health and wellbeing. Eating a healthy diet contributes to preventing future illness and improving quality and length of life. Your nutritional status is the state of your health as determined by what you eat. There are several ways of assessing nutritional status, including anthropometric (i.e. physical body measurement), food intake and biochemical measurement.

Nutrients

Some precursors (i.e., the substances from which other substances are formed) of cell materials can be synthesized by the cell from other materials, while others must be supplied in foods. All the inorganic materials required for growth, together with an assortment of organic compounds whose number may vary from 1 to 30 or more, depending on the organism, fall into the latter category. Although organisms are able to synthesize nonessential nutrients, such nutrients are frequently utilized directly if present in food, thereby saving the organism the need to expend the energy required to synthesize them.

Inorganic Nutrients

A number of inorganic elements (minerals) are essential for the growth of living things. Boron, for example, has been demonstrated to be required for the growth of many—perhaps all—higher plants but has not been implicated as an essential element in the nutrition of either microorganisms or animals. Trace amounts of fluorine (as fluoride) are certainly beneficial, and perhaps essential, for proper tooth formation in higher animals. Similarly, iodine (as iodide) is required in animals for formation of thyroxine, the active component of an important regulatory hormone. Silicon (as silicate) is a prominent component of the outer skeletons of diatomaceous protozoans and similar organisms and is required in them for normal growth. In higher animals the requirement for silicon is much smaller. A less obvious example of a specialized mineral requirement is provided by calcium, which is required by higher animals in comparatively large amounts because it is a major component of bone and eggshells (in birds); for other organisms, calcium is an essential nutrient but only as a trace element. Mineral elements in wide variety are present in trace amounts in almost all foodstuffs. It cannot be assumed that the nonessential mineral elements play no useful role in metabolism.

Important antagonistic relationships between certain mineral nutrients also are known. A large excess of rubidium, for example, interferes with the utilization of potassium in some lactic-acid bacteria; zinc can interfere with manganese utilization in the same organism. In animal nutrition, excessive molybdenum or zinc (both of which are essential minerals) interferes with the utilization of copper, another essential mineral, and, in higher plants, excessive zinc can lead to a disorder that is known as iron chlorosis. Proper nutrient growth media for microorganisms and plants or diets for animals, therefore, require not only that the essential mineral elements be provided in sufficient amounts but also that they be used in the proper ratios to each other.

Organic Nutrients

The organic nutrients are the necessary building blocks of various cell components that certain organisms cannot synthesize and therefore must obtain preformed. These compounds include carbohydrates, protein, and lipids. Other organic nutrients include the vitamins, which are required in small amounts, because of either the catalytic role or the regulatory role they play in metabolism.

Interdependency of Nutritional Requirements

Competition for Sites of Absorption by the Cell

Since absorption of nutrients frequently occurs by way of active transport within cell membranes, an excess of one nutrient (A) may inhibit absorption of a second nutrient (B), if they share the same absorption pathway. In such cases, the apparent requirement for nutrient B increases; B, however, can sometimes be supplied in an alternate form that is able to enter the cell by a different route. Many examples of amino acid antagonism, in which inhibition of growth by one amino acid is counteracted by another amino acid, are best explained by this mechanism. For example, under some conditions Lactobacillus casei requires both D- and L-alanine, which differ from each other only in the position of the amino, or NH_2, group in the molecule, and the two forms of this amino acid share the same absorption pathway. Excess D-alanine inhibits growth of this species, but the inhibition can be alleviated either by supplying additional L-alanine or, more effectively, by supplying peptides of L-alanine. The peptides enter the cell by a pathway different from that of the two forms of alanine and, after they are in the cell, can be broken down to form L-alanine. Relationships of this type provide one explanation for the fact that peptides are frequently more effective than amino acids in promoting growth of bacteria.

Competition for Sites of Utilization within the Cell

This phenomenon is similar to that regarding competition for absorption sites, but it occurs inside the cell and only between structurally similar nutrients (e.g., leucine and valine; serine and threonine).

Precursor-product Relationships

The requirement of rats and humans for the essential amino acids phenylalanine and methionine is substantially reduced if tyrosine, which is formed from phenylalanine, or cysteine, which is formed from methionine, is added to the diet. These relationships are explained by the fact that tyrosine and cysteine are synthesized in animals from phenylalanine and methionine, respectively. When the former (product) amino acids are supplied preformed, the latter (precursor) amino acids are required in smaller amounts. Several instances of the sparing of one nutrient by another because they have similar precursor-product relationships have been identified in other organisms.

Changes in Metabolic Pathways within the Cell

Rats fed diets containing large amounts of fat require substantially less thiamin (vitamin B1) than do those fed diets high in carbohydrate. The utilization of carbohydrate as an energy source (i.e., for ATP formation) is known to involve an important thiamin-dependent step, which is bypassed when fat is used as an energy source, and it is assumed that the lessened requirement for thiamin results from the change in metabolic pathways.

Syntrophism

Since the nutritional requirements and metabolic activities of organisms differ, it is clear that two or more different organisms growing relatedly may produce different overall changes in the environment. A rough example is provided by a balanced aquarium, in which aquatic plants utilize light and the waste products of animals—e.g., carbon dioxide, water, ammonia—to synthesize cell materials and generate oxygen, which in turn provide the materials necessary for animal growth. Such relationships are common among microorganisms; i.e., intermediate or end products of metabolism of one organism may provide essential nutrients for another. The mixed populations that result in nature provide examples of this phenomenon, which is called syntrophism; in some instances, the relationship may be so close as to constitute nutritional symbiosis, or mutualism. Several examples of this phenomenon have been found among thiamin-requiring yeasts and fungi, certain of which (group A) synthesized the thiazole component of thiamin molecule but require the pyrimidine portion preformed; for a second group (group B), the relationship is reversed. When group A and group B are grown together in a thiamin-free medium, both types of organisms survive, since each organism synthesizes the growth factor required by its partner; neither organism grows alone under these same conditions. Thus, two or more types of microorganisms frequently grow in situations in which only one species would not.

Such nutritional interrelationships may explain the fact that the nutritionally demanding lactic-acid bacteria are able to coexist with the nutritionally non-demanding coliform bacteria in the intestinal tracts of animals. It is known that the bacterial flora of the intestinal tract synthesize sufficient amounts of certain vitamins (e.g., vitamin K, folic acid) so that detection of deficiency symptoms in rats requires special measures, and the role of rumen bacteria in ruminant animals (e.g., cows, sheep) in rendering otherwise indigestible cellulose and other materials available to the host animal is well-known. These few examples indicate that syntrophic interrelationships are widespread in nature and may contribute substantially to the nutrition of a wide variety of species.

Nutritional Evolution of Organisms

Little is known about the nutritional evolution of living organisms. Nucleic acids, proteins, carbohydrates, and fats, which are present in all living cells, are formed by specific reaction sequences from a limited number of smaller compounds, most of which are common to all living organisms and, according to current theories, were available on Earth before life arose. Since less complex metabolic organization and less energy are required to synthesize cellular proteins from preformed amino acids than from carbon dioxide and other precursors, it is assumed that the simplest early forms of life were heterotrophic organisms requiring many organic nutrients for growth and that they selected such nutrients from their surroundings. As the supply of these preformed substances was exhausted, the organisms presumably developed the capacity to synthesize these preformed substances from simpler (precursor) materials present in the environment; in some organisms, this synthesizing capacity eventually evolved to the extent that carbon from carbon dioxide could be utilized to synthesize organic compounds.

At this point, autotrophy, as it now is known, became possible; autotrophy, in fact, may have evolved as a result of the exhaustion of the supply of preformed organic materials in the environment and the consequent necessity of organisms to synthesize the requirements themselves in order to survive. Implicit in this theory is the demonstrable assumption that autotrophic cells contain the most complex biosynthetic organization found in living things and that heterotrophic cells are simpler in that certain biosynthetic pathways do not occur. After the evolution of photosynthesis, a constantly renewable source of the organic compounds necessary for heterotrophic cell growth became available. It became feasible that those organisms whose environments provided a constantly available supply of a given compound could lose, through changes in their genetic material (mutations), the ability to synthesize that compound and still survive. Entire biosynthetic pathways may have been lost in this way; as long as such mutant organisms remained in an environment that supplied the necessary compound, the simplification in cellular organization and the energy saved by using preformed cell components would have given them a competitive advantage over the more complex parents from which they were derived and permitted stabilization of the mutation within the cell type. A theory that the requirements of modern organisms for essential organic nutrients arose through the loss of synthetic abilities present in more complex parent organisms was confirmed by the discovery that artificially produced mutant offspring of microorganisms can be readily obtained and may require the presence of one or more preformed organic compounds that the parent microorganisms could synthesize.

Human Nutrition

Human nutrition is the process by which substances in food are transformed into body tissues and provide energy for the full range of physical and mental activities that make up human life.

Utilization of Food by the Body

Calories and kilocalories: Energy Supply

The human body can be thought of as an engine that releases the energy present in the foods that it digests. This energy is utilized partly for the mechanical work performed by the muscles and in the secretory processes and partly for the work necessary to maintain the body's structure and functions. The performance of work is associated with the production of heat; heat loss is controlled so as to keep body temperature within a narrow range. Unlike other engines, however, the human body is continually breaking down (catabolizing) and building up (anabolizing) its component parts. Foods supply nutrients essential to the manufacture of the new material and provide energy needed for the chemical reactions involved.

Carbohydrate, fat, and protein are, to a large extent, interchangeable as sources of energy. Typically, the energy provided by food is measured in kilocalories, or Calories. One kilocalorie is equal to 1,000 gram-calories (or small calories), a measure of heat energy. However, in common parlance, kilocalories are referred to as "calories." In other words, a 2,000-calorie diet actually has 2,000 kilocalories of potential energy. One kilocalorie is the amount of heat energy required to raise one kilogram of water from 14.5 to 15.5 °C at one atmosphere of pressure. Another unit of energy widely used is the joule, which measures energy in terms of mechanical work. One joule is the energy expended when one kilogram is moved a distance of one metre by a force of one newton. The relatively higher levels of energy in human nutrition are more likely to be measured in kilojoules (1 kilojoule = 10^3 joules) or megajoules (1 megajoule = 10^6 joules). One kilocalorie is equivalent to 4.184 kilojoules.

The energy present in food can be determined directly by measuring the output of heat when the food is burned (oxidized) in a bomb calorimeter. However, the human body is not as efficient as a calorimeter, and some potential energy is lost during digestion and metabolism. Corrected physiological values for the heats of combustion of the three energy-yielding nutrients, rounded to whole numbers, are as follows: carbohydrate, 4 kilocalories (17 kilojoules) per gram; protein, 4 kilocalories (17 kilojoules) per gram; and fat, 9 kilocalories (38 kilojoules) per gram. Beverage alcohol (ethyl alcohol) also yields energy—7 kilocalories (29 kilojoules) per gram—although it is not essential in the diet. Vitamins, minerals, water, and other food constituents have no energy value, although many of them participate in energy-releasing processes in the body.

The energy provided by a well-digested food can be estimated if the gram amounts of energy-yielding substances (non-fibre carbohydrate, fat, protein, and alcohol) in that food are known. For example, a slice of white bread containing 12 grams of carbohydrate, 2 grams of protein, and 1 gram of fat supplies 67 kilocalories (280 kilojoules) of energy. Food composition tables and food labels provide useful data for evaluating energy and nutrient intake of an individual diet. Most foods provide a mixture of energy-supplying nutrients, along with vitamins, minerals, water, and other substances. Two notable exceptions are table sugar and vegetable oil, which are virtually pure carbohydrate (sucrose) and fat, respectively.

Throughout most of the world, protein supplies between 8 and 16 percent of the energy in the diet, although there are wide variations in the proportions of fat and carbohydrate in different populations. In more prosperous communities about 12 to 15 percent of energy is typically derived from protein, 30 to 40 percent from fat, and 50 to 60 percent from carbohydrate. On the other hand, in many poorer agricultural societies, where cereals comprise the bulk of the diet, carbohydrate provides an even larger percentage of energy, with protein and fat providing less. The human body is remarkably adaptable and can survive, and even thrive, on widely divergent diets. However, different dietary patterns are associated with particular health consequences.

BMR and REE: Energy Balance

Energy is needed not only when a person is physically active but even when the body is lying motionless. Depending on an individual's level of physical activity, between 50 and 80 percent of the energy expended each day is devoted to basic metabolic processes (basal metabolism), which enable the body to stay warm, breathe, pump blood, and conduct numerous physiological and biosynthetic activities, including synthesis of new tissue in growing children and in pregnant and lactating women. Digestion and subsequent processing of food by the body also uses energy and produces heat. This phenomenon, known as the thermic effect of food (or diet-induced thermogenesis), accounts for about 10 percent of daily energy expenditure, varying somewhat with the composition of the diet and prior dietary practices. Adaptive thermogenesis, another small but important component of energy expenditure, reflects alterations in metabolism due to changes in ambient temperature, hormone production, emotional stress, or other factors. Finally, the most variable component in energy expenditure is physical activity, which includes exercise and other voluntary activities as well as involuntary activities such as fidgeting, shivering, and maintaining posture. Physical activity accounts for 20 to 40 percent of the total energy expenditure, even less in a very sedentary person and more in someone who is extremely active.

Basal or resting energy expenditure is correlated primarily with lean body mass (fat-free mass and essential fat, excluding storage fat), which is the metabolically active tissue in the body. At rest, organs such as the liver, brain, heart, and kidney have the highest metabolic activity and, therefore, the highest need for energy, while muscle and bone require less energy, and body fat even less. Besides body composition, other factors affecting basal metabolism include age, sex, body temperature, and thyroid hormone levels.

The basal metabolic rate (BMR), a precisely defined measure of the energy expenditure necessary to support life, is determined under controlled and standardized conditions—shortly after awakening in the morning, at least 12 hours after the last meal, and with a comfortable room temperature. Because of practical considerations, the BMR is rarely measured; the resting energy expenditure (REE) is determined under less stringent conditions, with the individual resting comfortably about 2 to 4 hours after a meal. In practice, the BMR and REE differ by no more than 10 percent—the REE is usually slightly higher—and the terms are used interchangeably.

Energy expenditure can be assessed by direct calorimetry, or measurement of heat dissipated from the body, which employs apparatuses such as water-cooled garments or insulated chambers large enough to accommodate a person. However, energy expenditure is usually measured by the less cumbersome techniques of indirect calorimetry, in which heat produced by the body is calculated from measurements of oxygen inhaled, carbon dioxide exhaled, and urinary nitrogen excreted. The BMR (in kilocalories per day) can be roughly estimated using the following formula: $\text{BMR} = 70 \times (\text{body weight in kilograms})^{3/4}$.

The energy costs of various activities have been measured. While resting may require as little as 1 kilocalorie per minute, strenuous work may demand 10 times that much. Mental activity, though it may seem taxing, has no appreciable effect on energy requirement. A 70-kg (154-pound) man, whose REE over the course of a day might be 1,750 kilocalories, could expend a total of 2,400 kilocalories on a very sedentary day and up to 4,000 kilocalories on a very active day. A 55-kg (121-pound) woman, whose daily resting energy expenditure might be 1,350 kilocalories, could use from 1,850 to more than 3,000 total kilocalories, depending on level of activity.

The law of conservation of energy applies: If one takes in more energy than is expended, over time one will gain weight; insufficient energy intake results in weight loss, as the body taps its energy stores to provide for immediate needs. Excess food energy is stored in small amounts as glycogen, a short-term storage form of carbohydrate in muscle and liver, and as fat, the body's main energy reserve found in adipose tissue. Adipose tissue is mostly fat (about 87 percent), but it also contains some protein and water. In order to lose 454 grams (one pound) of adipose tissue, an energy deficit of about 3,500 kilocalories (14.6 megajoules) is required.

Body Mass, Body Fat and Body Water

The human body consists of materials similar to those found in foods; however, the relative proportions differ, according to genetic dictates as well as to the unique life experience of the individual. The body of a healthy lean man is composed of roughly 62 percent water, 16 percent fat, 16 percent protein, 6 percent minerals, and less than 1 percent carbohydrate, along with very small amounts of vitamins and other miscellaneous substances. Females usually carry more fat (about 22 percent in a healthy lean woman) and slightly less of the other components than do males of comparable weight.

The body's different compartments—lean body mass, body fat, and body water—are constantly adjusting to changes in the internal and external environment so that a state of dynamic equilibrium (homeostasis) is maintained. Tissues in the body are continuously being broken down (catabolism) and built up (anabolism) at varying rates. For example, the epithelial cells lining the digestive tract are replaced at a dizzying speed of every three or four days, while the life span of red blood cells is 120 days, and connective tissue is renewed over the course of several years.

Although estimates of the percentage of body fat can be made by direct inspection, this approach is imprecise. Body fat can be measured indirectly using fairly precise but costly methods, such as underwater weighing, total body potassium counting, and dual-energy X-ray absorptiometry (DXA). However, more practical, albeit less accurate, methods are often used, such as anthropometry, in which subcutaneous fat at various sites is measured using skinfold calipers; bioelectrical impedance, in which resistance to a low-intensity electrical current is used to estimate body fat; and near infrared interactance, in which an infrared light aimed at the biceps is used to assess fat and protein interaction. Direct measurement of the body's various compartments can only be performed on cadavers.

The composition of the body tends to change in somewhat predictable ways over the course of a lifetime—during the growing years, in pregnancy and lactation, and as one ages—with corresponding changes in nutrient needs during different phases of the life cycle. Regular physical exercise can help attenuate the age-related loss of lean tissue and increase in body fat.

Food Groups

The following nine food groups reflect foods with generally similar nutritional characteristics:

(1) Cereals,

(2) Starchy roots,

(3) Legumes,

(4) Vegetables and fruits,

(5) Sugars, preserves, and syrups,

(6) Meat, fish, and eggs,

(7) Milk and milk products,

(8) Fats and oils, and

(9) Beverages.

Cereals

The cereals are all grasses that have been bred over millennia to bear large seeds (i.e., grain). The most important cereals for human consumption are rice, wheat, and corn (maize). Others include barley, oats, and millet. The carbohydrate-rich cereals compare favourably with the protein-rich foods in energy value; in addition, the cost of production (per calorie) of cereals is less than that of almost all other foods and they can be stored dry for many years. Therefore, most of the world's diets are arranged to meet main calorie requirements from the cheaper carbohydrate foods. The major component of all grains is starch. Cereals contain little fat, with oats having an exceptional 9 percent. The amount of protein in cereals ranges from 6 to 16 percent but does not have as high a nutritive value as that of many animal foods because of the low lysine content.

Controversy exists as to the relative merits of white bread and bread made from whole wheat flour. White flour consists of about 72 percent of the grain but contains little of the germ (embryo) and of the outer coverings (bran). Since the B vitamins are concentrated mainly in the scutellum (covering of the germ), and to a lesser extent in the bran, the vitamin B content of white flour, unless artificially enriched, is less than that of brown flour. Dietary fibre is located mostly in the bran, so that white flour contains only about one-third of that in whole wheat flour. White flour is compulsorily enriched with synthetic vitamins in a number of countries, including the United States and the United Kingdom, so that the vitamin content is similar to that of the darker flours. White flour still lacks fibre and any yet unidentified beneficial factors that may be present in the outer layers of the wheat.

B vitamins are also lost when brown rice is polished to yield white rice. People living on white rice and little else are at risk for developing the disease beriberi, which is caused by a deficiency of thiamin (vitamin B1). Beriberi was formerly common in poor Asian communities in which a large proportion of the diet consisted of polished rice. The disease has almost completely disappeared from Asia with the advent of greater availability of other foods and, in some areas, fortification of the rice with thiamin.

Yellow corn differs from other cereals in that it contains carotenoids with vitamin A activity. (Another exception is a genetically modified so-called golden rice, which contains carotene, the precursor for vitamin A.) Corn is also lower in the amino acid tryptophan than other cereals. The niacin in corn is in a bound form that cannot be digested or absorbed by humans unless pretreated with lime (calcium hydroxide) or unless immature grains are eaten at the so-called milky stage (usually as sweet corn). Niacin is also formed in the body as a metabolite of the amino acid tryptophan, but this alternative source is not available when the tryptophan content is too low.

Starchy Roots

Starchy roots consumed in large quantities include potatoes, sweet potatoes, yams, taro, and cassava. Their nutritive value in general resembles that of cereals. The potato, however, provides some protein (2 percent) and also contains vitamin C. The yellow-fleshed varieties of sweet potato contain the pigment beta-carotene, convertible in the body into vitamin A. Cassava is extremely low in protein, and most varieties contain cyanide-forming compounds that make them toxic unless processed correctly.

Legumes

Beans and peas are the seeds of leguminous crops that are able to utilize atmospheric nitrogen via parasitic microorganisms attached to their roots. Legumes contain at least 20 percent protein, and they are a good source of most of the B vitamins and of iron. Like cereals, most legumes are low in fat; an important exception is the soybean (17 percent), a major commercial source of edible oil.

Vegetables and Fruits

Vegetables and fruits have similar nutritive properties. Because 70 percent or more of their weight is water, they provide comparatively little energy or protein, but many contain vitamin C and carotene. However, cooked vegetables are an uncertain source of vitamin C, as this vitamin is easily destroyed by heat. The dark-green leafy vegetables are particularly good sources of vitamin A activity. Vegetables also provide calcium and iron but often in a form that is poorly absorbed. The more typical fruits, such as apples, oranges, and berries, are rich in sugar. Bananas are a good source of potassium. Vegetables and fruits also contain fibre, which adds bulk to the intestinal content and is useful in preventing constipation.

Botanically, nuts are actually a kind of fruit, but they are quite different in character with their hard shell and high fat content. The coconut, for example, contains some 60 percent fat when dried. Olives are another fruit rich in fat and are traditionally grown for their oil.

Sugars, Preserves and Syrups

One characteristic of diets of affluent societies is their high content of sugar. This is due in part to sugar added at the table or as an ingredient in candy, preserves, and sweetened colas or other beverages. There are also naturally occurring sugars in foods (lactose in milk and fructose, glucose, and sucrose in fruits and some vegetables). Sugar, however, contains no protein, minerals, or vitamins and thus has been called the source of "empty calories."

Because sugar adsorbs water and prevents the growth of microorganisms, it is an excellent preservative. Making jam or marmalade is a way of preserving fruit, but most of the vitamin C is destroyed, and the products contain up to 70 percent sugar. Honey and natural syrups (e.g., maple syrup) are composed of more than 75 percent sugar.

Meat, Fish and Eggs

Generally meats consist of about 20 percent protein, 20 percent fat, and 60 percent water. The amount of fat present in a particular portion of meat varies greatly, not only with the kind of meat but also with the quality; the "energy value" varies in direct proportion with the fat content. Meat is valuable for its protein, which is of high biological value. Pork is an excellent source of thiamin. Meat is also a good source of niacin, vitamin B_{12}, vitamin B_6, and the mineral nutrients iron, zinc, phosphorus, potassium, and magnesium. Liver is the storage organ for, and is very rich in, vitamin A, riboflavin, and folic acid. In many cultures the organs (offal) of animals—including the kidneys, the heart, the tongue, and the liver—are considered delicacies. Liver is a particularly rich source of many vitamins.

The muscular tissue of fishes consists of 13 to 20 percent protein, fat ranging from less than 1 to more than 20 percent, and 60 to 82 percent water that varies inversely with fat content. Many species of fish, such as cod and haddock, concentrate fat in the liver and as a result have extremely lean muscles. The tissues of other fish, such as salmon and herring, may contain 15 percent fat or more. However, fish oil, unlike the fat in land animals, is rich in essential long-chain fatty acids, particularly eicosapentaenoic acid.

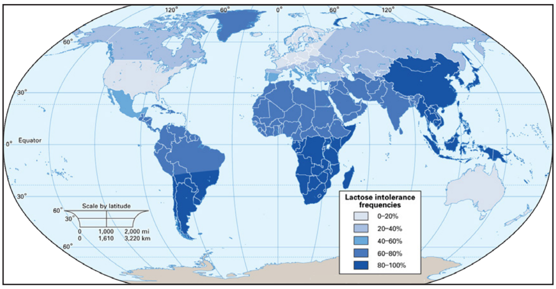
The egg has a deservedly high reputation as a food. Its white contains protein, and its yolk is rich in both protein and vitamin A. An egg also provides calcium and iron. Egg yolk, however, has a high cholesterol content.

Milk and Milk Products

The milk of each species of animal is a complete food for its young. Moreover, one pint of cow's milk contributes about 90 percent of the calcium, 30 to 40 percent of the riboflavin, 25 to 30 percent of the protein, 10 to 20 percent of the calories and vitamins A and B, and up to 10 percent of the iron and vitamin D needed by a human adult.

Human breast milk is the perfect food for infants, provided it comes from a healthy, well-nourished mother and the infant is full-term. Breast milk contains important antibodies, white blood cells, and nutrients. In communities where hygiene is poor, breastfed babies have fewer infections than formula-fed babies. In the past, infants who could not be breast-fed were given cow's milk that was partially "humanized" with the addition of water and a small amount of sugar or wheat flour. However, this was far from an ideal substitute for breast milk, being lower in iron and containing undenatured proteins that could produce allergic reactions with bleeding into the gut and, in some cases, eczema.

Lactose, the characteristic sugar of milk, is a disaccharide made of the monosaccharides glucose and galactose. Some adults can break down the lactose of large quantities of milk into galactose and glucose, but others have an inherited lactose intolerance as a result of the lactase enzyme no longer being secreted into the gut after the age of weaning. As a result, unabsorbed lactose is fermented by bacteria and produces bloating and gas. People who have little lactase in their bodies can still consume large amounts of milk if it has been allowed to go sour, if lactobacilli have split most of the lactose into lactic acid (as in yogurt), or if the lactose has been treated with commercially available lactase. People originating in northern Europe usually retain full intestinal lactase activity into adult life.



Lactose intolerance, global distribution: Global distribution of lactose intolerance in humans

Most commercially available milk has been pasteurized with heat to kill bovine tuberculosis organisms and other possible pathogens. The most widely used method for pasteurizing milk is the high-temperature, short-time (HTST) sterilization treatment. If products are to be stored under refrigeration, or even at room temperature, for long periods of time, they may be processed by ultrahigh temperature (UHT) pasteurization. Another method of preserving milk without refrigeration involves the removal of water to form condensed milk, which can be exposed to air for several days without deterioration. Milk, either whole or defatted, can also be dried to a powder. In some countries, such as the United States, milk is homogenized so that fat particles are broken up and evenly distributed throughout the product.

Cow's milk is good food for human adults, but the cream (i.e., the fat) contains 52 percent saturated fatty acids as compared with only 3 percent polyunsaturated fat. This fat is either drunk with the milk or eaten in butter or cream. Because milk fat is regarded as undesirable by people who want to reduce their energy intake or cholesterol level, the dairy industry has developed low-fat cow's milk (with 2 percent fat instead of the almost 4 percent of whole milk), very low-fat skim milk, and skim milk with extra nonfat milk solids (lactose, protein, and calcium) that give more body to the milk. Buttermilk, originally the watery residue of butter making, is now made from either low-fat or skim milk that has been inoculated with nonpathogenic bacteria.

Cheese making is an ancient art formerly used on farms to convert surplus milk into a food that could be stored without refrigeration. Rennet, an enzyme found in a calf's stomach, is added to milk, causing the milk protein casein to coagulate into a semisolid substance called curd, thus trapping most of the fat. The remaining watery liquid (whey) is then drained, and the curd is salted, inoculated with nonpathogenic organisms, and allowed to dry and mature. Cheese is rich in protein and calcium and is a good source of vitamin A and riboflavin. Most cheeses, however, contain about 25 to 30 percent fat (constituting about 70 percent of the calories of the cheese), which is mostly saturated, and they are usually high in sodium.

Fats and Oils

The animal fats used by humans are butter, suet (beef fat), lard (pork fat), and fish oils. Important vegetable oils include olive oil, peanut (groundnut) oil, coconut oil, cottonseed oil, sunflower seed oil, soybean oil, safflower oil, rape oil, sesame (gingelly) oil, mustard oil, red palm oil, and corn oil. Fats and oils provide more calories per gram than any other food, but they contain no protein and few micronutrients. Only butter and the previously mentioned fish-liver oils contain any vitamin A or D, though red palm oil does contain carotene, which is converted to vitamin A in the body. Vitamins A and D are added to margarines. All natural fats and oils contain variable amounts of vitamin E, the fat-soluble vitamin antioxidant.

The predominant substances in fats and oils are triglycerides, chemical compounds containing any three fatty acids combined with a molecule of glycerol. When no double bonds are present, a fatty acid is said to be saturated; with the presence of one or more double bonds, a fatty acid is said to be unsaturated. Fats with a high percentage of saturated fatty acids, e.g., butter and lard, tend to be solid at room temperature. Those with a high percentage of unsaturated fatty acids are usually liquid oils, e.g., sunflower, safflower, and corn oils. The process of hydrogenation is used by the food industry to convert unsaturated oils to saturated solid fats, which are more resistant to rancidity. However, hydrogenation also causes the formation of trans-fatty acids. These appear to have some of the same undesirable effects on blood cholesterol as saturated fatty acids.

A small group of fatty acids is essential in the diet. They occur in body structures, especially the different membranes inside and around cells, and cannot be synthesized in the body from other fats. Linoleic acid is the most important of these fatty acids because it is convertible to other essential fatty acids. Linoleic acid has two double bonds and is a polyunsaturated fatty acid. As well as being an essential fatty acid, it tends to lower the cholesterol level in the blood. Linoleic acid occurs in moderate to high proportions in many of the seed oils, e.g., corn, sunflower, cottonseed, and safflower oils. Some margarines (polyunsaturated margarines) use a blend of oils selected to provide a moderately high linoleic acid content.

Beverages

Although most adults drink one to two litres (about one to two quarts) of water a day, much of this is in the form of liquids such as coffee, tea, fruit juices, and soft drinks. In general, these are appreciated more for their taste or for their effects than for their nutritive value. Fruit juices are useful for their vitamin C content and are good sources of potassium; however, they tend to be very high in sugar. Coffee and tea by themselves are of little nutritive value; coffee contains some niacin, and tea contains fluoride and manganese. These beverages also contain natural caffeine, which has a stimulating effect. Caffeine is added to colas, and so-called diet soft drinks contain small quantities of artificial sweeteners in place of sugars so that their overall calorie value is reduced.

Since ethyl alcohol (ethanol) has an energy value of 7 kilocalories per gram, very significant amounts of energy can be obtained from alcoholic drinks. Beer contains 2 to 6 percent alcohol, wines 10 to 13 percent, and most spirits up to 40 percent. Fermented drinks also include significant amounts of residual sugars, and champagne and dessert wines may have sugar added to them. With one or two exceptions, alcoholic beverages contain no nutrients and are only a source of "empty calories." The only vitamin present in significant amounts in beer is riboflavin. Wines are devoid of vitamins but sometimes contain large amounts of iron, probably acquired from iron vessels used in their preparation. Heavy alcohol consumption is known to lead to a greater risk of malnutrition, in part because it can damage the absorptive power of the gut and also because heavy drinkers commonly neglect to follow a normal pattern of meals. On the other hand, evidence from a number of studies shows that persons consuming one to two drinks per day are healthier than are those who abstain from drinking alcohol. This might be due in part to substances in red wine, such as flavonoids and tannins, which may protect against heart disease.

Nutrition throughout the Life Cycle

Nutritional needs and concerns vary during different stages of life. Selected issues are discussed below:

Pregnancy and Lactation

A woman's nutritional status before and during pregnancy affects not only her own health but also the health and development of her baby. If a woman is underweight before becoming pregnant or fails to gain sufficient weight during pregnancy, her chance of having a premature or low-birth-weight infant is increased. Overweight women, on the other hand, have a high risk of complications during pregnancy, such as high blood pressure (hypertension) and gestational diabetes, and of having a poorly developed infant or one with birth defects. Weight loss during pregnancy is never recommended. Recommended weight gain during pregnancy is 11.5 to 16 kg (25 to 35 pounds) for a woman of normal weight—slightly more for an underweight woman and slightly less for an overweight woman.

Overall nutritional requirements increase with pregnancy. In the second and third trimesters, pregnant women need additional food energy—about 300 kilocalories above non-pregnant needs. Most additional nutrient needs can be met by selecting food wisely, but an iron supplement (30 milligrams per day) is usually recommended during the second and third trimesters, in addition to a folic acid supplement throughout pregnancy. Other key nutrients of particular concern are protein, vitamin D, calcium, and zinc.

Heavy alcohol consumption or “binge drinking” during pregnancy can cause fetal alcohol syndrome, a condition with irreversible mental and physical retardation. Even lighter social drinking during pregnancy may result in milder damage—growth retardation, behavioral or learning abnormalities, or motor impairments—sometimes described as fetal alcohol effects. Until a completely safe level of intake can be determined, pregnant women are advised not to drink at all, especially during the first trimester. Caffeine consumption is usually limited as a precautionary measure, and cigarette smoking is not advised under any circumstances. Limiting intake of certain fish, such as swordfish and shark, which may be contaminated with methylmercury, is also recommended.

An extra 500 kilocalories of food per day is needed to meet the energy demands of lactation. Because pregnancy depletes maternal iron stores, iron supplementation during lactation may be advised. Breast-fed infants may be sensitive to the constituents and flavours of foods and beverages consumed by the mother. In general, lactating women are advised to consume little, if any, alcohol.

Infancy, Childhood and Adolescence

Breast-fed infants, in general, have fewer infections and a reduced chance of developing allergies and food intolerances. For these and other reasons, breast-feeding is strongly recommended for at least the first four to six months of life. However, if a woman is unable to breast-feed or chooses not to, infant formulas (altered forms of cow’s milk) can provide safe and adequate nourishment for an infant. Goat’s milk, evaporated milk, and sweetened condensed milk are inappropriate for infants. Soy formulas and hydrolyzed protein formulas can be used if a milk allergy is suspected. In developing countries with poor sanitation, over-diluted formulas or those prepared with contaminated water can cause malnutrition and infection, resulting in diarrhea, dehydration, and even death. Breast-fed infants may need supplements of iron and vitamin D during the first six months of life and fluoride after six months. A vitamin B_{12} supplement is advised for breast-fed infants whose mothers are strict vegetarians (vegans).

Solid foods, starting with iron-fortified infant cereals, can be introduced between four and six months to meet nutrient needs that breast milk or infant formulas can no longer supply alone. Other foods can be introduced gradually, one every few days. Infants should not be given honey (which may contain bacteria that can cause botulism), foods that are too salty or sweet, foods that may cause choking, or large amounts of fruit juice.

Starting at one year of age, whole cow's milk can be an excellent source of nutrients for children. However, because cow's milk is associated with gastrointestinal blood loss, iron deficiency, and an allergic response in some young infants, some medical societies do not recommend giving unmodified whole cow's milk to children less than one year old. Low-fat or nonfat milk is inappropriate for children less than two years of age.

The rapid growth rate of infancy slows down in early childhood. During childhood—but not before the age of two—a gradual transition to lower-fat foods is recommended, along with regular exercise. Establishing healthful practices in childhood will reduce the risk of childhood obesity as well as obesity in adulthood and related chronic diseases (e.g., heart disease, diabetes, and high blood pressure).

Vegetarian children can be well nourished but care is needed for them to receive sufficient energy (calories), good-quality protein, vitamins B_{12} and D, and the minerals iron, zinc, and calcium. It is difficult for children who do not drink milk to obtain enough calcium from their food, and supplements may be required. Because of possible toxicity, iron supplements should be taken only under medical supervision.

A small percentage of school-age children who have difficulty sitting still and paying attention are diagnosed with attention-deficit/ hyperactivity disorder (ADHD). Studies have found no convincing evidence that ADHD is caused by sugar or food additives in the diet or that symptoms can be alleviated by eliminating these substances.

Because of unusual eating practices, skipped meals, and concerns about body image, many teenagers, especially girls, have a less than optimal diet. Teenage girls, in particular, need to take special care to obtain adequate amounts of calcium so that bones can be properly mineralized. Iron-deficiency anemia is a concern not only for teenage girls, who lose iron periodically in menstrual blood, but also for teenage boys.

Adulthood

No matter which nutritional and health practices are followed, the body continues to age, and there appears to be a strong genetic component to life expectancy. Nevertheless, healthful dietary practices and habits such as limited alcohol use, avoidance of tobacco products, and regular physical activity can help reduce the chance of premature death and increase the chance of vitality in the older years. For the most part, a diet that is beneficial for adults in general is also beneficial for people as they age, taking into account possible changes in energy needs.

In elderly people, common problems that contribute to inadequate nutrition are tooth loss, decreased sense of taste and smell, and a sense of isolation—all of which result in decreased food intake and weight loss. The elderly may have gastrointestinal ailments, such as poor absorption of vitamin B_{12}, and digestion difficulties, such as constipation. Inadequate fluid intake may lead to dehydration. Nutritional deficiency may further compromise declining immune function. Prescription and over-the-counter drugs may interact with nutrients and exacerbate the nutritional deficits of the elderly. In addition, decreasing physical activity, loss of muscle tissue, and increasing body fat are associated with type 2 diabetes, hypertension, and risk of cardiovascular disease and other diseases. Older people, especially those with reduced sun exposure or low intakes of fatty fish or vitamin D-fortified food, may need supplemental vitamin D to help preserve bone mass. Adequate calcium intake and weight-bearing exercise are also important, but these measures cannot completely stop the decline in bone density with age that makes both men and women vulnerable to bone fractures (due to osteoporosis), which could leave them bedridden and could even be life-threatening. Treatment with various bone-conserving drugs has been found to be effective in slowing bone loss. Staying physically fit as one ages can improve strength and balance, thereby preventing falls, contributing to overall health, and reducing the impact of aging.

There is evidence that intake of the antioxidants vitamin C, vitamin E, and beta-carotene as well as the mineral zinc may slow the progression of age-related macular degeneration, a leading cause of blindness in people older than 65 years. Two carotenoids, lutein and zeaxanthin, also are being studied for their possible role in protecting against age-related vision loss. Research suggests that the dietary supplement glucosamine, a substance that occurs naturally in the body and contributes to cartilage formation, may be useful in lessening the pain and disability of osteoarthritis. Aerobic exercise and strength training, as well as losing excess weight, also may provide some relief from arthritis pain.

Elevated blood levels of the amino acid homocysteine have been associated with an increased risk of cardiovascular disease and with Alzheimer disease, the most common form of dementia; certain B vitamins, particularly folic acid, may be effective in lowering homocysteine levels. High concentrations of aluminum in the brains of persons with Alzheimer disease are most likely a result of the disease and not a cause, as correspondingly high levels of aluminum are not found in blood and hair. There is ongoing research into the possible value of dietary supplements for the normal memory problems that beset healthy older people.

Eating a healthful diet, obtaining sufficient sleep, avoiding smoking, keeping physically fit, and maintaining an active mind are among the practices that may increase not only life expectancy but also the chance of a full and productive life in one's later years. The so-called free-radical theory of aging—the notion that aging is accelerated by highly reactive substances that damage cellular components, and that intake of various antioxidants can repair free-radical damage and thereby slow aging—has generated much interest and is a promising area of research, but it has not been scientifically established. On the contrary, the life spans of various mammalian species have not been extended significantly by antioxidant therapy. Ongoing studies are investigating whether the consumption of 30 percent fewer calories (undernutrition, not malnutrition) slows aging and age-related disease and extends life spans in nonhuman primates. There is no evidence that severe energy restriction would extend the human life span beyond its current maximum of 115 to 120 years.

Sports Nutrition

Sports nutrition is the foundation of athletic success. It is a well-designed nutrition plan that allows active adults and athletes to perform at their best. It supplies the right food type, energy, nutrients, and fluids to keep the body well hydrated and functioning at peak levels. A sports nutrition diet may vary day to day, depending on specific energy demands.

Sports Nutrition Basics

Macronutrients

The energy required for living and physical activity comes from the food we eat and fluid intake. Macronutrients in the following food groups supply the energy essential to optimal body function:

- Carbohydrates are either simple or complex, and the most important energy source for the human body. Simple carbs include sugars naturally occurring in foods like fruits, vegetables, and milk. Whole grain bread, potatoes, most vegetables, and oats are examples of healthy complex carbs. Your digestive system breaks down carbohydrates into glucose or blood sugar which feeds energy to your cells, tissues, and organs.
- Proteins are made up of a chain of amino acids and are essential to every cell of the human body. Protein can either be complete or incomplete. A complete protein contains all the amino acids needed by the body, and include animal sources like meat, fish, poultry, and milk. Incomplete protein sources (typically plant-based proteins) often lack one or more of the essential amino acids. Essential amino acids can't be made by the body and must be supplied by food. Protein plays an important role in muscle recovery and growth.

- Fats can be saturated or unsaturated, and they play a vital role in the human body. Unsaturated fats are considered healthy and come from plant sources like olive oil and nuts. Saturated fats are found in animal products like red meats and high-fat dairy, which are indicated to increase the risk of disease. Healthy fats provide energy, help with body development, protect our organs, and maintain cell membranes.

The Goal of Sports Nutrition

Active adults and competitive athletes turn to sports nutrition to help them achieve their goals. Examples of individual goals could include gaining lean mass, improving body composition, or enhancing athletic performance. These sport-specific scenarios require differing nutritional programs. Research findings indicate the right food type, caloric intake, nutrient timing, fluids, and supplementation are essential and specific to each individual. The following are different states of training and competitive sport benefiting from sports nutrition:

Eating for Exercise/Athletic Performance

Training programs require a well-designed diet for active adults and competitive athletes. Research shows a balanced nutrition plan should include sufficient calories and healthy macronutrients to optimize athletic performance. The body will use carbohydrates or fats as the main energy source, depending on exercise intensity and duration. Inadequate caloric intake can impede athletic training and performance.

For example, and according to research, energy expenditure for extreme cyclists competing in the Tour de France is approximately 12,000 calories per day.

- Carbohydrates are the main fuel source for an active adult or competitive athlete. General guidelines for carbohydrate intake are based on body size and training characteristics. Carbohydrate needs in a daily diet can range from 45 to 65 percent of total food intake depending on physical demands.

- Proteins are responsible for muscle growth and recovery in the active adult or athlete. Sufficient amounts of protein per individual help maintain a positive nitrogen balance in the body, which is vital to muscle tissue. Protein requirements can vary significantly ranging from 0.8g to 2g per kilogram of body weight per day.

- Fats help maintain energy balance, regulate hormones, and restore muscle tissue. Omega-3 and omega-6 are essential fatty acids that are especially important to a sports nutrition diet. Research findings recommend an athlete consume approximately 30 percent of their total daily caloric intake as a healthy fat.

Eating for Endurance

Endurance programs are defined as one to three hours per day of moderate to high-intensity exercise. High-energy intake in the form of carbohydrates is essential. According to research, target carbohydrate consumption for endurance athletes ranges from 6g to 10g per kilogram of body weight per day. Fat is a secondary source of energy used during long-duration training sessions. Endurance athletes are more at risk for dehydration. Replacing fluids and electrolytes lost through sweat are necessary for peak performance.

Eating for Strength

Resistance training programs are designed to gradually build the strength of skeletal muscle. Strength training is high-intensity work. It requires sufficient amounts of all macronutrients for muscle development. Protein intake is especially vital to increase and maintain lean body mass. Research indicates protein requirements can vary from 1.2g to 3.1g per kilogram of body weight per day.

Eating for Competition

Preparing for a competitive sport will vary in sports nutrition requirements. For example, strength athletes strive to increase lean mass and body size for their sport. Endurance runners focus on reduced body weight/fat for peak body function during their event. Athletic goals will determine the best sports nutrition strategy. Pre and post-workout meal planning are unique for each athlete and essential for optimal performance.

Hydration and Sports Performance

Adequate hydration and electrolytes are essential for health and athletic performance. We all lose water throughout the day, but active adults and athletes lose additional body water (and a significant amount of sodium) sweating during intense workouts.

Dehydration is the process of losing body water, and fluid deficits greater than 2 percent of body weight can compromise athletic performance and cognitive function. Athletes are recommended to use fluid replacement strategies as part of their sports nutrition to maintain optimal body functioning. Rehydration with water and sports drinks containing sodium are often consumed depending on the athlete and sporting event. Lack of sufficient hydration for athletes may lead to the following:

- Hypohydration (dehydration)
- Hypovolemia (decreased plasma/blood volume)
- Hyponatremia (low blood sodium levels/water intoxication)

Supplements in Sports Nutrition

Sports supplements and foods are unregulated products marketed to enhance athletic performance. According to the Academy of Sports Medicine, “the ethical use of sports supplements is a personal choice and remains controversial." There are limited supplements backed by clinical research. The Institute of Sport has provided a general guide ranking sports performance supplements and foods according to the significance of scientific evidence:

- Sports food: sports drinks, bars, and gels, electrolyte supplements, protein supplements, liquid meal supplements.
- Medical supplements: iron, calcium, vitamin D, multi-vitamin/mineral, omega-3 fatty acids.
- Performance supplements: creatine, caffeine, sodium bicarbonate, beta-alanine, nitrate.

Sports Nutrition for Special Populations and Environments

Sports nutrition covers a wide spectrum of needs for athletes. Certain populations and environments require additional guidelines and information to enhance athletic performance.

- Vegetarian athlete: A vegetarian diet contains high intakes of plant proteins, fruits, vegetables, whole grains, and nuts. It can be nutritionally adequate, but insufficient evidence exists on long-term vegetarianism and athletic performance. Dietary assessments are recommended to avoid deficiencies and to ensure adequate nutrients to support athletic demands.
- High altitude: Specialized training and nutrition are required for athletes training at high altitude. Increasing red blood cells to carry more oxygen is essential. Iron-rich foods are an important component for this athlete as well. Increased risk of illness is indicated with chronic high altitude exposure. Foods high in antioxidants and protein are essential. Fluid requirements will vary per athlete, and hydration status should be individually monitored.
- Hot environments: Athletes competing in hot conditions are at greater risk of heat illness. Heat illness can have adverse health complications. Fluid and electrolyte balance is crucial for these athletes. Hydration strategies are required to maintain peak performance while exercising in the heat.
- Cold environments: Primary concerns for athletes exercising in the cold are adequate hydration and body temperature. Leaner athletes are at higher risk of hypothermia. Modifying caloric and carbohydrate intake are important for this athlete. Appropriate foods and fluids that withstand cold temperatures will promote optimal athletic performance.

Special Topics in Sports Nutrition

Eating disorders in athletes are not uncommon. Many athletes are required to maintain lean bodies and low body weight and exhibit muscular development. Chronic competitive pressure can create psychological and physical stress of the athlete leading to disordered eating habits. Without proper counseling, adverse health effects may eventually develop. The most common eating disorders among athletes may include:

- Anorexia nervosa
- Bulimia
- Compulsive exercise disorder
- Orthorexia

Obviously, the nutritional needs of these individuals greatly differ from that of other active adults or athletes. Until someone with an eating disorder is considered well again, the primary focus should be put on treating and managing the eating disorder and consuming the nutrition needed to achieve and maintain good health, rather than athletic performance.

Micronutrient deficiencies are a concern for active adults and athletes. Exercise stresses important body functions where micronutrients are required. Additionally, athletes often restrict calories and certain food groups, which may potentially lead to deficiencies of essential micronutrients. Research indicates the most common micronutrient deficiencies include:

- Iron deficiency: can impair muscle function and compromise athletic performance.
- Vitamin D deficiency: can result in decreased bone strength and reduced muscle metabolic function.
- Calcium deficiency: can impair the repair of bone tissue, decrease regulation of muscle contraction, and reduce nerve conduction.

Roles of a Sports Dietitian

Athletes and active adults are seeking guidance from sports professionals to enhance their athletic performance. Sports dietitians are increasingly hired to develop nutrition and fluid programs catered to the individual athlete or teams. A unique credential has been created for sports nutrition professionals: Board Certified Specialist in Sports Dietetics (CSSD). Sports dietitians should have knowledge in the following areas:

- Clinical nutrition,
- Nutrition science,
- Exercise physiology,

- Evidence-based research,
- Safe and effective nutrition assessments,
- Sports nutrition guidance,
- Counseling for health and athletic performance,
- Medical nutrition therapy,
- Design and management of effective nutrition strategies and
- Effective nutrition programming for health, fitness, and optimal physical performance.

Malnutrition

Malnutrition is a state of nutrition in which a deficiency or excess (or imbalance) of energy, protein and other nutrients causes measurable adverse effects on tissue / body form (body shape, size and composition) and function and clinical outcome.

Symptoms of Malnutrition

Malnutrition can often be very difficult to recognise, particularly in patients who are overweight or obese to start with. Malnutrition can happen very gradually, which can make it very difficult to spot in the early stages. Some of the symptoms and signs to watch out for include:

- Loss of appetite,
- Weight loss – clothes, rings, jewellery, dentures may become loose,
- Tiredness, loss of energy,
- Reduced ability to perform normal tasks,
- Reduced physical performance – for example, not being able to walk as far or as fast as usual,
- Altered mood – malnutrition can be associated with lethargy and depression,
- Poor concentration and
- Poor growth in children.

People who are at the risk of malnutrition:

- Older people over the age of 65, particularly if they are living in a care home or nursing home or have been admitted to hospital.
- People with long-term conditions, such as diabetes, kidney disease, chronic lung disease.
- People with chronic progressive conditions – for example, dementia or cancer.
- People who abuse drugs or alcohol.

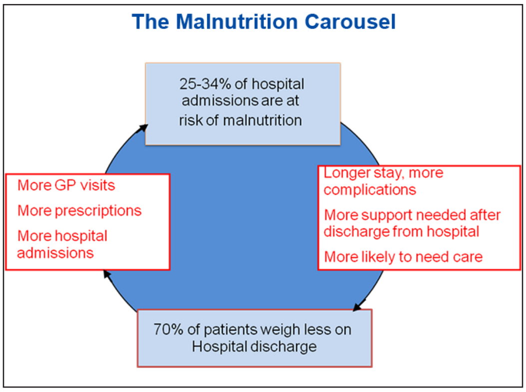
There are also social factors that can increase the risk of malnutrition including:

- Poverty,
- Social isolation and
- Cultural norms – for example, hospitals and care homes may not always provide food that meets particular religious or cultural needs and so increase the risk of malnutrition whilst a person is away from their normal environment.

Physical factors can also increase the risk of malnutrition. For example:

- Eating may be difficult because of a painful mouth or teeth.
- Swallowing may be more difficult (a stroke can affect swallowing) or painful.
- Losing your sense of smell or taste may affect your appetite.
- Being unable to cook for yourself may result in reduced food intake.
- Limited mobility or lack of transport may make it difficult to get food.

It is also important to realise that if an older person is less able to feed themselves and becomes malnourished, this will make them more susceptible to disease, which in turn will make their nutritional state worse and impair recovery. The Malnutrition Carousel below describes this downward vicious spiral.



Consequences of Malnutrition

Malnutrition affects every system in the body and always results in increased vulnerability to illness, increased complications and in very extreme cases even death.

1. Immune system: Reduced ability to fight infection.

2. Muscles:

- Inactivity and reduced ability to work, shop, cook and self-care,
- Inactivity may also lead to pressure ulcers and blood clots,
- Falls,
- Reduced ability to cough may predispose to chest infections and pneumonia and
- Heart failure.

Impaired wound Healing

1. Kidneys: Inability to regulate salt and fluid can lead to over-hydration or dehydration.

2. Brain: Malnutrition causes apathy, depression, introversion, self-neglect and deterioration in social interactions.

3. Reproduction: Malnutrition reduces fertility and if present during pregnancy can predispose to problems with diabetes, heart disease and stroke in the baby in later life.

4. Impaired temperature regulation: This can lead to hypothermia.

Consequences of malnutrition in children and adolescents:

- Growth failure and stunting,
- Delayed sexual development,
- Reduced muscle mass and strength,
- Impaired intellectual development,
- Rickets and
- Increased lifetime risk of osteoporosis.

Consequences of specific micronutrient deficiencies:

- Iron deficiency can cause anaemia,

- Zinc deficiency causes skin rashes and decreased ability to fight infection,
- Vitamin B12 deficiency can cause anaemia and problems with nerves,
- Vitamin D deficiency causes rickets in children and osteomalacia in adults,
- Vitamin C deficiency causes scurvy and
- Vitamin A deficiency causes night blindness.

Detection of Malnutrition

The best way to detect malnutrition is by the use of malnutrition screening tools, such as the 'Malnutrition Universal Screening Tool' ('MUST').This tool consists of three parts:

- Body Mass Index (BMI) – this is calculated from an individual's weight and height. A BMI of less than 18.5kg/m^2 suggests a significant risk of malnutrition.
- A history of recent weight loss that has happened without any intention to lose weight. The unintentional loss of more than 10% of normal body weight in the last 3 -6 months suggests a significant risk of malnutrition.
- An "acute disease effect" associated with being acutely ill and unable to eat for more than five days.

This tool assesses patients as being at low, medium or high risk of malnutrition and guides the user to develop individualised care plans for treatment if required and further monitoring.

Treatment of Malnutrition

Recognising the problem is the most important first step. Once individuals and those involved in their care are aware of the problem, often simple measures to increase food intake may be enough to reverse the downward cycle. We know for example that giving nutritional supplements to malnourished patients reduces complications such as wound breakdown by 70% and death by 40%.

Once an individual has been assessed as being at risk of malnutrition it is always necessary to assess the problem in more detail and identify any other factors that are contributing to the problem. Treatment should always be tailored to the needs of the individual, but in general, if a person is able to eat and does not have a diminished appetite, then the first step would to encourage this with a "Food first" approach. This may be in the form of advice on meals, snacks, nourishing drinks and food fortification, but should include setting goals of treatment and a plan for monitoring to ensure that these goals are met.

If simple measures are not working or where the patient has a reduced appetite, then an assessment and support from a dietitian may be needed. In addition to fortifying food and increasing what you eat, there are many different oral nutritional supplements. These should usually only be used under the supervision of a dietitian or doctor. It is also possible that you may need to take a vitamin and mineral supplement – but only if advised to do so by the professional treating you.

For patients who are unable to eat, nutrition can be provided by tube feeding – either into the gut (Enteral) or directly into the bloodstream via a vein (Parenteral).

Enteral Tube Feeding (Enteral Nutrition/EN)

The commonest type of enteral tube feeding uses a tube that is passed through the nose and down into the stomach (a nasogastric tube). This is usually for short term feeding (less than 4 weeks) to provide nutritional support as a patient recovers from illness.

For longer term feeding into the stomach, a tube is usually placed through the abdomen directly into the stomach (a PEG tube, or Percutaneous Gastrostomy tube).

If it is not possible to use the stomach, it is also possible to place tubes into the jejunum (the first part of the small intestine or small bowel). These can either be nasojejunal tubes (short –term) or jejunostomy tubes which are place through the abdominal wall directly (long-term).

Parenteral Nutrition (PN)

If it is not possible to use your gut for nutrition (for example in patients who have lost part of their gut or in whom the gut does not work properly to be able to absorb nutrients) then nutrients can be given directly into the blood stream. The sterile feed is given through a small tube (catheter) placed in a vein and is tailored to ensure that you are given all the nutrients that your body needs. For most patients this is done whilst they are in hospital for no more than a few weeks as they recover from illness. However for some patients, this is their only means of nutrition and they learn to do this themselves at home (Home Parenteral Nutrition or HPN).

Types of Malnutrition

Malnutrition is a group of conditions in children and adults generally related to poor quality or insufficient quantity of nutrient intake, absorption, or utilization.

There are two major types of malnutrition:

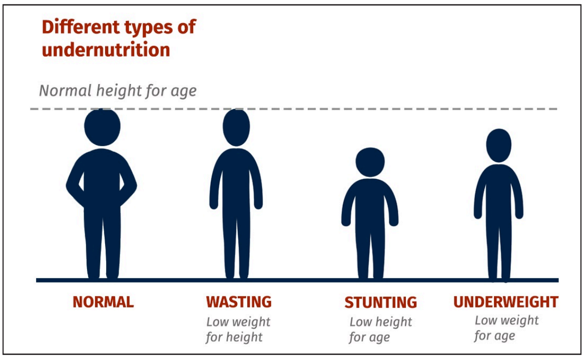
- Protein-energy malnutrition - resulting from deficiencies in any or all nutrients.
- Micronutrient deficiency diseases - resulting from a deficiency of specific micronutrients.

Protein-energy Malnutrition

There are three types of protein-energy malnutrition in children:

Type	Appearance	Cause
Acute malnutrition	Wasting or thinness	Acute inadequate nutrition leading to rapid weight loss or failure to gain weight normally.
Chronic malnutrition	Stunting or shortness	Inadequate nutrition over long period of time leading to failure of linear growth.
Acute and chronic malnutrition	Underweight	A combination measure, therefore, it could occur as a result of wasting, stunting, or both.

These forms of protein-energy malnutrition in children can be pictured like this:



Wasting and stunting are very different forms of malnutrition. Stunting is chronic and its causative factors are poorly understood. Stunting usually does not pose an immediate threat to life and is relatively common in many populations in less-developed countries. This is not to say that it is unimportant, just less important than wasting in humanitarian emergencies. Wasting results from an acute shortage of food, is reversible with refeeding, and has a relatively high mortality rate. For these reasons, wasting is the highest priority form of malnutrition in humanitarian emergencies.

Nutritional Disease

Nutritional disease is any of the nutrient-related diseases and conditions that cause illness in humans. Nutritional diseases also include developmental abnormalities that can be prevented by diet, hereditary metabolic disorders that respond to dietary treatment, the interaction of foods and nutrients with drugs, food allergies and intolerances, and potential hazards in the food supply.

Vitamin A

Vitamin A deficiency is the leading cause of preventable blindness. Night blindness is an early sign of vitamin A deficiency, followed by abnormal dryness of the eye and ultimately scarring of the cornea, a condition known as xerophthalmia. Other symptoms include dry skin, hardening of epithelial cells elsewhere in the body (such as mucous membranes), and impaired growth and development. In many areas where vitamin A deficiency is endemic, the incidence is being reduced by giving children a single large dose of vitamin A every six months. A genetically modified form of rice containing beta-carotene, a precursor of vitamin A, has the potential to reduce greatly the incidence of vitamin A deficiency, but the use of this so-called golden rice is controversial.

Vitamin D

Vitamin D (also known as vitamin D hormone) is synthesized in the body in a series of steps, starting in the skin by the action of sunlight's ultraviolet rays on a precursor compound; thus, without adequate food sources of vitamin D, a deficiency of the vitamin can occur when exposure to sunlight is limited. Lack of vitamin D in children causes rickets, a disease characterized by inadequate mineralization of bone, growth retardation, and skeletal deformities such as bowed legs. The adult form of rickets, known as osteomalacia, results in weak muscles as well as weak bones. Inadequate vitamin D may also contribute to the thinning of bones seen in osteoporosis. Individuals with limited sun exposure (including women who completely cover their bodies for religious reasons), elderly or homebound persons, and those with dark skin, particularly those who live in northern latitudes, are at risk of vitamin D deficiency. Vitamin D is found in very few foods naturally; thus fortification of milk and other foods (e.g., margarine, cereals, and breads) with the vitamin has helped protect those populations in which sun exposure is inadequate. Supplemental vitamin D also may help protect against bone fractures in the elderly, who make and activate vitamin D less efficiently even if exposed to sunlight.

Vitamin E

Vitamin E deficiency is rare in humans, although it may develop in premature infants and in people with impaired fat absorption or metabolism. In the former, fragility of red blood cells (hemolysis) is seen; in the latter, where deficiency is more prolonged, neuromuscular dysfunction involving the spinal cord and retina may result in loss of reflexes, impaired balance and coordination, muscle weakness, and visual disturbances. No specific metabolic function has been established for vitamin E; however, it is an important part of the antioxidant system that inhibits lipid peroxidation; i.e., it protects cells and their membranes against the damaging effects of free radicals (reactive oxygen and nitrogen species) that are produced metabolically or enter the body from the environment. The requirement for vitamin E is increased with increasing consumption of polyunsaturated fatty acids. People who smoke or are subjected to air pollution may also need more of the vitamin to protect against oxidative damage to the lungs.

Vitamin K

Vitamin K is necessary for the formation of prothrombin and other blood-clotting factors in the liver, and it also plays a role in bone metabolism. A form of the vitamin is produced by bacteria in the colon and can be utilized to some degree. Vitamin K deficiency causes impaired clotting of the blood and internal bleeding, even without injury. Due to poor transport of vitamin K across the placenta, newborn infants in developed countries are routinely given the vitamin intramuscularly or orally within six hours of birth to protect against a condition known as hemorrhagic disease of the newborn. Vitamin K deficiency is rare in adults, except in syndromes with poor fat absorption, in liver disease, or during treatment with certain anticoagulant drugs, which interfere with vitamin K metabolism. Bleeding due to vitamin K deficiency may be seen in patients whose gut bacteria have been killed by antibiotics.

Thiamin

Prolonged deficiency of thiamin (vitamin B_1) results in beriberi, a disease that has been endemic in populations where white rice has been the staple. Thiamin deficiency is still seen in areas where white rice or flour constitutes the bulk of the diet and thiamin lost in milling is not replaced through enrichment. Symptoms of the form known as dry beriberi include loss of appetite, confusion and other mental symptoms, muscle weakness, painful calf muscles, poor coordination, tingling and paralysis. In wet beriberi there is edema and the possibility of an enlarged heart and heart failure. Thiamin deficiency can also occur in populations eating large quantities of raw fish harbouring intestinal microbes that contain the enzyme thiaminase. In the developed world, thiamin deficiency is linked primarily to chronic alcoholism with poor diet, manifesting as Wernicke-Korsakoff syndrome, a condition with rapid eye movements, loss of muscle coordination, mental confusion, and memory loss.

Riboflavin

Riboflavin (vitamin B_2) deficiency, known as ariboflavinosis, is unlikely without the simultaneous deficiency of other nutrients. After several months of riboflavin deprivation, symptoms include cracks in the skin at the corners of the mouth, fissures of the lips, and an inflamed, magenta-coloured tongue. Because riboflavin is readily destroyed by ultraviolet light, jaundiced infants who are treated with light therapy are administered the vitamin. Milk, milk products, and cereals, major sources of riboflavin in the diet, are packaged to prevent exposure to light.

Niacin

Symptoms of pellagra develop about two months after niacin is withdrawn from the diet. Pellagra is characterized by the so-called three Ds—diarrhea, dermatitis, and dementia—and, if it is allowed to progress untreated, death ensues. The niacin in corn and other cereal grains is largely in bound form, unable to be absorbed well. Soaking corn in lime water, as practiced by Native American populations for centuries, frees bound niacin and thus protects against pellagra. In addition, unlike other cereals, corn is low in the amino acid tryptophan, which can be converted in part to niacin. Sufficient high-quality protein (containing tryptophan) in the diet can protect against niacin deficiency even if intake of niacin itself is inadequate.

Vitamin B_6

Vitamin B_6 (pyridoxine and related compounds) is essential in protein metabolism, the synthesis of neurotransmitters, and other critical functions in the body. Deficiency symptoms include dermatitis, microcytic hypochromic anemia (small, pale red blood cells), impaired immune function, depression, confusion, and convulsions. Although full-blown vitamin B_6 deficiency is rare, marginal inadequacy is more widespread, especially among the elderly, who may have a reduced ability to absorb the vitamin. People with alcoholism, especially those with the liver diseases cirrhosis and hepatitis, are at risk of deficiency. A number of drugs, including the tuberculosis drug isoniazid, interfere with vitamin B_6 metabolism.

Folic Acid

Vitamin B_{12} and folic acid (folate) are two B vitamins with many closely related functions, notably participation in DNA synthesis. As a result, people with deficiencies of either vitamin show many of the same symptoms, such as weakness and fatigue due to megaloblastic anemia, a condition in which red blood cells, lacking sufficient DNA for cell division, are large and immature. Deficiency of folic acid also causes disruption of cell division along the gastrointestinal tract, which results in persistent diarrhea, and impaired synthesis of white blood cells and platelets. Inadequate intake of the vitamin in early pregnancy may cause neural tube defects in the fetus. Thus, women capable of becoming pregnant are advised to take 400 micrograms (µg) of folic acid daily from supplements, fortified foods (such as fortified cereals), or both—in addition to consuming foods rich in folic acid such as fresh fruits and vegetables (especially leafy greens) and legumes. The cancer drug methotrexate interferes with folic acid metabolism, causing side effects such as hair loss and diarrhea. Folic acid deficiency may also result from heavy use of alcohol, which interferes with absorption of the vitamin.

Vitamin B_{12}

Deficiency of vitamin B_{12} (cobalamin), like folic acid, results in megaloblastic anemia (large, immature red blood cells), due to interference with normal DNA synthesis. Additionally, vitamin B_{12} maintains the myelin sheath that protects nerve fibres; therefore, an untreated deficiency of the vitamin can result in nerve degeneration and eventually paralysis. Large amounts of folic acid (over 1,000 µg per day) may conceal, and possibly even exacerbate, an underlying vitamin B_{12} deficiency. Only animal foods are reliable sources of vitamin B_{12}. Vegans, who eat no foods of animal origin, are at risk of vitamin B_{12} deficiency and must obtain the vitamin through fortified food or a supplement. For people who regularly eat animal products, deficiency of the vitamin is unlikely, unless there is a defect in absorption. In order to be absorbed, vitamin B_{12} must be bound to intrinsic factor, a substance secreted by the stomach. If intrinsic factor is absent (due to an autoimmune disorder known as pernicious anemia) or if there is insufficient production of hydrochloric acid by the stomach, absorption of the vitamin will be limited. Pernicious anemia, which occurs most often in the elderly, can be treated by injections or massive oral doses (1,000 μg) of vitamin B_{12}.

Pantothenic Acid

Pantothenic acid is so widespread in foods that deficiency is unlikely under normal circumstances. Deficiency has been seen only in individuals fed semisynthetic diets deficient in the vitamin or in subjects given a pantothenic acid antagonist. Symptoms of deficiency include fatigue, irritability, sleep disturbances, abdominal distress, and neurological symptoms such as tingling in the hands. Deficiency of the vitamin was suspected during World War II when prisoners of war in Asia who exhibited “burning feet” syndrome, characterized by numbness and tingling in the toes and other neurological symptoms, responded only to the administration of pantothenic acid.

Biotin

Deficiency of biotin is rare, and this may be due in part to synthesis of the vitamin by bacteria in the colon, although the importance of this source is unclear. Biotin deficiency has been observed in people who regularly eat large quantities of raw egg white, which contains a glycoprotein (avidin) that binds biotin and prevents its absorption. A rare genetic defect that renders some infants unable to absorb a form of biotin in food can be treated with a supplement of the vitamin. Long-term use of certain anticonvulsant drugs may also impair biotin absorption. Symptoms of deficiency include skin rash, hair loss, and eventually neurological abnormalities.

Vitamin C

Vitamin C, also known as ascorbic acid, functions as a water-soluble antioxidant and as a cofactor in various enzyme systems, such as those involved in the synthesis of connective tissue components and neurotransmitters. Symptoms of scurvy, a disease caused by vitamin C deficiency, include pinpoint hemorrhages (petechiae) under the skin, bleeding gums, joint pain, and impaired wound healing. Although rare in developed countries, scurvy is seen occasionally in people consuming restricted diets, particularly those containing few fruits and vegetables, or in infants fed boiled cow’s milk and no source of vitamin C. Scurvy can be prevented with relatively small quantities of vitamin C (10 milligrams [mg] per day), although recommended intakes, which aim to provide sufficient antioxidant protection, are closer to 100 mg per day. Disease states, environmental toxins, drugs, and other stresses can increase an individual’s vitamin C needs. Smokers, for example, may require an additional 35 mg of the vitamin daily to maintain vitamin C levels comparable to nonsmokers.

Minerals

Iron

Iron deficiency is the most common of all nutritional deficiencies, with much of the world's population being deficient in the mineral to some degree. Young children and premenopausal women are the most vulnerable. The main function of iron is in the formation of hemoglobin, the red pigment of the blood that carries oxygen from the lungs to other tissues. Since each millilitre of blood contains 0.5 mg of iron (as a component of hemoglobin), bleeding can drain the body's iron reserves. When iron stores are depleted a condition arises known as microcytic hypochromic anemia, characterized by small red blood cells that contain less hemoglobin than normal. Symptoms of severe iron deficiency anemia include fatigue, weakness, apathy, pale skin, difficulty breathing on exertion, and low resistance to cold temperatures. During childhood, iron deficiency can affect behaviour and learning ability as well as growth and development. Severe anemia increases the risk of pregnancy complications and maternal death. Iron deficiency anemia is most common during late infancy and early childhood, when iron stores present from birth are exhausted and milk, which is poor in iron, is a primary food; during the adolescent growth spurt; and in women during the childbearing years, because of blood loss during menstruation and the additional iron needs of pregnancy. Intestinal blood loss and subsequent iron deficiency anemia in adults may also stem from ulcers, hemorrhoids, tumours, or chronic use of certain drugs such as aspirin. In developing countries, blood loss due to hookworm and other infections, coupled with inadequate dietary iron intake, exacerbates iron deficiency in both children and adults.

Iodine

Iodine deficiency disorders are the most common cause of preventable brain damage, which affects an estimated 50 million people worldwide. During pregnancy, severe iodine deficiency may impair fetal development, resulting in cretinism (irreversible mental retardation with short stature and developmental abnormalities) as well as in miscarriage and stillbirth. Other more pervasive consequences of chronic iodine deficiency include lesser cognitive and neuromuscular deficits. The ocean is a dependable source of iodine, but away from coastal areas iodine in food is variable and largely reflects the amount in the soil. In chronic iodine deficiency the thyroid gland enlarges as it attempts to trap more iodide (the form in which iodine functions in the body) from the blood for synthesis of thyroid hormones, and it eventually becomes a visible lump at the front of the neck known as a goitre. Some foods, such as cassava, millet, sweet potato, certain beans, and members of the cabbage family, contain substances known as goitrogens that interfere with thyroid hormone synthesis; these substances, which are destroyed by cooking, can be a significant factor in persons with coexisting iodine deficiency who rely on goitrogenic foods as staples.

Zinc

A constituent of numerous enzymes, zinc plays a structural role in proteins and regulates gene expression. Deficiency of the mineral was attributed to the regional diet, which was low in meat and high in legumes, unleavened breads, and whole-grain foods that contain fibre, phytic acid, and other factors that inhibit zinc absorption. Also contributing to zinc deficiency was the practice of clay eating, which interferes with the absorption of zinc, iron, and other minerals. Severe zinc deficiency has also been described in patients fed intravenous solutions inadequate in zinc and in the inherited zinc-responsive syndrome known as acrodermatitis enteropathica. Symptoms of zinc deficiency may include skin lesions, diarrhea, increased susceptibility to infections, night blindness, reduced taste and smell acuity, poor appetite, hair loss, slow wound healing, low sperm count, and impotence. Zinc is highest in protein-rich foods, especially red meat and shellfish, and zinc status may be low in protein-energy malnutrition. Even in developed countries, young children, pregnant women, the elderly, strict vegetarians, people with alcoholism, and those with malabsorption syndromes are vulnerable to zinc deficiency.

Calcium

Almost all the calcium in the body is in the bones and teeth, the skeleton serving as a reservoir for calcium needed in the blood and elsewhere. During childhood and adolescence, adequate calcium intake is critical for bone growth and calcification. A low calcium intake during childhood, and especially during the adolescent growth spurt, may predispose one to osteoporosis, a disease characterized by reduced bone mass, later in life. As bones lose density, they become fragile and unable to withstand ordinary strains; the resulting fractures, particularly of the hip, may cause incapacitation and even death. Osteoporosis is particularly common in postmenopausal women in industrial societies. Not a calcium-deficiency disease per se, osteoporosis is strongly influenced by heredity; risk of the disease can be lessened by ensuring adequate calcium intake throughout life and engaging in regular weight-bearing exercise. Sufficient calcium intake in the immediate postmenopausal years does appear to slow bone loss, although not to the same extent as do bone-conserving drugs.

Fluoride

Fluoride also contributes to the mineralization of bones and teeth and protects against tooth decay. In areas where fluoride levels in the drinking water are low, prescription fluoride supplements are recommended for children older than six months of age; dentists also may apply fluoride rinses or gels periodically to their patients' teeth. Fluoridated toothpastes are an important source of fluoride for children and also for adults, who continue to benefit from fluoride intake.

Sodium

Sodium is usually provided in ample amounts by food, even without added table salt (sodium chloride). Furthermore, the body’s sodium-conservation mechanisms are highly developed, and thus sodium deficiency is rare, even for those on low-sodium diets. Sodium depletion may occur during prolonged heavy sweating, vomiting, or diarrhea or in the case of kidney disease. Symptoms of hyponatremia, or low blood sodium, include muscle cramps, nausea, dizziness, weakness, and eventually shock and coma. After prolonged high-intensity exertion in the heat, sodium balance can be restored by drinking beverages containing sodium and glucose (so-called sports drinks) and by eating salted food. Drinking a litre of water containing two millilitres (one-third teaspoon) of table salt also should suffice.

Chloride is lost from the body under conditions that parallel those of sodium loss. Severe chloride depletion results in a condition known as metabolic alkalosis (excess alkalinity in body fluids).

Potassium

Potassium is widely distributed in foods and is rarely deficient in the diet. However, some diuretics used in the treatment of hypertension deplete potassium. The mineral is also lost during sustained vomiting or diarrhea or with chronic use of laxatives. Symptoms of potassium deficiency include weakness, loss of appetite, muscle cramps, and confusion. Severe hypokalemia (low blood potassium) may result in cardiac arrhythmias. Potassium-rich foods, such as bananas or oranges, can help replace losses of the mineral, as can potassium chloride supplements, which should be taken only under medical supervision.

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Chapter 3

Essential Nutrients

The nutrients are broadly classified into two categories, namely, micronutrients and macronutrients. Some of the micronutrients are vitamins and minerals while carbohydrates, proteins and fats fall under macronutrients. The topics elaborated in this chapter will help in gaining a better perspective about these different types of essential nutrients.

Essential nutrients are compounds that the body can't make or can't make in sufficient quantity. According to the World Health Organization, these nutrients must come from food, and they're vital for disease prevention, growth, and good health.

While there are many essential nutrients, they can be broken into two categories: macronutrients and micronutrients.

Macronutrients are eaten in large amounts and include the primary building blocks of your diet — protein, carbohydrates, and fat — which provide your body with energy.

Vitamins and minerals are micronutrients, and small doses go a long way. There are six main groups of essential micronutrients and macronutrients.

Carbohydrate

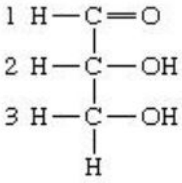
Carbohydrate is the class of naturally occurring compounds and derivatives formed from them. Carbohydrates are probably the most abundant and widespread organic substances in nature, and they are essential constituents of all living things. Carbohydrates are formed by green plants from carbon dioxide and water during the process of photosynthesis. Carbohydrates serve as energy sources and as essential structural components in organisms; in addition, part of the structure of nucleic acids, which contain genetic information, consists of carbohydrate.

Structural Arrangements and Properties

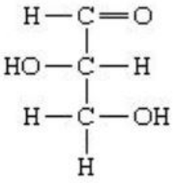
Stereoisomerism

Studies by German chemist Emil Fischer showed that carbohydrates, such as fructose and glucose, with the same molecular formulas but with different structural arrangements and properties (i.e., isomers) can be formed by relatively simple variations of their spatial, or geometric, arrangements. This type of isomerism, which is called stereoisomerism, exists in all biological systems. Among carbohydrates, the simplest example is provided by the three-carbon aldose sugar glyceraldehyde. There is no way by which the structures of the two isomers of glyceraldehyde, which can be distinguished by the so-called Fischer projection formulas, can be made identical, excluding breaking and reforming the linkages, or bonds, of the hydrogen (-H) and hydroxyl (-OH) groups attached to the carbon at position 2. The isomers are, in fact, mirror images akin to right and left hands; the term enantiomorphism is frequently employed for such isomerism. The chemical and physical properties of enantiomers are identical except for the property of optical rotation.

Optical rotation is the rotation of the plane of polarized light. Polarized light is light that has been separated into two beams that vibrate at right angles to each other; solutions of substances that rotate the plane of polarization are said to be optically active, and the degree of rotation is called the optical rotation of the solution. In the case of the isomers of glyceraldehyde, the magnitudes of the optical rotation are the same, but the direction in which the light is rotated-generally designated as plus, or d for dextrorotatory (to the right), or as minus, or l for levorotatory (to the left)-is opposite; i.e., a solution of D-(d)-glyceraldehyde causes the plane of polarized light to rotate to the right, and a solution of L-(l)-glyceraldehyde rotates the plane of polarized light to the left. Fischer projection formulas for the two isomers of glyceraldehyde are given below.



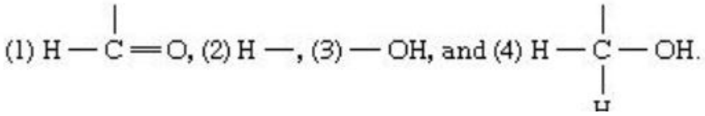
D-(*d*)-glyceraldehyde



L-(*l*)-glyceraldehyde

Configuration

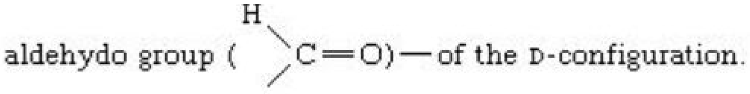
Molecules, such as the isomers of glyceraldehyde-the atoms of which can have different structural arrangements-are known as asymmetrical molecules. The number of possible structural arrangements for an asymmetrical molecule depends on the number of centres of asymmetry; i.e., for n (any given number of) centres of asymmetry, 2^n different isomers of a molecule are possible. An asymmetrical centre in the case of carbon is defined as a carbon atom to which four different groups are attached. In the three-carbon aldose sugar, glyceraldehyde, the asymmetrical centre is located at the central carbon atom.



$$(1)\ \mathrm{H}-\overset{|}{\mathrm{C}}=\mathrm{O},\ (2)\ \mathrm{H}-,\ (3)-\mathrm{OH},\ \text{and}\ (4)\ \mathrm{H}-\underset{\mathrm{H}}{\overset{|}{\underset{|}{\mathrm{C}}}}-\mathrm{OH}.$$

The position of the hydroxyl group (-OH) attached to the central carbon atom—i.e., whether -OH projects from the left or the right—determines whether the molecule rotates the plane of polarized light to the left or to the right. Since glyceraldehyde has one asymmetrical centre, n is one in the relationship 2^n, and there thus are two possible glyceraldehyde isomers. Sugars containing four carbon atoms have two asymmetrical centres; hence, there are four possible isomers (2^2). Similarly, sugars with five carbon atoms have three asymmetrical centres and thus have eight possible isomers (2^3). Keto sugars have one less asymmetrical centre for a given number of carbon atoms than do aldehyde sugars.

A convention of nomenclature states that the form of glyceraldehyde whose asymmetrical carbon atom has a hydroxyl group projecting to the right is designated as of the D-configuration; that form, whose asymmetrical carbon atom has a hydroxyl group projecting to the left, is designated as L. All sugars that can be derived from D-glyceraldehyde—i.e., hydroxyl group attached to the asymmetrical carbon atom most remote from the aldehyde or keto end of the molecule projects to the right—are said to be of the D-configuration; those sugars derived from L-glyceraldehyde are said to be of the L-configuration.



Representative disaccharides and oligosaccharides

Common name	Component sugars	Linkages	Sources
Cellobiose	Glucose, glucose	B1 → 4*	Hydrolysis of cellulose
Gentiobiose	Glucose, glucose	B1 → 6	Plant glycosides, amygdalin
Isomaltose	Glucose, glucose	A1 → 6	Hydrolysis of glycogen, amylopectin
Raffinose**	Galactose, glucose, fructose	A1 → 6, α1 → 2	Sugarcane, beets, seeds
Stachyose**	Galactose, galactose, glucose, fructose	A1 → 6, α1 → 6, α1 → 2	Soybeans, jasmine, twigs, lentils

*The linkage joins carbon atom 1 (in the β configuration) of one glucose molecule and carbon atom 4 of the second glucose molecule; the linkage may also be abbreviated β-1, 4.

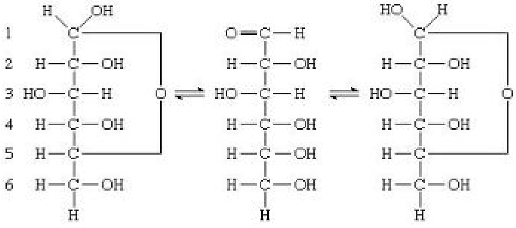
**Raffinose and stachyose are galactosyl sucroses.

The configurational notation D or L is independent of the sign of the optical rotation of a sugar in solution. It is common, therefore, to designate both, as, for example, D-(l)-fructose or D-(d)-glucose; i.e., both have a D-configuration at the centre of asymmetry most remote from the aldehyde end (in glucose) or keto end (in fructose) of the molecule, but fructose is levorotatory and glucose is dextrorotatory—hence the latter has been given the alternative name dextrose. Although the initial assignments of configuration for the glyceraldehydes were made on purely arbitrary grounds, studies that were carried out nearly half a century later established them as correct in an absolute spatial sense. In biological systems, only the D or L form may be utilized.

When more than one asymmetrical centre is present in a molecule, as is the case with sugars having four or more carbon atoms, a series of DL pairs exists, and they are functionally, physically, and chemically distinct. Thus, although D-xylose and D-lyxose both have five carbon atoms and are of the D-configuration, the spatial arrangement of the asymmetrical centres (at carbon atoms 2, 3, and 4) is such that they are not mirror images.

Hemiacetal and Hemiketal Forms

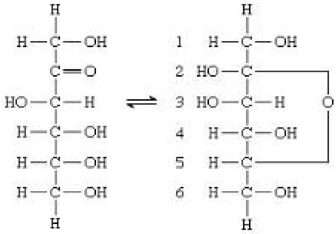
Although optical rotation has been one of the most frequently determined characteristics of carbohydrates, the rotational behaviour of freshly prepared solutions of many sugars differs from that of solutions that have been allowed to stand. This phenomenon, known as mutarotation, is demonstrable even with apparently identical sugars and is caused by a type of stereoisomerism involving formation of an asymmetrical centre at the first carbon atom (aldehyde carbon) in aldoses and the second one (keto carbon) in ketoses.



α-D-glucose (hemiacetal form)

D-glucose (linear, open-chain, or free form)

β-D-glucose (hemiacetal form)

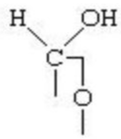


D-fructose (linear, open-chain, or free form)

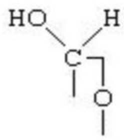
α-D-fructose (hemiketal form)

Most pentose and hexose sugars, therefore, do not exist as linear, or open-chain, structures in solution but form cyclic, or ring, structures in hemiacetal or hemiketal forms, respectively. As illustrated for glucose and fructose, the cyclic structures are formed by the addition of the hydroxyl group (—OH) from either the fourth, fifth, or sixth carbon atom to the carbonyl group $>C=O$ at position 1 in glucose or 2 in fructose. In the case of five-membered cyclic ketohexose or six-membered cyclic aldohexose, the cyclic forms are in equilibrium with (i.e., the rate of conversion from one form to another is stable) the open-chain structure—a free aldehyde if the solution contains glucose, a free ketone if it contains fructose; each form has a different optical rotation value. Since the forms are in equilibrium with each other, a constant value of optical rotation is measurable; the two cyclic forms represent more than 99.9 percent of the sugar in the case of a glucose solution.

By definition, the carbon atom containing the aldehyde or keto group is called the anomeric carbon atom; similarly, carbohydrate stereoisomers that differ in configuration only at this carbon atom are called anomers. When a cyclic hemiacetal or hemiketal structure forms, the structure with the new hydroxyl group projecting on the same side as that of the oxygen involved in forming the ring is called the alpha anomer; that with the hydroxyl group projecting on the opposite side from that of the oxygen ring is called the beta anomer.

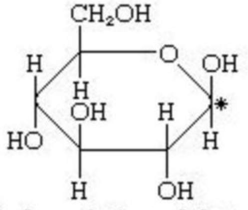


alpha anomer



beta anomer

The spatial arrangements of the atoms in these cyclic structures are better shown (glucose is used as an example) in the representation devised by British organic chemist Sir Norman Haworth they are still in widespread use. In the formulation the asterisk indicates the position of the anomeric carbon atom; the carbon atoms, except at position 6, usually are not labelled.



Haworth formulation of β-D-glucose

The large number of asymmetrical carbon atoms and the consequent number of possible isomers considerably complicates the structural chemistry of carbohydrates.

Classes of Carbohydrates

Monosaccharides

The most common naturally occurring monosaccharides are D-glucose, D-mannose, D-fructose, and D-galactose among the hexoses and D-xylose and L-arabinose among the pentoses. In a special sense, D-ribose and 2-deoxy-D-ribose are ubiquitous because they form the carbohydrate component of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), respectively; these sugars are present in all cells as components of nucleic acids.

Some naturally occurring monosaccharides

Sugar	**Sources**
L-arabinose	mesquite gum, wheat bran
D-ribose	all living cells; as component of ribonucleic acid
D-xylose	corncobs, seed hulls, straw
D-ribulose	as an intermediate in photosynthesis
2-deoxy-D-ribose	as constituent of deoxyribonucleic acid
D-galactose	lactose, agar, gum arabic, brain glycolipids
D-glucose	sucrose, cellulose, starch, glycogen
D-mannose	seeds, ivory nut
D-fructose	sucrose, artichokes, honey
L-fucose	marine algae, seaweed
L-rhamnose	poison-ivy blossom, oak bark
D-mannoheptulose	avocado
D-altroheptulose	numerous plants

D-Xylose, found in most plants in the form of a polysaccharide called xylan, is prepared from corncobs, cottonseed hulls, or straw by chemical breakdown of xylan. D-Galactose, a common constituent of both oligosaccharides and polysaccharides, also occurs in carbohydrate-containing lipids, called glycolipids, which are found in the brain and other nervous tissues of most animals. Galactose is generally prepared by acid hydrolysis (breakdown involving water) of lactose, which is composed of galactose and glucose. Since the biosynthesis of galactose in animals occurs through intermediate compounds derived directly from glucose, animals do not require galactose in the diet. In fact, in most human populations the majority of people do not retain the ability to manufacture the enzyme necessary to metabolize galactose after they reach the age of four, and many individuals possess a hereditary defect known as galactosemia and never have the ability to metabolize galactose.

D-Glucose the naturally occurring form, is found in fruits, honey, blood, and, under abnormal conditions, in urine. It is also a constituent of the two most common naturally found disaccharides, sucrose and lactose, as well as the exclusive structural unit of the polysaccharides cellulose, starch, and glycogen. Generally, D-glucose is prepared from either potato starch or cornstarch.

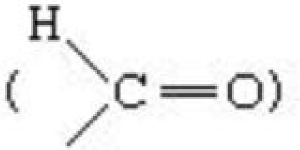
D-Fructose, a ketohexose, is one of the constituents of the disaccharide sucrose and is also found in uncombined form in honey, apples, and tomatoes. Fructose, generally considered the sweetest monosaccharide, is prepared by sucrose hydrolysis and is metabolized by humans.

Chemical Reactions

The reactions of the monosaccharides can be conveniently subdivided into those associated with the aldehyde or keto group and those associated with the hydroxyl groups.

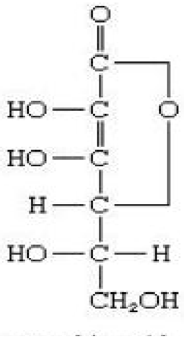
The relative ease with which sugars containing a free or potentially free aldehyde or keto group can be oxidized to form products has been known for a considerable time and once was the basis for the detection of these so-called reducing sugars in a variety of sources. For many years, analyses of blood glucose and urinary glucose were carried out by a procedure involving the use of an alkaline copper compound. Because the reaction has undesirable features—extensive destruction of carbohydrate structure occurs, and the reaction is not very specific (i.e., sugars other than glucose give similar results) and does not result in the formation of readily identifiable products—blood and urinary glucose now are analyzed by using the enzyme glucose oxidase, which catalyzes the oxidation of glucose to products that include hydrogen peroxide. The hydrogen peroxide then is used to oxidize a dye present in the reaction mixture; the intensity of the colour is directly proportional to the amount of glucose initially present. The enzyme, glucose oxidase, is highly specific for β-D-glucose.

In another reaction, the aldehyde group of glucose:



Reacts with alkaline iodine to form a class of compounds called aldonic acids. One important aldonic acid is ascorbic acid (vitamin C), an essential dietary component for humans and guinea pigs. The formation of similar acid derivatives does not occur with the keto sugars.

Either the aldehyde or the keto group of a sugar may be reduced (i.e., hydrogen added) to form an alcohol; compounds formed in this way are called alditols, or sugar alcohols. The product formed as a result of the reduction of the aldehyde carbon of D-glucose is called sorbitol (D-glucitol). D-Glucitol also is formed when L-sorbose is reduced. The reduction of mannose results in mannitol, that of galactose in dulcitol.

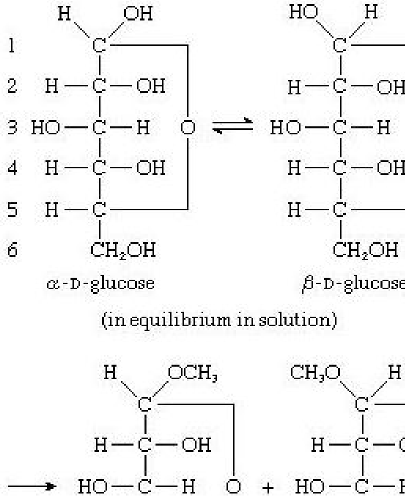


Ascorbic acid, vitamin C (L-gulonolactone-2, 3-enediol)

Sugar alcohols that are of commercial importance include sorbitol (D-glucitol), which is commonly used as a sweetening agent, and D-mannitol, which is also used as a sweetener, particularly in chewing gums, because it has a limited water solubility and remains powdery and granular on long storage.

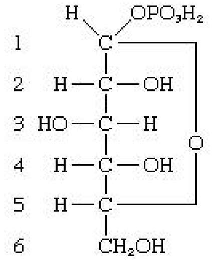
Formation of Glycosides

The hydroxyl group that is attached to the anomeric carbon atom (i.e., the carbon containing the aldehyde or keto group) of carbohydrates in solution has unusual reactivity, and derivatives, called glycosides, can be formed; glycosides formed from glucose are called glucosides. It is not possible for equilibration between the α- and β-anomers of a glycoside in solution (i.e., mutarotation) to occur. The reaction by which a glycoside is formed involves the hydroxyl group (-OH) of the anomeric carbon atom (numbered 1) of both α and β forms of D-glucose—α and β forms of D-glucose are shown in equilibrium in the reaction sequence—and the hydroxyl group of an alcohol (methyl alcohol in the reaction sequence); methyl α-D-glucosides and β-D-glucosides are formed as products, as is water.



Among the wide variety of naturally occurring glycosides are a number of plant pigments, particularly those red, violet, and blue in colour; these pigments are found in flowers and consist of a pigment molecule attached to a sugar molecule, frequently glucose. Plant indican (from Indigofera species), composed of glucose and the pigment indoxyl, was important in the preparation of indigo dye before synthetic dyes became prevalent. Of a number of heart muscle stimulants that occur as glycosides, digitalis is still used. Other naturally occurring glycosides include vanillin, which is found in the vanilla bean, and amygdalin (oil of bitter almonds); a variety of glycosides found in mustard have a sulfur atom at position 1 rather than oxygen.

A number of important antibiotics are glycosides; among the best known are streptomycin and erythromycin. Glucosides-i.e., glycosides formed from glucose-in which the anomeric carbon atom (at position 1) has phosphoric acid linked to it, are extremely important biological compounds. For example, α-D-glucose-1-phosphate is an intermediate product in the biosynthesis of cellulose, starch, and glycogen; similar glycosidic phosphate derivatives of other monosaccharides participate in the formation of naturally occurring glycosides and polysaccharides.

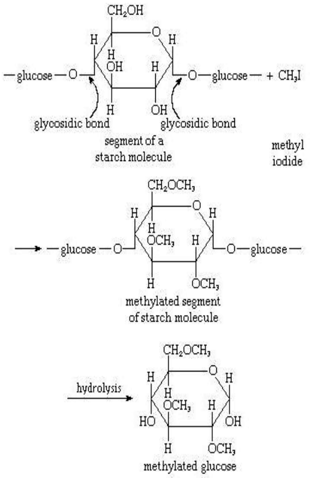


α-D-glucose-1-phosphate

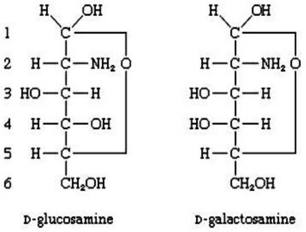
The hydroxyl groups other than the one at the anomeric carbon atom can undergo a variety of reactions. Esterification, which consists of reacting the hydroxyl groups with an appropriate acidic compound, results in the formation of a class of compounds called sugar esters. Among the common ones are the sugar acetates, in which the acid is acetic acid. Esters of phosphoric acid and sulfuric acid are important biological compounds; glucose-6-phosphate, for example, plays a central role in the energy metabolism of most living cells, and D-ribulose 1,5-diphosphate is important in photosynthesis.

Formation of Methyl Ethers

Treatment of a carbohydrate with methyl iodide or similar agents under appropriate conditions results in the formation of compounds in which the hydroxyl groups are converted to methyl groups ($-CH_3$). Called methyl ethers, these compounds are employed in structural studies of oligosaccharides and polysaccharides because their formation does not break the bonds, called glycosidic bonds, that link adjacent monosaccharide units. An example is the etherification of a starch molecule carried out using methyl iodide, in which methyl groups become attached to the glucose molecules, forming a methylated segment in the starch molecule; note that the glycosidic bonds are not broken by the reaction with methyl iodide. When the methylated starch molecule then is broken down (hydrolyzed), hydroxyl groups are located at the positions in the molecule previously involved in linking one sugar molecule to another, and a methylated glucose, in this case named 2,3,6 tri-O-methyl-D-glucose, forms. The linkage positions (which are not methylated) in a complex carbohydrate can be established by analyzing the locations of the methyl groups in the monosaccharides. This technique is useful in determining the structural details of polysaccharides, particularly since the various methylated sugars are easily separated by techniques involving gas chromatography, in which a moving gas stream carries a mixture through a column of a stationary liquid or solid, the components thus being resolved.



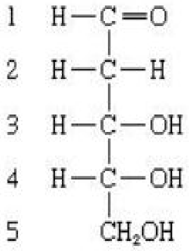
When the terminal group (CH_2OH) of a monosaccharide is oxidized chemically or biologically, a product called a uronic acid is formed. Glycosides that are derived from D-glucuronic acid (the uronic acid formed from D-glucose) and fatty substances called steroids appear in the urine of animals as normal metabolic products; in addition, foreign toxic substances are frequently converted in the liver to glucuronides before excretion in the urine. D-Glucuronic acid also is a major component of connective tissue polysaccharides, and D-galacturonic acid and D-mannuronic acid, formed from D-galactose and D-mannose, respectively, are found in several plant sources.



Other compounds formed from monosaccharides include those in which one hydroxyl group, usually at the carbon at position 2, is replaced by an amino group (-NH_2); these compounds, called amino sugars, are widely distributed in nature. The two most important ones are glucosamine (2-amino-2-deoxy-D-glucose) and galactosamine (2-amino-2-deoxy-D-galactose).

Neither amino sugar is found in the uncombined form. Both occur in animals as components of glycolipids or polysaccharides; e.g., the primary structural polysaccharide (chitin) of insect outer skeletons and various blood group substances.

In a number of naturally occurring sugars, known as deoxy sugars, the hydroxyl group at a particular position is replaced by a hydrogen atom. By far the most important representative is 2-deoxy-D-ribose, the pentose sugar found in deoxyribonucleic acid (DNA); the hydroxyl group at the carbon atom at position 2 has been replaced by a hydrogen atom.



2-deoxy-D-ribose

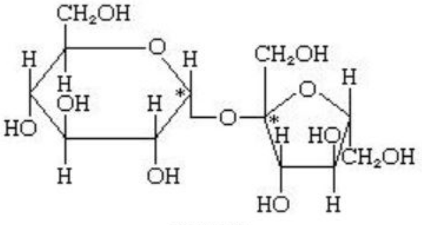
Other naturally occurring deoxy sugars are hexoses, of which L-rhamnose (6-deoxy-L-mannose) and L-fucose (6-deoxy-L-galactose) are the most common; the latter, for example, is present in the carbohydrate portion of blood group substances and on the outer surface of red blood cells.

Disaccharides and Oligosaccharides

Disaccharides are a specialized type of glycoside in which the anomeric hydroxyl group of one sugar has combined with the hydroxyl group of a second sugar with the elimination of the elements of water. Although an enormous number of disaccharide structures are possible, only a limited number are of commercial or biological significance.

Sucrose and Trehalose

Sucrose, or common table sugar, is a major commodity worldwide. The unusual type of linkage between the two anomeric hydroxyl groups of glucose and fructose means that neither a free aldehyde group (on the glucose moiety) nor a free keto group (on the fructose moiety) is available to react unless the linkage between the monosaccharides is destroyed; for this reason, sucrose is known as a nonreducing sugar. Sucrose solutions do not exhibit mutarotation, which involves formation of an asymmetrical centre at the aldehyde or keto group. If the linkage between the monosaccharides composing sucrose is broken, the optical rotation value of sucrose changes from positive to negative; the new value reflects the composite rotation values for D-glucose, which is dextrorotatory (+52°), and D-fructose, which is levorotatory (−92°). The change in the sign of optical rotation from positive to negative is the reason sucrose is sometimes called invert sugar.



sucrose

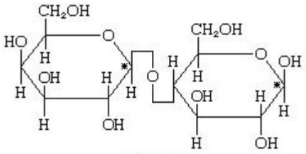
The commercial preparation of sucrose takes advantage of the alkaline stability of the sugar, and a variety of impurities are removed from crude sugarcane extracts by treatment with alkali. After this step, syrup preparations are crystallized to form table sugar. Successive "crops" of sucrose crystals are "harvested," and the later ones are known as brown sugar. The residual syrupy material is called either cane final molasses or blackstrap molasses; both are used in the preparation of antibiotics, as sweetening agents, and in the production of alcohol by yeast fermentation. Sucrose is formed following photosynthesis in plants by a reaction in which sucrose phosphate first is formed.

The disaccharide trehalose is similar in many respects to sucrose but is much less widely distributed. It is composed of two molecules of α-D-glucose and is also a nonreducing sugar. Trehalose is present in young mushrooms and in the resurrection plant (Selaginella); it is of considerable biological interest because it is also found in the circulating fluid (hemolymph) of many insects. Since trehalose can be converted to a glucose phosphate compound by an enzyme-catalyzed reaction that does not require energy, its function in hemolymph may be to provide an immediate energy source, a role similar to that of the carbohydrate storage forms (i.e., glycogen) found in higher animals.

Lactose and Maltose

Lactose is one of the sugars (sucrose is another) found most commonly in human diets throughout the world; it constitutes about 7 percent of human milk and about 4–5 percent of the milk of mammals such as cows, goats, and sheep. Lactose consists of two aldohexoses—β-D-galactose and glucose—linked so that the aldehyde group at the anomeric carbon of glucose is free to react; i.e., lactose is a reducing sugar.

A variety of metabolic disorders related to lactose may occur in infants; in some cases, they are the result of a failure to metabolize properly the galactose portion of the molecule.



β-lactose

Although not found in uncombined form in nature, the disaccharide maltose is biologically important because it is a product of the enzymatic breakdown of starches during digestion. Maltose consists of α-D-glucose linked to a second glucose unit in such a way that maltose is a reducing sugar. Maltose, which is readily hydrolyzed to glucose and can be metabolized by animals, is employed as a sweetening agent and as a food for infants whose tolerance for lactose is limited.

Polysaccharides

Polysaccharides, or glycans, may be classified in a number of ways; the following scheme is frequently used. Homopolysaccharides are defined as polysaccharides formed from only one type of monosaccharide. Homopolysaccharides may be further subdivided into straight-chain and branched-chain representatives, depending upon the arrangement of the monosaccharide units. Heteropolysaccharides are defined as polysaccharides containing two or more different types of monosaccharides; they may also occur in both straight-chain and branched-chain forms. In general, extensive variation of linkage types does not occur within a polysaccharide structure, nor are there many polysaccharides composed of more than three or four different monosaccharides; most contain one or two.

Representative homopolysaccharides				
Homopolysaccharide	Sugar Component	Linkage	Function	Sources
Cellulose	Glucose	β, 1 → 4	Structural	Throughout plant kingdom
Amylose	Glucose	α, 1 → 4	Food storage	Starches, especially corn, potatoes, rice
Chitin	N-acetylglucosamine	β, 1 → 4	Structural	Insect and crustacean skeleton
Inulin	Fructose	β, 2 → 1	Food storage	Artichokes, chicory
Xylan	Xylose	β, 1 → 4	Structural	All land plants
Glycogen	Glucose	α, 1 → 4, 6 ← 1, α	Food storage	Liver and muscle cells of all animals
Amylopectin	Glucose	α, 1 → 4, 6 ← 1, α	Food storage	Starches, especially corn, potatoes, rice

Representative homopolysaccharides				
Dextran	Glucose	α, 1 → 6, 4 ← 1, α	Unknown	Primarily bacterial
Agar*	Galactose	α, 1 → 3	Structural	Seaweeds
*May contain sulfate groups.				

Homopolysaccharides

In general, homopolysaccharides have a well-defined chemical structure, although the molecular weight of an individual amylose or xylan molecule may vary within a particular range, depending on the source; molecules from a single source also may vary in size, because most polysaccharides are formed biologically by an enzyme-catalyzed process lacking genetic information regarding size.

The basic structural component of most plants, cellulose, is widely distributed in nature. It has been estimated that 50 billion to 100 billion tons of cellulose are synthesized yearly as a result of photosynthesis by higher plants. The proportion of cellulose to total carbohydrate found in plants may vary in various types of woods from 30 to 40 percent, and to more than 98 percent in the seed hair of the cotton plant. Cellulose, a large, linear molecule composed of 3,000 or more β-D-glucose molecules, is insoluble in water.

The chains of glucose units composing cellulose molecules are frequently aligned within the cell-wall structure of a plant to form fibre-like or crystalline arrangements. This alignment permits very tight packing of the chains and promotes their structural stability but also makes structural analysis difficult. The relationships between cellulose and other polysaccharides present in the cell wall are not well established; in addition, the presence of unusual chemical linkages or nonglucose units within the cellulose structure has not yet been established with certainty.

During the preparation of cellulose, raw plant material is treated with hot alkali; this treatment removes most of the lignin, the hemicelluloses, and the mucilaginous components. The cellulose then is processed to produce papers and fibres. The high resistance of cellulose to chemical or enzymatic breakdown is important in the manufacture of paper and cloth. Cellulose also is modified chemically for other purposes; e.g., compounds such as cellulose acetate are used in the plastics industry, in the production of photographic film, and in the rayon-fibre industry. Cellulose nitrate (nitrocellulose) is employed in the lacquer and explosives industries.

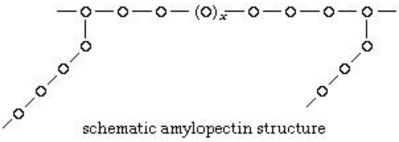
The noteworthy biological stability of cellulose is dramatically illustrated by trees, the life-span of which may be several thousand years. Enzymes capable of breaking down cellulose are generally found only among several species of bacteria and molds. The apparent ability of termites to utilize cellulose as an energy source depends on the presence in their intestinal tracts of protozoans that can break it down. Similarly, the single-celled organisms present in the rumina of sheep and cattle are responsible for the ability of these animals to utilize the cellulose present in typical grasses and other feeds.

Xylans are almost as ubiquitous as cellulose in plant cell walls and contain predominantly β-D-xylose units linked as in cellulose. Some xylans contain other sugars, such as L-arabinose, but they form branches and are not part of the main chain. Xylans are of little commercial importance.

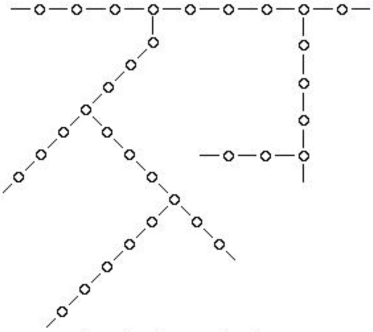
The term starch refers to a group of plant reserve polysaccharides consisting almost exclusively of a linear component (amylose) and a branched component (amylopectin). The use of starch as an energy source by humans depends on the ability to convert it completely to individual glucose units; the process is initiated by the action of enzymes called amylases, synthesized by the salivary glands in the mouth, and continues in the intestinal tract. The primary product of amylase action is maltose, which is hydrolyzed to two component glucose units as it is absorbed through the walls of the intestine.

A characteristic reaction of the amylose component of starch is the formation with iodine of a complex compound with a characteristic blue colour. About one iodine molecule is bound for each seven or eight glucose units, and at least five times that many glucose units are needed in an amylose chain to permit the effective development of the colour.

The amylopectin component of starch is structurally similar to glycogen in that both are composed of glucose units linked together in the same way, but the distance between branch points is greater in amylopectin than in glycogen, and the former may be thought of as occupying more space per unit weight.



schematic amylopectin structure



schematic glycogen structure

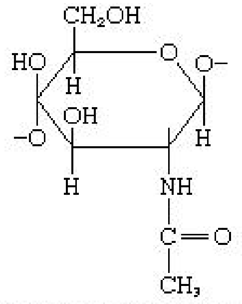
The applications of starches other than as foods are limited. Starches are employed in adhesive manufacture, and starch nitrate has some utility as an explosive.

Glycogen, which is found in all animal tissues, is the primary animal storage form of carbohydrate and, indirectly, of rapidly available energy. The distance between branch points in a glycogen molecule is only five or six units, which results in a compact tree-like structure. The ability of higher animals to form and break down this extensively branched structure is essential to their well-being; in conditions known as glycogen storage diseases, these activities are abnormal, and the asymmetrical glycogen molecules that are formed have severe, often fatal, consequences. Glycogen synthesis and breakdown are controlled by substances called hormones.

Large molecules—e.g., pectins and agars—composed of galactose or its uronic-acid derivative (galacturonic acid) are important because they can form gels. Pectins, which are predominantly galacturonans, are produced from citrus fruit rinds; they are used commercially in the preparation of jellies and jams. Agar is widely employed in biological laboratories as a solidifying agent for growth media for microorganisms and in the bakery industry as a gelling agent; it forms a part of the diet of people in several areas of East Asia.

Dextrans, a group of polysaccharides composed of glucose, are secreted by certain strains of bacteria as slimes. The structure of an individual dextran varies with the strain of microorganism. Dextrans can be used as plasma expanders (substitutes for whole blood) in cases of severe shock. In addition, a dextran derivative compound is employed medically as an anticoagulant for blood.

Chitin is structurally similar to cellulose, but the repeating sugar is 2-deoxy-2-acetamido-D-glucose (N-acetyl-D-glucosamine) rather than glucose.



N-acetyl-D-glucosamine

Sometimes referred to as animal cellulose, chitin is the major component of the outer skeletons of insects, crustaceans, and other arthropods, as well as annelid and nematode worms, mollusks, and coelenterates. The cell walls of most fungi also are predominantly chitin, which comprises nearly 50 percent of the dry weight of some species. Since chitin is nearly as chemically inactive as cellulose and easily obtained, numerous attempts, none of which has thus far been successful, have been made to develop it commercially. The nitrogen content of the biosphere, however, is stabilized by the ability of soil microorganisms to degrade nitrogen-containing compounds such as those found in insect skeletons; these microorganisms convert the nitrogen in complex molecules to a form usable by plants. If such microorganisms did not exist, much of the organic nitrogen present in natural materials would be unavailable to plants.

Heteropolysaccharides

In general, heteropolysaccharides (heteroglycans) contain two or more different monosaccharide units. Although a few representatives contain three or more different monosaccharides, most naturally occurring heteroglycans contain only two different ones and are closely associated with lipid or protein. The complex nature of these substances has made detailed structural studies extremely difficult. The major heteropolysaccharides include the connective-tissue polysaccharides, the blood group substances, glycoproteins (combinations of carbohydrates and proteins) such as gamma globulin, and glycolipids (combinations of carbohydrates and lipids), particularly those found in the central nervous system of animals and in a wide variety of plant gums.

Representative heteropolysaccharides

Heteropolysaccharide	Component Sugars	Functions	Fistribution
Hyaluronic acid	D-glucuronic acid and *N*-acetyl-D-glucosamine	Lubricant, shock absorber, water binding	Connective tissue, skin
Chondroitin-4-sulfate*	D-glucuronic acid and *N*-acetyl-D-galactosamine-4-*O*-sulfate	Calcium accumulation, cartilage and bone formation	Cartilage
Heparin*	D-glucuronic acid, *L*-iduronic acid, *N*-sulfo-D-glucosamine	Anticoagulant	Mast cells, blood
Gamma globulin*	*N*-acetyl-hexosamine, D-mannose, D-galactose	Antibody	Blood
Blood group substance*	D-glucosamine, D-galactosamine, *L*-fucose, D-galactose	Blood group specificity	Cell surfaces, especially red blood cells

*Covalently linked to protein; the proportion of protein to carbohydrate in such complex molecules varies from about 10% protein in the case of chondroitin-4-sulfate to better than 95% for gamma globulin.

The most important heteropolysaccharides are found in the connective tissues of all animals and include a group of large molecules that vary in size, shape, and interaction with other body substances. They have a structural role, and the structures of individual connective-tissue polysaccharides are related to specific animal functions; hyaluronic acid, for example, the major component of joint fluid in animals, functions as a lubricating agent and shock absorber.

The connective-tissue heteropolysaccharides contain acidic groups (uronic acids or sulfate groups) and can bind both water and inorganic metal ions. They can also play a role in other physiological functions; e.g., in the accumulation of calcium before bone formation. Ion-binding ability also appears to be related to the anticoagulant activity of the heteropolysaccharide heparin.

The size of the carbohydrate portion of glycoproteins such as gamma globulin or hen-egg albumin is usually between five and 10 monosaccharide units; several such units occur in some glycoprotein molecules. The function of the carbohydrate component has not yet been established except for glycoproteins associated with cell surfaces; in this case, they appear to act as antigenic determinants—i.e., they are capable of inducing the formation of specific antibodies.

Preparation and Analysis

In general, monosaccharides are prepared by breakdown with acids of the polysaccharides in which they occur. Sugars usually are difficult to obtain in crystalline form, and the crystallization process usually is begun by “seeding” a concentrated solution of the sugar with crystals. The techniques employed for separation of monosaccharides depend to some extent on their physical and chemical properties; chromatographic procedures are often used.

Oligosaccharides and polysaccharides are prepared from natural sources by techniques that take advantage of size, alkaline stability, or some combination of these and other properties of the molecule of interest. It should be noted that preparation of an oligosaccharide or polysaccharide usually results in a range of molecular sizes of the desired molecule. The purity of a carbohydrate preparation, which is frequently based on an analysis of its composition, is more easily established for monosaccharides and disaccharides than for large, insoluble molecules such as cellulose.

Analytical Techniques

A variety of organic chemical analytical techniques are generally applicable to studies involving carbohydrates. Optical rotation, for example, once was frequently used to characterize carbohydrates. The ability to measure the rotation of the plane of polarized light transmitted through a solution containing a carbohydrate depends on finding a suitable solvent; water usually is used, with light at a wavelength of 589 mμ (millimicrons). Optical rotation is no longer widely used to characterize monosaccharides. The magnitude and sign of the optical rotation of glycosides, however, is useful in assigning configuration (α or β) to the hydroxyl group at the anomeric centre; glycosides of the α-configuration generally have rotations of higher magnitude than do the same glycosides of the β-configuration. Optical rotation is not a completely additive property; a trisaccharide composed of three glucose residues, for example, does not have a rotation three times that of one glucose molecule. Sugar alcohols cannot form ring structures; their rotation values are extremely small, suggesting a relationship between ring structure and the ability of a carbohydrate to rotate the plane of polarized light. Certain types of reactions (e.g., glycoside hydrolysis) can be monitored by measuring the change in optical rotation as a function of time. This technique is frequently used to examine the breakdown of disaccharides or oligosaccharides to monosaccharide units, especially if a large change in the net optical rotation may be expected, as occurs in the hydrolysis of sucrose.

Spectroscopic Techniques

Several other optical techniques used in chemistry have been applied to the analysis of carbohydrates. Infrared spectroscopy, used to measure vibrational and rotational excitation of molecules, and nuclear magnetic-resonance spectroscopy, which measures the excitation of certain components of molecules in a magnetic field induced by radio-frequency radiation, are valuable, although the similarity of the functional groups (i.e., the hydroxyl groups) limits use of the former technique for most sugars. Proton magnetic-resonance spectroscopy, nuclear magnetic resonance applied to protons (H atoms), is employed to identify the relative spatial arrangements of individual hydrogen atoms in a molecule. When they are precisely placed, the corresponding positions of the hydroxyl groups attached to the same carbon atom can be deduced. An extension of this technique utilizes the resonance spectroscopy of carbon-13, a nonradioactive isotope of carbon, so that ring structures can be established with great accuracy. Both the proton and carbon magnetic resonance methods are best applied to monosaccharides; they are less valuable in studying polysaccharides because an individual hydrogen atom in a large molecule is too small for accurate detection.

Identification of Subunits

The study of polysaccharide structure usually focuses on the chemical composition, the linkage between the monosaccharide units, and the size and shape of the molecule. The size and shape of a polysaccharide can be ascertained by techniques that are usually applied to large molecules; e.g., the most accurate molecular weight determination measures the sedimentation properties of the molecule in an applied gravitational field (e.g., the rate at which a solid material is deposited from a state of suspension or solution in a liquid). Indications of the shape of polysaccharide molecules in solution are obtained from viscosity measurements, in which the resistance of the molecules to flow (viscosity) is equated with the end-to-end length of the molecule; the viscosity of hyaluronic acid, for example, shows a marked dependence on both concentration of the acid and the salt content of the solution, and, under conditions approximating those found in biological systems, a hyaluronic acid molecule may be thought of as occupying a great deal of space. Alternatively, the compact nature of a glycogen molecule of molecular weight equal to that of a molecule of hyaluronic acid results in its accommodation to a much smaller space than the latter molecule.

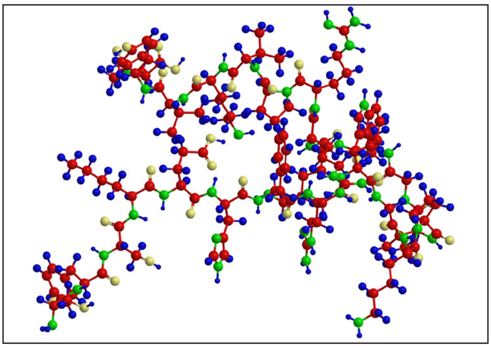
The identification of sugars in a mixture resulting from the hydrolytic breakdown of a heteropolysaccharide is most often carried out by chromatography of the mixture on paper, silica gel, or cellulose. Ready separations can be achieved between pentoses, hexoses and, for example, deoxy sugars; closely related compounds such as D-glucose and D-galactose also can be separated using chromatographic techniques. The linkage positions in polysaccharides are usually determined using the methylation procedure. The various monosaccharide methyl ethers produced by the methylation are separated by gas–liquid chromatography.

Detailed statements about polysaccharide structure and function are limited by the statistical nature of some measurements (e.g., branching frequency), the biological variability of parameters such as size and molecular weight, and incomplete information about associative interactions in living things.

Protein

Protein is a highly complex substance that is present in all living organisms. Proteins are of great nutritional value and are directly involved in the chemical processes essential for life.

A protein molecule is very large compared with molecules of sugar or salt and consists of many amino acids joined together to form long chains, much as beads are arranged on a string. There are about 20 different amino acids that occur naturally in proteins. Proteins of similar function have similar amino acid composition and sequence. Although it is not yet possible to explain all of the functions of a protein from its amino acid sequence, established correlations between structure and function can be attributed to the properties of the amino acids that compose proteins.



Peptide: The molecular structure of a peptide (a small protein) consists of a sequence of amino acids

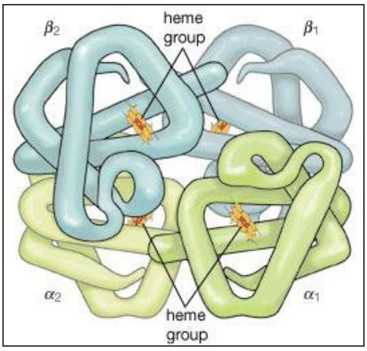
Plants can synthesize all of the amino acids; animals cannot, even though all of them are essential for life. Plants can grow in a medium containing inorganic nutrients that provide nitrogen, potassium, and other substances essential for growth. They utilize the carbon dioxide in the air during the process of photosynthesis to form organic compounds such as carbohydrates. Animals, however, must obtain organic nutrients from outside sources. Because the protein content of most plants is low, very large amounts of plant material are required by animals, such as ruminants (e.g., cows), that eat only plant material to meet their amino acid requirements. Nonruminant animals, including humans, obtain proteins principally from animals and their products-e.g., meat, milk, and eggs. The seeds of legumes are increasingly being used to prepare inexpensive protein-rich food.

Legume (amino acid) Legumes—such as beans, lentils, and peas—are high in protein and contain many essential amino acids

The protein content of animal organs is usually much higher than that of the blood plasma. Muscles, for example, contain about 30 percent protein, the liver 20 to 30 percent, and red blood cells 30 percent. Higher percentages of protein are found in hair, bones, and other organs and tissues with a low water content. The quantity of free amino acids and peptides in animals is much smaller than the amount of protein; protein molecules are produced in cells by the stepwise alignment of amino acids and are released into the body fluids only after synthesis is complete.

The high protein content of some organs does not mean that the importance of proteins is related to their amount in an organism or tissue; on the contrary, some of the most important proteins, such as enzymes and hormones, occur in extremely small amounts. The importance of proteins is related principally to their function. All enzymes identified thus far are proteins. Enzymes, which are the catalysts of all metabolic reactions, enable an organism to build up the chemical substances necessary for life—proteins, nucleic acids, carbohydrates, and lipids—to convert them into other substances, and to degrade them. Life without enzymes is not possible. There are several protein hormones with important regulatory functions. In all vertebrates, the respiratory protein hemoglobin acts as oxygen carrier in the blood, transporting oxygen from the lung to body organs and tissues. A large group of structural proteins maintains and protects the structure of the animal body.

In figure, each chain is attached to a heme group composed of porphyrin (an organic ringlike compound) attached to an iron atom. These iron-porphyrin complexes coordinate oxygen molecules reversibly, an ability directly related to the role of hemoglobin in oxygen transport in the blood.

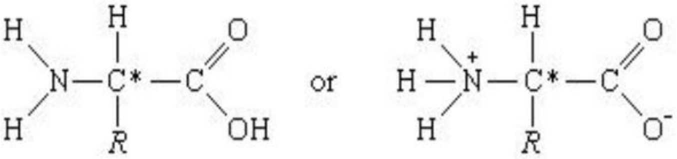


Hemoglobin is a protein made up of four polypeptide chains (α_1, α_2, β_1, and β_2)

General Structure and Properties of Proteins

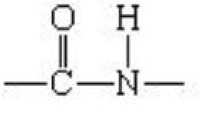
The Amino Acid Composition of Proteins

The common property of all proteins is that they consist of long chains of α-amino (alpha amino) acids. The general structure of α-amino acids is shown in . The α-amino acids are so called because the α-carbon atom in the molecule carries an amino group ($-NH_2$); the α-carbon atom also carries a carboxyl group (-COOH).

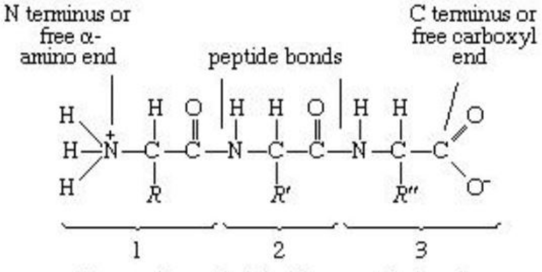


In acidic solutions, when the pH is less than 4, the -COO groups combine with hydrogen ions (H^+) and are thus converted into the uncharged form (-COOH). In alkaline solutions, at pH above 9, the ammonium groups ($-NH^+_3$) lose a hydrogen ion and are converted into amino groups ($-NH_2$). In the pH range between 4 and 8, amino acids carry both a positive and a negative charge and therefore do not migrate in an electrical field. Such structures have been designated as dipolar ions, or zwitterions (i.e., hybrid ions).

Although more than 100 amino acids occur in nature, particularly in plants, only 20 types are commonly found in most proteins. In protein molecules the α-amino acids are linked to each other by peptide bonds between the amino group of one amino acid and the carboxyl group of its neighbour.



The condensation (joining) of three amino acids yields the tripeptide.



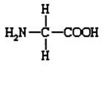
three amino acids joined by peptide bonds

It is customary to write the structure of peptides in such a way that the free α-amino group (also called the N terminus of the peptide) is at the left side and the free carboxyl group (the C terminus) at the right side. Proteins are macromolecular polypeptides—i.e., very large molecules (macromolecules) composed of many peptide-bonded amino acids. Most of the common ones contain more than 100 amino acids linked to each other in a long peptide chain. The average molecular weight (based on the weight of a hydrogen atom as 1) of each amino acid is approximately 100 to 125; thus, the molecular weights of proteins are usually in the range of 10,000 to 100,000 daltons (one dalton is the weight of one hydrogen atom). The species-specificity and organ-specificity of proteins result from differences in the number and sequences of amino acids. Twenty different amino acids in a chain 100 amino acids long can be arranged in far more than 10^{100} ways (10^{100} is the number one followed by 100 zeroes).

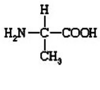
Structures of Common Amino Acids

The amino acids present in proteins differ from each other in the structure of their side (R) chains. The simplest amino acid is glycine, in which R is a hydrogen atom. In a number of amino acids, R represents straight or branched carbon chains. One of these amino acids is alanine, in which R is the methyl group ($-CH_3$). Valine, leucine, and isoleucine, with longer R groups, complete the alkyl side-chain series. The alkyl side chains (R groups) of these amino acids are nonpolar; this means that they have no affinity for water but some affinity for each other. Although plants can form all of the alkyl amino acids, animals can synthesize only alanine and glycine; thus valine, leucine, and isoleucine must be supplied in the diet.

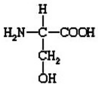
Two amino acids, each containing three carbon atoms, are derived from alanine; they are serine and cysteine. Serine contains an alcohol group ($-CH_2OH$) instead of the methyl group of alanine, and cysteine contains a mercapto group ($-CH_2SH$). Animals can synthesize serine but not cysteine or cystine. Cysteine occurs in proteins predominantly in its oxidized form (oxidation in this sense meaning the removal of hydrogen atoms), called cystine. Cystine consists of two cysteine molecules linked by the disulfide bond (-S-S-) that results when a hydrogen atom is removed from the mercapto group of each of the cysteines. Disulfide bonds are important in protein structure because they allow the linkage of two different parts of a protein molecule to-and thus the formation of loops in-the otherwise straight chains. Some proteins contain small amounts of cysteine with free sulfhydryl (-SH) groups.



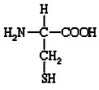
glycine
(Gly, G)



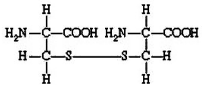
alanine
(Ala, A)



serine
(Ser, S)



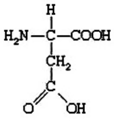
cysteine
(CysH, C)



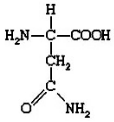
cystine
(Cys-S-S-Cys, C-C)

Four amino acids, each consisting of four carbon atoms, occur in proteins; they are aspartic acid, asparagine, threonine, and methionine. Aspartic acid and asparagine, which occur in large amounts, can be synthesized by animals. Threonine and methionine cannot be synthesized and thus are essential amino acids; i.e., they must be supplied in the diet. Most proteins contain only small amounts of methionine.

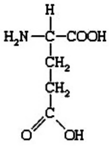
Proteins also contain an amino acid with five carbon atoms (glutamic acid) and a secondary amine (in proline), which is a structure with the amino group (-NH_2) bonded to the alkyl side chain, forming a ring. Glutamic acid and aspartic acid are dicarboxylic acids; that is, they have two carboxyl groups (-COOH).



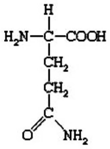
aspartic acid
(Asp, D; Asx or B)



asparagine
($AspNH_2$ or Asn, N; Asx or B)



glutamic acid
(Glu, E; Glx or Z)



glutamine
($GluNH_2$, GluN, or Gln, Q; Glx or Z)

Glutamine is similar to asparagine in that both are the amides of their corresponding dicarboxylic acid forms; i.e., they have an amide group (-$CONH_2$) in place of the carboxyl (-COOH) of the side chain. Glutamic acid and glutamine are abundant in most proteins; e.g., in plant proteins they sometimes comprise more than one-third of the amino acids present. Both glutamic acid and glutamine can be synthesized by animals.

Amino acid content of some proteins*

Amino Acid	Protein					
	Alpha-casein	Gliadin	Edestin	Collagen (oxhide)	Keratin (wool)	Myosin
lysine	60.9	4.45	19.9	27.4	6.2	85
histidine	18.7	11.7	18.6	4.5	19.7	15

Amino acid content of some proteins*

Amino Acid	Protein					
	Alpha-casein	Gliadin	Edestin	Collagen (oxhide)	Keratin (wool)	Myosin
arginine	24.7	15.7	99.2	47.1	56.9	41
aspartic acid**	63.1	10.1	99.4	51.9	51.5	85
threonine	41.2	17.6	31.2	19.3	55.9	41
serine	63.1	46.7	55.7	41.0	79.5	41
glutamic acid**	153.1	311.0	144.9	76.2	99.0	155
proline	71.3	117.8	32.9	125.2	58.3	22
glycine	37.3	—	68.0	354.6	78.0	39
alanine	41.5	23.9	57.7	115.7	43.8	78
half-cystine	3.6	21.3	10.9	0.0	105.0	86
valine	53.8	22.7	54.6	21.4	46.6	42
methionine	16.8	11.3	16.4	6.5	4.0	22
isoleucine	48.8	90.8***	41.9	14.5	29.0	42
leucine	60.3		60.0	28.2	59.9	79
tyrosine	44.7	17.7	26.9	5.5	28.7	18
phenylalanine	27.9	39.0	38.4	13.9	22.4	27
tryptophan	7.8	3.2	6.6	0.0	9.6	—
hydroxyproline	0.0	0.0	0.0	97.5	12.2	—
hydroxylysine	—	—	—	8.0	1.2	—
total	839	765	883	1,058	863	832
average residual weight	119	131	113	95	117	120

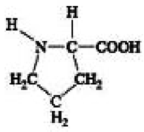
*Number of gram molecules of amino acid per 100,000 grams of protein.

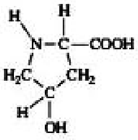
**The values for aspartic acid and glutamic acid include asparagine and glutamine, respectively.

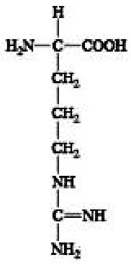
***Isoleucine plus leucine.

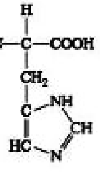
The amino acids proline and hydroxyproline occur in large amounts in collagen, the protein of the connective tissue of animals. Proline and hydroxyproline lack free amino (-NH_2) groups because the amino group is enclosed in a ring structure with the side chain; they thus cannot exist in a zwitterion form. Although the nitrogen-containing group (>NH) of these amino acids can form a peptide bond with the carboxyl group of another amino acid, the bond so formed gives rise to a kink in the peptide chain; i.e., the ring structure alters the regular bond angle of normal peptide bonds.

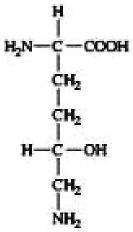
Proteins usually are almost neutral molecules; that is, they have neither acidic nor basic properties. This means that the acidic carboxyl (-COO^-) groups of aspartic and glutamic acid are about equal in number to the amino acids with basic side chains. Three such basic amino acids, each containing six carbon atoms, occur in proteins. The one with the simplest structure, lysine, is synthesized by plants but not by animals. Even some plants have a low lysine content. Arginine is found in all proteins; it occurs in particularly high amounts in the strongly basic protamines (simple proteins composed of relatively few amino acids) of fish sperm. The third basic amino acid is histidine. Both arginine and histidine can be synthesized by animals. Histidine is a weaker base than either lysine or arginine. The imidazole ring, a five-membered ring structure containing two nitrogen atoms in the side chain of histidine, acts as a buffer (i.e., a stabilizer of hydrogen ion concentration) by binding hydrogen ions (H^+) to the nitrogen atoms of the imidazole ring.

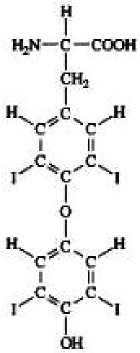

proline
(Pro, P)


hydroxyproline (Hypro)

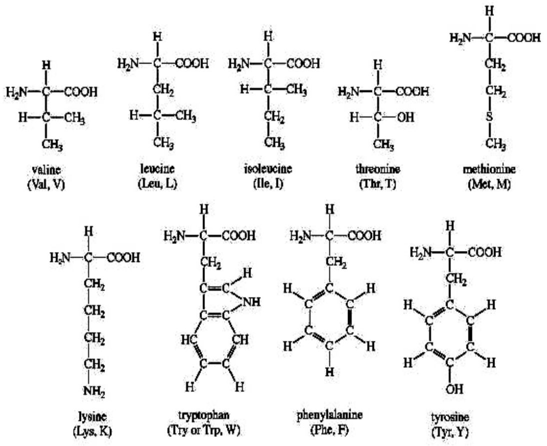

arginine
(Arg, R)


histidine
(His, H)


hydroxylysine
(Hylys or Lys–OH)


thyroxine (Thy)
occurs only in the
hormone protein
thyroglobulin:
I=iodine

The remaining amino acids—phenylalanine, tyrosine, and tryptophan—have in common an aromatic structure; i.e., a benzene ring is present. These three amino acids are essential, and, while animals cannot synthesize the benzene ring itself, they can convert phenylalanine to tyrosine.



Because these amino acids contain benzene rings, they can absorb ultraviolet light at wavelengths between 270 and 290 nanometres (nm; 1 nanometre = 10^{-9} metre = 10 angstrom units). Phenylalanine absorbs very little ultraviolet light; tyrosine and tryptophan, however, absorb it strongly and are responsible for the absorption band most proteins exhibit at 280–290 nanometres. This absorption is often used to determine the quantity of protein present in protein samples.

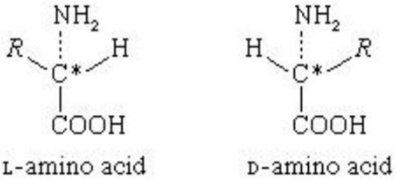
Most proteins contain only the amino acids described above; however, other amino acids occur in proteins in small amounts. For example, the collagen found in connective tissue contains, in addition to hydroxyproline, small amounts of hydroxylysine. Other proteins contain some monomethyl-, dimethyl-, or trimethyllysine-i.e., lysine derivatives containing one, two, or three methyl groups ($-CH_3$). The amount of these unusual amino acids in proteins, however, rarely exceeds 1 or 2 percent of the total amino acids.

Physicochemical Properties of the Amino Acids

The physicochemical properties of a protein are determined by the analogous properties of the amino acids in it.

The α-carbon atom of all amino acids, with the exception of glycine, is asymmetric; this means that four different chemical entities (atoms or groups of atoms) are attached to it. As a result, each of the amino acids, except glycine, can exist in two different spatial, or geometric, arrangements (i.e., isomers), which are mirror images akin to right and left hands.

These isomers exhibit the property of optical rotation. Optical rotation is the rotation of the plane of polarized light, which is composed of light waves that vibrate in one plane, or direction, only. Solutions of substances that rotate the plane of polarization are said to be optically active, and the degree of rotation is called the optical rotation of the solution. The direction in which the light is rotated is generally designed as plus, or d, for dextrorotatory (to the right), or as minus, or l, for levorotatory (to the left). Some amino acids are dextrorotatory, others are levorotatory. With the exception of a few small proteins (peptides) that occur in bacteria, the amino acids that occur in proteins are L-amino acids.



In bacteria, D-alanine and some other D-amino acids have been found as components of gramicidin and bacitracin. These peptides are toxic to other bacteria and are used in medicine as antibiotics. The D-alanine has also been found in some peptides of bacterial membranes.

In contrast to most organic acids and amines, the amino acids are insoluble in organic solvents. In aqueous solutions they are dipolar ions (zwitterions, or hybrid ions) that react with strong acids or bases in a way that leads to the neutralization of the negatively or positively charged ends, respectively. Because of their reactions with strong acids and strong bases, the amino acids act as buffers-stabilizers of hydrogen ion (H^+) or hydroxide ion (OH^-) concentrations. In fact, glycine is frequently used as a buffer in the pH range from 1 to 3 (acid solutions) and from 9 to 12 (basic solutions). In acid solutions, glycine has a positive charge and therefore migrates to the cathode (negative electrode of a direct-current electrical circuit with terminals in the solution). Its charge, however, is negative in alkaline solutions, in which it migrates to the anode (positive electrode). At pH 6.1 glycine does not migrate, because each molecule has one positive and one negative charge. The pH at which an amino acid does not migrate in an electrical field is called the isoelectric point. Most of the monoamino acids (i.e., those with only one amino group) have isoelectric points similar to that of glycine. The isoelectric points of aspartic and glutamic acids, however, are close to pH 3, and those of histidine, lysine, and arginine are at pH 7.6, 9.7, and 10.8, respectively.

Amino Acid Sequence in Protein Molecules

Since each protein molecule consists of a long chain of amino acid residues, linked to each other by peptide bonds, the hydrolytic cleavage of all peptide bonds is a prerequisite for the quantitative determination of the amino acid residues. Hydrolysis is most frequently accomplished by boiling the protein with concentrated hydrochloric acid. The quantitative determination of the amino acids is based on the discovery that amino acids can be separated from each other by chromatography on filter paper and made visible by spraying the paper with ninhydrin. The amino acids of the protein hydrolysate are separated from each other by passing the hydrolysate through a column of adsorbents, which adsorb the amino acids with different affinities and, on washing the column with buffer solutions, release them in a definite order. The amount of each of the amino acids can be determined by the intensity of the colour reaction with ninhydrin.

To obtain information about the sequence of the amino acid residues in the protein, the protein is degraded stepwise, one amino acid being split off in each step. This is accomplished by coupling the free α-amino group ($-NH_2$) of the N-terminal amino acid with phenyl isothiocyanate; subsequent mild hydrolysis does not affect the peptide bonds. The procedure, called the Edman degradation, can be applied repeatedly; it thus reveals the sequence of the amino acids in the peptide chain.

Unavoidable small losses that occur during each step make it impossible to determine the sequence of more than about 30 to 50 amino acids by this procedure. For this reason the protein is usually first hydrolyzed by exposure to the enzyme trypsin, which cleaves only peptide bonds formed by the carboxyl groups of lysine and arginine.

Levels of Structural organization in Proteins

Primary Structure

Analytical and synthetic procedures reveal only the primary structure of the proteins-that is, the amino acid sequence of the peptide chains. They do not reveal information about the conformation (arrangement in space) of the peptide chain-that is, whether the peptide chain is present as a long straight thread or is irregularly coiled and folded into a globule. The configuration, or conformation, of a protein is determined by mutual attraction or repulsion of polar or nonpolar groups in the side chains (R groups) of the amino acids. The former have positive or negative charges in their side chains; the latter repel water but attract each other. Some parts of a peptide chain containing 100 to 200 amino acids may form a loop, or helix; others may be straight or form irregular coils.

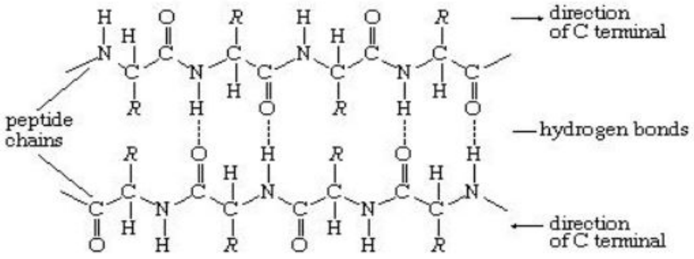
The primary structure of a protein is determined by its amino acid sequence without any regard for the arrangement of the peptide chain in space. The secondary structure is determined by the spatial arrangement of the main peptide chain without any regard for the conformation of side chains or other segments of the main chain. The tertiary structure is determined by both the side chains and other adjacent segments of the main chain, without regard for neighbouring peptide chains. Finally, the term quaternary structure is used for the arrangement of identical or different subunits of a large protein in which each subunit is a separate peptide chain.

Secondary Structure

The nitrogen and carbon atoms of a peptide chain cannot lie on a straight line, because of the magnitude of the bond angles between adjacent atoms of the chain; the bond angle is about 110°. Each of the nitrogen and carbon atoms can rotate to a certain extent, however, so that the chain has a limited flexibility. Because all of the amino acids, except glycine, are asymmetric L-amino acids, the peptide chain tends to assume an asymmetric helical shape; some of the fibrous proteins consist of elongated helices around a straight screw axis. Such structural features result from properties common to all peptide chains. The product of their effects is the secondary structure of the protein.

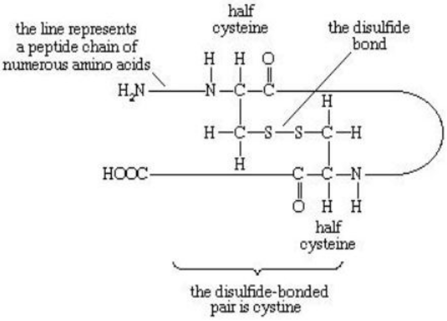
Tertiary Structure

The tertiary structure is the product of the interaction between the side chains (R) of the amino acids composing the protein. Some of them contain positively or negatively charged groups, others are polar, and still others are nonpolar. The number of carbon atoms in the side chain varies from zero in glycine to nine in tryptophan. Positively and negatively charged side chains have the tendency to attract each other; side chains with identical charges repel each other. The bonds formed by the forces between the negatively charged side chains of aspartic or glutamic acid on the one hand, and the positively charged side chains of lysine or arginine on the other hand, are called salt bridges. Mutual attraction of adjacent peptide chains also results from the formation of numerous hydrogen bonds.



Hydrogen bonds form as a result of the attraction between the nitrogen-bound hydrogen atom (the imide hydrogen) and the unshared pair of electrons of the oxygen atom in the double bonded carbon–oxygen group (the carbonyl group). The result is a slight displacement of the imide hydrogen toward the oxygen atom of the carbonyl group. Although the hydrogen bond is much weaker than a covalent bond (i.e., the type of bond between two carbon atoms, which equally share the pair of bonding electrons between them), the large number of imide and carbonyl groups in peptide chains results in the formation of numerous hydrogen bonds. Another type of attraction is that between nonpolar side chains of valine, leucine, isoleucine, and phenylalanine; the attraction results in the displacement of water molecules and is called hydrophobic interaction.

In proteins rich in cystine, the conformation of the peptide chain is determined to a considerable extent by the disulfide bonds (-S-S-) of cystine. The halves of cystine may be located in different parts of the peptide chain and thus may form a loop closed by the disulfide bond.

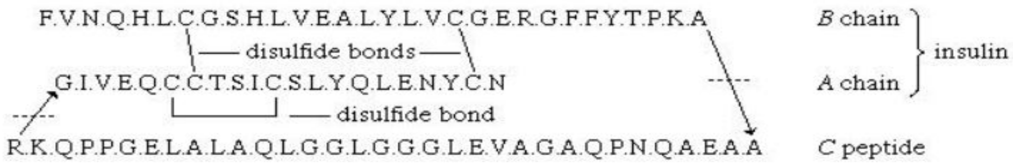


If the disulfide bond is reduced (i.e., hydrogen is added) to two sulfhydryl (-SH) groups, the tertiary structure of the protein undergoes a drastic change—closed loops are broken and adjacent disulfide-bonded peptide chains separate.

Quaternary Structure

The nature of the quaternary structure is demonstrated by the structure of hemoglobin. Each molecule of human hemoglobin consists of four peptide chains, two α-chains and two β-chains; i.e., it is a tetramer. The four subunits are linked to each other by hydrogen bonds and hydrophobic interaction. Because the four subunits are so closely linked, the hemoglobin tetramer is called a molecule, even though no covalent bonds occur between the peptide chains of the four subunits. In other proteins, the subunits are bound to each other by covalent bonds (disulfide bridges).

The amino acid sequence of porcine proinsulin is shown below. The arrows indicate the direction from the N terminus of the β-chain (B) to the C terminus of the α-chain (A).



The Isolation and Determination of Proteins

Animal material usually contains large amounts of protein and lipids and small amounts of carbohydrate; in plants, the bulk of the dry matter is usually carbohydrate. If it is necessary to determine the amount of protein in a mixture of animal foodstuffs, a sample is converted to ammonium salts by boiling with sulfuric acid and a suitable inorganic catalyst, such as copper sulfate (Kjeldahl method). The method is based on the assumption that proteins contain 16 percent nitrogen, and that nonprotein nitrogen is present in very small amounts. The assumption is justified for most tissues from higher animals but not for insects and crustaceans, in which a considerable portion of the body nitrogen is present in the form of chitin, a carbohydrate. Large amounts of nonprotein nitrogen are also found in the sap of many plants. In such cases, the precise quantitative analyses are made after the proteins have been separated from other biological compounds.

Proteins are sensitive to heat, acids, bases, organic solvents, and radiation exposure; for this reason, the chemical methods employed to purify organic compounds cannot be applied to proteins. Salts and molecules of small size are removed from protein solutions by dialysis—i.e., by placing the solution into a sac of semipermeable material, such as cellulose or acetylcellulose, which will allow small molecules to pass through but not large protein molecules, and immersing the sac in water or a salt solution. Small molecules can also be removed either by passing the protein solution through a column of resin that adsorbs only the protein or by gel filtration. In gel filtration, the large protein molecules pass through the column, and the small molecules are adsorbed to the gel.

Groups of proteins are separated from each other by salting out-i.e., the stepwise addition of sodium sulfate or ammonium sulfate to a protein solution. Some proteins, called globulins, become insoluble and precipitate when the solution is half-saturated with ammonium sulfate or when its sodium sulfate content exceeds about 12 percent. Other proteins, the albumins, can be precipitated from the supernatant solution (i.e., the solution remaining after a precipitation has taken place) by saturation with ammonium sulfate. Water-soluble proteins can be obtained in a dry state by freeze-drying (lyophilization), in which the protein solution is deep-frozen by lowering the temperature below −15 °C (5 °F) and removing the water; the protein is obtained as a dry powder.

Most proteins are insoluble in boiling water and are denatured by it-i.e., irreversibly converted into an insoluble material. Heat denaturation cannot be used with connective tissue because the principal structural protein, collagen, is converted by boiling water into water-soluble gelatin.

Fractionation (separation into components) of a mixture of proteins of different molecular weight can be accomplished by gel filtration. The size of the proteins retained by the gel depends upon the properties of the gel. The proteins retained in the gel are removed from the column by solutions of a suitable concentration of salts and hydrogen ions.

Many proteins were originally obtained in crystalline form, but crystallinity is not proof of purity; many crystalline protein preparations contain other substances. Various tests are used to determine whether a protein preparation contains only one protein. The purity of a protein solution can be determined by such techniques as chromatography and gel filtration. In addition, a solution of pure protein will yield one peak when spun in a centrifuge at very high speeds (ultracentrifugation) and will migrate as a single band in electrophoresis (migration of the protein in an electrical field). After these methods and others (such as amino acid analysis) indicate that the protein solution is pure, it can be considered so. Because chromatography, ultracentrifugation, and electrophoresis cannot be applied to insoluble proteins, little is known about them; they may be mixtures of many similar proteins.

Very small (microheterogeneous) differences in some of the apparently pure proteins are known to occur. They are differences in the amino acid composition of otherwise identical proteins and are transmitted from generation to generation; i.e., they are genetically determined. For example, some humans have two hemoglobins, hemoglobin A and hemoglobin S, which differ in one amino acid at a specific site in the molecule. In hemoglobin A the site is occupied by glutamic acid and in hemoglobin S by valine. Refinement of the techniques of protein analysis has resulted in the discovery of other instances of microheterogeneity.

The quantity of a pure protein can be determined by weighing or by measuring the ultraviolet absorbancy at 280 nanometres. The absorbency at 280 nanometres depends on the content of tyrosine and tryptophan in the protein. Sometimes the slightly less sensitive biuret reaction, a purple colour given by alkaline protein solutions upon the addition of copper sulfate, is used; its intensity depends only on the number of peptide bonds per gram, which is similar in all proteins.

Physicochemical Properties of Proteins

The Molecular Weight of Proteins

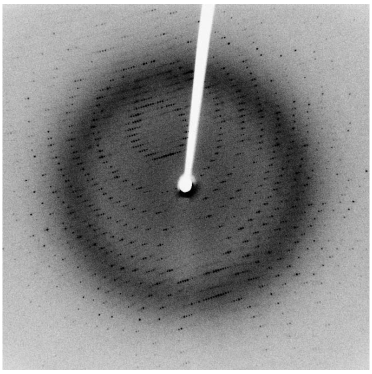
The molecular weight of proteins cannot be determined by the methods of classical chemistry (e.g., freezing-point depression), because they require solutions of a higher concentration of protein than can be prepared.

If a protein contains only one molecule of one of the amino acids or one atom of iron, copper, or another element, the minimum molecular weight of the protein or a subunit can be calculated; for example, the protein myoglobin contains 0.34 gram of iron in 100 grams of protein. The atomic weight of iron is 56; thus the minimum molecular weight of myoglobin is $(56 \times 100)/0.34$ = about 16,500. Direct measurements of the molecular weight of myoglobin yield the same value. The molecular weight of hemoglobin, however, which also contains 0.34 percent iron, has been found to be 66,000 or 4 × 16,500; thus hemoglobin contains four atoms of iron.

The method most frequently used to determine the molecular weight of proteins is ultracentrifugation-i.e., spinning in a centrifuge at velocities up to about 60,000 revolutions per minute. Centrifugal forces of more than 200,000 times the gravitational force on the surface of Earth are achieved at such velocities. The first ultracentrifuges, built in 1920, were used to determine the molecular weight of proteins. The molecular weights of a large number of proteins have been determined. Most consist of several subunits, the molecular weight of which is usually less than 100,000 and frequently ranges from 20,000 to 30,000. Proteins of very high molecular weights are found among hemocyanins, the copper-containing respiratory proteins of invertebrates; some range as high as several million. Although there is no definite lower limit for the molecular weight of proteins, short amino acid sequences are usually called peptides.

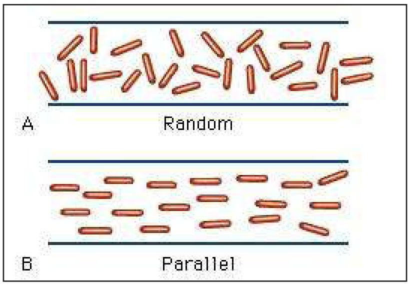
The Shape of Protein Molecules

In the technique of X-ray diffraction, the X-rays are allowed to strike a protein crystal. The X-rays, diffracted (bent) by the crystal, impinge on a photographic plate, forming a pattern of spots. This method reveals that peptide chains can assume very complicated, apparently irregular shapes. Two extremes in shape include the closely folded structure of the globular proteins and the elongated, unidimensional structure of the threadlike fibrous proteins; both were recognized many years before the technique of X-ray diffraction was developed. Solutions of fibrous proteins are extremely viscous (i.e., sticky); those of the globular proteins have low viscosity (i.e., they flow easily). A 5 percent solution of a globular protein-ovalbumin, for example-easily flows through a narrow glass tube; a 5 percent solution of gelatin, a fibrous protein, however, does not flow through the tube, because it is liquid only at high temperatures and solidifies at room temperature. Even solutions containing only 1 or 2 percent of gelatin are highly viscous and flow through a narrow tube either very slowly or only under pressure.



X-ray diffraction: X-ray diffraction pattern of a crystallized enzyme

The elongated peptide chains of the fibrous proteins can be imagined to become entangled not only mechanically but also by mutual attraction of their side chains, and in this way they incorporate large amounts of water. Most of the hydrophilic (water-attracting) groups of the globular proteins, however, lie on the surface of the molecules, and, as a result, globular proteins incorporate only a few water molecules. If a solution of a fibrous protein flows through a narrow tube, the elongated molecules become oriented parallel to the direction of the flow, and the solution thus becomes birefringent like a crystal; i.e., it splits a light ray into two components that travel at different velocities and are polarized at right angles to each other. Globular proteins do not show this phenomenon, which is called flow birefringence. Solutions of myosin, the contractile protein of muscles, show very high flow birefringence; other proteins with very high flow birefringence include solutions of fibrinogen, the clotting material of blood plasma, and solutions of tobacco mosaic virus. The gamma-globulins of the blood plasma show low flow birefringence, and none can be observed in solutions of serum albumin and ovalbumin.



Flow birefringence: Orientation of elongated, rodlike macromolecules (A) in resting solution, or (B) during flow through a horizontal tube

Hydration of Proteins

When dry proteins are exposed to air of high water content, they rapidly bind water up to a maximum quantity, which differs for different proteins; usually it is 10 to 20 percent of the weight of the protein. The hydrophilic groups of a protein are chiefly the positively charged groups in the side chains of lysine and arginine and the negatively charged groups of aspartic and glutamic acid. Hydration (i.e., the binding of water) may also occur at the hydroxyl (-OH) groups of serine and threonine or at the amide ($-CONH_2$) groups of asparagine and glutamine.

The binding of water molecules to either charged or polar (partly charged) groups is explained by the dipolar structure of the water molecule; that is, the two positively charged hydrogen atoms form an angle of about 105°, with the negatively charged oxygen atom at the apex. The centre of the positive charges is located between the two hydrogen atoms; the centre of the negative charge of the oxygen atom is at the apex of the angle. The negative pole of the dipolar water molecule binds to positively charged groups; the positive pole binds negatively charged ones. The negative pole of the water molecule also binds to the hydroxyl and amino groups of the protein.

The water of hydration is essential to the structure of protein crystals; when they are completely dehydrated, the crystalline structure disintegrates. In some proteins this process is accompanied by denaturation and loss of the biological function.

In aqueous solutions, proteins bind some of the water molecules very firmly; others are either very loosely bound or form islands of water molecules between loops of folded peptide chains. Because the water molecules in such an island are thought to be oriented as in ice, which is crystalline water, the islands of water in proteins are called icebergs. Water molecules may also form bridges between the carbonyl and imino groups of adjacent peptide chains, resulting in structures similar to those of the pleated sheet but with a water molecule in the position of the hydrogen bonds of that configuration. The extent of hydration of protein molecules in aqueous solutions is important, because some of the methods used to determine the molecular weight of proteins yield the molecular weight of the hydrated protein. The amount of water bound to one gram of a globular protein in solution varies from 0.2 to 0.5 gram. Much larger amounts of water are mechanically immobilized between the elongated peptide chains of fibrous proteins; for example, one gram of gelatin can immobilize at room temperature 25 to 30 grams of water.

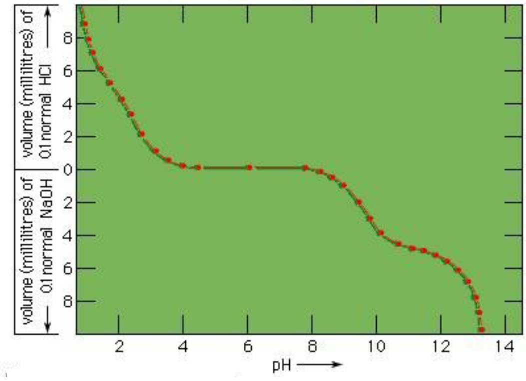
Hydration of proteins is necessary for their solubility in water. If the water of hydration of a protein dissolved in water is reduced by the addition of a salt such as ammonium sulfate, the protein is no longer soluble and is salted out, or precipitated. The salting-out process is reversible because the protein is not denatured (i.e., irreversibly converted to an insoluble material) by the addition of such salts as sodium chloride, sodium sulfate, or ammonium sulfate. Some globulins, called euglobulins, are insoluble in water in the absence of salts; their insolubility is attributed to the mutual interaction of polar groups on the surface of adjacent molecules, a process that results in the formation of large aggregates of molecules. Addition of small amounts of salt causes the euglobulins to become soluble. This process, called salting in, results from a combination between anions (negatively charged ions) and cations (positively charged ions) of the salt and positively and negatively charged side chains of the euglobulins. The combination prevents the aggregation of euglobulin molecules by preventing the formation of salt bridges between them. The addition of more sodium or ammonium sulfate causes the euglobulins to salt out again and to precipitate.

Electrochemistry of Proteins

Because the α-amino group and α-carboxyl group of amino acids are converted into peptide bonds in the protein molecule, there is only one α-amino group (at the N terminus) and one α-carboxyl group (at the C terminus) in a given protein molecule. The electrochemical character of a protein is affected very little by these two groups. Of importance, however, are the numerous positively charged ammonium groups ($-NH_3^+$) of lysine and arginine and the negatively charged carboxyl groups ($-COO^-$) of aspartic acid and glutamic acid. In most proteins, the number of positively and negatively charged groups varies from 10 to 20 per 100 amino acids.

Electrometric Titration

When measured volumes of hydrochloric acid are added to a solution of protein in salt-free water, the pH decreases in proportion to the amount of hydrogen ions added until it is about 4. Further addition of acid causes much less decrease in pH because the protein acts as a buffer at pH values of 3 to 4. The reaction that takes place in this pH range is the protonation of the carboxyl group-i.e., the conversion of $-COO^-$ into $-COOH$. Electrometric titration of an isoelectric protein with potassium hydroxide causes a very slow increase in pH and a weak buffering action of the protein at pH 7; a very strong buffering action occurs in the pH range from 9 to 10. The buffering action at pH 7, which is caused by loss of protons (positively charged hydrogen) from the imidazolium groups (i.e., the five-member ring structure in the side chain) of histidine, is weak because the histidine content of proteins is usually low. The much stronger buffering action at pH values from 9 to 10 is caused by the loss of protons from the hydroxyl group of tyrosine and from the ammonium groups of lysine. Finally, protons are lost from the guanidinium groups (i.e., the nitrogen-containing terminal portion of the arginine side chains) of arginine at pH 12. Electrometric titrations of proteins yield similar curves. Electrometric titration makes possible the determination of the approximate number of carboxyl groups, ammonium groups, histidines, and tyrosines per molecule of protein.



Electrometric titration of glycine

Electrophoresis

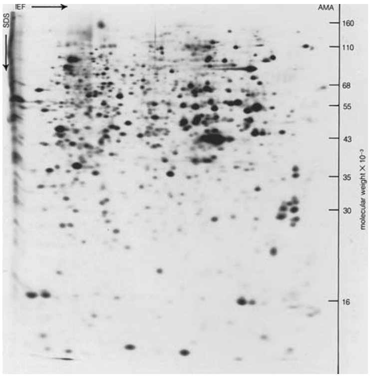
The positively and negatively charged side chains of proteins cause them to behave like amino acids in an electrical field; that is, they migrate during electrophoresis at low pH values to the cathode (negative terminal) and at high pH values to the anode (positive terminal). The isoelectric point, the pH value at which the protein molecule does not migrate, is in the range of pH 5 to 7 for many proteins. Proteins such as lysozyme, cytochrome c, histone, and others rich in lysine and arginine, however, have isoelectric points in the pH range between 8 and 10. The isoelectric point of pepsin, which contains very few basic amino acids, is close to 1.

Number of amino acids per protein molecule							
Amino Acid	Protein*						
	Cyto	Hb alpha	Hb beta	RNase	Lys	Chgen	Fdox
Lysine	18	11	11	10	6	14	4
Histidine	3	10	9	4	1	2	1
Arginine	2	3	3	4	11	4	1
Aspartic acid**	8	12	13	15	21	23	13
Threonine	7	9	7	10	7	23	8
Serine	2	11	5	15	10	28	7

Number of amino acids per protein molecule							
Amino Acid	Protein*						
	Cyto	Hb alpha	Hb beta	RNase	Lys	Chgen	Fdox
Glutamic acid**	10	5	11	12	5	15	13
Proline	4	7	7	4	2	9	4
Glycine	13	7	13	3	12	23	6
Alanine	6	21	15	12	12	22	9
Half-cystine	2	1	2	8	8	10	5
Valine	3	13	18	9	6	23	7
Methionine	3	2	1	4	2	2	0
Isoleucine	8	0	0	3	6	10	4
Leucine	6	18	18	2	8	19	8
Tyrosine	5	3	3	6	3	4	4
Phenylalanine	3	7	8	3	3	6	2
Tryptophan	1	1	2	0	6	8	1
Total	104	141	146	124	129	245	97

*Cyto = human cytochrome c; Hb alpha = human hemoglobin A, alpha-chain; Hb beta = human hemoglobin A, beta-chain; RNase = bovine ribonuclease; Lys = chicken lysozyme; Chgen = bovine chymotrypsinogen; Fdox = spinach ferredoxin.

**The values recorded for aspartic acid and glutamic acid include asparagine and glutamine, respectively.



Two-dimensional gel electrophoresis

In figure, two-dimensional gel electrophoresis, proteins are separated based on charge and size. Approaches commonly employed include isoelectric focusing (IEF) sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) and immobilized pH gradient (IPG-Dalt) SDS-PAGE.

Free-boundary electrophoresis, the original method of determining electrophoretic migration, has been replaced in many instances by zone electrophoresis, in which the protein is placed in either a gel of starch, agar, or polyacrylamide or in a porous medium such as paper or cellulose acetate. The migration of hemoglobin and other coloured proteins can be followed visually. Colourless proteins are made visible after the completion of electrophoresis by staining them with a suitable dye.

Conformation of Globular Proteins

Results of X-ray Diffraction Studies

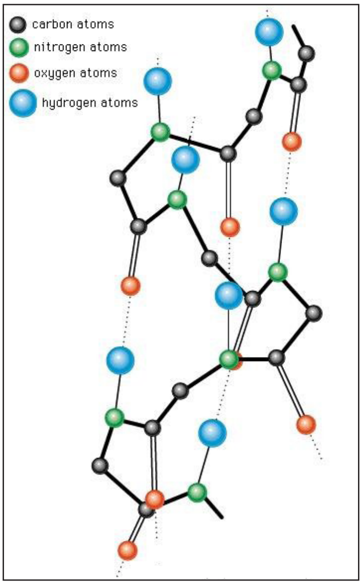
Most knowledge concerning secondary and tertiary structure of globular proteins has been obtained by the examination of their crystals using X-ray diffraction. In this technique, X-rays are allowed to strike the crystal; the X-rays are diffracted by the crystal and impinge on a photographic plate, forming a pattern of spots. The measured intensity of the diffraction pattern, as recorded on a photographic film, depends particularly on the electron density of the atoms in the protein crystal. This density is lowest in hydrogen atoms, and they do not give a visible diffraction pattern. Although carbon, oxygen, and nitrogen atoms yield visible diffraction patterns, they are present in such great number-about 700 or 800 per 100 amino acids-that the resolution of the structure of a protein containing more than 100 amino acids is almost impossible. Resolution is considerably improved by substituting into the side chains of certain amino acids very heavy atoms, particularly those of heavy metals. Mercury ions, for example, bind to the sulfhydryl (-SH) groups of cysteine. Platinum chloride has been used in other proteins. In the iron-containing proteins, the iron atom already in the molecule is adequate.

Although the X-ray diffraction technique cannot resolve the complete three-dimensional conformation (that is, the secondary and tertiary structure of the peptide chain), complete resolution has been obtained by combination of the results of X-ray diffraction with those of amino acid sequence analysis. In this way the complete conformation of such proteins as myoglobin, chymotrypsinogen, lysozyme, and ribonuclease has been resolved.

The X-ray diffraction method has revealed regular structural arrangements in proteins; one is an extended form of antiparallel peptide chains that are linked to each other by hydrogen bonds between the carbonyl and imino groups. This conformation, called the pleated sheet, or β-structure, is found in some fibrous proteins. Short strands of the β-structure have also been detected in some globular proteins.

A second important structural arrangement is the α-helix; it is formed by a sequence of amino acids wound around a straight axis in either a right-handed or a left-handed spiral. Each turn of the helix corresponds to a distance of 5.4 angstroms (= 0.54 nanometre) in the direction of the screw axis and contains 3.7 amino acids. Hence, the length of the α-helix per amino acid residue is 5.4 divided by 3.7, or 1.5 angstroms

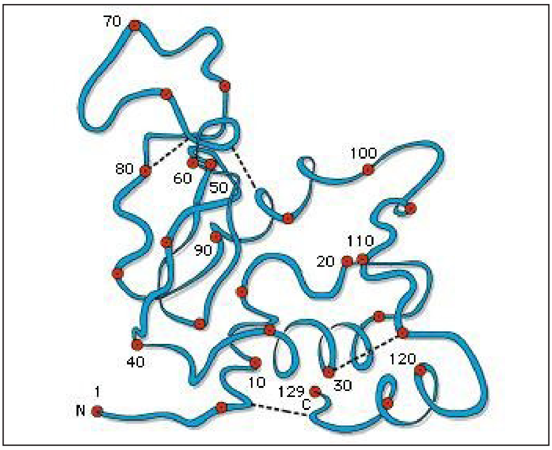
(1 angstrom = 0.1 nanometre). The stability of the α-helix is maintained by hydrogen bonds between the carbonyl and imino groups of neighbouring turns of the helix. It was once thought, based on data from analyses of the myoglobin molecule, more than half of which consists of α-helices, that the α-helix is the predominant structural element of the globular proteins; it is now known that myoglobin is exceptional in this respect. The other globular proteins for which the structures have been resolved by X-ray diffraction contain only small regions of α-helix. In most of them the peptide chains are folded in an apparently random fashion, for which the term random coil has been used. The term is misleading, however, because the folding is not random; rather, it is dictated by the primary structure and modified by the secondary and tertiary structures.



Protein structure (α-helix): The α-helix in the structural arrangement of a protein

The first proteins for which the internal structures were completely resolved are the iron-containing proteins myoglobin and hemoglobin. The investigation of the hydrated crystals of these proteins by Austrian-born British biochemist Max Perutz and British biochemist John C. Kendrew, revealed that the folding of the peptide chains is so tight that most of the water is displaced from the centre of the globular molecules. The amino acids that carry the ammonium (-NH_3^+) and carboxyl (-COO^-) groups were found to be shifted to the surface of the globular molecules, and the nonpolar amino acids were found to be concentrated in the interior.

The simplified structure of lysozyme from hen's egg white has a single peptide chain of 129 amino acids. The amino acid residues are numbered from the terminal α group (N) to the terminal carboxyl group (C). Circles indicate every fifth residue, and every tenth residue is numbered. Broken lines indicate the four disulfide bridges. Alpha-helices are visible in the ranges 25 to 35, 90 to 100, and 120 to 125.



Lysozyme; protein conformation

Other Approaches to the Determination of Protein Structure

None of the several other physical methods that have been used to obtain information on the secondary and tertiary structure of proteins provides as much direct information as the X-ray diffraction technique. Most of the techniques, however, are much simpler than X-ray diffraction, which requires, for the resolution of the structure of one protein, many years of work and equipment such as electronic computers. Some of the simpler techniques are based on the optical properties of proteins—refractivity, absorption of light of different wavelengths, rotation of the plane polarized light at different wavelengths, and luminescence.

Spectrophotometric Behaviour

Spectrophotometry of protein solutions (the measurement of the degree of absorbance of light by a protein within a specified wavelength) is useful within the range of visible light only with proteins that contain coloured prosthetic groups (the nonprotein components). Examples of such proteins include the red heme proteins of the blood, the purple pigments of the retina of the eye, green and yellow proteins that contain bile pigments, blue copper-containing proteins, and dark brown proteins called melanins. Peptide bonds, because of their carbonyl groups, absorb light energy at very short wavelengths (185–200 nanometres). The aromatic rings of phenylalanine, tyrosine, and tryptophan, however, absorb ultraviolet light between wavelengths of 280 and 290 nanometres. The absorbance of ultraviolet light by tryptophan is greatest, that of tyrosine is less, and that of phenylalanine is least. If the tyrosine or tryptophan content of the protein is known, therefore, the concentration of the protein solution can be determined by measuring its absorbance between 280 and 290 nanometres.

Optical Activity

It will be recalled that the amino acids, with the exception of glycine, exhibit optical activity. It is not surprising, therefore, that proteins also are optically active. They are usually levorotatory (i.e., they rotate the plane of polarization to the left) when polarized light of wavelengths in the visible range is used. Although the specific rotation (a function of the concentration of a protein solution and the distance the light travels in it) of most L-amino acids varies from −30° to +30°, the amino acid cystine has a specific rotation of approximately −300°. Although the optical rotation of a protein depends on all of the amino acids of which it is composed, the most important ones are cystine and the aromatic amino acids phenylalanine, tyrosine, and tryptophan. The contribution of the other amino acids to the optical activity of a protein is negligibly small.

Chemical Reactivity of Proteins

Information on the internal structure of proteins can be obtained with chemical methods that reveal whether certain groups are present on the surface of the protein molecule and thus able to react or whether they are buried inside the closely folded peptide chains and thus are unable to react. The chemical reagents used in such investigations must be mild ones that do not affect the structure of the protein.

The reactivity of tyrosine is of special interest. It has been found, for example, that only three of the six tyrosines found in the naturally occurring enzyme ribonuclease can be iodinated (i.e., reacted to accept an iodine atom). Enzyme-catalyzed breakdown of iodinated ribonuclease is used to identify the peptides in which the iodinated tyrosines are present. The three tyrosines that can be iodinated lie on the surface of ribonuclease; the others, assumed to be inaccessible, are said to be buried in the molecule. Tyrosine can also be identified by using other techniques-e.g., treatment with diazonium compounds or tetranitromethane. Because the compounds formed are coloured, they can easily be detected when the protein is broken down with enzymes.

Cysteine can be detected by coupling with compounds such as iodoacetic acid or iodoacetamide; the reaction results in the formation of carboxymethylcysteine or carbamidomethylcysteine, which can be detected by amino acid determination of the peptides containing them. The imidazole groups of certain histidines can also be located by coupling with the same reagents under different conditions. Unfortunately, few other amino acids can be labelled without changes in the secondary and tertiary structure of the protein.

Association of Protein Subunits

Many proteins with molecular weights of more than 50,000 occur in aqueous solutions as complexes: dimers, tetramers, and higher polymers-i.e., as chains of two, four, or more repeating basic structural units. The subunits, which are called monomers or protomers, usually are present as an even number. Less than 10 percent of the polymers have been found to have an odd number of monomers. The arrangement of the subunits is thought to be regular and may be cyclic, cubic, or tetrahedral. Some of the small proteins also contain subunits. Insulin, for example, with a molecular weight of about 6,000, consists of two peptide chains linked to each other by disulfide bridges (-S-S-). Similar interchain disulfide bonds have been found in the immunoglobulins. In other proteins, hydrogen bonds and hydrophobic bonds (resulting from the interaction between the amino acid side chains of valine, leucine, isoleucine, and phenylalanine) cause the formation of aggregates of the subunits. The subunits of some proteins are identical; those of others differ. Hemoglobin is a tetramer consisting of two α-chains and two β-chains.

Protein Denaturation

When a solution of a protein is boiled, the protein frequently becomes insoluble—i.e., it is denatured—and remains insoluble even when the solution is cooled. The denaturation of the proteins of egg white by heat—as when boiling an egg—is an example of irreversible denaturation. The denatured protein has the same primary structure as the original, or native, protein. The weak forces between charged groups and the weaker forces of mutual attraction of nonpolar groups are disrupted at elevated temperatures, however; as a result, the tertiary structure of the protein is lost. In some instances the original structure of the protein can be regenerated; the process is called renaturation.

Denaturation can be brought about in various ways. Proteins are denatured by treatment with alkaline or acid, oxidizing or reducing agents, and certain organic solvents. Interesting among denaturing agents are those that affect the secondary and tertiary structure without affecting the primary structure. The agents most frequently used for this purpose are urea and guanidinium chloride. These molecules, because of their high affinity for peptide bonds, break the hydrogen bonds and the salt bridges between positive and negative side chains, thereby abolishing the tertiary structure of the peptide chain. When denaturing agents are removed from a protein solution, the native protein re-forms in many cases. Denaturation can also be accomplished by reduction of the disulfide bonds of cystine-i.e., conversion of the disulfide bond (-S-S-) to two sulfhydryl groups (-SH). This, results in the formation of two cysteines. Reoxidation of the cysteines by exposure to air sometimes regenerates the native protein. In other cases, however, the wrong cysteines become bound to each other, resulting in a different protein. Finally, denaturation can also be accomplished by exposing proteins to organic solvents such as ethanol or acetone. It is believed that the organic solvents interfere with the mutual attraction of nonpolar groups.

Some of the smaller proteins, however, are extremely stable, even against heat; for example, solutions of ribonuclease can be exposed for short periods of time to temperatures of 90 °C (194 °F) without undergoing significant denaturation. Denaturation does not involve identical changes in protein molecules. A common property of denatured proteins, however, is the loss of biological activity-e.g., the ability to act as enzymes or hormones.

Although denaturation had long been considered an all-or-none reaction, it is now thought that many intermediary states exist between native and denatured protein. In some instances, however, the breaking of a key bond could be followed by the complete breakdown of the conformation of the native protein.

Although many native proteins are resistant to the action of the enzyme trypsin, which breaks down proteins during digestion, they are hydrolyzed by the same enzyme after denaturation. The peptide bonds that can be split by trypsin are inaccessible in the native proteins but become accessible during denaturation. Similarly, denatured proteins give more intense colour reactions for tyrosine, histidine, and arginine than do the same proteins in the native state. The increased accessibility of reactive groups of denatured proteins is attributed to an unfolding of the peptide chains.

If denaturation can be brought about easily and if renaturation is difficult, how is the native conformation of globular proteins maintained in living organisms, in which they are produced stepwise, by incorporation of one amino acid at a time? Experiments on the biosynthesis of proteins from amino acids containing radioactive carbon or heavy hydrogen reveal that the protein molecule grows stepwise from the N terminus to the C terminus; in each step a single amino acid residue is incorporated. As soon as the growing peptide chain contains six or seven amino acid residues, the side chains interact with each other and thus cause deviations from the straight or β-chain configuration. Depending on the nature of the side chains, this may result in the formation of an α-helix or of loops closed by hydrogen bonds or disulfide bridges. The final conformation is probably frozen when the peptide chain attains a length of 50 or more amino acid residues.

Conformation of Proteins in Interfaces

Like many other substances with both hydrophilic and hydrophobic groups, soluble proteins tend to migrate into the interface between air and water or oil and water; the term oil here means a hydrophobic liquid such as benzene or xylene. Within the interface, proteins spread, forming thin films. Measurements of the surface tension, or interfacial tension, of such films indicate that tension is reduced by the protein film. Proteins, when forming an interfacial film, are present as a monomolecular layer-i.e., a layer one molecule in height. Although it was once thought that globular protein molecules unfold completely in the interface, it has now been established that many proteins can be recovered from films in the native state. The application of lateral pressure on a protein film causes it to increase in thickness and finally to form a layer with a height corresponding to the diameter of the native protein molecule. Protein molecules in an interface, because of Brownian motions (molecular vibrations), occupy much more space than do those in the film after the application of pressure. The Brownian motion of compressed molecules is limited to the two dimensions of the interface, since the protein molecules cannot move upward or downward.

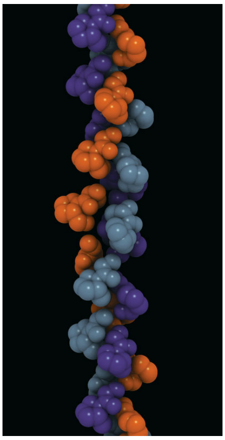
The motion of protein molecules at the air–water interface has been used to determine the molecular weight of proteins. The technique involves measuring the force exerted by the protein layer on a barrier.

When a protein solution is vigorously shaken in air, it forms a foam, because the soluble proteins migrate into the air–water interface and persist there, preventing or slowing the reconversion of the foam into a homogeneous solution. Some of the unstable, easily modified proteins are denatured when spread in the air–water interface. The formation of a permanent foam when egg white is vigorously stirred is an example of irreversible denaturation by spreading in a surface.

Classification of Proteins

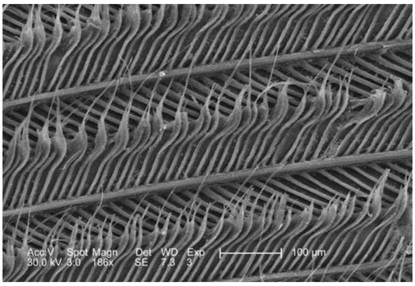
Classification by Solubility

After two German chemists, Emil Fischer and Franz Hofmeister, independently stated that proteins are essentially polypeptides consisting of many amino acids, an attempt was made to classify proteins according to their chemical and physical properties, because the biological function of proteins had not yet been established. Proteins were classified primarily according to their solubility in a number of solvents. This classification is no longer satisfactory, however, because proteins of quite different structure and function sometimes have similar solubilities; conversely, proteins of the same function and similar structure sometimes have different solubilities. The terms associated with the old classification, however, are still widely used.



Collagen (Collagen molecule)

Albumins are proteins that are soluble in water and in water half-saturated with ammonium sulfate. On the other hand, globulins are salted out (i.e., precipitated) by half-saturation with ammonium sulfate. Globulins that are soluble in salt-free water are called pseudoglobulins; those insoluble in salt-free water are euglobulins. Both prolamins and glutelins, which are plant proteins, are insoluble in water; the prolamins dissolve in 50 to 80 percent ethanol, the glutelins in acidified or alkaline solution. The term protamine is used for a number of proteins in fish sperm that consist of approximately 80 percent arginine and therefore are strongly alkaline. Histones, which are less alkaline, apparently occur only in cell nuclei, where they are bound to nucleic acids. The term scleroproteins has been used for the insoluble proteins of animal organs. They include keratin, the insoluble protein of certain epithelial tissues such as the skin or hair, and collagen, the protein of the connective tissue. A large group of proteins has been called conjugated proteins, because they are complex molecules of protein consisting of protein and nonprotein moieties. The nonprotein portion is called the prosthetic group. Conjugated proteins can be subdivided into mucoproteins, which, in addition to protein, contain carbohydrate; lipoproteins, which contain lipids; phosphoproteins, which are rich in phosphate; chromoproteins, which contain pigments such as iron-porphyrins, carotenoids, bile pigments, and melanin; and finally, nucleoproteins, which contain nucleic acid.



Keratin: Scanning electron micrograph showing strands of keratin in a feather, magnified 186×

The weakness of the above classification lies in the fact that many, if not all, globulins contain small amounts of carbohydrate; thus there is no sharp borderline between globulins and mucoproteins. Moreover, the phosphoproteins do not have a prosthetic group that can be isolated; they are merely proteins in which some of the hydroxyl groups of serine are phosphorylated (i.e., contain phosphate). Finally, the globulins include proteins with quite different roles-enzymes, antibodies, fibrous proteins, and contractile proteins.

Classification by Biological Functions

In view of the unsatisfactory state of the old classification, it is preferable to classify the proteins according to their biological function. Such a classification is far from ideal, however, because one protein can have more than one function. The contractile protein myosin, for example, also acts as an ATPase (adenosine triphosphatase), an enzyme that hydrolyzes adenosine triphosphate (removes a phosphate group from ATP by introducing a water molecule). Another problem with functional classification is that the definite function of a protein frequently is not known. A protein cannot be called an enzyme as long as its substrate (the specific compound upon which it acts) is not known. It cannot even be tested for its enzymatic action when its substrate is not known.

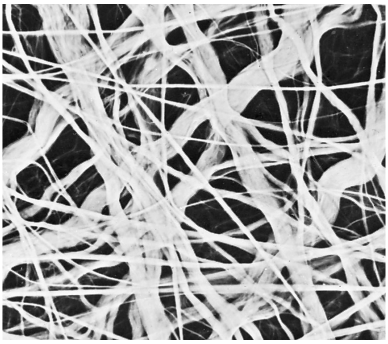
Special Structure and Function of Proteins

Despite its weaknesses, a functional classification is used here in order to demonstrate, whenever possible, the correlation between the structure and function of a protein. The structural, fibrous proteins are presented first, because their structure is simpler than that of the globular proteins and more clearly related to their function, which is the maintenance of either a rigid or a flexible structure.

Structural Proteins: Scleroproteins

Collagen

Collagen is the structural protein of bones, tendons, ligaments, and skin. For many years collagen was considered to be insoluble in water. Part of the collagen of calf skin, however, can be extracted with citrate buffer at pH 3.7. A precursor of collagen called procollagen is converted in the body into collagen. Procollagen has a molecular weight of 120,000. Cleavage of one or a few peptide bonds of procollagen yields collagen, which has three subunits, each with a molecular weight of 95,000; therefore, the molecular weight of collagen is 285,000 (3 × 95,000). The three subunits are wound as spirals around an elongated straight axis. The length of each subunit is 2,900 angstroms, and its diameter is approximately 15 angstroms. The three chains are staggered, so that the trimer has no definite terminal limits.



Randomly oriented collagenous fibres of varying size in a thin spread of loose areolar connective tissue (magnified about 370 ×)

Collagen differs from all other proteins in its high content of proline and hydroxyproline. Hydroxyproline does not occur in significant amounts in any other protein except elastin. Most of the proline in collagen is present in the sequence glycine-proline-X, in which X is frequently alanine or hydroxyproline. Collagen does not contain cystine or tryptophan and therefore cannot substitute for other proteins in the diet. The presence of proline causes kinks in the peptide chain and thus reduces the length of the amino acid unit from 3.7 angstroms in the extended chain of the β-structure to 2.86 angstroms in the collagen chain. In the intertwined triple helix, the glycines are inside, close to the axis; the prolines are outside.

Native collagen resists the action of trypsin but is hydrolyzed by the bacterial enzyme collagenase. When collagen is boiled with water, the triple helix is destroyed, and the subunits are partially hydrolyzed; the product is gelatin. The unfolded peptide chains of gelatin trap large amounts of water, resulting in a hydrated molecule.

When collagen is treated with tannic acid or with chromium salts, cross links form between the collagen fibres, and it becomes insoluble; the conversion of hide into leather is based on this tanning process. The tanned material is insoluble in hot water and cannot be converted to gelatin. On exposure to water at 62° to 63 °C (144° to 145 °F), however, the cross links formed by the tanning agents collapse, and the leather contracts irreversibly to about one-third its original volume.

Collagen seems to undergo an aging process in living organisms that may be caused by the formation of cross links between collagen fibres. They are formed by the conversion of some lysine side chains to aldehydes (compounds with the general structure RCHO), and the combination of the aldehydes with the ε-amino groups of intact lysine side chains. The protein elastin, which occurs in the elastic fibres of connective tissue, contains similar cross links and may result from the combination of collagen fibres with other proteins. When cross-linked collagen or elastin is degraded, products of the cross-linked lysine fragments, called desmosins and isodesmosins, are formed.

Keratin

Keratin, the structural protein of epithelial cells in the outermost layers of the skin, has been isolated from hair, nails, hoofs, and feathers. Keratin is completely insoluble in cold or hot water; it is not attacked by proteolytic enzymes (i.e., enzymes that break apart, or lyse, protein molecules), and therefore cannot replace proteins in the diet. The great stability of keratin results from the numerous disulfide bonds of cystine. The amino acid composition of keratin differs from that of collagen. Cystine may account for 24 percent of the total amino acids. The peptide chains of keratin are arranged in approximately equal amounts of antiparallel and parallel pleated sheets, in which the peptide chains are linked to each other by hydrogen bonds between the carbonyl and imino groups.

Reduction of the disulfide bonds to sulfhydryl groups results in dissociation of the peptide chains, the molecular weight of which is 25,000 to 28,000 each. The formation of permanent waves in the beauty treatment of hair is based on partial reduction of the disulfide bonds of hair keratin by thioglycol, or some other mild reducing agent, and subsequent oxidation of the sulfhydryl groups (-SH) in the reoriented hair to disulfide bonds (-S-S-) by exposure to the oxygen of the air.

The length of keratin fibres depends on their water content. They can bind approximately 16 percent of water; this hydration is accompanied by an increase in the length of the fibres of 10 to 12 percent.

The most thoroughly investigated keratin is hair keratin, particularly that of wool. It consists of a mixture of peptides with high and low cystine content. When wool is heated in water to about 90° C (190° F), it shrinks irreversibly. This is attributed to the breakage of hydrogen bonds and other noncovalent bonds; disulfide bonds do not seem to be affected.

Others

The most thoroughly investigated scleroprotein has been fibroin, the insoluble material of silk. The raw silk comprising the cocoon of the silkworm consists of two proteins. One, sericin, is soluble in hot water; the other, fibroin, is not. The amino acid composition of the latter differs from that of all other proteins. It contains large amounts of glycine, alanine, tyrosine, and serine; small amounts of the other amino acids; and no sulfur-containing ones. The peptide chains are arranged in antiparallel β-structures. Fibroin is partly soluble in concentrated solutions of lithium thiocyanate or in mixtures of cupric salts and ethylene diamine. Such solutions contain a protein of molecular weight 170,000, which is a dimer of two subunits.

Little is known about either the scleroproteins of the marine sponges or the insoluble proteins of the cellular membranes of animal cells. Some of the membranes are soluble in detergents; others, however, are detergent-insoluble.

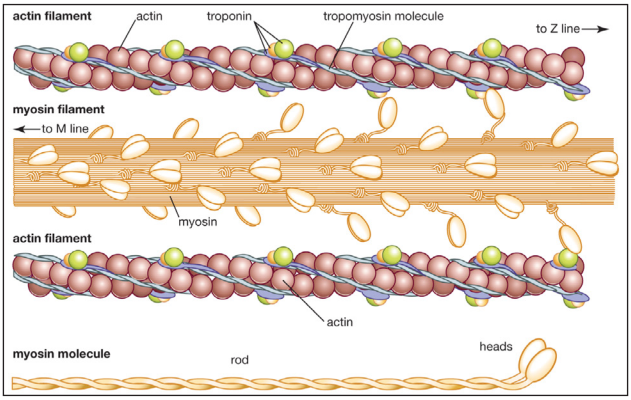
The Muscle Proteins

The total amount of muscle proteins in mammals, including humans, exceeds that of any other protein. About 40 percent of the body weight of a healthy human adult weighing about 70 kilograms (150 pounds) is muscle, which is composed of about 20 percent muscle protein. Thus, the human body contains about 5 to 6 kilograms (11 to 13 pounds) of muscle protein. An albumin-like fraction of these proteins, originally called myogen, contains various enzymes—phosphorylase, aldolase, glyceraldehyde phosphate dehydrogenase, and others; it does not seem to be involved in contraction. The globulin fraction contains myosin, the contractile protein, which also occurs in blood platelets, small bodies found in blood. Similar contractile substances occur in other contractile structures; for example, in the cilia or flagella (whiplike organs of locomotion) of bacteria and protozoans. In contrast to the scleroproteins, the contractile proteins are soluble in salt solutions and susceptible to enzymatic digestion.

The energy required for muscle contraction is provided by the oxidation of carbohydrates or lipids. The term mechanochemical reaction has been used for this conversion of chemical into mechanical energy. The molecular process underlying the reaction is known to involve the fibrous muscle proteins, the peptide chains of which undergo a change in conformation during contraction.

Myosin, which can be removed from fresh muscle by adding it to a chilled solution of dilute potassium chloride and sodium bicarbonate, is insoluble in water. Myosin, solutions of which are highly viscous, consists of an elongated-probably double-stranded-peptide chain, which is coiled at both ends in such a way that a terminal globule is formed. The length of the molecule is approximately 160 nanometres and its average diameter 2.6 nanometres. The equivalent weight of each of the two terminal globules is approximately 30,000; the molecular weight of myosin is close to 500,000. Trypsin splits myosin into large fragments called meromyosin. Myosin contains many amino acids with positively and negatively charged side chains; they form 18 and 16 percent, respectively, of the total number of amino acids. Myosin catalyzes the hydrolytic cleavage of ATP (adenosine triphosphate). A smaller protein with properties similar to those of myosin is tropomyosin. It has a molecular weight of 70,000 and dimensions of 45 by 2 nanometres. More than 90 percent of its peptide chains are present in the α-helix form.

Myosin combines easily with another muscle protein called actin, the molecular weight of which is about 50,000; it forms 12 to 15 percent of the muscle proteins. Actin can exist in two forms-one, G-actin, is globular; the other, F-actin, is fibrous. Actomyosin is a complex molecule formed by one molecule of myosin and one or two molecules of actin. In muscle, actin and myosin filaments are oriented parallel to each other and to the long axis of the muscle. The actin filaments are linked to each other lengthwise by fine threads called S filaments. During contraction the S filaments shorten, so that the actin filaments slide toward each other, past the myosin filaments, thus causing a shortening of the muscle.



Muscle (actin and myosin): The structure of actin and myosin filaments

Fibrinogen and Fibrin

Fibrinogen, the protein of the blood plasma, is converted into the insoluble protein fibrin during the clotting process. The fibrinogen-free fluid obtained after removal of the clot, called blood serum, is blood plasma minus fibrinogen. The fibrinogen content of the blood plasma is 0.2 to 0.4 percent.

Fibrinogen can be precipitated from the blood plasma by half-saturation with sodium chloride. Fibrinogen solutions are highly viscous and show strong flow birefringence. In electron micrographs the molecules appear as rods with a length of 47.5 nanometres and a diameter of 1.5 nanometres; in addition, two terminal and a central nodule are visible. The molecular weight is 340,000. An unusually high percentage, about 36 percent, of the amino acid side chains are positively or negatively charged.

The clotting process is initiated by the enzyme thrombin, which catalyzes the breakage of a few peptide bonds of fibrinogen; as a result, two small fibrinopeptides with molecular weights of 1,900 and 2,400 are released. The remainder of the fibrinogen molecule, a monomer, is soluble and stable at pH values less than 6 (i.e., in acid solutions). In neutral solution (pH 7) the monomer is converted into a larger molecule, insoluble fibrin; this results from the formation of new peptide bonds. The newly formed peptide bonds form intermolecular and intramolecular cross links, thus giving rise to a large clot, in which all molecules are linked to each other. Clotting, which takes place only in the presence of calcium ions, can be prevented by compounds such as oxalate or citrate, which have a high affinity for calcium ions.

Albumins, Globulins and other Soluble Proteins

The blood plasma, the lymph, and other animal fluids usually contain one to seven grams of protein per 100 millilitres of fluid, which includes small amounts of hundreds of enzymes and a large number of protein hormones.

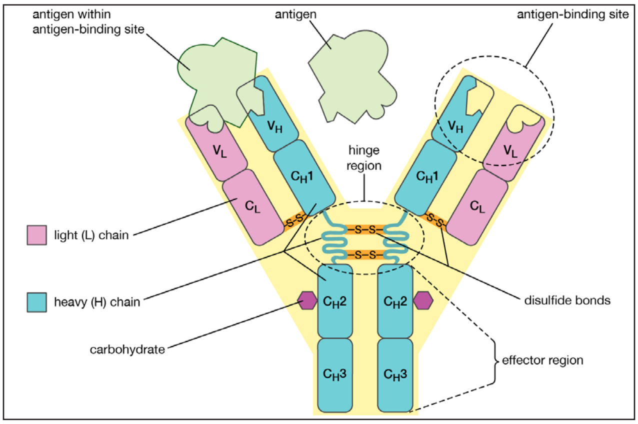
Proteins of the Blood Serum

Human blood serum contains about 7 percent protein, two-thirds of which is in the albumin fraction; the other third is in the globulin fraction. Electrophoresis of serum reveals a large albumin peak and three smaller globulin peaks, the alpha-, beta-, and gamma-globulins. The amounts of alpha-, beta-, and gamma-globulin in normal human serum are approximately 1.5, 1.9, and 1.1 percent, respectively. Each globulin fraction is a mixture of many different proteins, as has been demonstrated by immunoelectrophoresis. In this method, serum from an animal (e.g., a rabbit) injected with human serum is allowed to diffuse into the four protein bands-albumin, alpha-, beta-, and gamma-globulin-obtained from the electrophoresis of human serum. Because the animal has previously been injected with human serum, its blood contains antibodies (substances formed in response to a foreign substance introduced into the body) against each of the human serum proteins; each antibody combines with the serum protein (antigen) that caused its formation in the animal. The result is the formation of about 20 regions of insoluble antigen-antibody precipitate, which appear as white arcs in the transparent gel of the electrophoresis medium. Each region corresponds to a different human serum protein.

Serum albumin is much less heterogeneous (i.e., contains fewer distinct proteins) than are the globulins; in fact, it is one of the few serum proteins that can be obtained in a crystalline form. Serum albumin combines easily with many acidic dyes (e.g., Congo red and methyl orange); with bilirubin, the yellow bile pigment; and with fatty acids. It seems to act, in living organisms, as a carrier for certain biological substances. Present in blood serum in relatively high concentration, serum albumin also acts as a protective colloid, a protein that stabilizes other proteins. Albumin (molecular weight of 68,000) has a single free sulfhydryl (- SH) group, which on oxidation forms a disulfide bond with the sulfhydryl group of another serum albumin molecule, thus forming a dimer. The isoelectric point of serum albumin is pH 4.7.

The alpha-globulin fraction of blood serum is a mixture of several conjugated proteins. The best known are an α-lipoprotein (combination of lipid and protein) and two mucoproteins (combinations of carbohydrate and protein). One mucoprotein is called orosomucoid, or α1-acid glycoprotein; the other is called haptoglobin because it combines specifically with globin, the protein component of hemoglobin. Haptoglobin contains about 20 percent carbohydrate. The beta-globulin fraction of serum contains, in addition to lipoproteins and mucoproteins, two metal-binding proteins, transferrin and ceruloplasmin, which bind iron and copper, respectively. They are the principal iron and copper carriers of the blood.

The gamma-globulins are the most heterogeneous globulins. Although most have a molecular weight of approximately 150,000, that of some, called macroglobulins, is as high as 800,000. Because typical antibodies are of the same size and exhibit the same electrophoretic behaviour as γ-globulins, they are called immunoglobulins. The designation IgM or gamma M (γM) is used for the macroglobulins; the designation IgG or gamma G (γG) is used for γ–globulins of molecular weight 150,000.



The four-chain structure of an antibody, or immunoglobulin, molecule

In figure, the basic unit is composed of two identical light (L) chains and two identical heavy (H) chains, which are held together by disulfide bonds to form a flexible Y shape. Each chain is composed of a variable (V) region and a constant (C) region.

Milk Proteins

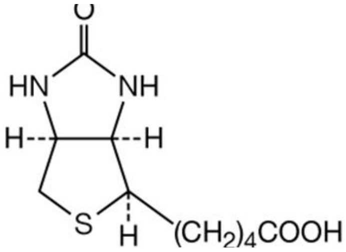
Milk contains the following: an albumin, α-lactalbumin; a globulin, beta-lactoglobulin; and a phosphoprotein, casein. If acid is added to milk, casein precipitates. The remaining watery liquid (the supernatant solution), or whey, contains α-lactalbumin and β-lactoglobulin. Both have been obtained in crystalline form; in bovine milk, their molecular weights are approximately 14,000 and 18,400, respectively. Lactoglobulin also occurs as a dimer of molecular weight 37,000. Genetic variations can produce small variations in the amino acid composition of lactoglobulin. The amino acid composition and the tertiary structure of lactalbumin resemble that of lysozyme, an egg protein.

Casein is precipitated not only by the addition of acid but also by the action of the enzyme rennin, which is found in gastric juice. Rennin from calf stomachs is used to precipitate casein, from which cheese is made. Milk fat precipitates with casein; milk sugar, however, remains in the supernatant (whey). Casein is a mixture of several similar phosphoproteins, called α-, β-, γ–, and κ-casein, all of which contain some serine side chains combined with phosphoric acid. Approximately 75 percent of casein is α-casein. Cystine has been found only in κ-casein. In milk, casein seems to form polymeric globules (micelles) with radially arranged monomers, each with a molecular weight of 24,000; the acidic side chains occur predominantly on the surface of the micelle, rather than inside.

Egg Proteins

About 50 percent of the proteins of egg white are composed of ovalbumin, which is easily obtained in crystals. Its molecular weight is 46,000 and its amino acid composition differs from that of serum albumin. Other proteins of egg white are conalbumin, lysozyme, ovoglobulin, ovomucoid, and avidin. Lysozyme is an enzyme that hydrolyzes the carbohydrates found in the capsules certain bacteria secrete around themselves; it causes lysis (disintegration) of the bacteria. The molecular weight of lysozyme is 14,100. Its three-dimensional structure is similar to that of α-lactalbumin, which stimulates the formation of lactose by the enzyme lactose synthetase. Lysozyme has also been found in the urine of patients suffering from leukemia, meningitis, and renal disease.

Avidin is a glycoprotein that combines specifically with biotin, a vitamin. In animals fed large amounts of raw egg white, the action of avidin results in "egg-white injury." The molecular weight of avidin, which forms a tetramer, is 16,200. Its amino acid sequence is known.



Egg-yolk proteins contain a mixture of lipoproteins and livetins. The latter are similar to serum albumin, α-globulin, and β-globulin. The yolk also contains a phosphoprotein, phosvitin. Phosvitin, which has also been found in fish sperm, has a molecular weight of 40,000 and an unusual amino acid composition; one third of its amino acids are phosphoserine.

Protamines and Histones

Protamines are found in the sperm cells of fish. The most thoroughly investigated protamines are salmine from salmon sperm and clupeine from herring sperm. The protamines are bound to deoxyribonucleic acid (DNA), forming nucleoprotamines. The amino acid composition of the protamines is simple; they contain, in addition to large amounts of arginine, small amounts of five or six other amino acids. The composition of the salmine molecule, for example, is: Arg_{51}, Ala_4, Val_4, Ile_1, Pro_7, and Ser_6, in which the subscript numbers indicate the number of each amino acid in the molecule. Because of the high arginine content, the isoelectric points of the protamines are at pH values of 11 to 12; i.e., the protamines are alkaline. The molecular weights of salmine and clupeine are close to 6,000. All of the protamines investigated thus far are mixtures of several similar proteins.

The histones are less basic than the protamines. They contain high amounts of either lysine or arginine and small amounts of aspartic acid and glutamic acid. Histones occur in combination with DNA as nucleohistones in the nuclei of the body cells of animals and plants, but not in animal sperm. The molecular weights of histones vary from 10,000 to 22,000. In contrast to the protamines, the histones contain most of the 20 amino acids, with the exception of tryptophan and the sulfur-containing ones. Like the protamines, histone preparations are heterogeneous mixtures. The amino acid sequence of some of the histones has been determined.

Plant Proteins

Plant proteins, mostly globulins, have been obtained chiefly from the protein-rich seeds of cereals and legumes. Small amounts of albumins are found in seeds. The best known globulins, insoluble in water, can be extracted from seeds by treatment with 2 to 10 percent solutions of sodium chloride. Many plant globulins have been obtained in crystalline form; they include edestin from hemp, molecular weight 310,000; amandin from almonds, 330,000; concanavalin A (42,000) and B (96,000); and canavalin (113,000) from jack beans. They are polymers of smaller subunits; edestin, for example, is a hexamer of a subunit with a molecular weight of 50,000, and concanavalin B a trimer of a subunit with a molecular weight of 30,000. After extraction of lipids from cereal seeds by ether and alcohol, further extraction with water containing 50 to 80 percent of alcohol yields proteins that are insoluble in water but soluble in water–ethanol mixtures and have been called prolamins. Their solubility in aqueous ethanol may result from their high proline and glutamine content. Gliadin, the prolamin from wheat, contains 14 grams of proline and 46 grams of glutamic acid in 100 grams of protein; most of the glutamic acid is in the form of glutamine. The total amounts of the basic amino acids (arginine, lysine, and histidine) in gliadin are only 5 percent of the weight of gliadin. Because the glysine content is either low or nonexistent, human populations dependent on grain as a sole protein source suffer from lysine deficiency.

Conjugated Proteins

Combination of Proteins with Prosthetic Groups

The link between a protein molecule and its prosthetic group is a covalent bond (an electron-sharing bond) in the glycoproteins, the biliproteins, and some of the heme proteins. In lipoproteins, nucleoproteins, and some heme proteins, the two components are linked by noncovalent bonds; the bonding results from the same forces that are responsible for the tertiary structure of proteins: hydrogen bonds, salt bridges between positively and negatively charged groups, disulfide bonds, and mutual interaction of hydrophobic groups. In the metalloproteins (proteins with a metal element as a prosthetic group), the metal ion usually forms a centre to which various groups are bound.

Some of the conjugated proteins have been mentioned in preceding sections because they occur in the blood serum, in milk, and in eggs.

Mucoproteins and Glycoproteins

The prosthetic groups in mucoproteins and glycoproteins are oligosaccharides (carbohydrates consisting of a small number of simple sugar molecules) usually containing from four to 12 sugar molecules; the most common sugars are galactose, mannose, glucosamine, and galactosamine. Xylose, fucose, glucuronic acid, sialic acid, and other simple sugars sometimes also occur. Some mucoproteins contain 20 percent or more of carbohydrate, usually in several oligosaccharides attached to different parts of the peptide chain. The designation mucoprotein is used for proteins with more than 3 to 4 percent carbohydrate; if the carbohydrate content is less than 3 percent, the protein is sometimes called a glycoprotein or simply a protein.

Mucoproteins, highly viscous proteins originally called mucins, are found in saliva, in gastric juice, and in other animal secretions. Mucoproteins occur in large amounts in cartilage, synovial fluid (the lubricating fluid of joints and tendons), and egg white. The mucoprotein of cartilage is formed by the combination of collagen with chondroitinsulfuric acid, which is a polymer of either glucuronic or iduronic acid and acetylhexosamine or acetylgalactosamine. It is not yet clear whether or not chondroitinsulfate is bound to collagen by covalent bonds.

Lipoproteins and Proteolipids

The bond between the protein and the lipid portion of lipoproteins and proteolipids is a noncovalent one. It is thought that some of the lipid is enclosed in a meshlike arrangement of peptide chains and becomes accessible for reaction only after the unfolding of the chains by denaturing agents. Although lipoproteins in the α- and β-globulin fraction of blood serum are soluble in water (but insoluble in organic solvents), some of the brain lipoproteins, because they have a high lipid content, are soluble in organic solvents; they are called proteolipids. The β-lipoprotein of human blood serum is a macroglobulin with a molecular weight of about 1,300,000, 70 percent of which is lipid; of the lipid, about 30 percent is phospholipid and 40 percent cholesterol and compounds derived from it. Because of their lipid content, the lipoproteins have the lowest density (mass per unit volume) of all proteins and are usually classified as low- and high-density lipoproteins (LDL and HDL).

Coloured lipoproteins are formed by the combination of protein with carotenoids. Crustacyanin, the pigment of lobsters, crayfish, and other crustaceans, contains astaxanthin, which is a compound derived from carotene. Among the most interesting of the coloured lipoproteins are the pigments of the retina of the eye. They contain retinal, which is a compound derived from carotene and which is formed by the oxidation of vitamin A. In rhodopsin, the red pigment of the retina, the aldehyde group (-CHO) of retinal forms a covalent bond with an amino ($-NH_2$) group of opsin, the protein carrier. Colour vision is mediated by the presence of several visual pigments in the retina that differ from rhodopsin either in the structure of retinal or in that of the protein carrier.

Metalloproteins

Proteins in which heavy metal ions are bound directly to some of the side chains of histidine, cysteine, or some other amino acid are called metalloproteins. Two metalloproteins, transferrin and ceruloplasmin, occur in the globulin fractions of blood serum; they act as carriers of iron and copper, respectively. Transferrin has a molecular weight of about 80,000 and consists of two identical subunits, each of which contains one ferric ion (Fe^{3+}) that seems to be bound to tyrosine. Several genetic variants of transferrin are known to occur in humans. Another iron protein, ferritin, which contains 20 to 22 percent iron, is the form in which iron is stored in animals; it has been obtained in crystalline form from liver and spleen. A molecule consisting of 20 subunits, its molecular weight is approximately 480,000. The iron can be removed by reduction from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state. The iron-free protein, apoferritin, is synthesized in the body before the iron is incorporated.

Green plants and some photosynthetic and nitrogen-fixing bacteria (i.e., bacteria that convert atmospheric nitrogen, N_2, into amino acids and proteins) contain various ferredoxins. They are small proteins containing 50 to 100 amino acids and a chain of iron and disulfide units (FeS_2), in which some of the sulfur atoms are contributed by cysteine; others are sulfide ions (S^{2-}). The number of FeS_2 units per ferredoxin molecule varies from five in the ferredoxin of spinach to 10 in the ferredoxin of certain bacteria. Ferredoxins act as electron carriers in photosynthesis and in nitrogen fixation.

Ceruloplasmin is a copper-containing globulin that has a molecular weight of 151,000; the molecule consists of eight subunits, each containing one copper ion. Ceruloplasmin is the principal carrier of copper in organisms, although copper can also be transported by the iron-containing globulin transferrin. Another copper-containing protein, copper-zinc superoxide dismutase (formerly known as erythrocuprein), has been isolated from red blood cells; it has also been found in the liver and in the brain. The molecule, which consists of two subunits of similar size, contains copper ions and zinc ions. Because of their copper content, ceruloplasmin and copper-zinc superoxide dismutase possess catalytic activity in oxidation-reduction reactions.

Many animal enzymes contain zinc ions, which are usually bound to the sulfur of cysteine.

Horse kidneys contain the protein metallothionein, which contain zinc and cadmium; both are bound to sulfur. A vanadium-protein complex (hemovanadin) has been found in surprisingly high amounts in yellowish-green cells (vanadocytes) of tunicates, which are marine invertebrates.

Heme Proteins and other Chromoproteins

Although the heme proteins contain iron, they are usually not classified as metalloproteins, because their prosthetic group is an iron-porphyrin complex in which the iron is bound very firmly. The intense red or brown colour of the heme proteins is not caused by iron but by porphyrin, a complex cyclic structure. All porphyrin compounds absorb light intensely at or close to 410 nanometres. Porphyrin consists of four pyrrole rings (five-membered closed structures containing one nitrogen and four carbon atoms) linked to each other by methine groups (-CH=). The iron atom is kept in the centre of the porphyrin ring by interaction with the four nitrogen atoms. The iron atom can combine with two other substituents; in oxyhemoglobin, one substituent is a histidine of the protein carrier, the other is an oxygen molecule. In some heme proteins, the protein is also bound covalently to the side chains of porphyrin.

The chromoprotein melanin, a pigment found in dark skin, dark hair, and melanotic tumours, occurs in every major group of living organisms and appears to be remarkably diverse in structure. In humans, melanin produced by melanocytes may be dark brown (eumelanin) or pale red or yellowish (phaeomelanin). The different types are synthesized via different pathways, though they share the same initial step—the oxidation of tyrosine.

Green chromoproteins called biliproteins are found in many insects, such as grasshoppers, and also in the eggshells of many birds. The biliproteins are derived from the bile pigment biliverdin, which in turn is formed from porphyrin; biliverdin contains four pyrrole rings and three of the four methine groups of porphyrin. Large amounts of biliproteins have been found in red algae and blue-green algae; the red protein is called phycoerythrin, the blue one phycocyanobilin.

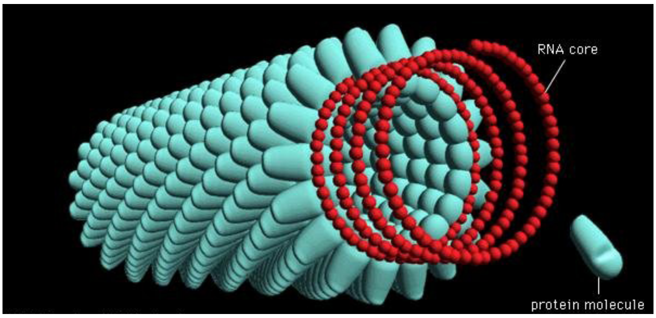
Blue-green algae in Morning Glory Pool, Yellowstone National Park, Wyoming

Nucleoproteins

When a protein solution is mixed with a solution of a nucleic acid, the phosphoric acid component of the nucleic acid combines with the positively charged ammonium groups ($-NH_3^+$) of the protein to form a protein–nucleic acid complex. The nucleus of a cell contains predominantly deoxyribonucleic acid (DNA) and the cytoplasm predominantly ribonucleic acid (RNA); both parts of the cell also contain protein. Protein–nucleic acid complexes, therefore, form in living cells.

The only nucleoproteins for which some evidence for specificity exists are nucleoprotamines, nucleohistones, and some RNA and DNA viruses. The nucleoprotamines are the form in which protamines occur in the sperm cells of fish; the histones of the thymus and of pea seedlings and other plant material apparently occur predominantly as nucleohistones. Both nucleoprotamines and nucleohistones contain only DNA.

Some of the simplest viruses consist of a specific RNA, which is coated by protein. One of the best known RNA viruses, tobacco mosaic virus (TMV), has the shape of a rod. RNA comprises only 5.1 percent of the mass of the virus. The complete sequence of the virus protein, which consists of about 2,130 identical peptide chains, each containing 158 amino acids, has been determined. The protein is arranged in a spiral around the RNA core.



Schematic structure of the tobacco mosaic virus: The cutaway section shows the helical ribonucleic acid associated with protein molecules in a ratio of three nucleotides per protein molecule

DNA has been found in most bacterial viruses (bacteriophages) and in some animal viruses. As in TMV, the core of DNA is surrounded by protein. Phage protein is a mixture of enzymes and therefore cannot be considered as the protein portion of only one nucleoprotein.

Respiratory Proteins

Hemoglobin

Hemoglobin is the oxygen carrier in all vertebrates and some invertebrates. In oxyhemoglobin (HbO_2), which is bright red, the ferrous ion (Fe^{2+}) is bound to the four nitrogen atoms of porphyrin; the other two substituents are an oxygen molecule and the histidine of globin, the protein component of hemoglobin. Deoxyhemoglobin (deoxy-Hb), as its name implies, is oxyhemoglobin minus oxygen (i.e., reduced hemoglobin); it is purple in colour. Oxidation of the ferrous ion of hemoglobin yields a ferric compound, methemoglobin, sometimes called hemiglobin or ferrihemoglobin. The oxygen of oxyhemoglobin can be displaced by carbon monoxide, for which hemoglobin has a much greater affinity, preventing oxygen from reaching the body tissues.

The hemoglobins of all mammals, birds, and many other vertebrates are tetramers of two α- and two β-chains. The molecular weight of the tetramer is 64,500; the molecular weight of the α- and β-chains is approximately 16,100 each, and the four subunits are linked to each other by noncovalent interactions. If hemin (the ferric porphyrin component) is removed from globin (the protein component), two molecules of globin, each consisting of one α- and one β-chain, are obtained; the molecular weight of globin is 32,200. In contrast to hemoglobin, globin is an unstable protein that is easily denatured. If native globin is incubated with a solution of hemin at pH values of 8 to 9, native hemoglobin is reconstituted. Myoglobin, the red pigment of mammalian muscles, is a monomer with a molecular weight of 16,000.

The mammalian hemoglobins differ from each other in their amino acid composition and therefore in their secondary and tertiary structure. Rat and horse hemoglobins crystallize very easily, but those of humans, cattle, and sheep, because they are more soluble, are difficult to crystallize. The shape of hemoglobin crystals varies in different species; moreover, decomposition and denaturation occur at different rates in different species. It was also found that the blood of human newborns contains two different hemoglobins: about 20 percent of their hemoglobin is an adult hemoglobin (hemoglobin A) and 80 percent is a fetal hemoglobin (hemoglobin F). Hemoglobin F persists in the infant for the first seven months of life. The same hemoglobin F has also been found in the blood of patients suffering from thalassemia, an anemia with a high incidence in regions surrounding the Mediterranean Sea. Hemoglobin F contains, as does hemoglobin A, two α-chains; the two β-chains, however, have been replaced by two quite different γ-chains. When the technique of electrophoresis was first applied to the hemoglobin of blacks suffering from sickle cell anemia in 1949, a new hemoglobin (hemoglobin S) was discovered. More than 200 different human hemoglobins have been discovered since. They differ from normal hemoglobin A in the amino acid composition of either the α- or the β-chain.

The hemoglobins of some of the lowest fishes are monomers containing one iron atom per molecule. Hemoglobin-like respiratory proteins have been found in some invertebrates. The red hemoglobin of insects, mollusks, and protozoans is called erythrocruorin. It differs from vertebrate hemoglobin by its high molecular weight.

Although green plants contain no hemoglobin, a red protein, called leghemoglobin, has been discovered in the root nodules of leguminous plants. It seems to be produced by the nitrogen-fixing bacteria of the root nodules and may be involved in the reduction of atmospheric nitrogen to ammonia and amino acids.

Other Respiratory Proteins

A green respiratory protein, chlorocruorin, has been found in the blood of marine worms in the genera Serpula and Spirographis. It has the same high molecular weight as erythrocruorin but differs from hemoglobin in its prosthetic group. A red metalloprotein, hemerythrin, acts as a respiratory protein in marine worms of the phylum Sipuncula. The molecule consists of eight subunits with a molecular weight of 13,500 each. Hemerythrin contains no porphyrins and therefore is not a heme protein.

A metalloprotein containing copper is the respiratory protein of crustaceans (shrimps, crabs, etc.) and of some gastropods (snails). The protein, called hemocyanin, is pale yellow when not combined with oxygen, and blue when combined with oxygen. The molecular weights of hemocyanins vary from 300,000 to 9,000,000. Each animal investigated thus far apparently has a species-specific hemocyanin.

Protein Hormones

Some hormones that are products of endocrine glands are proteins or peptides, others are steroids. None of the hormones has any enzymatic activity. Each has a target organ in which it elicits some biological action-e.g., secretion of gastric or pancreatic juice, production of milk, production of steroid hormones. The mechanism by which the hormones exert their effects is not fully understood. Cyclic adenosine monophosphate is involved in the transmittance of the hormonal stimulus to the cells whose activity is specifically increased by the hormone.

Hormones of the Thyroid Gland

Thyroglobulin, the active groups of which are two molecules of the iodine-containing compound thyroxine, has a molecular weight of 670,000. Thyroglobulin also contains thyroxine with two and three iodine atoms instead of four and tyrosine with one and two iodine atoms. Injection of the hormone causes an increase in metabolism; lack of it results in a slowdown.

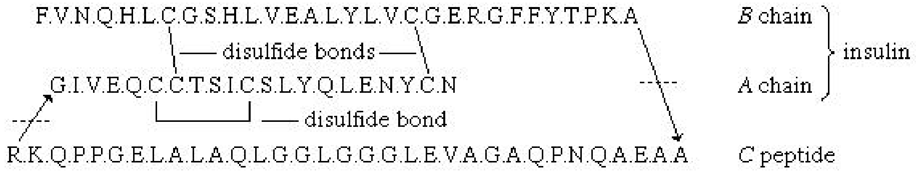
Another hormone, calcitonin, which lowers the calcium level of the blood, occurs in the thyroid gland. The amino acid sequences of calcitonin from pig, beef, and salmon differ from human calcitonin in some amino acids. All of them, however, have the half-cystines (C) and the prolinamide (P) in the same position.

C.S.N.L.S.T.C.V.L.S.A.Y.W.K.D.L.N.N.Y.H.R.F.S.G.M.G.F.G.P.E.T.P(CONH$_2$)

Parathyroid hormone (parathormone), produced in small glands that are embedded in or lie behind the thyroid gland, is essential for maintaining the calcium level of the blood. A decrease in its production results in hypocalcemia (a reduction of calcium levels in the bloodstream below the normal range). Bovine parathormone has a molecular weight of 8,500; it contains no cystine or cysteine and is rich in aspartic acid, glutamic acid, or their amides.

Hormones of the Pancreas

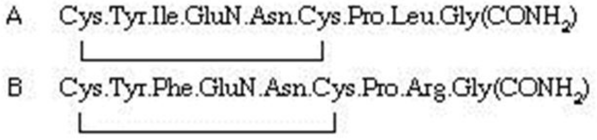
Although the amino acid structure of insulin has been known since 1949, repeated attempts to synthesize it gave very poor yields because of the failure of the two peptide chains to combine forming the correct disulfide bridge. The ease of the biosynthesis of insulin is explained by the discovery in the pancreas of proinsulin, from which insulin is formed. The single peptide chain of proinsulin loses a peptide consisting of 33 amino acids and called the connecting peptide, or C peptide, during its conversion to insulin. The disulfide bridges of proinsulin connect the A and B chains.



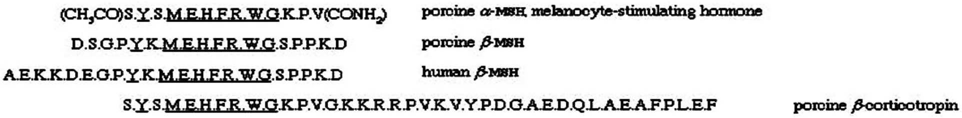
In aqueous solutions, insulin exists predominantly as a complex of six subunits, each of which contains an A and a B chain. The insulins of several species have been isolated and analyzed; their amino acid sequences have been found to differ somewhat, but all apparently contain the same disulfide bridges between the two chains.

Although the injection of insulin lowers the blood sugar, administration of glucagon, another pancreas hormone, raises the blood sugar level. Glucagon consists of a straight peptide chain of 29 amino acids. It has been synthesized; the synthetic product has the full biological activity of natural glucagon. The structure of glucagon is free of cystine and isoleucine.

The pituitary gland has an anterior lobe, a posterior lobe, and an intermediate portion; they differ in cellular structure and in the structure and action of the hormones they form. The posterior lobe produces two similar hormones, oxytocin and vasopressin. The former causes contraction of the pregnant uterus; the latter raises the blood pressure. Both are octapeptides formed by a ring of five amino acids (the two cystine halves count as one amino acid) and a side chain of three amino acids. The two cystine halves are linked to each other by a disulfide bond, and the C terminal amino acid is glycinamide. The structure has been established and confirmed. Human vasopressin differs from oxytocin in that isoleucine is replaced by phenylalanine and leucine by arginine.



The intermediate part of the pituitary gland produces the melanocyte-stimulating hormone (MSH), which causes expansion of the pigmented melanophores (cells) in the skin of frogs and other batrachians. Two hormones, called α-MSH and β-MSH, have been prepared from hog pituitary glands. The first, α-MSH, consists of 13 amino acids; its N terminal serine is acetylated (i.e., the acetyl group, CH_3CO, of acetic acid is attached), and its C terminal valine residue is present as valinamide. The second, β-MSH, contains in its 18 amino acids many of those occurring in α-MSH.



(CH_3CO)S.Y.S.M.E.H.F.R.W.G.K.P.V$(CONH_2)$	porcine α-MSH, melanocyte-stimulating hormone
D.S.G.P.Y.K.M.E.H.F.R.W.G.S.P.P.K.D	porcine β-MSH
A.E.K.K.D.E.G.P.Y.K.M.E.H.F.R.W.G.S.P.P.K.D	human β-MSH
S.Y.S.M.E.H.F.R.W.G.K.P.V.G.K.K.R.R.P.V.K.V.Y.P.D.G.A.E.D.Q.L.A.E.A.F.P.L.E.F	porcine β-corticotropin

The anterior pituitary lobe produces several protein hormones-a thyroid-stimulating hormone (thyrotropin), molecular weight 28,000; a lactogenic hormone, molecular weight 22,500; a growth hormone, molecular weight 21,500; a luteinizing hormone, molecular weight 30,000; and a follicle-stimulating hormone, molecular weight 29,000. The thyroid-stimulating hormone consists of α and β subunits with a composition similar to the subunits of luteinizing hormone. When separated, neither of the two subunits has hormonal activity; when combined, however, they regain about 50 percent of the original activity. The lactogenic hormone (prolactin) from sheep pituitary glands contains 190 amino acids. Their sequence has been elucidated; a similar peptide chain of 188 amino acids that has been synthesized not only has 10 percent of the biological activity of the natural hormone but also some activity of the growth hormone. The amino acid sequence of the growth hormone (somatotropic hormone) is also known; it seems to stimulate the synthesis of RNA and in this way to accelerate growth. The luteinizing hormone, a mucoprotein containing about 12 percent carbohydrate, consists of two subunits, each with a molecular weight of approximately 15,000; when separated, the subunits recombine spontaneously. The urine of pregnant women contains chorionic gonadotropin, the presence of which makes possible early diagnosis of pregnancy. The amino acid sequence is known. The sequence of 160 of its 190 amino acids is identical with those of the growth hormone; 100 of these also occur in the same sequence as in lactogenic hormone. The different pituitary hormones and the chorionic gonadotropin thus may have been derived from a common substance that, during evolution, underwent differentiation.

Peptides with Hormonelike Activity

Small peptides have been discovered that, like hormones, act on certain target organs. One peptide, angiotensin (angiotonin or hypertensin), is formed in the blood from angiotensinogen by the action of renin, an enzyme of the kidney. It is an octapeptide and increases blood pressure. Similar peptides include bradykinin, which stimulates smooth muscles; gastrin, which stimulates secretion of hydrochloric acid and pepsin in the stomach; secretin, which stimulates the flow of pancreatic juice; and kallikrein, the activity of which is similar to bradykinin.

Immunoglobulins and Antibodies

Antibodies, proteins that combat foreign substances in the body, are associated with the globulin fraction of the immune serum. when the serum globulins are separated into α-, β-, and γ- fractions, antibodies are associated with the γ-globulins. Antibodies can be purified by precipitation with the antigen (i.e., the foreign substance) that caused their formation, followed by separation of the antigen-antibody complex. Antibodies prepared in this way consist of a mixture of many similar antibody molecules, which differ in molecular weight, amino acid composition, and other properties. The same differences are found in the γ-globulins of normal blood serums. The γ-globulin of normal blood serum is thought to consist of a mixture of hundreds of different γ-globulins, each of which occurs in amounts too small for isolation. Because the physical and chemical properties of normal γ-globulins are the same as those of antibodies, the γ-globulins are frequently called immunoglobulins. They may be considered to be antibodies against unknown antigens. If solutions of γ-globulin are resolved by gel filtration through dextran, the first fraction has a molecular weight of 900,000. This fraction is called IgM or γM; Ig is an abbreviation for immunoglobulin and M for macroglobulin. The next two fractions are IgA (γA) and IgG (γG), with molecular weights of about 320,000 and 150,000 respectively. Two other immunoglobulins, known as IgD and IgE, have also been detected in much smaller amounts in some immune sera.

The bulk of the immunoglobulins is found in the IgG fraction, which also contains most of the antibodies. The IgM molecules are apparently pentamers—aggregates of five of the IgG molecules. Electron microscopy shows their five subunits to be linked to each other by disulfide bonds in the form of a pentagon. The IgA molecules are found principally in milk and in secretions of the intestinal mucosa. Some of them contain, in addition to a dimer of IgG, a “secretory piece” that enables the passage of IgA molecules between tissue and fluid; the structure of the secretory piece is not yet known. The IgM and IgA immunoglobulins and antibodies contain 10 to 15 percent carbohydrate; the carbohydrate content of the IgG molecules is 2 to 3 percent.

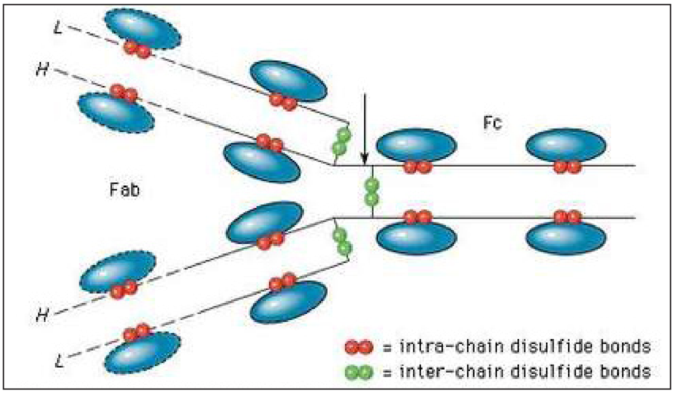


Diagram of an IgG immunoglobulin

IgG molecules treated with the enzyme papain split into three fragments of almost identical molecular weight of 50,000. Two of these, called Fab fragments, are identical; the third is abbreviated Fc. Reduction to sulfhydryl groups of some of the disulfide bonds of IgG results in the formation of two heavy, or H, chains (molecular weight 55,000) and two light, or L, chains (molecular weight 22,000). They are linked by disulfide bonds in the order $L-H-H-L$. Each H chain contains four intrachain disulfide bonds, and each L chain contains two.

Antibody preparations of the IgG type, even after removal of IgM and IgA antibodies, are heterogeneous. The H and L chains consist of a large number of different L chains and a variety of H chains. Pure IgG, IgM, and IgA immunoglobulins, however, occur in the blood serum of patients suffering from myelomas, which are malignant tumours of the bone marrow. The tumours produce either an IgG, an IgM, or an IgA protein, but rarely more than one class. A protein called the Bence-Jones protein, which is found in the urine of patients suffering from myeloma tumours, is identical with the L chains of the myeloma protein. Each patient has a different Bence-Jones protein; no two of the more than 100 Bence-Jones proteins that have been analyzed thus far are identical. It is thought that one lymphoid cell among hundreds of thousands becomes malignant and multiplies rapidly, forming the mass of a myeloma tumour that produces one γ-globulin.

Analyses of the Bence-Jones proteins have revealed that the L chains of humans and other mammals are of two quite different types, kappa (κ) and lambda (λ). Both consist of approximately 220 amino acids. The N–terminal halves of κ- and λ-chains are variable, differing in each Bence-Jones protein. The C–terminal halves of these same L chains have a constant amino acid sequence of either the κ- or the λ-type. The fact that one half of a peptide chain is variable and the other half invariant is contradictory to the view that the amino acid sequence of each peptide chain is determined by one gene. Evidently, two genes, one of them variable, the other invariant, fuse to form the gene for the single peptide chain of the L chains. Whereas the normal human L chains are always mixtures of the κ- and λ-types, the H chains of IgG, IgM, and IgA are different. They have been designated as gamma (γ), mu (μ), and alpha (α) chains, respectively. The N-terminal quarter of the H chains has a variable amino acid sequence; the C-terminal three-quarters of the H chains have a constant amino acid sequence.

Fat

Fats are nutrients in food that the body uses to build nerve tissue (including the brain and nerves) and hormones. The body also uses fat as fuel. If fats eaten aren’t burned as energy or used as building blocks, they’re stored by the body in fat cells. This is the body’s way of thinking ahead: By saving fat for future use, it plans for times when food might be scarce.

Fat gives food flavor and texture, but it's also high in calories and excess amounts of fatty foods can cause many health problems.

For kids and teens, desserts and snacks (including potato chips, chocolate, cakes, doughnuts, pastries, and cookies) are a significant source of fat. Kids also get fat from whole-milk products and high-fat meats, such as bacon, hot dogs, and fattier cuts of red meat.

Fast-food and takeout meals tend to have more fat than home cooking; and in restaurants, fried dishes are the highest in fat content. Fat also often "hides" in foods in the form of creamy, cheesy, or buttery sauces or dressings.

However, healthy fats at the recommended daily amounts are an important part of a nutritious diet for both kids and adults.

Getting enough healthy fats is essential for growth and development. Young kids, in particular, need enough of them in their diet to help the brain and nervous system develop normally.

Besides supplying fuel for the body, fats:

- Help the body absorb some vitamins (vitamins A, D, E, and K are fat soluble, meaning they can only be absorbed if there's fat in a person's diet).
- Are the building blocks of hormones.
- Insulate all nervous system tissues in the body.
- Help people feel full, so they're less likely to overeat.

Fat is a great source of energy, but does have twice the amount of calories as carbohydrates or protein. For example, 1 gram of fat provides 9 calories, whereas 1 gram of carbohydrates or protein provide 4 calories.

Advantages and Disadvantages of Fat

Advantages of Fat

- Fat is a concentrated source of energy – 1 gram of fat contains 9 calories, much more than a gram of protein or carbohydrate which both contain 4 calories. The body can pull on its fat reserves during lean times for energy, converting fat into glucose.
- Fat provides a cushion to help protect our vital organs – without fat our organs would be more prone to damage. Furthermore, fat acts as an insulator, helping us to maintain the correct body temperature.
- Fat enables our bodies to process vitamins A, D, E and K, which are all fat soluble and vital to good health.

- Like amino acids in protein, fat contains essential fatty acids (EFA's). These EFA's are, as their name suggests, essential to good health and likely to help the heart and immune system. The human body cannot make its own (synthesize) these EFA's and therefore has to get them from consumption of fat.
- Some fatty acids – like omega 3 – may provide other health benefits such as complimenting the cognitive processes of the brain.
- Fat makes food taste better. Hot buttered crumpets, double cream on trifle, gravy made from dripping.

Disadvantages of Fat

- Due to its high calorific value (1 gram of fat = 9 calories) it is easy to consume too many calories when eating fatty foods. Unused calories can be stored by the body as fat and will cause weight gain.
- Our bodies store fat for lean times and have evolved to cope with seasonal availability of food – storing fat when food is plentiful and burning it off when food is scarce. In the modern world, and for most people, food is plentiful all year round – our bodies store fat but never burn it off, as fat accumulates we become overweight.
- Fat can cushion and protect our internal organs; however too much cushioning means more bulk and weight which in turn increases the workload of the heart and other organs.
- Your body (the liver) produces cholesterol which is vital to a healthy body and a building block for other essential chemicals that the body produces. Cholesterol is a waxy substance that, in low levels, flows freely around your body in the blood. Higher levels of cholesterol mean a higher risk of developing coronary heart disease.
- Some fats are worse than others. Saturated fats are worse for you than unsaturated fats – this is to do with their chemical structure and how the body processes them. Trans or hydrogenated fats – which are almost exclusively manufactured (although do occur naturally in small quantities in meat and dairy produce) and are used in many processed foods are particularly bad and are linked to an increased risk of high cholesterol levels and coronary heart disease.

Types of Fats

Saturated Fat

Saturated fats have no double bonds in their chemical structure. They are "saturated" with hydrogen atoms. Because of their chemical structure, they have a solid consistency at room temperature.

Saturated fats are often called 'bad fats' – they are not considered essential for good health, and have been linked with an increased risk of high blood cholesterol levels in the body which in turn is a risk factor for heart disease.

Saturated fat is a type of fat that is solid at room temperature. It is mainly found in animal products but can be found in some plant sources.

Animal-based sources of saturated fat include:

- Dairy foods – such as butter, cream, ghee, regular-fat milk and cheese.
- Meat – such as fatty cuts of beef, pork and lamb, processed meats like salami, sausages and the skin on chicken.
- Lard.
- Plant-derived sources of saturated fat include:
- Palm oil,
- Cooking margarine and copha, and
- Coconut oil, milk and cream.

Saturated fats are also commonly found in many manufactured and packaged foods such as:

- Fatty snack foods,
- Deep fried take away foods,
- Cakes,
- Biscuits, and
- Pastries and pies.

All fats are composed of molecules called fatty acids, which are chains of carbon atoms. The different types of saturated fatty acids can be distinguished by the length of their carbon chains.

Here are the most common saturated fatty acids in the human diet:

- Stearic acid: 18 carbon atoms long,
- Palmitic acid: 16 carbon atoms long,
- Myristic acid: 14 carbon atoms long,
- Lauric acid: 12 carbon atoms long,
- Capric acid: 10 carbon atoms long,

- Caprylic acid: 8 carbon atoms long, and
- Caproic acid: 6 carbon atoms long.

It's rare to find saturated fatty acids other than these in the diet.

Saturated fatty acids that are less than six carbon atoms long are collectively known as short-chain fatty acids.

These are produced when gut bacteria ferment fiber. They're created in your gut from the fiber you eat and can also be found in trace amounts in some fermented food products.

Stearic Acid

- Stearic acid is the second most common saturated fat in diet.
- Compared with carbs or other saturated fats, stearic acid lowers LDL (bad) cholesterol slightly or has neutral effects. As such, it may be healthier than many other saturated fats.
- Our body partly converts stearic acid to oleic acid, a healthy unsaturated fat. However, according to some estimates, the conversion rate is only 14% and may not have much health relevance.
- The main dietary source of stearic acid is animal fat. The levels of stearic acid are usually low in plant fat, with the exception of coconut oil, cocoa butter, and palm kernel oil.
- Stearic acid is considered a healthy saturated fat and does not appear to raise your risk of heart disease.
- This held true even in a 40-day study in people whose stearic acid intake constituted up to 11% of their total calorie intake.

Palmitic Acid

- Palmitic acid is the most common saturated fat in plants and animals.
- The richest dietary source is palm oil, but palmitic acid also makes up approximately a quarter of the fat in red meat and dairy.
- Compared with carbs and unsaturated fats, palmitic acid raises levels of total cholesterol and LDL (bad) cholesterol without affecting HDL (good) cholesterol.
- High levels of LDL cholesterol are a well-known risk factor for heart disease.
- Still, not all LDL cholesterol is the same. More accurate markers of heart disease are the presence of a large number of LDL particles and of small, dense LDL particles.

- Though palmitic acid raises total LDL cholesterol, this is mainly due to an increase in large LDL particles. Many researchers consider high levels of large LDL particles to be less of a concern, though others disagree.
- When linoleic acid, a type of unsaturated fat, is eaten at the same time, it can offset some of palmitic acid's effects on cholesterol.
- Palmitic acid may also affect other aspects of your metabolism. Studies in both mice and humans indicate that a high-palmitic-acid diet may adversely affect mood and reduce physical activity.
- Several human studies suggest that eating higher amounts of palmitic acid reduces the number of calories you burn, compared with eating more unsaturated fats, such as oleic acid.

Myristic Acid

- Myristic acid causes a significant increase in total cholesterol and LDL (bad) cholesterol compared with palmitic acid or carbs. However, it doesn't appear to affect HDL (good) cholesterol levels.
- These effects are much stronger than those of palmitic acid. Yet, similar to palmitic acid, myristic acid appears to increase your levels of large LDL particles, which many scientists consider to be less of a concern.
- Myristic acid is a relatively rare fatty acid, not found in high amounts in most foods. Yet certain oils and fats contain a decent amount.
- Though coconut oil and palm kernel oil boast relatively high amounts of myristic acid, they also provide other types of fats, which may offset the effects of myristic acid on your blood lipid profile.

Lauric Acid

- With 12 carbon atoms, lauric acid is the longest of the medium-chain fatty acids.
- It raises total cholesterol more than most other fatty acids. Still, this increase is largely due to an increase in HDL (good) cholesterol.
- In other words, lauric acid reduces the amount of total cholesterol relative to HDL cholesterol. These changes are associated with a reduced risk of heart disease.
- In fact, lauric acid appears to have more beneficial effects on HDL cholesterol levels than any other saturated fatty acid.
- Lauric acid makes up approximately 47% of palm kernel oil and 42% of coconut oil. In comparison, other commonly eaten oils or fats provide only trace amounts.

Caproic, Caprylic and Capric Acid

- Caproic, caprylic, and capric acid are medium-chain fatty acids (MCFAs).
- Their names are derived from the Latin “capra,” which means “female goat.” They’re sometimes referred to as capra fatty acids, due to their abundance in goat’s milk.
- MCFAs are metabolized differently than long-chain fatty acids. They’re more easily absorbed and transported straight to your liver, where they’re rapidly metabolized.
- Evidence suggests that MCFAs may have the following benefits:
    - Weight loss: Several studies indicate that they may slightly increase the number of calories you burn and promote weight loss, especially when compared with long-chain fatty acids.
    - Increased insulin sensitivity: Some evidence suggests that MCFAs increase insulin sensitivity, compared with long-chain fatty acids.
    - Antiseizure effects: MCFAs, especially capric acid, may have antiseizure effects, especially when combined with a ketogenic diet.
- Due to their potential health benefits, MCFAs are sold as supplements, known as MCT oils. These oils usually consist primarily of capric acid and caprylic acid.
- Capric acid is the most common of these. It constitutes around 5% of palm kernel oil and 4% of coconut oil. Smaller amounts are found in animal fat. Otherwise, it is rare in foods.

Short-chain Fatty Acids

- Saturated fatty acids that contain fewer than six carbon atoms are known as short-chain fatty acids (SCFAs).
- The most important SCFAs are:
    - Butyric acid: 4 carbon atoms long.
    - Propionic acid: 3 carbon atoms long.
    - Acetic acid: 2 carbon atoms long.
- SCFAs are formed when beneficial gut bacteria ferment fiber in your colon.
- Their dietary intake is minimal compared with the amounts of SCFAs produced in your colon. They’re uncommon in food and only found in small amounts in dairy fat and certain fermented foods.

- SCFAs are responsible for many of the health benefits associated with fiber intake. For instance, butyric acid is an important source of nutrition for the cells lining your colon.
- Types of fiber that promote the formation of short-chain fatty acids are known as prebiotics. They include resistant starch, pectin, inulin, and arabinoxylan.

Unsaturated Fat

Unsaturated fats are defined by their chemical structure. Those that are visible (as in oils and spreads) are liquid or soft at room temperature and can be classified as monounsaturated or polyunsaturated. Different types of unsaturated fatty acids may have different health benefits but unsaturated fats are generally healthier than saturated fats.

When they replace saturated fats, unsaturated fats can help reduce blood cholesterol levels and thus lower the risk of heart disease. Extra virgin olive oil, with its monounsaturated fat plus its wide range of other protective factors, forms a staple of the Mediterranean diet, a healthy balanced diet that reduces the risk of heart disease and some cancers.

Sources of Unsaturated Fats

Foods that contain unsaturated fat include:

- Avocados: This delicious fruit, which has become very popular in recent years, is chock-full of monounsaturated fats. Avocados can be added to many recipes without too much prep time– as a spread on your sandwich or sliced onto your favorite soup, salad, or entrée.
- Olives: Green, black, Kalamata – olives are not only high in flavor, but they are also high in monounsaturated fats. Whether you slice, dice, or use them whole, there are many opportunities to add olives to your cholesterol-friendly diet.
- Nuts: These delicious foods come in a wide variety of types and most people like at least a few varieties. Nuts are high in both PUFAs and monounsaturated fats. Walnuts are typically higher in PUFAs in comparison to other nuts, whereas pistachios, almonds, and pecans are higher in monounsaturated fat content. Nuts are also high in other healthy ingredients, such as fiber, phytosterols, vitamins, minerals, and protein. Nuts are versatile and can be included in your diet in a number of ways. A handful of nuts can make a satisfying snack, or they can be added to a salad.
- Fatty fish: Fish are generally lean and good to include in your lipid-lowering diet. Some fish are high in omega-3 fats, a type of PUFA. Fish in this category would include salmon, mackerel, herring, tuna, and anchovy. If you include this type of fish in your diet, you can keep it heart-healthy by grilling, baking, or poaching. However, you should avoid frying the fish, as this can introduce calories and unhealthy trans fats into your diet.

- Certain oils: Oils can be added to dips and dressings and they can also be used in cooking your favorite sautéed or baked goods. If you're following a lipid-lowering diet, you can switch out butter or margarine for oils high in unsaturated fat. These oils include olive, canola, vegetable, safflower, corn and soybean oils.
- Seeds: Besides nuts, seeds can also make a good go-to snack that is high in filling fiber, protein, and unsaturated fat. Sesame seeds are higher in monounsaturated fats, whereas pumpkin, sunflower, flax and chia seeds are higher in polyunsaturated fats. Seeds can be included in your sides, in your cereal, or as a topper for yogurt or salads. However, you should watch out for salt content – since some seeds may be prepared with a lot of salt.
- Eggs: Eggs contain saturated fats and unsaturated fats. When they are prepared without frying, they are considered a healthy addition to your diet.
- Dark chocolate: Chocolate contains a small portion of monounsaturated fats, and, in low to moderate amounts, has been considered healthy. However, eating a lot of heavily sweetened chocolate can be high in calories and in unhealthy fats.

Monounsaturated Fat

Monounsaturated fats have only one double bond in their molecular structure. They may help in maintaining the overall health of cells. Further, they can lower bad cholesterol, which reduces the risk for heart disease and stroke in the long run.

There are several healthy foods containing monounsaturated fats, including:

- Cooking oils, including olive oil, sesame oil, and canola oil,
- Peanut butter,
- Nuts, including peanuts and cashews,
- Avocados,
- Olives,
- Sesame seeds and
- Healthy spreads labeled "high oleic".

Health Benefits of Monounsaturated Fats

1. May Help with Weight Loss:

- Diets high in monounsaturated fatty acids (MUFAs), such as the Mediterranean diet, may help with weight loss.

- In obese women, a diet high in MUFAs led to more weight and fat loss than high polyunsaturated fat diet or their habitual diet.
- However, in overweight/obese type 2 diabetics, a high-MUFA diet was as effective as a low-fat high-carbohydrate diet in terms of weight loss (4% of body weight lost), HDL levels, blood pressure, and blood sugar improvements (RCT).

2. Can Help Lower Cholesterol and Triglycerides:

- A diet high in monounsaturated fats can help lower cholesterol levels. The replacement of saturated fat with MUFA decreases LDL cholesterol, which is the main risk factor for heart disease.
- Consuming oleic acid in olive oil helps prevent LDL oxidation and, thus, atherosclerosis (hardening of the arteries).
- Palmitoleic acid can:
  - Reduce triglycerides and cholesterol levels in people with abnormally high levels (DB-RCT).
  - Increase HDL cholesterol.
  - Decrease LDL cholesterol.

3. Protect Against Heart Disease:

- Diets high in MUFAs were associated with reduced risk for heart disease, heart attack, and stroke. Oleic acid, olive oil, and Mediterranean diets all help prevent heart disease-related deaths.
- MUFAs coexist with saturated fatty acids in several food sources. Since saturated fat may worsen heart health, it is important to consume food that contains high MUFA levels and low saturated fat levels.
- A high-MUFA Mediterranean diet may prevent heart attack-related deaths.
- Diets high in oleic acid can also help lower blood pressure, cholesterol, glucose, and insulin levels, which protects against heart disease.

4. Can Prevent Diabetes:

- Diets high in monounsaturated fatty acids (MUFAs) may be healthier for diabetic patients than low-fat, high-carbohydrate diets. The Mediterranean diet, which is high in MUFAs, improve glucose control and insulin sensitivity.
- Overweight/obese type 2 diabetes patients can better control their diabetes with a high-MUFA diet than a high-carbohydrate diet (RT).
- In healthy subjects, replacing saturated fats by MUFAs in the diet helped improve insulin sensitivity (RCT).

- However, this favorable effect on insulin sensitivity only occurs along with a low total fat intake. Also, the high-MUFA diet had no effect on insulin secretion.
- In rats, supplementation with fish oil (high in MUFAs) can help reduce blood sugar. It also helps improve insulin function and reduce inflammation, which reduces insulin resistance.
- Olive oil intake is associated with a decreased risk of developing type 2 diabetes (meta-analysis of 29 RCTs). However, some other substances in olive oil other than MUFAs may also be responsible for these effects.

5. Benefit the Immune System and Lower Inflammation:

- Oleic acid has various effects on the immune system and inflammation. MUFA-rich oils might have similar anti-inflammatory effects as fish oil.
- Like fish oil, olive oil helps to:
  - Increase nitric oxide (in rats).
  - Decrease arachidonic acid and the production of the inflammatory mediator prostaglandin E2.
  - Suppress inflammatory cytokines.
- Consumption of almond (which contains MUFAs), can help to:
  - Decrease E-selectin (a marker of inflamed blood vessels).
  - Decrease CRP (a marker of inflammation).
  - Reduce oxidative damage, although this could also be due to other antioxidant compounds in nuts.

6. Increase Bone Strength:

- Monounsaturated fats can help with bone health. High monounsaturated fatty acid (MUFA) intake is associated with higher bone density and lower bone fracture risk.
- In a study of 187 women, high oleic acid diets were associated with increased bone strength.
- A higher amount of MUFA compared to polyunsaturated fat in their diets also reduced the risk of bone fractures in elderly patients.
- By reducing prostaglandin levels and reducing bone resorption (the breakdown and loss of bone tissue to release minerals into the blood), MUFAs prevent osteoporosis.

- In mice, a high-MUFA diet increased bone thickness and volume in comparison to a high saturated fat and a normal fat diet. In addition, the high saturated fat diet also increased calcium absorption in the intestines, although it was unclear if calcium absorption had any effect on bone mineral density.

7. May Improve Mood and Reduce Risks of Depression:

- A high monounsaturated fat (MUFA) diet can help improve mood. The Mediterranean diet is associated with lower levels of depression.
- 14 young adults who consumed high amounts of oleic acid over the course of 3 weeks reported less anger compared to those in the high palmitic acid group.
- Thus, replacing palmitic acid (a saturated fatty acid) with oleic acid (a monounsaturated fatty acid) reduces feelings of anger and hostility. However, it wasn't clear if this was due to increased MUFAs or decreased saturated fatty acids.

8. Reduce Cancer Risk:

- Consuming monounsaturated fats (MUFAs) instead of saturated fats can help reduce cancer risk. Diets containing oleic acid reduced breast cancer risk.
- Breast cancer risk and MUFA intake also depend on the food source of the fats. Olive oil is associated with reduced breast cancer risk. However, margarine, a highly processed source of MUFAs, does not help reduce breast cancer risk.
- Possibly, the anti-cancer effects of olive oil were due to antioxidant content rather than to oleic acid.

9. Suppress Rheumatoid Arthritis Symptoms:

- A Mediterranean diet can help suppress rheumatoid arthritis symptoms.
- Compared to healthy people, rheumatoid arthritis patients ate significantly fewer MUFAs, suggesting that MUFAs may prevent the disease.
- In addition, olive oil consumption was associated with a lower risk of rheumatoid arthritis.

10. Protect the Liver:

- MUFAs can protect against liver injury due to a drug overdose.
- In rats, a high-MUFA diet protected against acetaminophen (Tylenol)-induced liver injuries. The presence of monounsaturated fats in the cell membrane of liver cells reduces the cells' susceptibility to oxidative damage.
- In addition, diets rich in MUFAs tend to decrease liver fat content, which may protect against nonalcoholic fatty liver disease.

11. Increase Good Gut Bacteria:

- Gut bacteria can lead to obesity and, conversely, obesity can disrupt gut bacteria.
- An oleic acid-derived compound can help with weight loss by restoring gut flora. Oleic acid reduces a family of bacteria called Enterobacteriaceae, which is associated with inflammation. In addition, it increases good bacteria (Bifidobacteria).

12. Stop Skin Damage:

- High olive oil intake, which is rich in MUFAs, lowers the risk of severe skin damage from the sun.

Omega-7 fatty Acid

Omega-7 fatty acids are a class of unsaturated fatty acids in which the site of unsaturation is seven carbon atoms from the end of the carbon chain. The two most common omega-7 fatty acids in nature are palmitoleic acid and vaccenic acid. They are widely used in cosmetics due to their moisturizing properties. Diets rich in omega-7 fatty acids have been shown to have beneficial health effects, such as increasing levels of HDL cholesterol and lowering levels of LDL cholesterol.

Rich sources include macadamia nut oil and sea buckthorn oil in the form of palmitoleic acid, while dairy products are the primary sources of vaccenic acid and rumenic acid. A lesser but useful source of palmitoleic acid is avocado fruit (25,000ppm).

The monounsaturated omega-7 fatty acids have the general chemical structure CH_3-$(CH_2)_5$-CH=CH-$(CH_2)_n$-CO_2H.

Common name	Lipid name	Chemical name
none	12:1 (n−7)	5-Dodecenoic acid
none	14:1 (n−7)	7-Tetradecenoic acid
Palmitoleic acid	16:1 (n−7)	9-Hexadecenoic acid
Vaccenic acid	18:1 (n−7)	11-Octadecenoic acid
Rumenic acid	18:2 (n−7)	Octadeca-9,11-dienoic acid
Paullinic acid	20:1 (n−7)	13-Eicosenoic acid
none	22:1 (n−7)	15-Docosenoic acid
none	24:1 (n−7)	17-Tetracosenoic acid

Metabolism

16- and 18-carbon omega-7 unsaturated fatty acids are known to be converted into 18- or 20-carbon highly unsaturated fatty acids in the body by nonselective desaturating enzymes. The same enzymes also act on omega-3, omega-6, and omega-9 fatty acids. As a result, while proportions of individual highly unsaturated fatty acids may vary greatly in different tissue types due to factors such as diet, the overall concentration of highly unsaturated fatty acids is kept stable in a living organism. These individual concentrations are highly influential in determining what fatty acids will be used by a given tissue type in phospholipid synthesis such as that required for the maintenance of the cellular membrane.

Research

Diabetes

Omega-7 fatty acids, especially palmitoleic acid, have been shown *in vitro* to decrease glucose-sensitive apoptosis in beta cells in the pancreas, a condition associated with diabetes. In adult organisms, new beta cells are most commonly the result of replication rather than from direct stem cell differentiation, meaning that preventing apoptosis of beta cells is crucial for maintaining a stable population of beta cells. The cytoprotective effect of omega-7 fatty acids makes them a candidate for diabetes treatment. Omega-7 fatty acids have also been found to improve insulin sensitivity, and a diet rich in omega-7 fatty acids is correlated with a greatly decreased incidence of diabetes.

Cholesterol

In an animal study, dietary omega-7 fatty acids were found to result in an increase in HDL cholesterol in comparison to a diet rich in coconut or canola oils.

Production

In Cows

Dairy products are one of the primary sources of dietary omega-7 fatty acids. However, the production of omega-7 fatty acids in cows is heavily diet-dependent. Specifically, a reduction in the proportion of herbage consumed by a cow is correlated with a significant decrease in the omega-7 fatty acid content of the cow's milk. Rumenic and vaccenic acid concentrations declined significantly within one week of removing herbage from the cow's diet, suggesting that modern dairy farming methods may lead to decreases in beneficial fatty acid content of dairy products.

Algal Extraction

Traditional sources of omega-7 fatty acids such as macadamia nuts have proved expensive on the industrial scale, prompting the discovery of new omega-7 rich sources such as algae. Alterations to algal growing conditions such as carbon dioxide or dipotassium phosphate enrichment have been shown to potential bias algal biosynthesis towards lipids. Up to 90% of their dry weight may be harvested as lipids. In this process, raw algae is dewatered to yield algal oil. Algal oil gets degummed, typically via washing with acid, to removing polar lipids and metals. Degummed algal oil is then transesterified and purified to yield a mixture of omega-7 esters and eicosapentaenoic acids, which can be hydrodeoxygenated to form algae jet fuel and algae green diesel, respectively. These products are then crystallized and separated to yield the desired omega-7 fatty acid.

Omega-9 fatty Acid

Omega-9 fatty acids (ω−9 fatty acids or *n*−9 fatty acids) are a family of unsaturated fatty acids which have in common a final carbon–carbon double bond in the omega−9 position; that is, the ninth bond from the methyl end of the fatty acid.

Some omega−9 fatty acids are common components of animal fat and vegetable oil. Two omega−9 fatty acids important in industry are:

- Oleic acid (18:1, *n*−9), which is a main component of olive oil, macadamia oil and other monounsaturated fats.
- Erucic acid (22:1, *n*−9), which is found in rapeseed, wallflower seed, and mustard seed. Rapeseed with high erucic acid content is grown for commercial use in paintings and coatings as a drying oil. Canola oil comes from a cultivar of the rapeseed plant that has been bred, or in some cases genetically modified, to contain very little erucic acid.

Unlike omega-3 fatty acids and omega-6 fatty acid, omega−9 fatty acids are not classed as essential fatty acids (EFA). This is both because they can be created by the human body from unsaturated fat, and are therefore not essential in the diet, and because the lack of an omega−6 double bond keeps them from participating in the reactions that form the eicosanoids.

Under severe conditions of EFA deprivation, mammals will elongate and desaturate oleic acid to make mead acid, (20:3, *n*−9). This has been documented to a lesser extent in one study following vegetarians and semi-vegetarians who followed diets without substantial sources of EFA.

Omega−9 fatty acids		
Common name	Lipid name	Chemical name
Oleic acid	18:1 (*n*−9)	(*Z*)-octadec-9-enoic acid
Elaidic acid	18:1 (*n*−9)	(*E*)-octadec-9-enoic acid
Gondoic acid	20:1 (*n*−9)	(*Z*)-eicos-11-enoic acid
Mead acid	20:3 (*n*−9)	(5*Z*,8*Z*,11*Z*)-eicosa-5,8,11-trienoic acid
Erucic acid	22:1 (*n*−9)	(*Z*)-docos-13-enoic acid
Nervonic acid	24:1 (*n*−9)	(*Z*)-tetracos-15-enoic acid
Ximenic acid	26:1 (*n*−9)	

Foods High in Omega-9 Fats

Omega-9 fats are also common in vegetable and seed oils, as well as nuts and seeds.

There are no adequate intake recommendations for omega-9s, since they are non-essential.

Here are the amounts of omega-9s in 100 grams of the following foods:

- Olive oil: 83 grams
- Cashew nut oil: 73 grams
- Almond oil: 70 grams
- Avocado oil: 60 grams
- Peanut oil: 47 grams
- Almonds: 30 grams
- Cashews: 24 grams
- Walnuts: 9 grams

Polyunsaturated Fat

Polyunsaturated fats differ from monounsaturated fats because they have more than one double bond in their structure. Much like monounsaturated fats, polyunsaturated fats can help lower bad cholesterol. Polyunsaturated fats also contain omega-3 and omega-6 fatty acids, which your body needs for brain function and cell growth.

Foods high in polyunsaturated fats include:

- Seeds including sunflower seeds and pumpkin seeds.
- Cooking oils including corn oil, safflower oil, and soybean oil.
- Nuts such as pine nuts and walnuts.

A certain type of polyunsaturated fat, omega-3 fats, have specifically been studied regarding their effects on heart health and ability to lower lipid levels. Studies have shown omega-3 fats can lower triglyceride levels and slightly increase HDL (good cholesterol) levels. In fact, eating fish high in omega-3 fatty acids, such as salmon, tuna, and sardines, at least twice a week significantly decreases blood triglyceride levels.

The following foods contain this specific type of polyunsaturated fat:

- Fatty fish, including salmon, mackerel, herring, and tuna,
- Seeds, including flax seeds and chia seeds,

- Walnuts and
- Algae (e.g., seaweed, spirulina, nori).

Benefits and Advantages of Polyunsaturated Fats

- Omega-3 fatty acids are known to reduce the risk of heart attacks.
- Omega-6 fatty acids reduce the risk of heart disease.
- Omega-3 fatty acids inhibit the growth of a prostate tumor.
- High levels of Omega-3 fatty acids are associated with the reduced risk of breast cancer.
- Polyunsaturated fat intake reduces the risk of sclerosis.

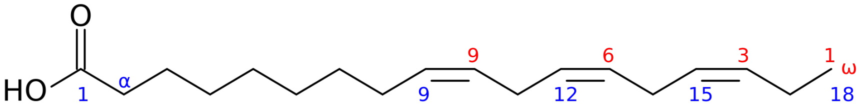
Omega-3 fatty Acid

Omega–3 fatty acids, also called ω–3 fatty acids or *n*–3 fatty acids, are polyunsaturated fatty acids (PUFAs) characterized by the presence of a double bond three atoms away from the terminal methyl group in their chemical structure. They are widely distributed in nature, being important constituents of animal lipid metabolism, and they play an important role in the human diet and in human physiology. The three types of omega–3 fatty acids involved in human physiology are α-linolenic acid (ALA), found in plant oils, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both commonly found in marine oils. Marine algae and phytoplankton are primary sources of omega–3 fatty acids. Common sources of plant oils containing ALA include walnut, edible seeds, clary sage seed oil, algal oil, flaxseed oil, Sacha Inchi oil, *Echium* oil, and hemp oil, while sources of animal omega–3 fatty acids EPA and DHA include fish, fish oils, eggs from chickens fed EPA and DHA, squid oils, and krill oil.

Mammals are unable to synthesize the essential omega–3 fatty acid ALA and can only obtain it through diet, which they can then use to form the long-chain omega–3 fatty acids, EPA (20 carbons and 5 double bonds) and then from EPA make DHA (22 carbons and 6 double bonds). The ability to make the longer-chain omega–3 fatty acids from ALA may be impaired in aging. In foods exposed to air, unsaturated fatty acids are vulnerable to oxidation and rancidity. Dietary supplementation with omega–3 fatty acids does not appear to affect the risk of death, cancer or heart disease. Furthermore, fish oil supplement studies have failed to support claims of preventing heart attacks or strokes or any vascular disease outcomes.

Nomenclature

The highest locant is assigned to the terminal methyl group as carbon 18, while the double bond closest to the end begins at carbon 15 = 18–3. Hence, ALA is a *n*–3 fatty acid with $n = 18$. This classification scheme is useful since most chemical changes occur at the carboxyl end of the molecule, while the methyl group and its nearest double bond are unchanged in most chemical or enzymatic reactions.



Chemical structure of α-linolenic acid (ALA), an essential omega−3 fatty acid, (18:3Δ9c,12c,15c, which means a chain of 18 carbons with three cis double bonds on carbons numbered 9, 12, and 15).

The terms *ω−3 ("omega−3") fatty acid* and *n−3 fatty acid* are derived from organic nomenclature. One way in which a fatty acid is named is determined by the location of the first double bond, counted from the methyl end, that is, the omega (ω-) or the *n*- end. In general terminology using either *n−x* or ω−*x*, the dash is a minus sign (although it is never read as such), and the number *n−x* (or ω−*x*) refers to the locant of the double bond linking two carbon atoms. Thus, in omega−3 fatty acids in particular, there is a double bond located on the carbon numbered 3 lower than the highest carbon number. By example, α-linolenic acid (ALA; illustration) is an 18-carbon chain having three double bonds, the first being located at the third carbon from the methyl end of the fatty acid chain at carbon 15. α-Linolenic acid is polyunsaturated (containing more than one double bond) and is also described by a lipid number, 18:3, meaning that there are 18 carbon atoms and 3 double bonds (at carbons 9, 12, and 15).

Although *n* and ω (omega) are synonymous, the IUPAC recommends that *n* be used to identify the highest carbon number of a fatty acid. Nevertheless, the more common name – omega−3 fatty acid – is used in both the lay media and scientific literature.

Health Effects

Supplementation does not appear to be associated with a lower risk of all-cause mortality.

Cancer

The evidence linking the consumption of marine omega−3 fats to a lower risk of cancer is poor. With the possible exception of breast cancer, there is insufficient evidence that supplementation with omega−3 fatty acids has an effect on different cancers. The effect of consumption on prostate cancer is not conclusive. There is a decreased risk with higher blood levels of DPA, but an increased risk of more aggressive prostate cancer was shown with higher blood levels of combined EPA and DHA. In people with advanced cancer and cachexia, omega−3 fatty acids supplements may be of benefit, improving appetite, weight, and quality of life.

Cardiovascular Disease

Evidence in the population generally does not support a beneficial role for omega−3 fatty acid supplementation in preventing cardiovascular disease (including myocardial infarction and sudden cardiac death) or stroke. A 2018 meta-analysis found no support that daily intake of one gram of omega-3 fatty acid in individuals with a history of coronary heart disease prevents fatal coronary heart disease, nonfatal myocardial infarction or any other vascular event. However, omega−3 fatty acid supplementation greater than one gram daily for at least a year may be protective against cardiac death, sudden death, and myocardial infarction in people who have a history of cardiovascular disease. No protective effect against the development of stroke or all-cause mortality was seen in this population. Eating a diet high in fish that contain long chain omega−3 fatty acids does appear to decrease the risk of stroke. Fish oil supplementation has not been shown to benefit revascularization or abnormal heart rhythms and has no effect on heart failure hospital admission rates. Furthermore, fish oil supplement studies have failed to support claims of preventing heart attacks or strokes.

Evidence suggests that omega−3 fatty acids modestly lower blood pressure (systolic and diastolic) in people with hypertension and in people with normal blood pressure. Some evidence suggests that people with certain circulatory problems, such as varicose veins, may benefit from the consumption of EPA and DHA, which may stimulate blood circulation and increase the breakdown of fibrin, a protein involved in blood clotting and scar formation. Omega−3 fatty acids reduce blood triglyceride levels but do not significantly change the level of LDL cholesterol or HDL cholesterol in the blood. The American Heart Association position (2011) is that borderline elevated triglycerides, defined as 150–199 mg/dL, can be lowered by 0.5-1.0 grams of EPA and DHA per day; high triglycerides 200–499 mg/dL benefit from 1-2 g/day; and >500 mg/dL be treated under a physician's supervision with 2-4 g/day using a prescription product.

ALA does not confer the cardiovascular health benefits of EPA and DHAs.

The effect of omega−3 polyunsaturated fatty acids on stroke is unclear, with a possible benefit in women.

Inflammation

A 2013 systematic review found tentative evidence of benefit for lowering inflammation levels in healthy adults and in people with one or more biomarkers of metabolic syndrome. Consumption of omega−3 fatty acids from marine sources lowers blood markers of inflammation such as C-reactive protein, interleukin 6, and TNF alpha.

For rheumatoid arthritis, one systematic review found consistent, but modest, evidence for the effect of marine n−3 PUFAs on symptoms such as "joint swelling and pain, duration of morning stiffness, global assessments of pain and disease activity" as well as the use of non-steroidal anti-inflammatory drugs. The American College of Rheumatology has stated that there may be modest benefit from the use of fish oils, but that it may take months for effects to be seen, and cautions for possible gastrointestinal side effects and the possibility of the supplements containing mercury or vitamin A at toxic levels. The National Center for Complementary and Integrative Health has concluded that “supplements containing omega-3 fatty acids, gamma-Linolenic acid (GLA), or the herb thunder god vine may help relieve rheumatoid arthritis symptoms”.

Developmental Disabilities

Although not supported by current scientific evidence as a primary treatment for attention deficit hyperactivity disorder (ADHD), autism, and other developmental disabilities, omega–3 fatty acid supplements are being given to children with these conditions.

One meta-analysis concluded that omega–3 fatty acid supplementation demonstrated a modest effect for improving ADHD symptoms. A Cochrane review of PUFA (not necessarily omega–3) supplementation found “there is little evidence that PUFA supplementation provides any benefit for the symptoms of ADHD in children and adolescents”, while a different review found “insufficient evidence to draw any conclusion about the use of PUFAs for children with specific learning disorders”. Another review concluded that the evidence is inconclusive for the use of omega–3 fatty acids in behavior and non-neurodegenerative neuropsychiatric disorders such as ADHD and depression.

Fish oil has only a small benefit on the risk of premature birth. A 2015 meta-analysis of the effect of omega–3 supplementation during pregnancy did not demonstrate a decrease in the rate of preterm birth or improve outcomes in women with singleton pregnancies with no prior preterm births. A systematic review and meta-analysis published the same year reached the opposite conclusion, specifically, that omega–3 fatty acids were effective in “preventing early and any preterm delivery”.

Mental Health

There is some evidence that omega–3 fatty acids are related to mental health, including that they may tentatively be useful as an add-on for the treatment of depression associated with bipolar disorder. Significant benefits due to EPA supplementation were only seen, however, when treating depressive symptoms and not manic symptoms suggesting a link between omega–3 and depressive mood. There is also preliminary evidence that EPA supplementation is helpful in cases of depression. The link between omega–3 and depression has been attributed to the fact that many of the products of the omega–3 synthesis pathway play key roles in regulating inflammation (such as prostaglandin E3) which have been linked to depression. This link to inflammation regulation has been supported in both in vivo studies and in a meta-analysis.

There is, however, significant difficulty in interpreting the literature due to participant recall and systematic differences in diets. There is also controversy as to the efficacy of omega–3, with many meta-analysis papers finding heterogeneity among results which can be explained mostly by publication bias. A significant correlation between shorter treatment trials was associated with increased omega−3 efficacy for treating depressed symptoms further implicating bias in publication. One review found that “Although evidence of benefits for any specific intervention is not conclusive, these findings suggest that it might be possible to delay or prevent transition to psychosis.”

Cognitive Aging

Epidemiological studies are inconclusive about an effect of omega−3 fatty acids on the mechanisms of Alzheimer’s disease. There is preliminary evidence of effect on mild cognitive problems, but none supporting an effect in healthy people or those with dementia.

Brain and Visual Functions

Brain function and vision rely on dietary intake of DHA to support a broad range of cell membrane properties, particularly in grey matter, which is rich in membranes. A major structural component of the mammalian brain, DHA is the most abundant omega−3 fatty acid in the brain. It is under study as a candidate essential nutrient with roles in neurodevelopment, cognition, and neurodegenerative disorders.

Atopic Diseases

Results of studies investigating the role of LCPUFA supplementation and LCPUFA status in the prevention and therapy of atopic diseases (allergic rhinoconjunctivitis, atopic dermatitis and allergic asthma) are controversial; therefore, at the present stage of our knowledge (as of 2013) we cannot state either that the nutritional intake of n−3 fatty acids has a clear preventive or therapeutic role, or that the intake of n-6 fatty acids has a promoting role in context of atopic diseases.

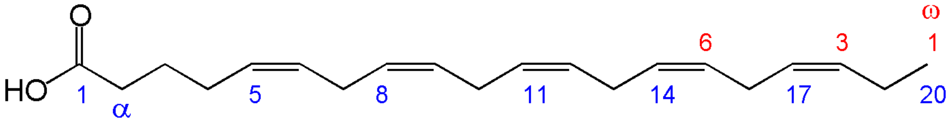
Risk of Deficiency

People with PKU often have low intake of omega−3 fatty acids, because nutrients rich in omega−3 fatty acids are excluded from their diet due to high protein content.

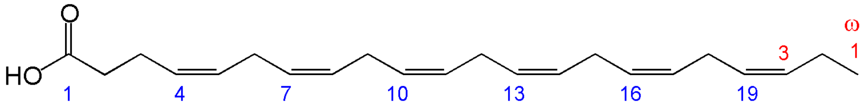
Asthma

As of 2015, there was no evidence that taking omega−3 supplements can prevent asthma attacks in children.

Chemistry



Chemical structure of eicosapentaenoic acid (EPA)



Chemical structure of docosahexaenoic acid (DHA)

An omega–3 fatty acid is a fatty acid with multiple double bonds, where the first double bond is between the third and fourth carbon atoms from the end of the carbon atom chain. "Short chain" omega–3 fatty acids have a chain of 18 carbon atoms or less, while "long chain" omega–3 fatty acids have a chain of 20 or more.

Three omega–3 fatty acids are important in human physiology, α-linolenic acid (18:3, *n*-3; ALA), eicosapentaenoic acid (20:5, *n*-3; EPA), and docosahexaenoic acid (22:6, *n*-3; DHA). These three polyunsaturates have either 3, 5, or 6 double bonds in a carbon chain of 18, 20, or 22 carbon atoms, respectively. As with most naturally-produced fatty acids, all double bonds are in the *cis*-configuration, in other words, the two hydrogen atoms are on the same side of the double bond; and the double bonds are interrupted by methylene bridges ($-CH_2-$), so that there are two single bonds between each pair of adjacent double bonds.

List of Omega–3 fatty Acids

This table lists several different names for the most common omega–3 fatty acids found in nature.

Common name	Lipid number	Chemical name
Hexadecatrienoic acid (HTA)	16:3 (*n*-3)	*all-cis*-7,10,13-hexadecatrienoic acid
α-Linolenic acid (ALA)	18:3 (*n*-3)	*all-cis*-9,12,15-octadecatrienoic acid
Stearidonic acid (SDA)	18:4 (*n*-3)	*all-cis*-6,9,12,15-octadecatetraenoic acid
Eicosatrienoic acid (ETE)	20:3 (*n*-3)	*all-cis*-11,14,17-eicosatrienoic acid
Eicosatetraenoic acid (ETA)	20:4 (*n*-3)	*all-cis*-8,11,14,17-eicosatetraenoic acid
Eicosapentaenoic acid (EPA)	20:5 (*n*-3)	*all-cis*-5,8,11,14,17-eicosapentaenoic acid
Heneicosapentaenoic acid (HPA)	21:5 (*n*-3)	*all-cis*-6,9,12,15,18-heneicosapentaenoic acid
Docosapentaenoic acid (DPA), Clupanodonic acid	22:5 (*n*-3)	*all-cis*-7,10,13,16,19-docosapentaenoic acid
Docosahexaenoic acid (DHA)	22:6 (*n*-3)	*all-cis*-4,7,10,13,16,19-docosahexaenoic acid
Tetracosapentaenoic acid	24:5 (*n*-3)	*all-cis*-9,12,15,18,21-tetracosapentaenoic acid
Tetracosahexaenoic acid (Nisinic acid)	24:6 (*n*-3)	*all-cis*-6,9,12,15,18,21-tetracosahexaenoic acid

Forms

Omega–3 fatty acids occur naturally in two forms, triglycerides and phospholipids. In the triglycerides, they, together with other fatty acids, are bonded to glycerol; three fatty acids are attached to glycerol. Phospholipid omega–3 is composed of two fatty acids attached to a phosphate group via glycerol.

The triglycerides can be converted to the free fatty acid or to methyl or ethyl esters, and the individual esters of omega–3 fatty acids are available.

Biochemistry

Transporters

DHA in the form of lysophosphatidylcholine is transported into the brain by a membrane transport protein, MFSD2A, which is exclusively expressed in the endothelium of the blood–brain barrier.

Mechanism of Action

The ‘essential’ fatty acids were given their name when researchers found that they are essential to normal growth in young children and animals. The omega–3 fatty acid DHA, also known as docosahexaenoic acid, is found in high abundance in the human brain. It is produced by a desaturation process, but humans lack the desaturase enzyme, which acts to insert double bonds at the ω_6 and ω_3 position. Therefore, the ω_6 and ω_3 polyunsaturated fatty acids cannot be synthesized, are appropriately called essential fatty acids, and must be obtained from the diet.

In 1964, it was discovered that enzymes found in sheep tissues convert omega–6 arachidonic acid into the inflammatory agent, prostaglandin E_2, which is involved in the immune response of traumatized and infected tissues. By 1979, eicosanoids were further identified, including thromboxanes, prostacyclins, and leukotrienes. The eicosanoids typically have a short period of activity in the body, starting with synthesis from fatty acids and ending with metabolism by enzymes. If the rate of synthesis exceeds the rate of metabolism, the excess eicosanoids may have deleterious effects. Researchers found that certain omega–3 fatty acids are also converted into eicosanoids and docosanoids, but at a slower rate. If both omega–3 and omega–6 fatty acids are present, they will “compete” to be transformed, so the ratio of long-chain omega–3:omega–6 fatty acids directly affects the type of eicosanoids that are produced.

Interconversion

Conversion Efficiency of ALA to EPA and DHA

Humans can convert short-chain omega–3 fatty acids to long-chain forms (EPA, DHA) with an efficiency below 5%. The omega–3 conversion efficiency is greater in women than in men, but less studied. Higher ALA and DHA values found in plasma phospholipids of women may be due to the higher activity of desaturases, especially that of delta-6-desaturase.

These conversions occur competitively with omega–6 fatty acids, which are essential closely related chemical analogues that are derived from linoleic acid. They both utilize the same desaturase and elongase proteins in order to synthesize inflammatory regulatory proteins. The products of both pathways are vital for growth making a balanced diet of omega–3 and omega–6 important to an individual's health. A balanced intake ratio of 1:1 was believed to be ideal in order for proteins to be able to synthesize both pathways sufficiently, but this has been controversial as of recent research.

The conversion of ALA to EPA and further to DHA in humans has been reported to be limited, but varies with individuals. Women have higher ALA-to-DHA conversion efficiency than men, which is presumed to be due to the lower rate of use of dietary ALA for beta-oxidation. One preliminary study showed that EPA can be increased by lowering the amount of dietary linoleic acid, and DHA can be increased by elevating intake of dietary ALA.

Omega–6 to Omega–3 Ratio

Human diet has changed rapidly in recent centuries resulting in a reported increased diet of omega–6 in comparison to omega–3. The rapid evolution of human diet away from a 1:1 omega–3 and omega–6 ratio, such as during the Neolithic Agricultural Revolution, has presumably been too fast for humans to have adapted to biological profiles adept at balancing omega–3 and omega–6 ratios of 1:1. This is commonly believed to be the reason why modern diets are correlated with many inflammatory disorders. While omega–3 polyunsaturated fatty acids may be beneficial in preventing heart disease in humans, the level of omega–6 polyunsaturated fatty acids (and, therefore, the ratio) does not matter.

Both omega–6 and omega–3 fatty acids are essential: humans must consume them in their diet. Omega–6 and omega–3 eighteen-carbon polyunsaturated fatty acids compete for the same metabolic enzymes, thus the omega–6:omega–3 ratio of ingested fatty acids has significant influence on the ratio and rate of production of eicosanoids, a group of hormones intimately involved in the body's inflammatory and homeostatic processes, which include the prostaglandins, leukotrienes, and thromboxanes, among others. Altering this ratio can change the body's metabolic and inflammatory state. In general, grass-fed animals accumulate more omega–3 than do grain-fed animals, which accumulate relatively more omega–6. Metabolites of omega–6 are more inflammatory (esp. arachidonic acid) than those of omega–3. This necessitates that omega–6 and omega–3 be consumed in a balanced proportion; healthy ratios of omega–6:omega–3, according to some authors, range from 1:1 to 1:4. Other authors believe that a ratio of 4:1 (4 times as much omega–6 as omega–3) is already healthy. Studies suggest the evolutionary human diet, rich in game animals, seafood, and other sources of omega–3, may have provided such a ratio.

Typical Western diets provide ratios of between 10:1 and 30:1 (i.e., dramatically higher levels of omega–6 than omega–3). The ratios of omega–6 to omega–3 fatty acids in some common vegetable oils are: canola 2:1, hemp 2–3:1, soybean 7:1, olive 3–13:1, sunflower (no omega–3), flax 1:3, cottonseed (almost no omega–3), peanut (no omega–3), grapeseed oil (almost no omega–3) and corn oil 46:1.

Dietary Sources

Dietary Recommendations

Grams of omega–3 per 3oz (85g) serving	
Common name	grams omega–3
Flax	11.4
Hemp	11.0
Herring, sardines	1.3–2
Mackerel: Spanish/Atlantic/Pacific	1.1–1.7
Salmon	1.1–1.9
Halibut	0.60–1.12
Tuna	0.21–1.1
Swordfish	0.97
Greenshell/lipped mussels	0.95
Tilefish	0.9
Tuna (canned, light)	0.17–0.24
Pollock	0.45
Cod	0.15–0.24
Catfish	0.22–0.3
Flounder	0.48
Grouper	0.23
Mahi mahi	0.13
Red snapper	0.29
Shark	0.83
King mackerel	0.36
Hoki (blue grenadier)	0.41
Gemfish	0.40
Blue eye cod	0.31
Sydney rock oysters	0.30
Tuna, canned	0.23
Snapper	0.22
Eggs, large regular	0.109
Strawberry or Kiwifruit	0.10–0.20
Broccoli	0.10–0.20
Barramundi, saltwater	0.100
Giant tiger prawn	0.100
Lean red meat	0.031
Turkey	0.030
Milk, regular	0.00

In the United States, the Institute of Medicine publishes a system of Dietary Reference Intakes, which includes Recommended Dietary Allowances (RDAs) for individual nutrients, and Acceptable Macronutrient Distribution Ranges (AMDRs) for certain groups of nutrients, such as fats. When there is insufficient evidence to determine an RDA, the institute may publish an Adequate Intake (AI) instead, which has a similar meaning, but is less certain. The AI for α-linolenic acid is 1.6 grams/day for men and 1.1 grams/day for women, while the AMDR is 0.6% to 1.2% of total energy. Because the physiological potency of EPA and DHA is much greater than that of ALA, it is not possible to estimate one AMDR for all omega−3 fatty acids. Approximately 10 percent of the AMDR can be consumed as EPA and DHA. The Institute of Medicine has not established a RDA or AI for EPA, DHA or the combination, so there is no Daily Value (DVs are derived from RDAs), no labeling of foods or supplements as providing a DV percentage of these fatty acids per serving, and no labeling a food or supplement as an excellent source. As for safety, there was insufficient evidence as of 2005 to set an upper tolerable limit for omega−3 fatty acids, although the FDA has advised that adults can safely consume up to a total of 3 grams per day of combined DHA and EPA, with no more than 2 g from dietary supplements.

Fish

The most widely available dietary source of EPA and DHA is oily fish, such as salmon, herring, mackerel, anchovies, menhaden, and sardines. Oils from these fish have a profile of around seven times as much omega−3 as omega−6. Other oily fish, such as tuna, also contain *n*-3 in somewhat lesser amounts. Consumers of oily fish should be aware of the potential presence of heavy metals and fat-soluble pollutants like PCBs and dioxins, which are known to accumulate up the food chain. Although fish are a dietary source of omega−3 fatty acids, fish do not synthesize them; they obtain them from the algae (microalgae in particular) or plankton in their diets. In the case of farmed fish, omega-3 fatty acids is provided by fish oil; In 2009, 81% of the global fish oil production is used by aquaculture.

Fish Oil

Fish oil capsules

Marine and freshwater fish oil vary in content of arachidonic acid, EPA and DHA. They also differ in their effects on organ lipids.

Not all forms of fish oil may be equally digestible. Of four studies that compare bioavailability of the glyceryl ester form of fish oil vs. the ethyl ester form, two have concluded the natural glyceryl ester form is better, and the other two studies did not find a significant difference. No studies have shown the ethyl ester form to be superior, although it is cheaper to manufacture.

Krill

Krill oil is a source of omega−3 fatty acids. The effect of krill oil, at a lower dose of EPA + DHA (62.8%), was demonstrated to be similar to that of fish oil on blood lipid levels and markers of inflammation in healthy humans. While not an endangered species, krill are a mainstay of the diets of many ocean-based species including whales, causing environmental and scientific concerns about their sustainability.

Plant Sources

Chia is grown commercially for its seeds rich in ALA.

Flax seeds contain linseed oil which has high ALA content.

Table: ALA content as the percentage of the seed oil.

Common name	Alternative name	Linnaean name	% ALA
Kiwifruit seed oil	Chinese gooseberry	*Actinidia deliciosa*	63
Perilla	shiso	*Perilla frutescens*	61
Chia seed	chia sage	*Salvia hispanica*	58
Flax	linseed	*Linum usitatissimum*	53 – 59

Lingonberry	Cowberry	*Vaccinium vitis-idaea*	49
Fig seed oil	Common Fig	*Ficus carica*	47.7
Camelina	Gold-of-pleasure	*Camelina sativa*	36
Purslane	Portulaca	*Portulaca oleracea*	35
Black raspberry		*Rubus occidentalis*	33
Hemp		*Cannabis sativa*	19
Canola	Rapeseed oil	mostly *Brassica napus*	9 – 11

Table: ALA Content as the Percentage of the Whole Food.

Common name	Linnaean name	% ALA
Flaxseed	*Linum usitatissimum*	18.1
Hempseed	*Cannabis sativa*	8.7
Butternuts	*Juglans cinerea*	8.7
Persian walnuts	*Juglans regia*	6.3
Pecan nuts	*Carya illinoinensis*	0.6
Hazel nuts	*Corylus avellana*	0.1

Flaxseed (or linseed) (*Linum usitatissimum*) and its oil are perhaps the most widely available botanical source of the omega–3 fatty acid ALA. Flaxseed oil consists of approximately 55% ALA, which makes it six times richer than most fish oils in omega–3 fatty acids. A portion of this is converted by the body to EPA and DHA, though the actual converted percentage may differ between men and women.

In 2013 Rothamsted Research in the UK reported they had developed a genetically modified form of the plant Camelina that produced EPA and DHA. Oil from the seeds of this plant contained on average 11% EPA and 8% DHA in one development and 24% EPA in another.

Eggs

Eggs produced by hens fed a diet of greens and insects contain higher levels of omega–3 fatty acids than those produced by chickens fed corn or soybeans. In addition to feeding chickens insects and greens, fish oils may be added to their diets to increase the omega–3 fatty acid concentrations in eggs.

The addition of flax and canola seeds to the diets of chickens, both good sources of alpha-linolenic acid, increases the omega–3 content of the eggs, predominantly DHA.

The addition of green algae or seaweed to the diets boosts the content of DHA and EPA, which are the forms of omega–3 approved by the FDA for medical claims. A common consumer complaint is “Omega–3 eggs can sometimes have a fishy taste if the hens are fed marine oils”.

Meat

Omega–3 fatty acids are formed in the chloroplasts of green leaves and algae. While seaweeds and algae are the source of omega–3 fatty acids present in fish, grass is the source of omega–3 fatty acids present in grass fed animals. When cattle are taken off omega–3 fatty acid rich grass and shipped to a feedlot to be fattened on omega–3 fatty acid deficient grain, they begin losing their store of this beneficial fat. Each day that an animal spends in the feedlot, the amount of omega–3 fatty acids in its meat is diminished.

The omega–6:omega–3 ratio of grass-fed beef is about 2:1, making it a more useful source of omega–3 than grain-fed beef, which usually has a ratio of 4:1.

In a 2009 joint study by the USDA and researchers at Clemson University in South Carolina, grass-fed beef was compared with grain-finished beef. The researchers found that grass-finished beef is higher in moisture content, 42.5% lower total lipid content, 54% lower in total fatty acids, 54% higher in beta-carotene, 288% higher in vitamin E (alpha-tocopherol), higher in the B-vitamins thiamin and riboflavin, higher in the minerals calcium, magnesium, and potassium, 193% higher in total omega–3s, 117% higher in CLA (cis-9, trans-11 octadecenoic acid, a conjugated linoleic acid, which is a potential cancer fighter), 90% higher in vaccenic acid (which can be transformed into CLA), lower in the saturated fats, and has a healthier ratio of omega–6 to omega–3 fatty acids (1.65 vs 4.84). Protein and cholesterol content were equal.

The omega–3 content of chicken meat may be enhanced by increasing the animals' dietary intake of grains high in omega–3, such as flax, chia, and canola.

Kangaroo meat is also a source of omega–3, with fillet and steak containing 74 mg per 100 g of raw meat.

Seal Oil

Seal oil is a source of EPA, DPA, and DHA. According to Health Canada, it helps to support the development of the brain, eyes, and nerves in children up to 12 years of age. Like all seal products, it is not allowed to be imported into the European Union.

A trend in the early 21st century was to fortify food with omega–3 fatty acids. The microalgae *Crypthecodinium cohnii* and *Schizochytrium* are rich sources of DHA, but not EPA, and can be produced commercially in bioreactors for use as food additives. Oil from brown algae (kelp) is a source of EPA. The alga *Nannochloropsis* also has high levels of EPA.

Omega-6 Fatty Acid

Omega-6 fatty acids (also referred to as ω-6 fatty acids or *n*-6 fatty acids) are a family of polyunsaturated fatty acids that have in common a final carbon-carbon double bond in the *n*-6 position, that is, the sixth bond, counting from the methyl end. Members of the family can have pro-inflammatory or anti-inflammatory effects.

The biological effects of the omega-6 fatty acids are largely produced during and after physical activity for the purpose of promoting growth and during the inflammatory cascade to halt cell damage and promote cell repair by their conversion to omega-6 eicosanoids that bind to diverse receptors found in every tissue of the body.

Biochemistry

Linoleic acid (18:2, *n*−6), the shortest-chained omega-6 fatty acid is categorized as an essential fatty acid because the human body cannot synthesize it. Mammalian cells lack the enzyme omega-3 desaturase and therefore cannot convert omega-6 fatty acids to omega-3 fatty acids. Closely related omega-3 and omega-6 fatty acids act as competing substrates for the same enzymes. This outlines the importance of the proportion of omega-3 to omega-6 fatty acids in a diet.

Omega-6 fatty acids are precursors to endocannabinoids, lipoxins, and specific eicosanoids.

Medical research on humans found a correlation (though correlation does not imply causation) between the high intake of omega-6 fatty acids from vegetable oils and disease in humans. However, biochemistry research has concluded that air pollution, heavy metals, smoking, passive smoking, lipopolysaccharides, lipid peroxidation products (found mainly in vegetable oils, roasted/rancid nuts and roasted/rancid oily seeds) and other exogenous toxins initiate the inflammatory response in the cells which leads to the expression of the COX-2 enzyme and subsequently to the temporary production of inflammatory *promoting* prostaglandins from arachidonic acid for the purpose of alerting the immune system of the cell damage and eventually to the production of anti-inflammatory molecules (e.g. lipoxins & prostacyclin) during the resolution phase of inflammation, after the cell damage has been repaired.

Pharmacology

The conversion of cell membrane arachidonic acid (20:4n-6) to omega-6 prostaglandin and omega-6 leukotriene eicosanoids during the inflammatory cascade provides many targets for pharmaceutical drugs to impede the inflammatory process in atherosclerosis, asthma, arthritis, vascular disease, thrombosis, immune-inflammatory processes, and tumor proliferation. Competitive interactions with the omega-3 fatty acids affect the relative storage, mobilization, conversion and action of the omega-3 and omega-6 eicosanoid precursors.

Health Effects

Some medical research suggests that excessive levels of omega-6 fatty acids from seed oils relative to certain omega-3 fatty acids may increase the probability of a number of diseases. However, consumption of non-rancid nuts, which are high in omega 6, is associated with a lower risk for some diseases, such as cardiovascular diseases including coronary heart disease (CHD), cancer, stroke, heart attacks, and lower rates of premature death.

Modern Western diets typically have ratios of omega-6 to omega-3 in excess of 10, some as high as 30; the average ratio of omega-6 to omega-3 in the Western diet is 15–16.7. Humans are thought to have evolved with a diet of a 1-to-1 ratio of omega-6 to omega-3 and the optimal ratio is thought to be 4-to-1 or lower, although some sources suggest ratios as low as 1. A ratio of 2–3 omega-6 to omega-3 helped reduce inflammation in patients with rheumatoid arthritis. A ratio of 5-to-1 had a beneficial effect on patients with asthma but a ratio of 10-to-1 had a negative effect. A ratio of 2.5-to-1 reduced rectal cell proliferation in patients with colorectal cancer, whereas a ratio of 4-to-1 had no effect.

Excess omega-6 fatty acids from vegetable oils interfere with the health benefits of omega-3 fats, in part because they compete for the same rate-limiting enzymes. A high proportion of omega-6 to omega-3 fat in the diet shifts the physiological state in the tissues toward the pathogenesis of many diseases: prothrombotic, proinflammatory and proconstrictive.

Chronic excessive production of omega-6 eicosanoids is correlated with arthritis, inflammation, and cancer. Many of the medications used to treat and manage these conditions work by blocking the effects of the COX-2 enzyme. Many steps in formation and action of omega-6 prostaglandins from omega-6 arachidonic acid proceed more vigorously than the corresponding competitive steps in formation and action of omega-3 hormones from omega-3 eicosapentaenoic acid. The COX-1 and COX-2 inhibitor medications, used to treat inflammation and pain, work by preventing the COX enzymes from turning arachidonic acid into inflammatory compounds. The LOX inhibitor medications often used to treat asthma work by preventing the LOX enzyme from converting arachidonic acid into the leukotrienes. Many of the anti-mania medications used to treat bipolar disorder work by targeting the arachidonic acid cascade in the brain.

A high consumption of oxidized polyunsaturated fatty acids (PUFAs), which are found in most types of vegetable oil, may increase the likelihood that postmenopausal women will develop breast cancer. Similar effect was observed on prostate cancer, but the study was performed on mice. Another “analysis suggested an inverse association between total polyunsaturated fatty acids and breast cancer risk, but individual polyunsaturated fatty acids behaved differently [from each other]. Linoleic acid was inversely associated with the risk of breast cancer”.

Omega-6 Consumption

Industry-sponsored studies have suggested that omega-6 fatty acids should be consumed in a 1:1 ratio to omega-3, though it has been observed that the diet of many individuals today is at a ratio of about 16:1, mainly from vegetable oils. Omega-6 and omega-3 are essential fatty acids that are metabolized by some of the same enzymes, and therefore an imbalanced ratio can affect how the other is metabolized. In a study performed by Ponnampalam, it was noticed that feeding systems had a great effect on nutrient content on the meat sold to consumers. Cynthia Doyle conducted an experiment to observe the fatty acid content of beef raised through grass feeding versus grain feeding; she concluded that grass fed animals contain an overall omega-6:omega-3 ratio that is preferred by nutritionists. In today's modern agriculture, the main focus is on production quantity, which has decreased the omega-3 content, and increased the omega-6 content, due to simple changes such as grain-feeding cattle. In grain-feeding cattle, this is a way to increase their weight and prepare them for slaughter much quicker compared to grass-feeding. This modern way of feeding animals may be one of many indications as to why the omega-6:omega-3 ratio has increased.

List of Omega-6 fatty Acids

Common name	Lipid name	Chemical name
Linoleic acid (LA)	18:2 (*n*−6)	*all-cis*-9,12-octadecadienoic acid
Gamma-linolenic acid (GLA)	18:3 (*n*−6)	*all-cis*-6,9,12-octadecatrienoic acid
Calendic acid	18:3 (*n*−6)	8E,10E,12Z-octadecatrienoic acid
Eicosadienoic acid	20:2 (*n*−6)	*all-cis*-11,14-eicosadienoic acid
Dihomo-gamma-linolenic acid (DGLA)	20:3 (*n*−6)	*all-cis*-8,11,14-eicosatrienoic acid
Arachidonic acid (AA, ARA)	20:4 (*n*−6)	*all-cis*-5,8,11,14-eicosatetraenoic acid
Docosadienoic acid	22:2 (*n*−6)	*all-cis*-13,16-docosadienoic acid
Adrenic acid	22:4 (*n*−6)	*all-cis*-7,10,13,16-docosatetraenoic acid
Osbond acid	22:5 (*n*−6)	*all-cis*-4,7,10,13,16-docosapentaenoic acid
Tetracosatetraenoic acid	24:4 (*n*−6)	*all-cis*-9,12,15,18-tetracosatetraenoic acid
Tetracosapentaenoic acid	24:5 (*n*−6)	*all-cis*-6,9,12,15,18-tetracosapentaenoic acid

The melting point of the fatty acids increase as the number of carbons in the chain increases.

Dietary Linoleic Acid Requirement

Adding more controversy to the omega-6 fat issue is that the dietary requirement for linoleic acid has been questioned, because of a significant methodology error proposed by University of Toronto scientist Stephen Cunnane. Cunnane proposed that the seminal research used to determine the dietary requirement for linoleic acid was based on feeding animals linoleic acid-deficient diets, which were simultaneously deficient in omega-3 fats. The omega-3 deficiency was not taken into account. The omega-6 oils added back systematically to correct the deficiency also contained trace amounts of omega-3 fats. Therefore, the researchers were inadvertently correcting the omega-3 deficiency as well. Ultimately, it took more oil to correct both deficiencies. According to Cunnane, this error overestimates linoleic acid requirements by 5 to 15 times.

Dietary Sources

The evening primrose flower (*O. biennis*) produces an oil containing a high content of γ-linolenic acid, a type of omega-6 fatty acid

Vegetable oils are a major source of omega-6 linoleic acid. Worldwide, more than 100 million metric tons of vegetable oils are extracted annually from palm fruits, soybean seeds, rape seeds, and sunflower seeds, providing more than 32 million metric tons of omega-6 linoleic acid and 4 million metric tons of omega-3 alpha-linolenic acid.

Dietary sources of omega-6 fatty acids include:

- Poultry
- Eggs
- Nuts
- Hulled sesame seeds
- Cereals
- Durum wheat
- Whole-grain breads
- Pumpkin seeds
- Most vegetable oils including:
  - Grape seed oil
  - Evening primrose oil
  - Borage oil
  - Blackcurrant seed oil
  - Flax or linseed oil

- Rapeseed or canola oil
- Hemp oil
- Soybean oil
- Cottonseed oil
- Sunflower seed oil
- Corn oil
- Safflower oil
- Palm oil.

Trans Fat

Trans fats are unsaturated fats produced from vegetable oils. They are commonly used in the preparation of margarine and commercially baked or fried foods. There are two forms of trans fat - naturally-occurring and artificial trans fats. Artificial trans fats are man-made fats produced through a chemical process called hydrogenation. Naturally-occurring trans fats can be found in many animal products, including milk and meat.

Chemistry of Trans Fats

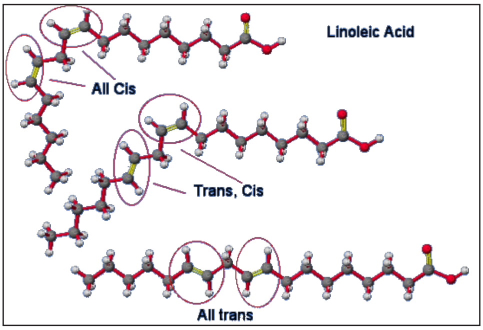
Trans fatty acids are made when manufacturers add hydrogen to vegetable oil -- in a process described as partial hydrogenation. If the hydrogenation process were allowed to go to completion, there would be no trans fatty acids left, but the resulting material would be too solid for practical use. Usually the hydrogen atoms at a double bond in a natural fatty acid are positioned on the same side of the carbon chain. However, partial hydrogenation reconfigures most of the double bonds that do not become chemically saturated, so that the hydrogen atoms end up on different sides of the chain. This type of configuration is called trans.

Biochemistry of Trans Fats

Trans fat behaves like saturated fat by raising low-density lipoprotein (LDL or “bad cholesterol”) that increases the risk of coronary heart disease (CHD). It also decreases levels of HDL in the blood, this is the “good” lipoprotein that helps remove cholesterol from arteries.

Some reports have suggested that trans fats may be worse for the body than saturated fats; in fact, some studies that have incriminated saturated fat made no distinction between the two.

Trans fats are the result of a side reaction with the catalyst of the hydrogenation process. This is the result of an unsaturated fat which is normally found as a cis isomer converts to a trans isomer of the unsaturated fat. Isomers are molecules that have the same molecular formula but are bonded together differently. Focusing on the sp2 double bonded carbons, a cis isomer has the hydrogens on the same side. Due to the added energy from the hydrogenation process, the activation energy is reached to convert the cis isomers of the unsaturated fat to a trans isomer of the unsaturated fat. The effect is putting one of the hydrogens on the opposite side of one of the carbons. This results in a trans configuration of the double bonded carbons. The human body does not recognize trans fats.



Although trans fatty acids are chemically “monounsaturated” or “polyunsaturated,” they are considered so different from the cis monounsaturated or polyunsaturated fatty acids that they can not be legally designated as unsaturated for purposes of labeling. Most of the trans fatty acids (although chemically still unsaturated) produced by the partial hydrogenation process are now classified in the same category as saturated fats.

The major negative is that trans fat tends to raise “bad” LDL- cholesterol and lower “good” HDL-cholesterol, although not as much as saturated fat. Trans fat are found in margarine, baked goods such as doughnuts and Danish pastry, deep-fried foods like fried chicken and French-fried potatoes, snack chips, imitation cheese, and confectionery fats.

Sources of Trans Fats

The manufactured form of trans fat, known as partially hydrogenated oil, is found in a variety of food products, including:

- Baked goods: Most cakes, cookies, pie crusts and crackers contain shortening, which is usually made from partially hydrogenated vegetable oil. Ready-made frosting is another source of trans fat.
- Snacks: Potato, corn and tortilla chips often contain trans fat. And while popcorn can be a healthy snack, many types of packaged or microwave popcorn use trans fat to help cook or flavor the popcorn.
- Fried food: Foods that require deep frying — french fries, doughnuts and fried chicken — can contain trans fat from the oil used in the cooking process.

- Refrigerator dough: Products such as canned biscuits and cinnamon rolls often contain trans fat, as do frozen pizza crusts.
- Creamer and margarine: Nondairy coffee creamer and stick margarines also may contain partially hydrogenated vegetable oils.

Vitamin

Vitamin is any of several organic substances that are necessary in small quantities for normal health and growth in higher forms of animal life. Vitamins are distinct in several ways from other biologically important compounds such as proteins, carbohydrates, and lipids. Although these latter substances also are indispensable for proper bodily functions, almost all of them can be synthesized by animals in adequate quantities. Vitamins, on the other hand, generally cannot be synthesized in amounts sufficient to meet bodily needs and therefore must be obtained from the diet or from some synthetic source. For this reason, vitamins are called essential nutrients. Vitamins also differ from the other biological compounds in that relatively small quantities are needed to complete their functions. In general these functions are of a catalytic or regulatory nature, facilitating or controlling vital chemical reactions in the body’s cells. If a vitamin is absent from the diet or is not properly absorbed by the body, a specific deficiency disease may develop.

Vitamins are usually designated by selected letters of the alphabet, as in vitamin D or vitamin C, though they are also designated by chemical names, such as niacin and folic acid. Biochemists traditionally separate them into two groups, the water-soluble vitamins and the fat-soluble vitamins.

Biological Significance of Vitamins

Regulatory Role

The vitamins regulate reactions that occur in metabolism, in contrast to other dietary components known as macronutrients (e.g., fats, carbohydrates, proteins), which are the compounds utilized in the reactions regulated by the vitamins. Absence of a vitamin blocks one or more specific metabolic reactions in a cell and eventually may disrupt the metabolic balance within a cell and in the entire organism as well.

With the exception of vitamin C (ascorbic acid), all of the water-soluble vitamins have a catalytic function; i.e., they act as coenzymes of enzymes that function in energy transfer or in the metabolism of fats, carbohydrates, and proteins. The metabolic importance of the water-soluble vitamins is reflected by their presence in most plant and animal tissues involved in metabolism.

Some of the fat-soluble vitamins form part of the structure of biological membranes or assist in maintaining the integrity (and therefore, indirectly, the function) of membranes. Some fat-soluble vitamins also may function at the genetic level by controlling the synthesis of certain enzymes. Unlike the water-soluble ones, fat-soluble vitamins are necessary for specific functions in highly differentiated and specialized tissues; therefore, their distribution in nature tends to be more selective than that of the water-soluble vitamins.

Vitamins, which are found in all living organisms either because they are synthesized in the organism or are acquired from the environment, are not distributed equally throughout nature. Some are absent from certain tissues or species; for example, beta-carotene, which can be converted to vitamin A, is synthesized in plant tissues but not in animal tissues. On the other hand, vitamins A and D_3 (cholecalciferol) occur only in animal tissues. Both plants and animals are important natural vitamin sources for human beings. Since vitamins are not distributed equally in foodstuffs, the more restricted the diet of an individual, the more likely it is that he will lack adequate amounts of one or more vitamins. Food sources of vitamin D are limited, but it can be synthesized in the skin through ultraviolet radiation (from the Sun); therefore, with adequate exposure to sunlight, the dietary intake of vitamin D is of little significance.

All vitamins can be either synthesized or produced commercially from food sources and are available for human consumption in pharmaceutical preparations. Commercial processing of food (e.g., milling of grains) frequently destroys or removes considerable amounts of vitamins. In most such instances, however, the vitamins are replaced by chemical methods. Some foods are fortified with vitamins not normally present in them (e.g., vitamin D is added to milk). Loss of vitamins may also occur when food is cooked; for instance, heat destroys vitamin A, and water-soluble vitamins may be extracted from food to water and lost. Certain vitamins (e.g., B vitamins, vitamin K) can be synthesized by microorganisms normally present in the intestines of some animals; however, the microorganisms usually do not supply the host animal with an adequate quantity of a vitamin.

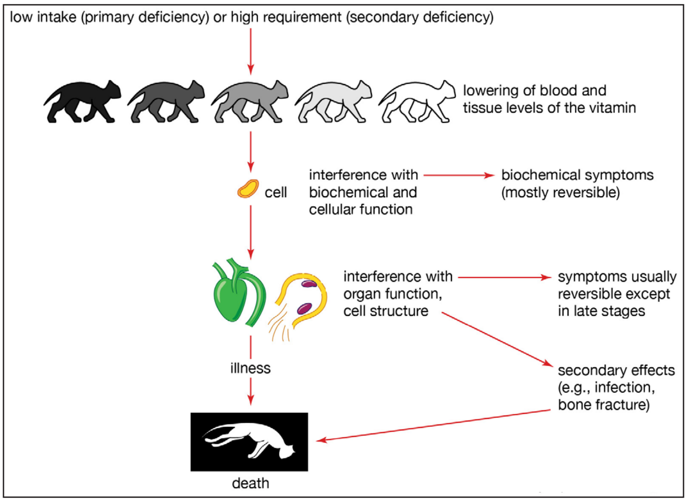
Requirements in Living Things

Vitamin requirements vary according to species, and the amount of a vitamin required by a specific organism is difficult to determine because of the numerous factors (e.g., genetic variation, relative proportions of other dietary constituents, environmental stresses). Although there is not uniform agreement concerning the human requirements of vitamins, recommended daily vitamin intakes are sufficiently high to account for individual variation and normal environmental stresses.

A number of interrelationships exist among vitamins and between vitamins and other dietary constituents. The interactions may be synergistic (i.e., cooperative) or antagonistic, reflecting, for example, overlapping metabolic roles (of the B vitamins in particular), protective roles (e.g., vitamins A and E), or structural dependency (e.g., cobalt in the vitamin B_{12} molecule).

Results of Deficiencies

Inadequate intake of a specific vitamin results in a characteristic deficiency disease (hypovitaminosis), the severity of which depends upon the degree of vitamin deprivation. Symptoms may be specific (e.g., functional night blindness of vitamin A deficiency) or nonspecific (e.g., loss of appetite, failure to grow). All symptoms for a specific deficiency disease may not appear; in addition, the nature of the symptoms may vary with the species. Some effects of vitamin deficiencies cannot be reversed by adding the vitamin to the diet, especially if damage to nonregenerative tissue (e.g., cornea of the eye, nerve tissue, calcified bone) has occurred.



Vitamin deficiency: Flow of events resulting from vitamin deficiency in animals

A vitamin deficiency may be primary (or dietary), in which case the dietary intake is lower than the normal requirement of the vitamin. A secondary (or conditioned) deficiency may occur (even though the dietary intake is adequate) if a preexisting disease or state of stress is present (e.g., malabsorption of food from the intestine, chronic alcoholism, repeated pregnancies and lactation).

Evolution of Vitamin-Dependent Organisms

Evolution of metabolic processes in primitive forms of life required the development of enzyme systems to catalyze the complex sequences of chemical reactions involved in metabolism. In the beginning, the environment presumably could supply all the necessary compounds (including the vitamin coenzymes); eventually, these compounds were synthesized within an organism. As higher forms of life evolved, however, the ability to synthesize certain of these vitamin coenzymes was gradually lost.

Since higher plants show no requirements for vitamins or other growth factors, it is assumed that they retain the ability to synthesize them. Among insects, however, niacin, thiamin, riboflavin, vitamin B_6, vitamin C, and pantothenic acid are required by a few groups. All vertebrates, including humans, require dietary sources of vitamin A, vitamin D, thiamin, riboflavin, vitamin B_6, and pantothenic acid; some vertebrates, particularly the more highly evolved ones, have additional requirements for other vitamins.

The Water-Soluble Vitamins

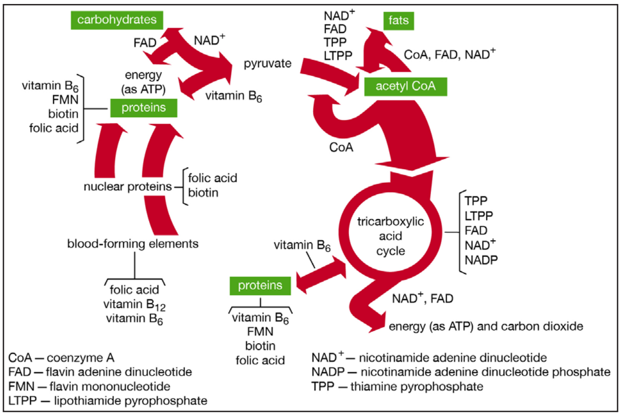
Basic Properties

Although the vitamins included in this classification are all water-soluble, the degree to which they dissolve in water is variable. This property influences the route of absorption, their excretion, and their degree of tissue storage and distinguishes them from fat-soluble vitamins, which are handled and stored differently by the body. The active forms and the accepted nomenclature of individual vitamins in each vitamin group. The water-soluble vitamins are vitamin C (ascorbic acid) and the B vitamins, which include thiamin (vitamin B_1), riboflavin (vitamin B_2), vitamin B_6, niacin (nicotinic acid), vitamin B_{12}, folic acid, pantothenic acid, and biotin. These relatively simple molecules contain the elements carbon, hydrogen, and oxygen; some also contain nitrogen, sulfur, or cobalt.

The water-soluble vitamins, inactive in their so-called free states, must be activated to their coenzyme forms; addition of phosphate groups occurs in the activation of thiamin, riboflavin, and vitamin B_6; a shift in structure activates biotin, and formation of a complex between the free vitamin and parts of other molecules is involved in the activation of niacin, pantothenic acid, folic acid, and vitamin B_{12}. After an active coenzyme is formed, it must combine with the proper protein component (called an apoenzyme) before enzyme-catalyzed reactions can occur.

Functions

The B-vitamin coenzymes function in enzyme systems that transfer certain groups between molecules; as a result, specific proteins, fats, and carbohydrates are formed and may be utilized to produce body tissues or to store or release energy. The pantothenic acid coenzyme functions in the tricarboxylic acid cycle (also called the Krebs, or citric acid, cycle), which interconnects carbohydrate, fat, and protein metabolism; this coenzyme (coenzyme A) acts at the hub of these reactions and thus is an important molecule in controlling the interconversion of fats, proteins, and carbohydrates and their conversion into metabolic energy. Thiamin and vitamin B_6 coenzymes control the conversion of carbohydrates and proteins respectively into metabolic energy during the citric acid cycle. Niacin and riboflavin coenzymes facilitate the transfer of hydrogen ions or electrons (negatively charged particles), which occurs during the reactions of the tricarboxylic acid cycle. All of these coenzymes also function in transfer reactions that are involved in the synthesis of structural compounds; these reactions are not part of the tricarboxylic acid cycle.



Functions of B-vitamin coenzymes in metabolism

Although vitamin C participates in some enzyme-catalyzed reactions, it has not yet been established that the vitamin is a coenzyme. Its function probably is related to its properties as a strong reducing agent (i.e., it readily gives electrons to other molecules).

Metabolism

The water-soluble vitamins are absorbed in the animal intestine, pass directly to the blood, and are carried to the tissues in which they will be utilized. Vitamin B_{12} requires a substance known as intrinsic factor in order to be absorbed.

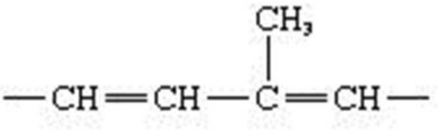
Some of the B vitamins can occur in forms that cannot be used by an animal. Most of the niacin in some cereal grains (wheat, corn, rice, barley, bran), for example, is bound to another substance, forming a complex called niacytin that cannot be absorbed in the animal intestine. Biotin can be bound by the protein avidin, which is found in raw egg white; this complex also cannot be absorbed or broken down by digestive-tract enzymes, and thus the biotin cannot be utilized. In animal products (e.g., meat), biotin, vitamin B_6, and folic acid are bound to other molecules to form complexes or conjugated molecules; although none is active in the complex form, the three vitamins normally are released from the bound forms by the enzymes of the intestinal tract (for biotin and vitamin B_6) or in the tissues (for folic acid) and thus can be utilized. The B vitamins are distributed in most metabolizing tissues of plants and animals.

Water-soluble vitamins usually are excreted in the urine of humans. Thiamin, riboflavin, vitamin B6, vitamin C, pantothenic acid, and biotin appear in urine as free vitamins (rather than as coenzymes); however, little free niacin is excreted in the urine. Products (also called metabolites) that are formed during the metabolism of thiamin, niacin, and vitamin B_6 also appear in the urine. Urinary metabolites of biotin, riboflavin, and pantothenic acid also are formed. Excretion of these vitamins (or their metabolites) is low when intake is sufficient for proper body function. If intake begins to exceed minimal requirements, excess vitamins are stored in the tissues. Tissue storage capacity is limited, however, and, as the tissues become saturated, the rate of excretion increases sharply. Unlike the other water-soluble vitamins, however, vitamin B_{12} is excreted solely in the feces. Some folic acid and biotin also are normally excreted in this way. Although fecal excretion of water-soluble vitamins (other than vitamin B_{12}, folic acid, and biotin) occurs, their source probably is the intestinal bacteria that synthesize the vitamins, rather than vitamins that have been eaten and utilized by the animal.

The water-soluble vitamins generally are not considered toxic if taken in excessive amounts. There is, however, one exception in humans: large amounts (50–100 mg; 1 mg = 0.001 gram) of niacin produce dilation of blood vessels; in larger amounts, the effects are more serious and may result in impaired liver function. Thiamin given to animals in amounts 100 times the requirement (i.e., about 100 mg) can cause death from respiratory failure. Therapeutic doses (100–500 mg) of thiamin have no known toxic effects in humans (except rare instances of anaphylactic shock in sensitive individuals). There is no known toxicity for any other B vitamins.

The Fat-Soluble Vitamins

The four fat-soluble vitamin groups are A, D, E, and K; they are related structurally in that all have as a basic structural unit of the molecule a five-carbon isoprene segment.



$$-CH{=}CH-\overset{\overset{\displaystyle CH_3}{|}}{C}{=}CH-$$

Each of the fat-soluble vitamin groups contains several related compounds that have biological activity. The active forms and the accepted nomenclature of individual vitamins in each vitamin group. The potency of the active forms in each vitamin group varies, and not all of the active forms now known are available from dietary sources; i.e., some are produced synthetically. The characteristics of each fat-soluble vitamin group are discussed below.

Chemical Properties

The chemical properties of fat-soluble vitamins determine their biological activities, functions, metabolism, and excretion. However, while the substances in each group of fat-soluble vitamins are related in structure, indicating that they share similar chemical properties, they do have important differences. These differences impart to the vitamins unique qualities, chemical and biological, that affect attributes ranging from the manner in which the vitamins are stored to the species in which they are active.

Vitamin A Group

Ten carotenes, coloured molecules synthesized only in plants, show vitamin A activity; however, only the alpha- and beta-carotenes and cryptoxanthin are important to humans, and beta-carotene is the most active. Retinol (vitamin A alcohol) is considered the primary active form of the vitamin, although retinal, or vitamin A aldehyde, is the form involved in the visual process in the retina of the eye. A metabolite of retinol with high biological activity may be an even more direct active form than retinol. The ester form of retinol is the storage form of vitamin A; presumably, it must be converted to retinol before it is utilized. Retinoic acid is a short-lived product of retinol; only retinoic acid of the vitamin A group is not supplied by the diet.

Vitamin D Group

Although about 10 compounds have vitamin D activity, the two most important ones are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D_3 represents the dietary source, while vitamin D_2 occurs in yeasts and fungi. Both can be formed from their respective provitamins by ultraviolet irradiation; in humans and other animals the provitamin (7-dehydrocholesterol), which is found in skin, can be converted by sunlight to vitamin D_3 and thus is an important source of the vitamin. Both vitamin D_2 and vitamin D_3 can be utilized by rats and humans; however, chicks cannot use vitamin D_2 effectively. The form of the vitamin probably active in humans is calcitriol.

Vitamin E Group

The tocopherols are a closely related group of biologically active compounds that vary only in number and position of methyl ($-CH_3$) groups in the molecule; however, these structural differences influence the biological activity of the various molecules. The active tocopherols are named in order of their potency; i.e., alpha-tocopherol is the most active. Some metabolites of alpha-tocopherol (such as alpha-tocopherolquinone and alphatocopheronolactone) have activity in some mammals (e.g., rats, rabbits); however, these metabolites do not support all the functions attributed to vitamin E.

Vitamin K Group

Vitamin K_1 (20), or phylloquinone, is synthesized by plants; the members of the vitamin K_2 (30), or menaquinone, series are of microbial origin. Vitamin K_2 (20) is the important form in mammalian tissue; all other forms are converted to K_2 (20) from vitamin K_3 (menadione). Since vitamin K_3 does not accumulate in tissue, it does not furnish any dietary vitamin K.

Functions

The vitamin A group is essential for the maintenance of the linings of the body surfaces (e.g., skin, respiratory tract, cornea), for sperm formation, and for the proper functioning of the immune system. In the retina of the eye, retinal is combined with a protein called opsin; the complex molecules formed as a result of this combination and known as rhodopsin (or visual purple) are involved in dark vision. The vitamin D group is required for growth (especially bone growth or calcification). The vitamin E group also is necessary for normal animal growth; without vitamin E, animals are not fertile and develop abnormalities of the central nervous system, muscles, and organs (especially the liver). The vitamin K group is required for normal metabolism, including the conversion of food into cellular energy in certain biological membranes; vitamin K also is necessary for the proper clotting of blood.

Metabolism

The fat-soluble vitamins are transported primarily by lymph from the intestines to the circulating blood. Bile salts are required for efficient absorption of fat-soluble metabolites in the intestine; anything that interferes with fat absorption, therefore, also inhibits absorption of the fat-soluble vitamins. Since a fatty acid (preferentially palmitic acid) is added to the retinol (vitamin A alcohol) molecule before it is transported by the lymph, this ester form predominates in the bloodstream during digestion. Vitamins D, E, and K do not require the addition of a fatty acid molecule for absorption. Small amounts of vitamin A (and possibly vitamin K) may be absorbed directly into the bloodstream; however, both vitamins A and D are bound to a protein during transport in the bloodstream.

Larger quantities of the fat-soluble vitamins than of water-soluble ones can be stored in the body. Vitamins A, D, and K are stored chiefly in the liver, with smaller amounts stored in other soft body tissues; however, most of the stored vitamin E is found in body fat, although large amounts also occur in the uterus of females and testis of males. The various forms of vitamin E are stored in tissues in different amounts; alpha-tocopherol is stored in higher concentrations than are the other forms. More vitamin A is stored than any other fat-soluble vitamin.

Excessive intakes of both vitamins A and D may produce toxicity (or hypervitaminosis A or D). Toxicity of both vitamin A and vitamin D can easily occur, however, if pharmaceutical vitamin preparations are used in excess.

Toxic levels of vitamin A exceed the normal requirement by 100 times-i.e., about 150,000 micrograms (μg; 1 μg = 0.000001 gram) each day for a period of several months. Toxicity in infants may occur with much smaller doses. Excessive doses of the natural vitamins K_1 and K_2 have no obvious effects except that resistance may develop to therapy with anticoagulant drugs; however, vitamin K_3 is toxic to newborn infants if given in large doses. Vitamin E, even if given in large excess of the normal requirement, has no apparent obvious adverse effects.

Vitamin groups E and K belong to a class of organic compounds called quinones. These substances are changed to sugarlike substances known as alpha-lactones, which are excreted in the urine. Some vitamin K_1 also is excreted in the bile and thus appears in the feces. Vitamin A is broken down and excreted in bile (and, therefore, feces) and urine. Vitamin D and its breakdown products are excreted only in the feces.

Vitamin-Like Substances

There are a number of organic compounds that, although related to the vitamins in activity, cannot be defined as true vitamins; normally they can be synthesized by humans in adequate amounts and therefore are not required in the diet. These substances usually are classified with the B vitamins, however, because of similarities in biological function or distribution in foods.

Choline

Choline appears to be an essential nutrient for a number of animals and microorganisms that cannot synthesize adequate quantities to satisfy their requirements.

Choline is a constituent of an important class of lipids called phospholipids, which form structural elements of cell membranes; it is a component of the acetylcholine molecule, which is important in nerve function. Choline also serves as a source of methyl groups ($-CH_3$ groups) that are required in various metabolic processes. The effects of a dietary deficiency of choline itself can be alleviated by other dietary compounds that can be changed into choline. Choline also functions in the transport of fats from the liver; for this reason, it may be called a lipotropic factor. A deficiency of choline in the rat results in an accumulation of fat in the liver. Choline-deficiency symptoms vary among species; it is not known if choline is an essential nutrient for humans since a dietary deficiency has not been demonstrated.

Myo-inositol

The biological significance of myo-inositol has not yet been established with certainty. It is present in large amounts—principally as a constituent of phospholipids—in humans. Inositol is a carbohydrate that closely resembles glucose in structure; inositol can be converted to phytic acid, which is found in grains and forms an insoluble (and thus unabsorbable) calcium salt in the intestines of mammals. Inositol has not been established as an essential nutrient for humans; however, it is a required factor for the growth of some yeasts and fungi.

Para-aminobenzoic Acid

Para-aminobenzoic acid (PABA) is required for the growth of several types of microorganisms; however, a dietary requirement by vertebrates has not been shown. The antimicrobial sulfa drugs (sulfanilamide and related compounds) inhibit the growth of bacteria by competing with PABA for a position in a coenzyme that is necessary for bacterial reproduction. Although a structural unit of folic acid, PABA is not considered a vitamin.

Carnitine

Carnitine is essential for the growth of mealworms. The role of carnitine in all organisms is associated with the transfer of fatty acids from the bloodstream to active sites of fatty acid oxidation within muscle cells. Carnitine, therefore, regulates the rate of oxidation of these acids; this function may afford means by which a cell can rapidly shift its metabolic patterns (e.g., from fat synthesis to fat breakdown). Synthesis of carnitine occurs in insects and in higher animals; therefore, it is not considered a true vitamin.

Lipoic Acid

Lipoic acid has a coenzyme function similar to that of thiamin. Although it is apparently an essential nutrient for some microorganisms, no deficiency in mammals has been observed; therefore, lipoic acid is not considered a true vitamin.

Mineral

Minerals are essential nutrients that are needed in small amounts to keep you healthy. The body does not make minerals. To meet your daily needs, minerals must be obtained through the food and beverages you eat and drink.

Minerals are the regulators and initiators of many of the processes occurring in the body. They are essential to muscle contractions, body structure, and fluid balance within the cells and nervous system communication. Minerals give strength and structure to your teeth, bones, muscles, blood and body tissues. They are inorganic matter that cannot be destroyed by cooking or heat. They are absorbed into your intestines and then transported, stored or utilized by your body in different ways. Some travel through the blood stream or are excreted in urine or even stored by your body to toxic levels if too much is consumed. Avoid excess supplementation of minerals.

Typically minerals are split into two separate categories, Major Minerals and Trace Minerals.

Major minerals include sodium, chloride, potassium, phosphorus, magnesium and calcium. You need these minerals in greater amounts than trace minerals.

Major Minerals

The Electrolytes: Sodium, Potassium and Chloride.

- Sodium helps regulate your blood pressure, the movement of fluid in and out of your cells, nerve transmissions and muscle relaxation. It is found in processed foods, table salt and small amounts naturally occurring in food. Too much sodium in your diet can cause fluid retention and swelling if your body is unable to excrete the excess. Prolonged vomiting, diarrhea or kidney problems can result in a sodium deficiency, causing muscle cramps, nausea and dizziness.

- Potassium assists in regulating the mineral and fluid balance both on the inside and surrounding outside of your cells. It is used to regulate sodium's impact on blood pressure, help muscles contract and transmit nerve signals. It is found in fruits, vegetables, legumes, meat, nuts and dairy products. Your body excretes excess amounts, however, and people with kidney problems might need to limit potassium in their diets, as heart problems and even sudden death can result. Not enough potassium can result in muscle cramps, nausea, weakness and fatigue.
- Chloride regulates the fluids in and out of body cells, transmits nerve impulses and helps your body digest food by absorbing nutrients as a component of stomach acid. It is found in salt (Sodium Chloride). Deficiency and excess levels are rare but have similar effects as sodium.

Other Major Minerals: Magnesium, Phosphorus, Calcium.

- Magnesium is part of 300 enzymes that regulate your body in making energy, protein and muscle contractions, as well as many other functions. It is part of bones. It helps signal muscles to relax and contract and maintains body cells in muscles and nerves. Magnesium is best found in whole grains, legumes, nuts and green vegetables. Deficiency is rare but irregular heartbeat and nausea can occur. Excess is excreted, unless kidney problems exist.
- Calcium is well known for its part in building bone strength, length and tissues. It continues to support bone health by slowing bone loss as you age. But calcium also helps clot your blood, supports nerve function and assists muscles in contracting, including your heart beat! It is found in green leafy vegetables, calcium-fortified foods, dairy products and tofu. Deficiency can lead to impaired growth in children and permanently affect height. Even a small deficiency over a long period can affect bone density and loss while increasing risk of osteoporosis.

Trace Minerals

- Iodine works as part of your thyroid hormones to regulate how your body uses energy. Iodine is found naturally occurring in saltwater fish, potatoes and cooked navy beans, but most people get their daily needs in iodized salt. People who are iodine deficient may experience weight gain or develop goiters. Goiters are enlarged thyroid glands and are frequently still seen in developing countries where the salt has not been iodized. Excess iodine can result in irregular heartbeat.
- Fluoride protects your teeth from decay, hardens tooth enamel and strengthens bones. Inadequate fluoride can result in weak tooth enamel but an excess can cause tooth mottling, or stains. It is found in tea, fish and drinking water.

- Chromium helps insulin in your body to use glucose. You are unlikely to consume excess from dietary sources but a deficiency could appear to look like diabetes. It is found in whole-grains, cheese, peas, eggs and meats.
- Copper serves as a part of many enzymes. It helps your body make hemoglobin and connective tissues as well as plays a part in producing energy in your cells. Copper is found in nuts, seeds, organ meats and seafood.
- Iron is an essential part of hemoglobin, needed to transport oxygen to every body cell and enzymes. It is needed for healthy brain development and immune function. A deficiency can lead to anemia, fatigue and infections. Excess amount can cause an enlarged liver, skin coloring, diabetes and internal damage. High iron foods include meats, beans, spinach, seeds and whole-wheat foods.
- Manganese helps in bone formation, metabolism of energy from foods, and is a part of many enzymes. It is rare to have a deficiency or consume excess from dietary sources. It is found in whole-grain products, lentils, and some fruits (pineapple and strawberries) and vegetables (kale).
- Selenium works with Vitamin E as an antioxidant, both protecting cells and supporting immune function. It is found in seeds, whole-grains, seafood, organ meats and eggs. Deficiency or overconsumption is rare in a normal diet.
- Zinc helps your body use food, support enzymatic reactions and promote cell reproduction and tissue growth and repair. Zinc is found in whole-grain products, meats, eggs, peas, nuts and seeds. A zinc deficiency can impair growth in children and cause birth defects during pregnancy. Avoid excess zinc supplementation.
- Molybdenum is a part of enzymes and works with riboflavin to help use iron to make red blood cells. Deficiency and excess consumption in a normal diet is rare.

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Chapter 4

Food Supplements

The purpose of food supplements is to provide nutrients which an individual is not able to obtain through normal diet, or is consuming in insufficient quantities. The diverse applications as well as the health benefits of food supplements have been thoroughly discussed in this chapter.

The idea behind food supplements, also called dietary or nutritional supplements, is to deliver nutrients that may not be consumed in sufficient quantities. Food supplements can be vitamins, minerals, amino acids, fatty acids, and other substances delivered in the form of pills, tablets, capsules, liquid, etc. Supplements are available in a range of doses, and in different combinations. However, only a certain amount of each nutrient is needed for our bodies to function, and higher amounts are not necessarily better. At high doses, some substances may have adverse effects, and may become harmful. For the reason of safeguarding consumers' health, supplements can therefore only be legally sold with an appropriate daily dose recommendation, and a warning statement not to exceed that dose.

Supplement use varies in Europe. For example it is common in Germany and Denmark (43% and 59% of the adult population respectively) but is less so in Ireland and Spain (23% and 9% respectively). Women use supplements more than men.

Need of Food Supplements

Supplements are not a substitute for a balanced healthy diet. A diet that includes plenty of fruits, vegetables, whole grains, adequate protein, and healthy fats should normally provide all the nutrients needed for good health. Most European countries agree that messages aimed at the general public should focus on food-based dietary guidelines. Supplements do not feature in these guidelines, but there are certain population groups or individuals who may need advice about supplements, even when they eat a healthy balanced diet, i.e. women of childbearing age, individuals on specific medications.

Partly due to our modern lifestyle, not everyone manages to eat a healthy diet. In Europe, dietary surveys have suggested that there are suboptimal intakes for several micronutrients. The EU-funded EURRECA project found inadequate intakes for vitamin C, vitamin D, folic acid, calcium, selenium and iodine. A recent comparison of national surveys showed widespread concern about vitamin D intakes, whereas certain age groups are more likely to have low intakes of minerals. For example, there is concern about adequate intakes of iron amongst teenage girls in Denmark, France, Poland, Germany and the UK. Poor iron status in young women also increases the risk of infants being born with low-birth weight, iron deficiency and delayed brain development. Folate status is also critical for women who may become pregnant. They are advised to take folic acid before conception, and continue for the first weeks of pregnancy. An adequate folate status can decrease the risk of having a baby with neural tube defects such as spina bifida. Recent research suggests that 50–70% of Europeans have poor vitamin D status. Since vitamin D status is dependent not only on dietary intake but also exposure to UV light, there may be a stronger case for advising supplements for vitamin D in Northern European countries. In some countries (including UK, Ireland, the Netherlands and Sweden) there are already recommendations for certain groups in the population to take a vitamin D supplement.

Other common concerns are shown in table, although groups considered at risk are not the same in different countries.

Table: Examples of population groups requiring specific advice about supplements.

Population group	Nutrients
People over age 50	Vitamin D, Vitamin B_{12}, folate Frail elderly may benefit from a low-dose multivitamin supplement.
Women of childbearing age	Folic acid and vitamin D, possibly iron
Children under age 5	Vitamin A, vitamin C, vitamin D, although children with a good appetite who eat a wide variety of food may not need them.
Breastfeeding women	Vitamin D
People who cover their skin, are dark-skinned, or are housebound	Vitamin D
Vegans	Vitamin $B_{12,}$ vitamin D_2

Particular risks for specific population groups despite having a role in the health of some individuals, not all supplements are useful for everybody. In fact, for some people, it is not advisable to take certain supplements, in particular in high doses. Some studies show multivitamins can contribute to an increased risk of excessive nutrient intakes, and it has been suggested that multivitamins should be formulated with greater consideration for the intakes of micronutrients from foods. Individuals should pay particular attention to reading the label and assure that a supplement is suitable for them. For pregnant woman, for example, supplements containing vitamin A (retinol) including fish liver oil may be harmful and cause birth defects if the recommended dose is greatly exceeded, or exceeded over an extended period of time.

Fish Oil

Fish oil is the fat or oil that's extracted from fish tissue. It usually comes from oily fish, such as herring, tuna, anchovies, and mackerel. Yet it's sometimes produced from the livers of other fish, as is the case with cod liver oil.

Fish oil is a dietary source of omega-3 fatty acids — substances your body needs for many functions, from muscle activity to cell growth.

Omega-3 fatty acids are derived from food. They can't be manufactured in the body. Fish oil contains two omega-3s called docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Dietary sources of DHA and EPA are fatty fish, such as salmon, mackerel and trout, and shellfish, such as mussels, oysters and crabs. Some nuts, seeds and vegetable oils contain another omega-3 called alpha-linolenic acid (ALA).

Fish oil supplements come in liquid, capsule and pill form.

People take fish oil to reduce the risk of heart attacks and strokes, to treat high triglycerides and high blood pressure, and to improve symptoms of rheumatoid arthritis.

Foods Containing Fish Oil

The fillets of oily fish contain up to 30 percent oil, but this figure varies. White fish, such as cod, contains high concentrations of oil in the liver but less oil overall. Oily fish that are rich in omega-3 fatty acids include anchovies, herring, sardines, salmon, trout, and mackerel.

Other animal sources of omega-3 fatty acids are eggs, especially those with "high in omega-3" written on the shell.

Vegetable-based alternatives to fish oil for omega 3 include:

- Flax
- Hempseed
- Perilla oil
- Spirulina
- Walnuts
- Chia seeds
- Radish seeds, sprouted raw
- Fresh basil

- Leafy dark green vegetables, such as spinach
- Dried tarragon

A person who consumes a healthful, balanced diet should not need to use supplements.

Benefits of Fish Oil

1. May Support Heart Health

- Heart disease is the leading cause of death worldwide.
- People who eat a lot of fish have much lower rates of heart disease.
- Multiple risk factors for heart disease appear to be reduced by consumption of fish or fish oil. The benefits of fish oil for heart health include:
    - Cholesterol levels: It can increase levels of “good” HDL cholesterol. However, it does not appear to reduce levels of “bad” LDL cholesterol.
    - Triglycerides: It can lower triglycerides by about 15–30%.
    - Blood pressure: Even in small doses, it helps reduce blood pressure in people with elevated levels.
    - Plaque: It may prevent the plaques that cause your arteries to harden, as well as make arterial plaques more stable and safer in those who already have them.
    - Fatal arrhythmias: In people who are at risk, it may reduce fatal arrhythmia events. Arrhythmias are abnormal heart rhythms that can cause heart attacks in certain cases.
- Although fish oil supplements can improve many of the risk factors for heart disease, there is no clear evidence that it can prevent heart attacks or strokes.

2. May Help Treat Certain Mental Disorders

- Your brain is made up of nearly 60% fat, and much of this fat is omega-3 fatty acids. Therefore, omega-3s are essential for normal brain function.
- In fact, people with certain mental disorders have lower omega-3 blood levels.
- Fish oil supplements can prevent the onset or improve the symptoms of some mental disorders. For example, it can reduce the chances of psychotic disorders in those who are at risk.
- In addition, supplementing with fish oil in high doses may reduce some symptoms of both schizophrenia and bipolar disorder.

3. May Aid Weight Loss

- Obesity is defined as having a body mass index (BMI) greater than 30. Globally, about 39% of adults are overweight, while 13% are obese. The numbers are even higher in high-income countries like the US.
- Obesity can significantly increase your risk of other diseases, including heart disease, type 2 diabetes, and cancer.
- Fish oil supplements may improve body composition and risk factors for heart disease in obese people.
- Furthermore, fish oil supplements, in combination with diet or exercise, can help you lose weight.

4. May Support Eye Health

- Like your brain, your eyes rely on omega-3 fats. Evidence shows that people who don't get enough omega-3s have a greater risk of eye diseases.
- Furthermore, eye health begins to decline in old age, which can lead to age-related macular degeneration (AMD). Eating fish is linked to a reduced risk of AMD, but the results on fish oil supplements are less convincing.
- Consuming a high dose of fish oil for 19 weeks improved vision in all AMD patients. However, this was a very small study.
- Combined effect of omega-3s and other nutrients on AMD. A positive effect, while the other exhibited no effect. Therefore, the results are unclear.

5. May Reduce Inflammation

- Inflammation is your immune system's way of fighting infection and treating injuries.
- However, chronic inflammation is associated with serious illnesses, such as obesity, diabetes, depression, and heart disease.
- Reducing inflammation can help treat symptoms of these diseases.
- Because fish oil has anti-inflammatory properties, it may help treat conditions involving chronic inflammation.
- For example, in stressed and obese individuals, fish oil can reduce the production and gene expression of inflammatory molecules called cytokines.
- Moreover, fish oil supplements can significantly reduce joint pain, stiffness, and medication needs in people with rheumatoid arthritis, which causes painful joints.
- While inflammatory bowel disease (IBD) is also triggered by inflammation, there is no clear evidence to suggest whether fish oil improves its symptoms.

6. May Support Healthy Skin

- Your skin is the largest organ in your body, and it contains a lot of omega-3 fatty acids.
- Skin health can decline throughout your life, especially during old age or after too much sun exposure.
- That said, there are a number of skin disorders that may benefit from fish oil supplements, including psoriasis and dermatitis.

7. May Support Pregnancy and Early Life

- Omega-3s are essential for early growth and development.
- Therefore, it's important for mothers to get enough omega-3s during pregnancy and while breastfeeding.
- Fish oil supplements in pregnant and breastfeeding mothers may improve hand-eye coordination in infants. However, it's unclear whether learning or IQ are improved.
- Taking fish oil supplements during pregnancy and breastfeeding may also improve infant visual development and help reduce the risk of allergies.

8. May Reduce Liver Fat

- Your liver processes most of the fat in your body and can play a role in weight gain.
- Liver disease is increasingly common — particularly non-alcoholic fatty liver disease (NAFLD), in which fat accumulates in your liver.
- Fish oil supplements can improve liver function and inflammation, which may help reduce symptoms of NAFLD and the amount of fat in your liver.

9. May Improve Symptoms of Depression

- Depression is expected to become the second-largest cause of illness by 2030.
- Interestingly, people with major depression appear to have lower blood levels of omega-3s.
- Fish oil and omega-3 supplements may improve symptoms of depression.
- Moreover, oils rich in EPA help reduce depressive symptoms more than DHA.

10. May Improve Attention and Hyperactivity in Children

- A number of behavioral disorders in children, such as attention deficit hyperactivity disorder (ADHD), involve hyperactivity and inattention.

- Given that omega-3s make up a significant proportion of the brain, getting enough of them may be important for preventing behavioral disorders in early life.
- Fish oil supplements may improve perceived hyperactivity, inattention, impulsiveness, and aggression in children. This may benefit early life learning.

11. May Help Prevent Symptoms of Mental Decline

- As you age, your brain function slows down, and your risk of Alzheimer's disease increases.
- People who eat more fish tend to experience a slower decline in brain function in old age.
- However, studies on fish oil supplements in older adults haven't provided clear evidence that they can slow the decline of brain function.
- Nevertheless, fish oil may improve memory in healthy, older adults.

12. May Improve Asthma Symptoms and Allergy Risk

- Asthma, which can cause swelling in the lungs and shortness of breath, is becoming much more common in infants.
- Fish oil may reduce asthma symptoms, especially in early life.
- In one review in nearly 100,000 people, a mother's fish or omega-3 intake was found to reduce the risk of asthma in children by 24–29%.
- Furthermore, fish oil supplements in pregnant mothers may reduce the risk of allergies in infants.

13. May Improve Bone Health

- During old age, bones can begin to lose their essential minerals, making them more likely to break. This can lead to conditions like osteoporosis and osteoarthritis.
- Calcium and vitamin D are very important for bone health.
- People with higher omega-3 intakes and blood levels may have better bone mineral density (BMD).
- However, it's unclear whether fish oil supplements improve BMD.
- Fish oil supplements reduce markers of bone breakdown, which may prevent bone disease.

Lactagen

Lactagen was a nutritional supplement produced by Ritter Pharmaceuticals that claimed to reduce the symptoms of lactose intolerance. In 2011, Ritter Pharmaceuticals ceased sales of Lactagen, and other dietary supplements, in order to pursue FDA approval for a treatment for lactose intolerance.

Symptoms said to be relieved by Lactagen include bloating, gas, diarrhea, and abdominal cramping which are associated with the symptoms of lactose intolerance. Lactagen is a powder ingested with water or juice in increasing amounts for a period of 38 days. The manufacturer claims that the mixture of prebiotics and probiotics will relieve all symptoms permanently for 8 out of 10 users. Gastroenterologists suggest that Lactagen can relieve symptoms of lactose intolerance, but relief may not be permanent.

Since Lactagen was a natural dietary supplement, it was not regulated by the U.S. Food and Drug Administration. Therefore, claims of effectiveness were not evaluated by the agency, and Ritter Pharmaceuticals can therefore not make any claims that Lactagen cures or prevents any disease.

Lactose Intolerance

Lactose intolerance (“LI”) is a widespread condition affecting over 1 billion people worldwide. Approximately 40 million Americans (or 15% of the US population) suffer from lactose intolerance, with an estimated 9 million of those individuals demonstrating moderate and severe symptoms. Lactose intolerance is caused by a lack of the naturally produced enzyme lactase in the stomach. Lactose intolerance is exemplified by the inability to tolerate and fully digest dairy products, such as milk, ice cream, cheese and pizza. Symptoms may include a painful combination of digestive discomfort, including gas, cramping, bloating or diarrhea. According to the American Academy of Pediatrics Committee on Nutrition, “the symptoms of lactose intolerance can lead to significant discomfort, disruption of the quality of life, and loss of school attendance, leisure and sports activities, and work time, all at a cost to individuals, families, and society.”

Currently, there is no approved prescription treatment for LI. Most persons with LI avoid ingestion of milk and dairy products, while others substitute non-lactose-containing foods in their diet. However, complete avoidance of lactose-containing foods is difficult to achieve (especially for those with moderate to severe symptoms), and can lead to significant long-term morbidity, i.e., dietary deficiencies of calcium and vitamin D.

At the 2010 National Institute of Health (“NIH”) Consensus Development Conference: Lactose Intolerance and Health, the NIH highlighted numerous health risks tied to lactose intolerance such as: osteoporosis; hypertension; and low bone density. There is substantial evidence indicating that lactose intolerance is a major factor in limiting calcium and nutrient intake in the diet of people who are lactose intolerant. Adequate calcium intake is essential to reducing the risks of osteoporosis and hypertension. In addition, chronic calcium depletion has been linked to increased arterial blood pressure, thereby establishing a relationship between hypertension and a low calcium intake. Moreover, there is evidence of a correlation between calcium intake and both colon and breast cancer.

Over 50% of lactose intolerant patients are very concerned that they may be susceptible to one of these health risks, as a result of their lactose intolerance.

Clinical Data

In a clinical study conducted by Ritter Pharmaceuticals medical board member Chris Landon, M.D., Lactagen showed a statistically significant reduction in lactose intolerance symptoms. The results were gathered among sixty-subjects in a double-blind clinical trial at the Ventura County Medical Center in Los Angeles, California. Specifically, 79% of subjects on Lactagen reported a substantial reduction of lactose intolerant symptoms after completion of the 38-day program, while those on a placebo showed little or no reduction in symptoms.

Effectiveness

At the National Institute of Health Consensus Development Conference on Lactose Intolerance and Health, experts stated that strategies such as colonic adaptation have intriguing preliminary data and may be useful for individuals with lactose intolerance, although additional research needs to be completed in the area.

In an interview with the Washington Post, gastroenterologist Theodore Bayless of Johns Hopkins School of Medicine suggested that such a course can relieve symptoms of lactose intolerance, but the relief would not be permanent, particularly if the user were to take antibiotics at a later date.

L-theanine

L-theanine is an amino acid. The human body does not produce this compound, and it is not essential for humans. Green tea, black tea, and certain types of mushroom naturally contain L-theanine.

L-theanine may affect the levels of certain chemicals in the brain. These include serotonin and dopamine, which influence mood, sleep, and emotion, and cortisol, which helps the body deal with stress.

A change in the balance of these chemicals can change a person's mood or stress levels.

Health Benefits of L-theanine

L-theanine Helps in Blocking Anxiety and Stress

L-theanine relieves anxiety in large part because it bears a close resemblance to the brain-signaling chemical glutamate. L-theanine produces the opposite effect in the brain.

While glutamate is the brain's most important excitatory neurotransmitter, L-theanine binds to the same brain cell receptors and blocks them to glutamate's effects. This action produces inhibitory effects. That inhibition to brain overactivity has a calming, relaxing effect in which anxiety fades.

In addition to blocking excitatory stimuli at glutamate receptors in the brain, L-theanine also stimulates production of the inhibitory, relaxing neurotransmitter GABA, adding to its calming, anti-anxiety effects.

Unlike prescription anti-anxiety drugs, however, some of which mimic GABA's effects, L-theanine produces its anti-anxiety effects without producing sleepiness or impairing motor behavior. In fact, L-theanine has been shown in human studies to moderately improve alertness and attention while exerting its anxiety-reducing effects.

L-theanine supplementation prevents the abrupt rise in blood pressure that some people experience under stress. The reason this is so critical is that many people have normal blood pressure readings at rest that spike up to dangerously high levels when subjected to stressful situations.

These periods of surging blood pressure inflict massive arterial damage and are the main reason why at-home and at-office blood pressure testing are so important.

L-theanine Protects Brain Cells and Promotes Cognitive Function

There's a link between anxiety, reaction to stress, and the brain's most fundamental function, maintaining cognition.

Stress has powerful negative effects on one's ability to think clearly and make smart decisions. This is demonstrated physiologically by animal experiments showing that stress significantly reduces animals' performance on standard tests of learning and memory, as well as by increased oxidative stress in the brain and elevated blood levels of stress-response hormones such as catecholamine and adrenaline. Treating animals with L-theanine before the stress is applied, however, results in reversal not only of cognitive impairment, but also of the elevation of stress hormones and oxidative damage.

L-theanine can specifically reduce the molecular impacts of acute stress, and the resulting excitotoxicity, on brain cells. The issue with chronic glutamate-driven excitotoxicity is profound and long-lasting cognitive dysfunction, including neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's diseases, and amyotrophic lateral sclerosis (ALS).

The protective effects of L-theanine have been shown in animal models for at least the first three of these disorders, suggesting that regular L-theanine supplementation might be important in fending off these tragic conditions by opposing the destructive effects of long-term glutamate excitotoxicity.

L-theanine Reduces Stroke Impact

A stroke is the result of a sudden blockage of blood (ischemia) to a part of the brain, resulting in massive chemical stresses, extreme excitotoxicity, and eventual death of brain cells. L-theanine has properties that may both help to prevent strokes and to mitigate the damage caused when they do occur.

L-theanine is capable of significantly improving nitric oxide production in endothelial (artery-lining) cells. This has the potential to lower stroke risk because nitric oxide is a signaling molecule that endothelial cells use to communicate information about blood flow and pressure to muscles in the artery walls, telling them to constrict or relax appropriately in response and distributing blood flow appropriately.

In another stroke-preventing mechanism, L-theanine has recently been shown to significantly reduce the expression of adhesion molecules to the endothelial wall by inhibiting tumor necrosis factor alpha (TNF-a), thereby reducing the risk of an artery-blocking clot or obstruction that produces a stroke.

L-theanine protects the body from the damage of blood reperfusing, or refilling that occurs after the abrupt loss of circulation during the stroke.

This ischemia-reperfusion injury results in massive release of glutamate and produces deadly excitotoxicity.

L-theanine may Play a Role in Ameliorating Schizophrenia

Schizophrenia, literally a "split mind" in which sufferers experience a cut-off from reality, is one of the most tragic and misunderstood disorders known. People with schizophrenia may experience positive symptoms such as hallucinations, delusions, and paranoid thinking, as well as negative symptoms including loss of ability to experience pleasure, blunted emotions, and diminished speech capacity.

Perhaps because schizophrenia may involve excitotoxic damage to brain cells, L-theanine has recently been the focus of human studies in patients with this disease.

Ginkgo Biloba

Ginkgo biloba is a popular supplement. Ginkgo biloba extract is collected from the dried green leaves of the plant and is available as liquid extracts, capsules, and tablets. People use it for a variety of reasons.

The therapeutic properties of the ginkgo plant are said to include treatment for blood disorders and memory problems, enhancement of cardiovascular function and to improve eye health.

Gingko contains high levels of flavonoids and terpenoids, antioxidants that provide protection against oxidative cell damage from harmful free radicals. In this way, antioxidants are believed to help reduce the risk of cancer.

Also known as the maidenhair tree, ginkgo is one of the oldest species of tree in the world. The trees can grow more than 130 feet tall and can live for over 1,000 years. Some trees in China are said to be over 2,500 years old.

The tree is considered to be a "living fossil," meaning that it has continued to survive even after major extinction events.

The extract can be taken as a supplement, and the dried leaves of the plant can be used to make tea.

Health Benefits of Ginkgo Biloba

1. Contains Powerful Antioxidants

- Ginkgo's antioxidant content may be the reason behind many of its health claims.
- Ginkgo contains high levels of flavonoids and terpenoids, which are compounds known for their strong antioxidant effects.
- Antioxidants combat or neutralize the damaging effects of free radicals.
- Free radicals are highly reactive particles that are produced in the body during normal metabolic functions, such as converting food to energy or detoxification.
- Yet, they also have the potential to damage healthy tissues, contributing to accelerated aging and disease development.

2. Can Help Fight Inflammation

- Inflammation is part of the body's natural response to injury or invasion by a foreign substance.

- In the inflammatory response, various components of the immune system are recruited to fight against the foreign invader or heal the injured area.
- Some chronic diseases trigger an inflammatory response even when there is no illness or injury present. Over time, this excessive inflammation can cause permanent damage to the body's tissues and DNA.
- Some specific conditions in which ginkgo extract has shown to reduce inflammation include:
    - Arthritis
    - Irritable bowel disease (IBD)
    - Cancer
    - Heart disease
    - Stroke

3. Improves Circulation and Heart Health

- In traditional Chinese medicine, ginkgo seeds were used to open "channels" of energy to different organ systems, including the kidneys, liver, brain and lungs.
- Ginkgo's apparent ability to increase blood flow to various parts of the body may be the origin of many of its supposed benefits.

4. Reduces Symptoms of Psychiatric Disorders and Dementia

- Ginkgo has been repeatedly evaluated for its ability to reduce anxiety, stress and other symptoms associated with Alzheimer's disease and cognitive decline associated with aging.

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Chapter 5

Food Additives

A chemical substance which is added to food in order to produce particular desirable effects is known as a food additive. Anticaking agents, food coloring and sugar substitutes are a few examples of commonly used food additives. The diverse applications of these additives have been thoroughly discussed in this chapter.

Food additive is any of various chemical substances added to foods to produce specific desirable effects. Additives such as salt, spices, and sulfites have been used since ancient times to preserve foods and make them more palatable. With the increased processing of foods in the 20th century, there came a need for both the greater use of and new types of food additives. Many modern products, such as low-calorie, snack, and ready-to-eat convenience foods, would not be possible without food additives.

Nutritional Additives

Nutritional additives are used for the purpose of restoring nutrients lost or degraded during production, fortifying or enriching certain foods in order to correct dietary deficiencies, or adding nutrients to food substitutes. Vitamins are commonly added to many foods in order to enrich their nutritional value. For example, vitamins A and D are added to dairy and cereal products, several of the B vitamins are added to flour, cereals, baked goods, and pasta, and vitamin C is added to fruit beverages, cereals, dairy products, and confectioneries. Other nutritional additives include the essential fatty acid linoleic acid, minerals such as calcium and iron, and dietary fibre.

Processing Agents

A number of agents are added to foods in order to aid in processing or to maintain the desired consistency of the product.

Processing additives and their uses

Function	Typical Chemical Agent	Typical Product
Anticaking	Sodium aluminosilicate	Salt
Bleaching	Benzoyl peroxide	Flour
Chelating	Ethylenediaminetetraacetic acid (EDTA)	Dressings, mayonnaise, sauces, dried bananas

Processing additives and their uses		
Function	Typical Chemical Agent	Typical Product
Clarifying	Bentonite, proteins	Fruit juices, wines
Conditioning	Potassium bromate	Flour
Emulsifying	Lecithin	Ice cream, mayonnaise, bakery products
Leavening	Yeast, baking powder, baking soda	Bakery products
Moisture control (humectants)	Glycerol	Marshmallows, soft candies, chewing gum
pH control	Citric acid, lactic acid	Certain cheeses, confections, jams and jellies
Stabilizing and thickening	Pectin, gelatin, carrageenan, gums (arabic, guar, locust bean)	Dressings, frozen desserts, confections, pudding mixes, jams and jellies

Emulsifiers are used to maintain a uniform dispersion of one liquid in another, such as oil in water. The basic structure of an emulsifying agent includes a hydrophobic portion, usually a long-chain fatty acid, and a hydrophilic portion that may be either charged or uncharged. The hydrophobic portion of the emulsifier dissolves in the oil phase, and the hydrophilic portion dissolves in the aqueous phase, forming a dispersion of small oil droplets. Emulsifiers thus form and stabilize oil-in-water emulsions (e.g., mayonnaise), uniformly disperse oil-soluble flavour compounds throughout a product, prevent large ice crystal formation in frozen products (e.g., ice cream), and improve the volume, uniformity, and fineness of baked products.

Stabilizers and thickeners have many functions in foods. Most stabilizing and thickening agents are polysaccharides, such as starches or gums, or proteins, such as gelatin. The primary function of these compounds is to act as thickening or gelling agents that increase the viscosity of the final product. These agents stabilize emulsions, either by adsorbing to the outer surface of oil droplets or by increasing the viscosity of the water phase. Thus, they prevent the coalescence of the oil droplets, promoting the separation of the oil phase from the aqueous phase (i.e., creaming). The formation and stabilization of foam in a food product occurs by a similar mechanism, except that the oil phase is replaced by a gas phase. The compounds also act to inhibit the formation of ice or sugar crystals in foods and can be used to encapsulate flavour compounds.

Chelating, or sequestering, agents protect food products from many enzymatic reactions that promote deterioration during processing and storage. These agents bind to many of the minerals that are present in food (e.g., calcium and magnesium) and are required as cofactors for the activity of certain enzymes.

Sensory Agents

Colorants

Colour is an extremely important sensory characteristic of foods; it directly influences the perception of both the flavour and quality of a product. The processing of food can cause degradation or loss of natural pigments in the raw materials. In addition, some formulated products, such as soft drinks, confections, ice cream, and snack foods, require the addition of colouring agents. Colorants are often necessary to produce a uniform product from raw materials that vary in colour intensity. Colorants used as food additives are classified as natural or synthetic. Natural colorants are derived from plant, animal, and mineral sources, while synthetic colorants are primarily petroleum-based chemical compounds.

Soft drink: Many soft drinks, including colas, contain colorants

Natural Colorants

Most natural colorants are extracts derived from plant tissues. The use of these extracts in the food industry has certain problems associated with it, including the lack of consistent colour intensities, instability upon exposure to light and heat, variability of supply, reactivity with other food components, and addition of secondary flavours and odours. In addition, many are insoluble in water and therefore must be added with an emulsifier in order to achieve an even distribution throughout the food product.

Natural food colorants				
Chemical Class	Colour	Plant Source	Pigment	Products
Anthocyanins	Red	Strawberry (fragaria species)	Pelargonidin 3-glucoside*	Beverages, confections, preserves, fruit products
	Blue	Grape (vitis species)	Malvidin 3-glucoside*	Beverages
Betacyanins	Red	Beetroot (beta vulgaris)	Betanin	Dairy products, desserts, icings

Natural food colorants				
Chemical Class	Colour	Plant Source	Pigment	Products
Carotenoids**	Yellow/orange	Annatto (bixa orellana)	Bixin	Dairy products, margarine
	Yellow	Saffron (crocus sativus)	Crocin	Rice dishes, bakery products
	Red/orange	Paprika (capsicum annuum)	Capsanthin	Soups, sauces
	Orange	Carrot (daucus carota)	Beta-carotene	Bakery products, confections
	Red	Mushroom (cantharellus cinnabarinus)	Canthaxanthin	Sauces, soups, dressings
Phenolics	Orange/yellow	Turmeric (cuycuma longa)	Curcumin	Dairy products, confections
*Plus other similar compounds.				
**Many carotenoids used as food colorants are chemically synthesized.				

Synthetic Colorants

Synthetic colorants are water-soluble and are available commercially as powders, pastes, granules, or solutions. Special preparations called lakes are formulated by treating the colorants with aluminum hydroxide. They contain approximately 10 to 40 percent of the synthetic dye and are insoluble in water and organic solvents. Lakes are ideal for use in dry and oil-based products. The stability of synthetic colorants is affected by light, heat, pH, and reducing agents. A number of dyes have been chemically synthesized and approved for usage in various countries. These colorants are designated according to special numbering systems specific to individual countries. For example, the United States uses FD&C numbers (chemicals approved for use in foods, drugs, and cosmetics), and the European Union (EU) uses E numbers.

Synthetic food colorants			
	Designation		
Common Name	United States	European Union	Products
Allura red AC	FD&C red no. 40	...	Gelatin, puddings, dairy products, confections, beverages
Brilliant blue FCF	FD&C blue no. 1	E133	Beverages, confections, icings, syrups, dairy products

Synthetic food colorants			
	Designation		
Common Name	United States	European Union	Products
Erythrosine	FD&C red no. 3	E127	Maraschino cherries
Fast green FCF	FD&C green no. 3	...	Beverages, puddings, ice cream, sherbet, confections
Indigo carmine	FD&C blue no. 2	E132	Confections, ice cream, bakery products
Sunset yellow FCF	FD&C yellow no. 6	E110	Bakery products, ice cream, sauces, cereals, beverages
Tartrazine	FD&C yellow no. 5	E102	Beverages, cereals, bakery products, ice cream, sauces

All synthetic colorants have undergone extensive toxicological analysis. Brilliant Blue FCF, Indigo Carmine, Fast Green FCF, and Erythrosine are poorly absorbed and show little toxicity. Extremely high concentrations (greater than 10 percent) of Allura Red AC cause psychotoxicity, and Tartrazine induces hypersensitive reactions in some persons. Synthetic colorants are not universally approved in all countries.

Flavourings

The flavour of food results from the stimulation of the chemical senses of taste and smell by specific food molecules. Taste reception is carried out in specialized cells located in the taste buds. The five basic taste sensations—sweet, salty, bitter, sour, and umami—are detected in regions of the tongue, mouth, and throat. Taste cells are specific for certain flavour molecules (e.g., sweeteners).

In addition to the basic tastes, the flavouring molecules in food stimulate specific olfactory (smell) cells in the nasal cavity. These cells can detect more than 10,000 different stimuli, thus fine-tuning the flavour sensation of a food.

A flavour additive is a single chemical or blend of chemicals of natural or synthetic origin that provides all or part of the flavour impact of a particular food. These chemicals are added in order to replace flavour lost in processing and to develop new products. Flavourings are the largest group of food additives, with more than 1,200 compounds available for commercial use. Natural flavourings are derived or extracted from plants, spices, herbs, animals, or microbial fermentations. Artificial flavourings are mixtures of synthetic compounds that may be chemically identical to natural flavourings. Artificial flavourings are often used in food products because of the high cost, lack of availability, or insufficient potency of natural flavourings.

Flavour enhancers are compounds that are added to a food in order to supplement or enhance its own natural flavour. The concept of flavour enhancement originated in Asia, where cooks added seaweed to soup stocks in order to provide a richer flavour to certain foods. The flavour-enhancing component of seaweed was identified as the amino acid L-glutamate, and monosodium glutamate (MSG) became the first flavour enhancer to be used commercially. The rich flavour associated with L-glutamate was called umami.

Other compounds that are used as flavour enhancers include the 5′-ribonucleotides, inosine monophosphate (IMP), guanosine monophosphate (GMP), yeast extract, and hydrolyzed vegetable protein. Flavour enhancers may be used in soups, broths, sauces, gravies, flavouring and spice blends, canned and frozen vegetables, and meats.

Sweeteners

Sucrose, or table sugar, is the standard on which the relative sweetness of all other sweeteners is based. Because sucrose provides energy in the form of carbohydrates, it is considered a nutritive sweetener. Other nutritive sweeteners include glucose, fructose, corn syrup, high-fructose corn syrup, and sugar alcohols (e.g., sorbitol, mannitol, and xylitol).

Efforts to chemically synthesize sweeteners began in the late 1800s with the discovery of saccharin. Since then, a number of synthetic compounds have been developed that provide few or no calories or nutrients in the diet and are called nonnutritive sweeteners. These sweeteners have significantly greater sweetening power than sucrose, and therefore a relatively low concentration may be used in food products. In addition to saccharin, the most commonly used nonnutritive sweeteners are cyclamates, aspartame, and acesulfame K.

The sensation of sweetness is transmitted through specific protein molecules, called receptors, located on the surface of specialized taste cells. All sweeteners function by binding to these receptors on the outside of the cells. The increased sweetness of the nonnutritive sweeteners relative to sucrose may be due to either tighter or longer binding of these synthetic compounds to the receptors.

Nonnutritive sweeteners are primarily used for the production of low-calorie products including baked goods, confectioneries, dairy products, desserts, preserves, soft drinks, and tabletop sweeteners. They are also used as a carbohydrate replacement for persons with diabetes mellitus and in chewing gum and candies to minimize the risk of dental caries (i.e., tooth decay). Unlike nutritive sweeteners, nonnutritive sweeteners do not provide viscosity or texture to products, so bulking agents such as polydextrose are often required for manufacture.

Anticaking Agent

Anticaking agents are food additives that keep powders or granulated materials such as milk powder, powdered sugar, tea and coffee powders used in vending machines, table salt etc. flowing freely. Anticaking agents, in fact, prevent the formation of lumps making these products manageable for packaging, transport, and for use by end consumer.

Anticaking Agent is the food additive that prevents agglomeration in certain solids, permitting a free-flowing condition. Anticaking agents consist of such substances as starch, magnesium carbonate, and silica and are added to fine-particle solids, such as food products like table salt, flours, coffee, and sugar.

Examples of Anticaking Agents Food

Some of the common examples of foods that contain anti-caking agents include:

- Vending machine powders (coffee, cocoa, soup)
- Milk and cream powders
- Grated cheese
- Icing sugar
- Baking powder
- Cake mixes
- Instant soup powders
- Drinking chocolate
- Table salt

Anticaking agents are also one of the most common flour treatment agents.

Functions of Anticaking Agents

Anticaking agents function either by adsorbing excess moisture, or by coating particles and making them water repellent. Some anticaking agents are soluble in water; others are soluble in alcohols or other organic solvents. Calcium silicate ($CaSiO_3$), a common anti-caking agent which is added to table salt etc. adsorbs both water and oil. Although they are food additives, anticaking agents have other applications too. For example, anticaking agents are popularly used in non-food items like road salt, fertilizers, cosmetics, synthetic detergents, and in other such manufacturing applications.

Manufactured and Natural Anticaking Agents

There are two types of anticaking agents-manufactured or man-made and natural anticaking agents. Most of the anti-caking agents are made from synthetic substances such as silicon dioxide or magnesium and calcium stearates (solid saturated fatty acids.) However, there are many ani caking agents that come from natural sources. Some of the natural anticaking agents include kaolin and bentonite. Some anticaking agents manufacturers also produce organic and hypoallergenic anti caking agents from such natural source as rice.

Man-made anticaking agents are manufactured from chemicals and other artificial substances like silicates, acids etc. Examples of manufactured anti-caking agents include calcium silicate (E552), magnesium carbonate (E504) and sodium aluminosilicate (E554) etc. Majority of anti-caking agents are found with E numbers from 500 to 599. However, apart from the list of E numbers anti caking agents, there are a few that fall into other categories too like the acidity regulators (because they serve two purposes.) Some of such anti caking agents having dual/multiple purposes include:

- E421 - Mannitol as it is also a texturising agent, a sweetening agent, and anti-sticking agent as well as a humectant.
- E460a - Microcrystalline cellulose as is also a bulking agent, binder and stabiliser.
- E460b - Alpha cellulose as it is also a bulking agent, binder and stabiliser.

Food Coloring

A food coloring is any substance that is added to food or drink to change its color. It is sometimes used in cooking.

Some food colorings are extracted from natural sources, others are artificially synthesized. They are used for various purposes, such as to enhance or mask natural food colors, to provide identity to foods, and to decorate cakes and desserts. They offset the loss of natural colors when foods are exposed to light, air, temperature extremes, and moisture. Some are thought to protect flavors and vitamins present in foods from damage by light. Recent studies indicate that certain artificial coloring agents (and synthetic food preservatives) aggravate symptoms of attention-deficit hyperactivity disorder (ADHD). Several countries have therefore banned the use of some colorants.

Food coloring spreading on a thin water film

Purpose of Food Coloring

People associate certain colors with certain flavors, and the color of food can influence the perceived flavor, in anything from candy to wine. For this reason, food manufacturers add dyes to their products. Sometimes, the aim is to simulate a color that is perceived by the consumer as natural, such as adding red coloring to glacé cherries (which would otherwise be beige). At other times, it is for effect, such as a variety of children's cereals or the green ketchup that Heinz launched in 2000.

Although most consumers are aware that foods with bright or unnatural colors likely contain food coloring, far fewer people know that seemingly "natural" foods such as oranges and salmon are sometimes also dyed to mask natural variations in color. Color variation in foods throughout the seasons and the effects of processing and storage often make color addition commercially advantageous to maintain the color expected or preferred by the consumer.

Some of the primary reasons for adding food coloring include:

- Offsetting color loss due to light, air, extremes of temperature, moisture, and storage conditions.
- Masking natural variations in color.
- Enhancing naturally occurring colors.
- Providing identity to foods.
- Protecting flavors and vitamins from damage by light.
- Decorating, such as cake icing.

Natural Food Dyes

Several food dyes are derived from natural sources. Prominent examples are given below:

- Caramel coloring is found in cola products. It is made from caramelized sugar. It is also used in cosmetics.
- Annatto is a reddish-orange dye made from the seed of the Achiote.
- Chlorella is green and is derived from algae.
- Cochineal is a red dye derived from cochineal insects.
- Beet juice, turmeric, saffron, and paprika are also used as colorants.

Dyes and Lakes

In the United States, certifiable color additives are available for use in food as either "dyes" or "lakes."

Dyes dissolve in water, but they are not soluble in oil. They are manufactured as powders, granules, liquids, or other special purpose forms. They can be used in beverages, dry mixes, baked goods, confections, dairy products, pet foods, and a variety of other products. Dyes also have side effects which lakes do not, including the fact that large amounts of dyes ingested can color stools.

Lakes are the combination of dyes and insoluble material. Lakes tint by dispersion. They are not oil soluble, but they are oil dispersible. Lakes are more stable than dyes and are ideal for coloring products containing fats and oils or items lacking sufficient moisture to dissolve dyes. Typical uses include coated tablets, cake and donut mixes, hard candies and chewing gums, lipsticks, soaps, shampoos, and talc.

Other uses

Because food dyes are generally safer to use than normal artistic dyes and pigments, some artists have been using food coloring for painting pictures, especially in forms such as body-painting. Also, food coloring can serve as a means of dyeing fabric. It can be fixed on nylon and animal fibers, but it is not wash-fast when used on cotton, hemp, and other plant fibers.

Flour Treatment Agent

Flour treatment agents are used to help the manageability of dough during baking. These improve flour performance in bread making.

Newly milled flour without further treatment does not make good bread. Flour treatment agents help mature the flour and give a smooth texture. This gives the flour a more uniform appearance and eases the handling of the dough under different conditions like fermentation.

These are used to ensure good loaf volume and improve the crumb structure, softness and color. Vitamin C, or ascorbic acid, is the most commonly used flour treatment agent.

Flour Bleaching Agent

Flour bleaching agent is a food additive added to flour in order to make it appear whiter (freshly milled flour has a yellowish tint) and to oxidize the surfaces of the flour grains and help with developing of gluten.

Usual flour bleaching agents are:

- Organic peroxides (benzoyl peroxide),
- Calcium peroxide,

- Chlorine,
- Chlorine dioxide,
- Azodicarbonamide,
- Nitrogen dioxide and
- Atmospheric oxygen, used during natural aging of flour.

Use of chlorine, bromates, and peroxides is not allowed in the European Union.

Bleached flour improves the structure-forming capacity, allowing the use of dough formulas with lower proportions of flour and higher proportions of sugar. In biscuit making, use of chlorinated flour reduces the spread of the dough, and provides a "tighter" surface. The changes of functional properties of the flour proteins are likely to be caused by their oxidation.

In countries where bleached flour is prohibited, microwaving plain flour produces similar chemical changes to the bleaching process. This improves the final texture of baked goods made to recipes intended for bleached flours.

Oxidizing agents are added to flour to help with gluten development. They may or may not also act as bleaching agents. Originally flour was naturally aged through exposure to the atmosphere. Oxidizing agents primarily affect sulfur-containing amino acids, ultimately helping to form disulfide bridges between the gluten molecules. The addition of these agents to flour will create a stronger dough.

Common oxidizing agents are:

- Ascorbic acid (Ascorbic acid converts into its oxidizing form, dehydroascorbic acid (DHAA) during mixing.),
- Azodicarbonamide,
- Potassium bromate, the component which gives bromated flour its name, used mainly in the U.S. East and Midwest, acts as a bleaching agent, banned in some areas) and
- Potassium iodate.

Reducing agents help to weaken the flour by breaking the protein network. This will help with various aspects of handling a strong dough. The benefits of adding these agents are reduced mixing time, reduced dough elasticity, reduced proofing time, and improved machinability.

Common reducing agents are:

- L-cysteine quantities in the tens of ppm range help soften the dough and thus reduce processing time),

- Fumaric acid,
- Sodium bisulfite and
- Non-leavening yeast (ruptured cells).
- Enzymes are also used to improve processing characteristics. Yeast naturally produces both amylases and proteinases, but additional quantities may be added to produce faster and more complete reactions.
- Amylases break down the starch in flours into simple sugars, thereby letting yeast ferment quickly. Malt is a natural source of amylase.
- Proteases improve extensibility of the dough by degrading some of the gluten.
- Lipoxygenases oxidize the flour.

Other additives may be used as yeast nutrients or as a source of enzymes:

- Carbamide (also known as urea)
- Phosphates
- Malted barley.

Humectant

A humectant is a substance used primarily in foods and cosmetic products to help retain moisture. These substances are called hygroscopic, which means that they are able to absorb ambient water. Some humectant additives are beneficial when consumed or used. Others, particularly in some foods, are less helpful, may cause abdominal distress, and should probably be avoided.

A common humectant in food products is sorbitol. This humectant is a sugar alcohol derived from sugar. It is used in dietetic or sugarless foods and is lower in calories than sugar, so it is a popular choice among those who are dieting. In doses larger than 1.76 ounces (50 g), it has a laxative effect and can cause diarrhea. In the 1990s, consumer advocates successfully lobbied to have product warnings placed on foods containing sorbitol, so people could keep their consumption below harmful levels.

Polydextrose is another humectant food additive used to replace sugar. It is not only found in sweet foods, but frequently in other foods like salad dressings. This particular humectant not only replaces sugar in some foods, but can also be used as a fat or starch replacement. It has a laxative effect as well, so reading the labels of one’s food flavoring can inform selection.

Sugar Substitute

Sugar substitutes are sweeteners that you use instead of regular table sugar (sucrose). Artificial sweeteners are just one type of sugar substitute.

Some manufacturers call their sweeteners “natural” even though they’re processed or refined. Stevia preparations are one example. And some artificial sweeteners are derived from naturally occurring substances — sucralose comes from sugar.

Artificial Sweeteners

Artificial sweeteners are synthetic sugar substitutes. But they may be derived from naturally occurring substances, such as herbs or sugar itself. Artificial sweeteners are also known as intense sweeteners because they are many times sweeter than sugar.

Artificial sweeteners can be attractive alternatives to sugar because they add virtually no calories to your diet. Also, you need only a fraction of artificial sweetener compared with the amount of sugar you would normally use for sweetness.

Uses for Artificial Sweeteners

Artificial sweeteners are widely used in processed foods, including:

- Soft drinks, powdered drink mixes and other beverages
- Baked goods
- Candy
- Puddings
- Canned foods
- Jams and jellies
- Dairy products

Artificial sweeteners are also popular for home use. Some can even be used in baking or cooking.

Certain recipes may need modification because unlike sugar, artificial sweeteners provide no bulk or volume. Check the labels on artificial sweeteners for appropriate home use.

Some artificial sweeteners may leave an aftertaste. A different artificial sweetener or a combination may be more appealing.

Possible Health Benefits of Artificial Sweeteners

Artificial sweeteners don’t contribute to tooth decay and cavities. Artificial sweeteners may also help with:

- Weight control: Artificial sweeteners have virtually no calories. In contrast, a teaspoon of sugar has 6 calories — so a can of sweetened cola with 10 teaspoons of added sugar has 160 calories. If you’re trying to lose weight or prevent weight gain, products sweetened with artificial sweeteners may be an attractive option, although their effectiveness for long-term weight loss isn’t clear.
- Diabetes: Artificial sweeteners aren’t carbohydrates. So unlike sugar, artificial sweeteners generally don’t raise blood sugar levels. If a person has disbetes, he should take doctor’s advice before adding any sugar substitute to his diet.

Possible Health Concerns with Artificial Sweeteners

Artificial sweeteners have been scrutinized intensely for decades.

Critics of artificial sweeteners say that they cause a variety of health problems, including cancer. That’s largely because of studies dating to the 1970s that linked the artificial sweetener saccharin to bladder cancer in laboratory rats. Because of those studies, saccharin once carried a label warning that it may be hazardous to your health.

But according to the National Cancer Institute and other health agencies, there’s no sound scientific evidence that any of the artificial sweeteners approved for use in the United States cause cancer or other serious health problems. Numerous studies confirm that artificial sweeteners are generally safe in limited quantities, even for pregnant women. As a result, the warning label for saccharin was dropped.

Artificial sweeteners are regulated by the Food and Drug Administration (FDA) as food additives. They must be reviewed and approved by the FDA before being made available for sale.

Sometimes the FDA declares a substance “generally recognized as safe” (GRAS). Substances receive this designation if they meet either of these criteria:

- Qualified professionals deem the substance safe for its intended use on the basis of scientific data. Stevia preparations are an example of this type of GRAS designation.
- The substances have such a lengthy history of common use in food that they’re considered generally safe.

The FDA has established an acceptable daily intake (ADI) for each artificial sweetener. ADI is the maximum amount considered safe to consume each day over the course of a lifetime. ADIs are set at very conservative levels.

Novel Sweeteners

Novel sweeteners are hard to fit into a particular category because of what they're made from and how they're made. Stevia is an example. The FDA has approved highly refined stevia preparations as novel sweeteners but hasn't approved whole-leaf stevia or crude stevia extracts for this use.

Tagatose is also considered a novel sweetener because of its chemical structure. Tagatose is a low-carbohydrate sweetener similar to fructose that occurs naturally but is manufactured from the lactose in dairy products. The FDA categorizes tagatose as a GRAS substance.

Sugar Alcohols

Sugar alcohols (polyols) are carbohydrates that occur naturally in certain fruits and vegetables- although they can also be manufactured. Despite their name, sugar alcohols aren't alcoholic because they don't contain ethanol, which is found in alcoholic beverages.

Sugar alcohols aren't considered intense sweeteners because they aren't sweeter than sugar. In fact, some are less sweet than sugar. As with artificial sweeteners, the FDA regulates the use of sugar alcohols.

Sugar alcohols contain calories. But they're lower in calories than sugar, making them an attractive alternative.

Uses for Sugar Alcohols

Sugar alcohols generally aren't used when you prepare food at home. But they're in many processed foods and other products, including chocolate, chewing gum and toothpaste. Sugar alcohols add sweetness, bulk and texture to food, as well as helping food to stay moist.

Sugar alcohols are often combined with artificial sweeteners to enhance sweetness. Food labels may use the general term “sugar alcohol” or list the specific name, such as sorbitol.

Possible Health Benefits of Sugar Alcohols

Like artificial sweeteners, sugar alcohols don't contribute to tooth decay and cavities, and may also help with:

- Weight control: Sugar alcohols contribute calories to your diet — but fewer calories than regular sugar. Sugar alcohols may help weight-control efforts.
- Diabetes: Unlike artificial sweeteners, sugar alcohols are carbohydrates and can raise blood sugar levels. But your body doesn't completely absorb sugar alcohols, so their effect on blood sugar is smaller than that of other sugars. Talk to your doctor or dietitian for guidance because sugar alcohols vary in their effects on blood sugar.

Possible Health Concerns with Sugar Alcohols

When eaten in large amounts, sugar alcohols can have a laxative effect, causing bloating, intestinal gas and diarrhea. Product labels may carry a warning this potential laxative effect.

Natural Sweeteners

Natural sweeteners are sugar substitutes that are often promoted as healthier options than sugar or other sugar substitutes. But even these "natural sweeteners" often undergo processing and refining.

Natural sweeteners that the FDA recognizes as generally safe include:

- Fruit juices and nectars
- Honey
- Molasses
- Maple syrup

Uses for Natural Sweeteners

Natural sweeteners have a variety of uses both at home and in processed foods. They're sometimes known as "added sugars" because they're added to foods during processing.

Possible Health Benefits of Natural Sweeteners

Natural sugar substitutes may seem healthier than sugar. But their vitamin and mineral content isn't significantly different. For example, honey and sugar are nutritionally similar, and your body processes both into glucose and fructose.

Possible Health Concerns with Natural Sweeteners

Natural sweeteners are generally safe. But there's no health advantage to consuming any particular type of added sugar.

Consuming too much added sugar, even natural sweeteners, can lead to health problems, such as tooth decay, weight gain, poor nutrition and increased triglycerides. Honey can contain small amounts of bacterial spores that can produce botulism toxin. Honey shouldn't be given to children younger than than 1 year old.

Examples of Sugar Substitutes

Birch Sugar

Xylitol is a sugar alcohol which is used as a sugar substitute and is better known as birch sugar. Xylitol is a natural constituent of some vegetables and fruits (e.g. cauliflower, berries and plums). Birch sugar can be produced industrially by hydrolyzing xylan (a hemicellulose) into xylose, which is then further processed to obtain xylitol. However, since this process is very expensive, birch sugar is most often produced from corn cobs these days.

- Pros: Birch sugar has 40% fewer calories than refined sugar and does not cause your blood sugar level to rise as high after eating. Plus, birch sugar doesn't cause cavities. The good thing is, this sugar substitute has the same sweetness as regular sugar.
- Cons: Xylitol is not calorie-free and provides 240 calories per 100 g. Eating large quantities of it (20-30 g) can lead to flatulence or severe diarrhea. As there is still not much research on xylitol, the health-promoting aspects of it should be treated with caution.

Honey

Honey is a natural product, contains a lot of valuable nutrients, and is considered one of the the oldest sweeteners in the world. It is produced by bees collecting flower nectar, breaking it down into simple sugars and then storing it in honeycombs.

- Pros: Honey has long been used as a healing agent in natural medicine. It has anti-inflammatory, antibacterial and antiseptic properties. Paired with ginger, honey can be used to fight colds.

- Cons: What you should know: 100 g of blossom honey contains 310 calories. That is almost as much as 100 g of white sugar. Therefore, honey isn't going to save you many calories. Due to the water content of honey, 100 g of honey is not as sweet as 100 g of sugar. In fact, honey is 80% as sweet as sugar. It has a similar impact on blood sugar levels as sugar (sucrose). For diabetics or those trying to manage blood sugar levels, there is no advantage to substituting honey for sugar.

Stevia

Steviol glycoside is a sugar substitute that is extracted from the South American plant species stevia rebaundiana. It has been an approved food additive in the EU since 2011. Before that the sugar substitute was known as a bath additive.

- Pros: Stevia has no calories and does not damage the teeth. It also has no effect on your blood sugar level, so stevia can also be used by diabetics.
- Cons: The stevia added to foods is a chemical extract and thus does not contain any essential nutrients. Stevia may be much sweeter than sugar, but it has a bitter aftertaste.

Agave Nectar

Agave nectar, also known as agave syrup, is produced from the juice of the agave plant found in Mexico. This sweetener tastes like honey but has a thinner consistency.

- Pros: In contrast to ordinary sugar, agave nectar contains metabolites, vitamins and minerals. The sugar substitute is runnier than honey and thus more soluble in foods and drinks. Plus, it is suitable for vegans.

- Cons: Agave nectar has the same number of calories as honey and is thus not much lower in calories than sugar. Due to its high fructose content, the sweetener has a lower glycemic index, but too much fructose intake over a long period of time can be bad for your health: it can elevate your blood fat level, lead to a fatty liver, contribute to insulin resistance, while also raising the risk of metabolic syndrome and type 2 diabetes.

Coconut Sugar

This sugar substitute is made from the nectar of the flower buds of the coconut palm. It tastes a lot like caramel.

- Pros: Coconut sugar is said to contain many vitamins and minerals, including magnesium, iron and zinc. The sweetness of coconut sugar is comparable to ordinary granulated sugar.
- Cons: Coconut sugar has a lower glycemic index than sugar, but this is due to its high fructose content like agave nectar. Despite having a lower glycemic index, it is a fairly pure form of sugar. So, individuals with type 2 diabetes should use it sparingly. Plus, this sugar substitute costs 20 euros per kilo, which isn't exactly cheap. But this isn't surprising when you consider that it is extracted by hand. Thus, this sugar substitute has to be classified as a luxury good.

Maple Syrup

Extracted from the sugar maple tree, maple syrup (also known as "liquid gold") is delicious on pancakes and also a good alternative to sugar in tea.

- Pros: Maple syrup is a natural product: unlike ordinary sugar, it contains more than 50 beneficial compounds including antioxidants and a molecule with anti-inflammatory properties. The darker the syrup, the higher the concentration of these compounds. Plus, 100 g of maple syrup has 90 mg of calcium, 185 mg of potassium, 25 mg of magnesium, and 2 mg of iron. However, the question remains how much of these benefits are actually derived when the syrup is consumed in the recommended (small) amounts.
- Cons: The sweetness of maple syrup (100 g has 260 cal) is 60 to 70% lower than refined sugar. So you need more maple syrup to achieve the sweetness of sugar. Pay attention to the purity and the ingredients when you buy it. Maple syrup (not a protected name in Europe) often contains sugar water – not recommended for a sugar-free diet. The ingredients of syrups, such as maple syrup, often contain various proportions of glucose and fructose depending on the producer. That's why diabetics need to be cautious using this syrup – as it can cause the same response as plain white sugar, depending on the amount and the quality.

Thickening Agent

Thickening agents, or thickeners, are substances which, when added to an aqueous mixture, increase its viscosity without substantially modifying its other properties, such as taste. They provide body, increase stability, and improve suspension of added ingredients.

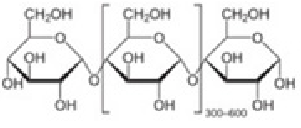
Examples of thickening agents include: polysaccharides (starches, vegetable gums, and pectin), proteins (eggs, collagen, gelatin, blood albumin) and fats (butter, oil and lards).

All purpose flour is the most popular food thickener, followed by cornstarch and arrowroot or tapioca. All of these thickeners are based on starch as the thickening agent.

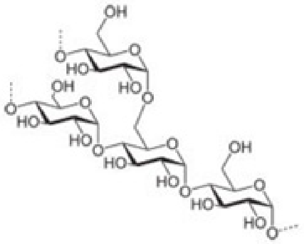
Starch

Starch or amylum is a carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. This polysaccharide is produced by all green plants as an energy store. It is the most important carbohydrate in the human diet and is contained in such staple foods as potatoes, wheat, maize (corn), rice, and cassava.

Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. 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.



Structure of the amylose molecule.



Structure of the amylopectin molecule.

Starches thicken by a process called gelatinization. Starch gelatinization is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water. This irreversibly dissolves the starch granule. Penetration of water increases randomness in the general granule structure and decreases the number and size of crystalline regions. Crystalline regions do not allow water entry. Heat causes such regions to be diffused, so that the chains begin to separate into an amorphous form. Some type of unmodified native starches start swelling at 55 °C, other types at 85 °C.

Gelatinization is also known as the thickening of a liquid. The starch grains/flour granules absorb the liquid. When heated the grains/granules swelling and then burst, releasing starch into the liquid. The granules/grains swell to 30 times their original size (swelling power, peak viscosity).

Different Types of Starches

1. Cornstarch - Cornstarch is actually a flour. It is the endosperm of corn kernels that has been dried and ground. Corn starch is used as a thickening agent in soups and liquid-based foods, such as sauces, gravies and custard. It is sometimes preferred over flour because it forms a translucent mixture, rather than an opaque one. As the starch is heated, the molecular chains unravel, allowing them to collide with other starch chains to form a mesh, thickening the liquid. It is usually included as an anti-caking agent in powdered sugar (10X or confectioner's sugar). For this reason, recipes calling for powdered sugar often call for at least light cooking to remove the raw corn starch taste. When using corn starch, first mix it with cold water (or another liquid) until it forms a smooth paste, and then add it to whatever is being thickened. If it is added directly into the cooking food it will form lumps that are then difficult to mash out for a smooth mixture. An easy way to make certain that all the lumps are gone from the corn starch/water mixture is to put the two into a jar with a screw on lid and vigorously shake the sealed jar until the lumps are gone. This also works with a flour/water mixture. This method also allows for better portion control when slowly adding it to a soup, sauce, or gravy.

2. Potato starch - Potato starch is not potato flour. Potato flour is dehydrated potatoes ground into powder and is much heavier and denser. Potato starch is the result of an extraction process removing the starch only from the potato. Potato starch has no gluten. Like cornstarch, you have to dissolve the potato starch in a little bit of water before adding it as a thickener. Many people especially prefer starch made from potatoes or corn when thickening sauces because it can help the sauces remain translucent; whereas flour creates a more cloudied appearing sauce. Potato starch is used to thicken soups and gravies. Liquids thickened with potato starch should never be boiled. It is used in recipes for those who are gluten intolerant. It is naturally gluten free. It is similar in use to Arrowroot and corn starch. Its main advantage over other starch thickeners is that it's a permitted ingredient for Passover, unlike cornstarch and other grain-based foods.

3. Tapioca -Tapioca is a starch extracted from the ground, dried root of the cassava plant, which grows in the tropics. Tapioca does not lose it's quality even on reheating and freezing. When tapioca starch is used as a thickening agent, it becomes clear and completely dissolves. The bad thing tapioca starch is that it breaks down a little quicker than corn starch, especially when you're on a very high temperature.

4. Arrowroot -Arrowroot is like other pure starches, however, arrowroot is almost pure carbohydrate and devoid of protein, thus it does not equal wheat flour nutritionally.

Arrowroot thickens at a lower temperature than does flour or cornstarch, is not weakened by acidic ingredients, has a more neutral taste, and is not affected by freezing. It doesn't mix well with dairy, forming a slimy mixture. For acidic foods, arrowroot is a better choice than cornstarch, which loses thickening potency in acidic mixtures.

It is recommended to mix arrowroot with a cool liquid before adding to a hot fluid. The mixture should be heated only until the mixture thickens and removed immediately to prevent the mixture from thinning. Overheating tends to break down arrowroot's thickening property. Substitute two teaspoons of arrowroot for one tablespoon of cornstarch, or one teaspoon of arrowroot for one tablespoon of wheat flour. The lack of gluten in arrowroot flour makes it useful as a replacement for wheat flour in baking.

5. Rice flour (also rice powder) - Rice flour is a form of flour made from finely milled rice. It is distinct from rice starch, which is usually produced by steeping rice in lye. In Japanese, rice flour is called either komeko or mochiko. Rice flour is a particularly good substitute for wheat flour, which causes irritation in the digestive systems of those who are gluten-intolerant. Rice flour is also used as a thickening agent in recipes that are refrigerated or frozen since it inhibits liquid separation.

6. All purpose flour - Flour is often used for thickening gravies, gumbos, and stews. It must be cooked thoroughly to avoid the taste of uncooked flour. Roux, a mixture of flour and fat (usually butter) cooked into a paste, is used for gravies, sauces and stews. Cornstarch and arrowroot will thicken more efficiently than flour since they contain no protein. They have 50 to 100% more thickening power than flour and thus, less of them

is needed. They also thicken at a somewhat lower temperature and do not need to be precooked, like roux. However, they do need to be dissolved in fluid first.

7. Kuzu (kudzo or japanese arrowroot) - Kuzu is a very high quality starch thickener with a smooth texture and neutral flavour. It is from the root of the Kudzo that is naturally extracted from the roots of the Kuzu plant (Pueraria montana) one of Japan's most vigorous mountain plants. In Japan, the plant is known as kuzu and the starch named kuzuko. Health benefits of Kuzu root --Kudzu contains a number of useful isoflavones, including puerarin, 60% of the total isoflavones, and also daidzein (an anti-inflammatory and antimicrobial agent) and daidzin (structurally related to genistein).

A half to one tablespoon of kuzu will thicken one cup of liquid (1 ¾ tbsp. of Kuzu can replace 1 tbsp of corn flour). Kuzu comes a solid and needs to be dissolved in a cold liquid before adding it to anything hot. Stir constantly when heating until the milky white becomes clear. Kuzu is Fat and sodium free. Kuzu although often refered to as japanese arrowroot is very different. Kuzu is far superior in jelling strength, taste, texture, and healing qualities. Chef David Bouley frequently uses Kuzu in place of other thickeners in many of his dishes.

Other Polysaccharide Thickeners

Polysaccharides as a thickener food includes the starches, vegetable gums and pectin. Food starch is a flavorless powder in which comes the cornstarch, potato starch, katakuri starch.

Polysaccharides are polymeric carbohydrate structures, formed of repeating units either mono- saccharides(e.g., glucose , fructose , galactose) or di-saccharides (e.g., sucrose, lactose) joined together by glycosidic bonds.

Natural/Vegetable Gums

Vegetable Gums are all polysaccharides of natural origin, capable of causing a large viscosity increase in solution, even at small concentrations. In the food industry they are used as thickening agents, gelling agents, emulsifying agents, and stabilizers.

1. Agar - Agar or agar-agar is a gelatinous substance derived from a polysaccharide that accumulates in the cell walls of agarophyte red algae. Historically and in a modern context, it is chiefly used as an ingredient in desserts throughout Asia and also as a solid substrate to contain culture medium for microbiological work. The gelling agent is an unbranched polysaccharide obtained from the cell walls of some species of red algae, primarily from the genera Gelidium and Gracilaria, or seaweed (Sphaerococcus euchema). Chemically, agar is a polymer made up of subunits of the sugar galactose.

2. Guar gum - Chemically, guar gum is a polysaccharide composed of the sugars galactose and mannose. The backbone is a linear chain of ß 1,4-linked mannose residues to which galactose residues are 1,6-linked at every second mannose, forming short side-branches. Guar gum is economical because it has almost 8 times the water-thickening potency of

cornstarch - only a very small quantity is needed for producing sufficient viscosity. Thus it can be used in various multi-phase formulations: as an emulsifier because it helps to prevent oil droplets from coalescing, and as a stabilizer because it helps to prevent solid particles from settling. . At (acidic) pH levels below 4.5, guar gum has sharply reduced aqueous solubility, thus also reducing its thickening capability.

3. Xanthan gum - Xanthan gum is a polysaccharide, derived from the bacterial coat of Xanthomonas commonly used as a food thickening agent (in salad dressings, for example). Xanthan gum is a long chain polysaccharide composed of the sugars glucose, mannose, and glucuronic acid. The backbone is similar to cellulose, with added sidechains of trisacharides (three sugars in a chain). One of the most remarkable properties of xanthan gum is its ability to produce a large increase in the viscosity of a liquid by adding a very small quantity of gum, on the order of one percent. In most foods, it is used at 0.5%, and can be used in lower concentrations. In foods, xanthan gum is most often found in salad dressings and sauces. It helps to prevent oil separation by stabilizing the emulsion, although it is not an emulsifier. Xanthan gum also helps suspend solid particles, such as spices. Also used in frozen foods and beverages, xanthan gum helps create the pleasant texture in many ice creams, along with guar gum and locust bean gum.

Pectin Pectin is a kind of polysaccharide (Polymer of D-Galacturonic Acid) that is obtained from plant such as citrus fruit peel, apple peel etc. Pectin is a vegetable gum and food thickener that is used to make gel. In human digestion, pectin goes through the small intestine more or less intact. Pectin is thus a soluble dietary fiber. The main use for pectin is as a gelling agent, thickening agent and stabilizer in food. Pectin does not add any flavor to a dishes but it does work extremely well as a thickening agent.

Proteins as Thickening Agents

1. Egg yolks - Egg yolks are the most efficient protein thickeners in part because they are so concentrated with protein, have a rich flavor and offer a velvety smooth texture. The difficulty in using egg yolks is the small window of temperature needed to thicken the sauce or soup but not allow the egg to set.

2. Collagen - A protein found in nearly all connective tissue, when cooked it will dissolve and thicken sauces.

3. Gelatin - A protein produced by partial hydrolysis of collagen extracted from the boiled bones, connective tissues, organs and some intestines of animals.

4. Yogurt - Yogurt is popular in Eastern Europe and Middle East for thickening soups.

Fats as Thickening Agents

Butter is the best thickener to use for wine and stock based sauces. Make sure that the butter is whisked in off the heat or the emulsion can separate.

Thickening by Reduction

Reduction: The most flavorful way to thicken a sauce is by reduction. Reduce the moisture content of a sauce by simmering over low heat and letting evaporation take over. As the water evaporates the remaining ingredients become more concentrated. Stocks made using meat bones and/or vegetables take on a thicker appearance. Adding fats toward the end of the reduction process can complete the thickening process. After a reduction to a syrup consistency butter as softened cubes can be whisked in either on a very low flame or off the heat. Too much heat can cause the emulsion to break.

Pureed Vegetables and Tomato Paste as a Thickener

Adding some pureed vegetables or tomato paste is another ideal way to thicken a soup or sauce. This can add both texture and additional flavor.

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Chapter 6

Allied Aspects of Food Science and Nutrition

Food science and nutrition deal with a lot of processes which are associated with food such as food safety, food preservation and food fortification. It also includes sub-disciplines like food chemistry, food biotechnology and food microbiology. This chapter discusses in detail these processes and disciplines related to food science and nutrition.

Food Safety

Food Safety refers to handling, preparing and storing food in a way to best reduce the risk individuals becoming sick from foodborne illnesses.

Food Safety refers to handling, preparing and storing food in a way to best reduce the risk of individuals becoming sick from foodborne illnesses.

Food safety is a global concern that covers a variety of different areas of everyday life.

The principles of food safety aim to prevent food from becoming contaminated and causing food poisoning. This is achieved through a variety of different avenues, some of which are:

- Properly cleaning and sanitising all surfaces, equipment and utensils.
- Maintaining a high level of personal hygiene, especially hand-washing.
- Storing, chilling and heating food correctly with regards to temperature, environment and equipment.
- Implementing effective pest control.
- Comprehending food allergies, food poisoning and food intolerance.

Importance of Food Safety

Food safety (hygiene) isn't just something for restaurants to worry about. It's important to know how to prepare food safely and hygienically at home too.

There are four important elements to good food hygiene:

- Cleaning,
- Storing,
- Preparing,
- Cooking.

Food safety is important for the following reasons:

- Every day people get ill from the food they eat. Bacteria, viruses and parasites found in food can cause food poisoning.
- Often, there's no way of telling if food is contaminated because it might not look, taste or smell any different from normal.
- Food poisoning can lead to gastroenteritis and dehydration or potentially even more serious health problems such as kidney failure.
- Food poisoning can be serious in babies, children, older people and pregnant women because they have a weaker immune system.

Food Preservation

Food preservation is any of a number of methods by which food is kept from spoilage after harvest or slaughter. Such practices date to prehistoric times. Among the oldest methods of preservation are drying, refrigeration, and fermentation. Modern methods include canning, pasteurization, freezing, irradiation, and the addition of chemicals. Advances in packaging materials have played an important role in modern food preservation.

Spoilage Mechanisms

Food spoilage may be defined as any change that renders food unfit for human consumption. These changes may be caused by various factors, including contamination by microorganisms, infestation by insects, or degradation by endogenous enzymes (those present naturally in the food). In addition, physical and chemical changes, such as the tearing of plant or animal tissues or the oxidation of certain constituents of food, may promote food spoilage. Foods obtained from plant or animal sources begin to spoil soon after harvest or slaughter. The enzymes contained in the cells of plant and animal tissues may be released as a result of any mechanical damage inflicted during postharvest handling. These enzymes begin to break down the cellular material. The chemical reactions catalyzed by the enzymes result in the degradation of food quality, such as the development of off-flavours, the deterioration of texture, and the loss of nutrients. The typical microorganisms that cause food spoilage are bacteria (e.g., Lactobacillus), yeasts (e.g., Saccharomyces), and molds (e.g., Rhizopus).

Microbial Contamination

Bacteria and fungi (yeasts and molds) are the principal types of microorganisms that cause food spoilage and food-borne illnesses. Foods may be contaminated by microorganisms at any time during harvest, storage, processing, distribution, handling, or preparation. The primary sources of microbial contamination are soil, air, animal feed, animal hides and intestines, plant surfaces, sewage, and food processing machinery or utensils.

Bacteria

Bacteria are unicellular organisms that have a simple internal structure compared with the cells of other organisms. The increase in the number of bacteria in a population is commonly referred to as bacterial growth by microbiologists. This growth is the result of the division of one bacterial cell into two identical bacterial cells, a process called binary fission. Under optimal growth conditions, a bacterial cell may divide approximately every 20 minutes. Thus, a single cell can produce almost 70 billion cells in 12 hours. The factors that influence the growth of bacteria include nutrient availability, moisture, pH, oxygen levels, and the presence or absence of inhibiting substances (e.g., antibiotics).

The nutritional requirements of most bacteria are chemical elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, magnesium, potassium, sodium, calcium, and iron. The bacteria obtain these elements by utilizing gases in the atmosphere and by metabolizing certain food constituents such as carbohydrates and proteins.

Temperature and pH play a significant role in controlling the growth rates of bacteria. Bacteria may be classified into three groups based on their temperature requirement for optimal growth: thermophiles (55–75 °C, or 130–170 °F), mesophiles (20–45 °C, or 70–115 °F), or psychrotrophs (10–20 °C, or 50–70 °F). In addition, most bacteria grow best in a neutral environment (pH equal to 7).

Bacteria also require a certain amount of available water for their growth. The availability of water is expressed as water activity and is defined by the ratio of the vapour pressure of water in the food to the vapour pressure of pure water at a specific temperature. Therefore, the water activity of any food product is always a value between 0 and 1, with 0 representing an absence of water and 1 representing pure water. Most bacteria do not grow in foods with a water activity below 0.91, although some halophilic bacteria (those able to tolerate high salt concentrations) can grow in foods with a water activity lower than 0.75. Growth may be controlled by lowering the water activity—either by adding solutes such as sugar, glycerol, and salt or by removing water through dehydration.

The oxygen requirements for optimal growth vary considerably for different bacteria. Some bacteria require the presence of free oxygen for growth and are called obligate aerobes, whereas other bacteria are poisoned by the presence of oxygen and are called obligate anaerobes. Facultative anaerobes are bacteria that can grow in either the presence or absence of oxygen. In addition to oxygen concentration, the oxygen reduction potential of the growth medium influences bacterial growth. The oxygen reduction potential is a relative measure of the oxidizing or reducing capacity of the growth medium.

When bacteria contaminate a food substrate, it takes some time before they start growing. This lag phase is the period when the bacteria are adjusting to the environment. Following the lag phase is the log phase, in which population grows in a logarithmic fashion. As the population grows, the bacteria consume available nutrients and produce waste products. When the nutrient supply is depleted, the growth rate enters a stationary phase in which the number of viable bacteria cells remains the same. During the stationary phase, the rate of bacterial cell growth is equal to the rate of bacterial cell death. When the rate of cell death becomes greater than the rate of cell growth, the population enters the decline phase.

A bacterial population is expressed either per gram or per square centimetre of surface area. Rarely does the total bacterial population exceed 1010 cells per gram. A population of less than 106 cells per gram does not cause any noticeable spoilage except in raw milk. Populations of between 106 and 107 cells per gram cause spoilage in some foods; for example, they can generate off-odours in vacuum-packaged meats. Populations of between 107 and 108 cells per gram produce off-odours in meats and some vegetables. At levels above 5 × 107 cells per gram, most foods exhibit some form of spoilage.

When the conditions for bacterial cell growth are unfavourable (e.g., low or high temperatures or low moisture content), several species of bacteria can produce resistant cells called endospores. Endospores are highly resistant to heat, chemicals, desiccation (drying out), and ultraviolet light. The endospores may remain dormant for long periods of time. When conditions become favourable for growth (e.g., thawing of meats), the endospores germinate and produce viable cells that can begin exponential growth.

Fungi

The two types of fungi that are important in food spoilage are yeasts and molds. Molds are multicellular fungi that reproduce by the formation of spores (single cells that can grow into a mature fungus). Spores are formed in large numbers and are easily dispersed through the air. Once these spores land on a food substrate, they can grow and reproduce if conditions are favourable. Yeasts are unicellular fungi that are much larger than bacterial cells. They reproduce by cell division (binary fission) or budding.

The conditions affecting the growth of fungi are similar to those affecting bacteria. Both yeasts and molds are able to grow in an acidic environment (pH less than 7). The pH range for yeast growth is 3.5 to 4.5 and for molds is 3.5 to 8.0. The low pH of fruits is generally unfavourable for the growth of bacteria, but yeasts and molds can grow and cause spoilage in fruits. For example, species of the fungal genus Colletotrichum cause crown rot in bananas. Yeasts promote fermentation in fruits by breaking down sugars into alcohol and carbon dioxide. The amount of available water in a food product is also critical for the growth of fungi. Yeasts are unable to grow at a water activity of less than 0.9, and molds are unable to grow at a water activity below 0.8.

Control of Microbial Contamination

The most common methods used either to kill or to reduce the growth of microorganisms are the application of heat, the removal of water, the lowering of temperature during storage, the reduction of pH, the control of oxygen and carbon dioxide concentrations, and the removal of the nutrients needed for growth.

Chemical Deterioration

Enzymatic Reactions

Enzymes are large protein molecules that act as biological catalysts, accelerating chemical reactions without being consumed to any appreciable extent themselves. The activity of enzymes is specific for a certain set of chemical substrates, and it is dependent on both pH and temperature.

The living tissues of plants and animals maintain a balance of enzymatic activity. This balance is disrupted upon harvest or slaughter. In some cases, enzymes that play a useful role in living tissues may catalyze spoilage reactions following harvest or slaughter. For example, the enzyme pepsin is found in the stomach of all animals and is involved in the breakdown of proteins during the normal digestion process. However, soon after the slaughter of an animal, pepsin begins to break down the proteins of the organs, weakening the tissues and making them more susceptible to microbial contamination. After the harvesting of fruits, certain enzymes remain active within the cells of the plant tissues. These enzymes continue to catalyze the biochemical processes of ripening and may eventually lead to rotting, as can be observed in bananas. In addition, oxidative enzymes in fruits continue to carry out cellular respiration (the process of using oxygen to metabolize glucose for energy). This continued respiration decreases the shelf life of fresh fruits and may lead to spoilage. Respiration may be controlled by refrigerated storage or modified-atmosphere packaging. Table lists a number of enzymes involved in the degradation of food quality.

Enzymes that Cause Food Spoilage

Enzyme	Food	Spoilage action
Ascorbic Acid Oxidase	Vegetables	Destruction Of Vitamin C
Lipase	Cereals	Discoloration
	Milk	Hydrolytic Rancidity
	Oils	Hydrolytic Rancidity
Lipoxygenase	Vegetables	Destruction Of Vitamin A, Off-Flavour
Pectic Enzyme	Citrus Juices	Destruction Of Pectic Substances
	Fruits	Excessive Softening
Peroxidase	Fruits	Browning
Polyphenoloxidase	Fruits, Vegetables	Browning, Off-Flavour, Vitamin Loss
Protease	Eggs	Reduction Of Shelf Life Of Fresh And Dried Whole Eggs
	Crab, Lobster	Overtenderization
	Flour	Reduction Of Gluten Formation
Thiaminase	Meats, Fish	Destruction Of Thiamine

Autoxidation

The unsaturated fatty acids present in the lipids of many foods are susceptible to chemical breakdown when exposed to oxygen. The oxidation of unsaturated fatty acids is autocatalytic; that is, it proceeds by a free-radical chain reaction. Free radicals contain an unpaired electron (represented by a dot in the molecular formula) and, therefore, are highly reactive chemical molecules. The basic mechanisms in a free-radical chain reaction involve initiation, propagation, and termination steps. Under certain conditions, in initiation a free-radical molecule (X·) present in the food removes a hydrogen (H) atom from a lipid molecule, producing a lipid radical (L.). This lipid radical reacts with molecular oxygen (O_2) to form a peroxy radical (LOO·). The peroxy radical removes a hydrogen atom from another lipid molecule and the reaction starts over again (propagation). During the propagation steps, hydroperoxide molecules (LOOH) are formed that may break down into alkoxy (LO·) and peroxy radicals plus water (H_2O).

The lipid, alkoxy, and peroxy radicals may combine with one another (or other radicals) to form stable, nonpropagating products (termination). These products result in the development of rancid off-flavours. In addition to promoting rancidity, the free radicals and peroxides produced in these reactions may have other negative effects, such as the bleaching of food colour and the destruction of vitamins A, C, and E. This type of deterioration is prevalent in fried snacks, nuts, cooking oils, and margarine.

Initiation: $X\cdot + LH \rightarrow L\cdot + XH$

$L\cdot + O_2 \rightarrow LOO\cdot$

Propagation: $LOO\cdot + LH \rightarrow LOOH + L\cdot$

$2LOOH \rightarrow LO\cdot + LOO\cdot + H_2O$

Termination: $L\cdot, LO\cdot, LOO\cdot \rightarrow$ a number of stable nonpropgating species

Maillard Reaction

Another chemical reaction that causes major food spoilage is nonenzymatic browning, also known as the Maillard reaction. This reaction takes place between reducing sugars (simple monosaccharides capable of carrying out reduction reactions) and the amino group of proteins or amino acids present in foods. The products of the Maillard reaction lead to a darkening of colour, reduced solubility of proteins, development of bitter flavours, and reduced nutritional availability of certain amino acids such as lysine. The rate of this reaction is influenced by the water activity, temperature, and pH of the food product. Non-enzymatic browning causes spoilage during the storage of dry milk, dry whole eggs, and breakfast cereals.

Light-induced Reactions

Light influences a number of chemical reactions that lead to spoilage of foods. These light-induced reactions include the destruction of chlorophyll (the photosynthetic pigment that gives plants their green colour), resulting in the bleaching of certain vegetables; the discoloration of fresh meats; the destruction of riboflavin in milk; and the oxidation of vitamin C and carotenoid pigments (a process called photosensitized oxidation). The use of packaging material that prevents exposure to light is one of the most effective means of preventing light-induced chemical spoilage.

Low Temperature Preservation

Storage at low temperatures prolongs the shelf life of many foods. In general, low temperatures reduce the growth rates of microorganisms and slow many of the physical and chemical reactions that occur in foods.

Refrigeration

The life of many foods may be increased by storage at temperatures below 4 °C (40 °F). Commonly refrigerated foods include fresh fruits and vegetables, eggs, dairy products, and meats. Some foods, such as tropical fruits (e.g., bananas), are damaged if exposed to low temperatures. Also, refrigeration cannot improve the quality of decayed food; it can only retard deterioration. One problem of modern mechanical refrigeration—that of dehydration of foods due to moisture condensation—has been overcome through humidity control mechanisms within the storage chamber and by appropriate packaging techniques.

Freezing

Freezing and frozen storage provide an excellent means of preserving the nutritional quality of foods. At subfreezing temperatures the nutrient loss is extremely slow for the typical storage period used in commercial trade.

The Freezing Process

The freezing of food involves lowering its temperature below 0 °C, resulting in the gradual conversion of water, present in the food, into ice. Freezing is a crystallization process that begins with a nucleus or a seed derived from either a non-aqueous particle or a cluster of water molecules (formed when the temperature is reduced below 0 °C). This seed must be of a certain size to provide an adequate site for the crystal to begin to grow. If physical conditions are conducive to the presence of numerous seeds for crystallization, then a large number of small ice crystals will form. However, if only a few seeds are initially available, then a few ice crystals will form and each will grow to a large size. The size and the number of ice crystals influence the final quality of many frozen foods; for example, the smooth texture of ice cream indicates the presence of a large number of small ice crystals.

In pure water, the freezing process is initiated by lowering the temperature to slightly below 0 °C, called supercooling. As ice crystals begin to grow, the temperature returns to the freezing point. During the conversion of liquid water to ice, the temperature of the system does not change. The heat removed during this step is called the latent heat of fusion (equivalent to 333 joules per gram of water). Once all the water is converted to ice, any additional removal of heat will result in a decrease in the temperature below 0 °C.

The freezing of foods exhibits a number of important differences from the freezing of pure water. Foods do not freeze at 0 °C. Instead, owing to the presence of different soluble particulates (solutes) in the water present in foods, most foods begin to freeze at a temperature between 0 and −5 °C (32 and 23 °F). In addition, the removal of latent heat in foods during freezing does not occur at a fixed temperature. As the water present in the food freezes into ice, the remaining water becomes more concentrated with solutes. As a result, the freezing point is further depressed. Therefore, foods have a zone of maximum ice crystal formation that typically extends from −1 to −4 °C (30 to 25 °F). Damage to food quality during freezing can be minimized if the temperature of the product is brought below this temperature range as quickly as possible.

Industrial Freezers

The rate at which heat is removed from a food during freezing depends on how fast heat can travel within the food and how efficiently it can be liberated from the surface of the food into the surrounding atmosphere. Industrial freezers remove heat from the surface of a food as rapidly as possible. There are several types of industrial freezers, including air-blast tunnel freezers, belt freezers, fluidized-bed freezers, plate freezers, and cryogenic freezers.

In air-blast tunnel freezers and belt freezers, precooled air at approximately −40 °C is blown over the food products. Packaged foods, such as fruits, vegetables, bakery goods, poultry, meats, and prepared meals, are usually frozen in air-blast tunnels. The packages are placed onto dollies or hand trucks and then rolled into the freezer tunnels. In a belt freezer, food is placed on a conveyor belt that moves through a freezing zone. Bakery goods, chicken parts, and meat patties are frozen using a belt freezer.

Fluidized-bed freezers are used to freeze particulate foods such as peas, cut corn, diced carrots, and strawberries. The foods are placed on a mesh conveyor belt and moved through a freezing zone in which cold air is directed upward through the mesh belt and the food particulates begin to tumble and float. This tumbling exposes all sides of the food to the cold air and minimizes the resistance to heat transfer at the surface of the food.

Plate freezers are used to freeze flat products, such as pastries, fish fillets, and beef patties, as well as irregular-shaped vegetables that are packaged in brick-shaped containers, such as asparagus, cauliflower, spinach, and broccoli. The food is firmly pressed between metal plates that are cooled to subfreezing temperatures by internally circulating refrigerants.

Cryogenic freezing is used to freeze food at an extremely fast rate. The food is moved through a spray of liquid nitrogen or directly immersed in liquid nitrogen. The liquid nitrogen boils around the food at a temperature of −196 °C (−321 °F) and extracts a large amount of heat.

Quality of Frozen Foods

Improper freezing or storage of foods may result in detrimental quality changes. When foods with high amounts of water are frozen slowly, they may experience a loss of fluid, called drip, upon thawing. This fluid loss causes dehydration and nutrient loss in frozen food products.

During frozen storage, the ice crystals present in foods may enlarge in size, producing undesirable changes in texture. This phenomenon is commonly observed when the storage temperature is allowed to fluctuate. For example, ice cream stored in an automatic defrosting domestic freezer becomes sandy in texture because the ice crystals increase in size as the temperature of the system fluctuates.

Improperly packaged frozen foods lose small amounts of moisture during storage, resulting in surface dehydration (commonly called freezer burn). Frozen meats with freezer burn have the appearance of brown paper and quickly become rancid. Freezer burn can be minimized by the use of tightly wrapped packages and the elimination of fluctuating temperatures during storage.

Thermal Processing

Thermal processing is defined as the combination of temperature and time required to eliminate a desired number of microorganisms from a food product.

Presterilization Procedures

Selected crop varieties are grown specially for canning purposes. The harvesting schedules of the crops are carefully selected to conform to the cannery operations. A typical canning operation involves cleaning, filling, exhausting, can sealing, heat processing, cooking, labeling, casing, and storage. Most of these operations are performed using high-speed, automatic machines.

Cleaning involves the use of shakers, rotary reel cleaners, air blasters, water sprayers (as shown in Figure below), or immersion washers. Any inedible or extraneous material is removed before washing, and only potable water is used in the cleaning systems.

Spray washing of harvested tomatoes prior to processing.

Automatic filling machines are used to place the cleaned food into cans or other containers, such as glass jars or plastic pouches. When foods containing trapped air, such as leafy vegetables, are canned, the air must be removed from the cans prior to closing and sealing the lids by a process called exhausting. Exhausting is accomplished using steam exhaust hoods or by creation of a vacuum.

Immediately after exhausting, the lids are placed on the cans and the cans are sealed. An airtight seal is achieved between the lid and the rim of the can using a thin layer of gasket or compound. The anaerobic conditions prevent the growth of oxygen-requiring microorganisms. In addition, many of the spores of anaerobic microorganisms are less resistant to heat and are easily destroyed during the heat treatment.

Sterilization

The time and temperature required for the sterilization of foods are influenced by several factors, including the type of microorganisms found on the food, the size of the container, the acidity or pH of the food, and the method of heating.

The thermal processes of canning are generally designed to destroy the spores of the bacterium C. botulinum. This microorganism can easily grow under anaerobic conditions, producing the deadly toxin that causes botulism. Sterilization requires heating to temperatures greater than 100 °C (212 °F). However, C. botulinum is not viable in acidic foods that have a pH less than 4.6. These foods can be adequately processed by immersion in water at temperatures just below 100 °C.

The sterilization of low-acid foods (pH greater than 4.6) is generally carried out in steam vessels called retorts at temperatures ranging from 116 to 129 °C (240 to 265 °F). The retorts are controlled by automatic devices, and detailed records are kept of the time and temperature treatments for each lot of processed cans. At the end of the heating cycle, the cans are cooled under water sprays or in water baths to approximately 38 °C (100 °F) and dried to prevent any surface rusting. The cans are then labeled, placed in fibreboard cases either by hand or machine, and stored in cool, dry warehouses.

Quality of Canned Foods

The sterilization process is designed to provide the required heat treatment to the slowest heating location inside the can, called the cold spot. The areas of food farthest from the cold spot get a more severe heat treatment that may result in overprocessing and impairment of the overall quality of the product. Flat, laminated pouches can reduce the heat damage caused by overprocessing.

A significant loss of nutrients, especially heat-labile vitamins, may occur during the canning process. In general, canning has no major effect on the carbohydrate, protein, or fat content of foods. Vitamins A and D and beta-carotene are resistant to the effects of heat. However, vitamin B1 is sensitive to thermal treatment and the pH of the food. Although the anaerobic conditions of canned foods have a protective effect on the stability of vitamin C, it is destroyed during long heat treatments.

The ends of processed cans are slightly concave because of the internal vacuum created during sealing. Any bulging of the ends of a can may indicate a deterioration in quality due to mechanical, chemical, or physical factors. This bulging may lead to swelling and possible explosion of the can.

Pasteurization

Pasteurization is the application of heat to a food product in order to destroy pathogenic (disease-producing) microorganisms, to inactivate spoilage-causing enzymes, and to reduce or destroy spoilage microorganisms. The relatively mild heat treatment used in the pasteurization process causes minimal changes in the sensory and nutritional characteristics of foods compared to the severe heat treatments used in the sterilization process.

The temperature and time requirements of the pasteurization process are influenced by the pH of the food. When the pH is below 4.5, spoilage microorganisms and enzymes are the main targets of pasteurization. For example, the pasteurization process for fruit juices is aimed at inactivating certain enzymes such as pectinesterase and polygalacturonase. The typical processing conditions for the pasteurization of fruit juices include heating to 77 °C (171 °F) and holding for 1 minute, followed by rapid cooling to 7 °C (45 °F). In addition to inactivating enzymes, these conditions destroy any yeasts or molds that may lead to spoilage. Equivalent conditions capable of reducing spoilage microorganisms involve heating to 65 °C (149 °F) and holding for 30 minutes or heating to 88 °C (190 °F) and holding for 15 seconds.

When the pH of a food is greater than 4.5, the heat treatment must be severe enough to destroy pathogenic bacteria. In the pasteurization of milk, the time and temperature conditions target the pathogenic bacteria Mycobacterium tuberculosis, Coxiella burnetti, and Brucella abortus. The typical heat treatment used for pasteurizing milk is 72 °C (162 °F) for 15 seconds, followed by rapid cooling to 7 °C. Other equivalent heat treatments include heating to 63 °C (145 °F) for 30 minutes, 90 °C (194 °F) for 0.5 second, and 94 °C (201 °F) for 0.1 second. The high-temperature–short-time (HTST) treatments cause less damage to the nutrient composition and sensory characteristics of foods and therefore are preferred over the low-temperature–long-time (LTLT) treatments.

Since the heat treatment of pasteurization is not severe enough to render a product sterile, additional method such as refrigeration, fermentation, or the addition of chemicals are often used to control microbial growth and to extend the shelf life of a product. For example, the pasteurization of milk does not kill thermoduric bacteria (those resistant to heat), such as Lactobacillus and Streptococcus, or thermophilic bacteria (those that grow at high temperatures), such as Bacillus and Clostridium. Therefore, pasteurized milk must be kept under refrigerated conditions.

Liquid foods such as milk, fruit juices, beers, wines, and liquid eggs are pasteurized using plate-type heat exchangers. Wine and fruit juices are normally deaerated prior to pasteurization in order to remove oxygen and minimize oxidative deterioration of the products. Plate-type heat exchangers consist of a large number of thin, vertical steel plates that are clamped together in a frame. The plates are separated by small gaskets that allow the liquid to flow between each successive plate. The liquid product and heating medium (e.g., hot water) are pumped through alternate channels, and the gaskets ensure that the liquid product and heating or cooling mediums are kept separate. Plate-type heat exchangers are effective in rapid heating and cooling applications. After the pasteurization process is completed, the product is packaged under aseptic conditions to prevent recontamination of the product.

Aseptic Processing

The aseptic process involves placing a sterilized product into a sterilized package that is then sealed under sterile conditions.

Commercial Sterility

In aseptic processing the thermal process is based on achieving commercial sterility—i.e., no more than 1 nonsterile package for every 10,000 processed packages. The aseptic process uses the high-temperature–short-time (HTST) method in which foods are heated at a high temperature for a short period of time. The time and temperature conditions depend on several factors, such as size, shape, and type of food. The HTST method results in a higher retention of quality characteristics, such as vitamins, odour, flavour, and texture, while achieving the same level of sterility as the traditional canning process in which food is heated at a lower temperature for a longer period of time.

The heating and cooling of liquid foods can be performed using metal plate heat exchangers. These heat exchangers have large surface areas that result in improved heating and cooling rates. Other types of heat exchangers involve surrounding the food with steam or directly injecting steam into the food. Products sterilized with steam are then pumped into a vacuum chamber, where they are cooled rapidly.

Liquid foods that contain large solid particles are heated in scraped-surface heat exchangers. These heat exchangers use blades to continuously scrape the inside surface of the heating chamber. The scraping action protects highly viscous foods from being burned on the heating surface.

An alternate method for heating foods, called ohmic heating, passes a low-frequency electric current of 50 to 60 hertz directly through the food. A liquid food containing solids, such as diced fruit, is pumped through a pipe surrounded by electrodes. The product is heated as long as the electrical conductivity of the food is uniform throughout the entire volume. This uniform rate of heating prevents the overprocessing of any individual region of the food. Ohmic heating yields a food product of higher quality than those processed using conventional systems.

Packaging Aseptically Processed Products

The packaging containers used in aseptic processing are sterilized separately before they are used. The packaging machinery is sterilized using steam, sterile gases, or hydrogen peroxide. The sterilization process is generally monitored by culturing a test organism. For example, the remaining presence of the highly heat-resistant bacterium Bacillus subtilis globigii can be used as a marker to measure the completeness of sterilization.

Packages must be sealed under sterile conditions, usually using high-temperature sealing plates. Foods that are aseptically processed do not require refrigeration for storage.

Blanching

Blanching is a thermal process used mostly for vegetable tissues prior to freezing, drying, or canning. Before canning, blanching serves several purposes, including cleaning of the product, reducing the microbial load, removing any entrapped gases, and wilting the tissues of leafy vegetables so that they can be easily put into the containers. Blanching also inactivates enzymes that cause deterioration of foods during frozen storage.

Blanching is carried out at temperatures close to 100 °C (212 °F) for two to five minutes in either a water bath or a steam chamber. Because steam blanchers use a minimal amount of water, extra care must be taken to ensure that the product is uniformly exposed to the steam. Steam blanching leafy vegetables is especially difficult because they tend to clump together. The effectiveness of the blanching treatment is usually determined by measuring the residual activity of an enzyme called peroxidase.

Controlling Water Activity

Foods containing high concentrations of water are generally more susceptible to deterioration by microbial contamination and enzymatic activity. The water content of foods can be controlled by removing water through dehydration or by adding solutes to the food. In both cases the concentration of solutes in the food increases and the concentration of water decreases.

Dehydration

Dehydration, or drying, of foods has long been practiced commercially in the production of spaghetti and other starch products.

Pathogenic (toxin-producing) bacteria occasionally withstand the unfavourable environment of dried foods, causing food poisoning when the product is rehydrated and eaten. Control of bacterial contaminants in dried foods requires high-quality raw materials having low contamination, adequate sanitation in the processing plant, pasteurization before drying, and storage conditions that protect from infection by dust, insects, and rodents or other animals.

Foodstuffs may be dried in air, superheated steam, vacuum, or inert gas or by direct application of heat. Air is the most generally used drying medium, because it is plentiful and convenient and permits gradual drying, allowing sufficient control to avoid overheating that might result in scorching and discoloration. Air may be used both to transport heat to the food being dried and to carry away liberated moisture vapour. The use of other gases requires special moisture recovery systems.

Loss of moisture content produced by drying results in increased concentration of nutrients in the remaining food mass. The proteins, fats, and carbohydrates in dried foods are present in larger amounts per unit weight than in their fresh counterparts, and the nutrient value of most reconstituted or rehydrated foods is comparable to that of fresh items. The biological value of dried protein is dependent, however, on the method of drying. Prolonged exposure to high temperatures can render the protein less useful in the diet. Low-temperature treatment, on the other hand, may increase the digestibility of protein. Some vitamins are sensitive to the dehydration process. For example, in dried meats significant amounts of vitamin C and the B vitamins—riboflavin, thiamine, and niacin—are lost during dehydration.

Dried eggs, meat, milk, and vegetables are ordinarily packaged in tin or aluminum containers. Fibreboard or other types of material may be employed but are less satisfactory than metal, which offers protection against insects and moisture loss or gain and which permits packaging with an inert gas.

In-package desiccants (drying agents) improve storage stability of dehydrated white potatoes, sweet potatoes, cabbage, carrots, beets, and onions and give substantial protection against browning. Retention of ascorbic acid (vitamin C) is markedly improved by packaging at temperatures up to 49 °C (120 °F); the packaging gas may be either nitrogen or air.

A related technique, freeze-drying, employs high vacuum conditions, permitting establishment of specific temperature and pressure conditions. The raw food is frozen, and the low pressure conditions cause the ice in the food to sublimate directly into vapour (i.e., it does not transit through the liquid state). Adequate control of processing conditions contributes to satisfactory rehydration, with substantial retention of nutrient, colour, flavour, and texture characteristics.

Concentration of Moist Foods

Foods with substantial acidity, when concentrated to 65 percent or more soluble solids, may be preserved by mild heat treatments. High acid content is not a requirement for preserving foods concentrated to over 70 percent solids.

Fruit jelly and preserve manufacture, an important fruit by-product industry, is based on the high-solids–high-acid principle, with its moderate heat-treatment requirements. Fruits that possess excellent qualities but are visually unattractive may be preserved and utilized in the form of concentrates, which have a pleasing taste and substantial nutritive value.

Jellies and other fruit preserves are prepared from fruit by adding sugar and concentrating by evaporation to a point where microbial spoilage cannot occur. The prepared product can be stored without hermetic sealing, although such protection is useful to control mold growth, moisture loss, and oxidation. In modern practice, vacuum sealing has replaced the use of a paraffin cover.

The jelly-forming characteristics of fruits and their extracts are due to pectin, a substance present in varying amounts in all fruits. The essential ingredients in a fruit gel are pectin, acid, sugar, and water. Flavouring and colouring agents may be added, and additional pectin and acid may be added to overcome any deficiencies in the fruit itself.

Candied and glacéed fruits are made by slow impregnation of the fruit with syrup until the concentration of sugar in the tissue is sufficiently high to prevent growth of spoilage microorganisms. The candying process is conducted by treating fruits with syrups of progressively increasing sugar concentrations, so that the fruit does not soften into jam or become tough and leathery. After sugar impregnation the fruit is washed and dried. The resulting candied fruit may be packaged and marketed in this condition or may be dipped into syrup, becoming coated with a thin glazing of sugar (glacéed) and again dried.

Pickling

Pickled Fruits and Vegetables

Fresh fruits and vegetables soften after 24 hours in a watery solution and begin a slow, mixed fermentation-putrefaction. The addition of salt suppresses undesirable microbial activity, creating a favourable environment for the desired fermentation. Most green vegetables and fruit may be preserved by pickling.

When the pickling process is applied to a cucumber, its fermentable carbohydrate reserve is turned into acid, its colour changes from bright green to olive or yellow-green, and its tissue becomes translucent. The salt concentration is maintained at 8 to 10 percent during the first week and is increased 1 percent a week thereafter until the solution reaches 16 percent. Under properly controlled conditions the salted, fermented cucumber, called salt stock, may be held for several years.

Salt stock is not a consumer commodity. It must be freshened and prepared into consumer items. In cucumbers this is accomplished by leaching the salt from the cured cucumber with warm water (43–54 °C [110–130 °F]) for 10 to 14 hours. This process is repeated at least twice, and, in the final wash, alum may be added to firm the tissue and turmeric to improve the colour.

Pickled Meat

Meat may be preserved by dry curing or with a pickling solution. The ingredients used in curing and pickling are sodium nitrate, sodium nitrite, sodium chloride, sugar, and citric acid or vinegar.

Various methods are used- the meat may be mixed with dry ingredients; it may be soaked in pickling solution; pickling solution may be pumped or injected into the flesh; or a combination of these methods may be used.

Curing may be combined with smoking. Smoke acts as a dehydrating agent and coats the meat surfaces with various chemicals, including small amounts of formaldehyde.

Deterioration of Fermented and Pickled Products

Fermented foods and pickled products require protection against molds, which metabolize the acid developed and allow the advance of other microorganisms. Fermented and pickled food products placed in cool storage can be expected to remain stable for several months. Longer storage periods demand more complete protection, such as canning.

Nutrient retention in fermented and pickled products is about equal to retention for products preserved by other methods. Carbohydrates usually undergo conversion to acid or to alcohol, but these are also of nutritive value. In some instances, nutrient levels are increased because of the presence of yeasts.

Chemical Preservation

Chemical food preservatives are substances which, under certain conditions, either delay the growth of microorganisms without necessarily destroying them or prevent deterioration of quality during manufacture and distribution. The former group includes some natural food constituents which, when added to foods, retard or prevent the growth of microorganisms. Sugar is used partly for this purpose in making jams, jellies, and marmalades and in candying fruit. The use of vinegar and salt in pickling and of alcohol in brandying also falls in this category. Some chemicals foreign to foods are added to prevent the growth of microorganisms. The latter group includes some natural food constituents such as ascorbic acid (vitamin C), which is added to frozen peaches to prevent browning, and a long list of chemical compounds foreign to foods and classified as antioxidants, bleaching agents, acidulants, neutralizers, stabilizers, firming agents, and humectants.

Organic Chemical Preservatives

Sodium benzoate and other benzoates are among the principal chemical preservatives. The use of benzoates in certain products in prescribed quantity (usually not exceeding 0.1 percent) is permitted in most countries, some of which require a declaration of its use on the label of the food container. Since free benzoic acid actually is the active agent, benzoates must be used in an acid medium in order to be effective. The ability of cranberries to resist rapid deterioration is attributed to their high benzoic acid content. Benzoic acid is more effective against yeasts than against molds and bacteria.

Other organic compounds used as preservatives include vanillic acid esters, monochloroacetic acid, propionates, sorbic acid, dehydroacetic acid, and glycols.

Inorganic Chemical Preservatives

Sulfur dioxide and sulfites are perhaps the most important inorganic chemical preservatives. Sulfites are more effective against molds than against yeasts and are widely used in the preservation of fruits and vegetables. Sulfur compounds are extensively used in wine making and, as in most other instances when this preservative is used, much care has to be exercised to keep the concentrations low in order to avoid undesirable effects on flavour.

Oxidizing agents such as nitrates and nitrites are commonly used in the curing of meats.

Food Irradiation

Food irradiation involves the use of either high-speed electron beams or high-energy radiation with wavelengths smaller than 200 nanometres, or 2000 angstroms (e.g., X-rays and gamma rays). These rays contain sufficient energy to break chemical bonds and ionize molecules that lie in their path. The two most common sources of high-energy radiation used in the food industry are cobalt-60 (^{60}Co) and cesium-137 (^{137}Cs). For the same level of energy, gamma rays have a greater penetrating power into foods than high-speed electrons.

The unit of absorbed dose of radiation by a material is denoted as the gray (Gy), one gray being equal to the absorption of one joule of energy by one kilogram of food. The energy possessed by an electron is called an electron volt (eV). One eV is the amount of kinetic energy gained by an electron as it accelerates through an electric potential difference of one volt. It is usually more convenient to use a larger unit such as megaelectron volt (MeV), which is equal to one million electron volts.

Biological Effects of Irradiation

Irradiation has both direct and indirect effects on biological materials. The direct effects are due to the collision of radiation with atoms, resulting in an ejection of electrons from the atoms. The indirect effects are due to the formation of free radicals (unstable molecules carrying an extra electron) during the radiolysis (radiation-induced splitting) of water molecules. The radiolysis of water molecules produces hydroxyl radicals, highly reactive species that interact with the organic molecules present in foods. The products of these interactions cause many of the characteristics associated with the spoilage of food, such as off-flavours and off-odours.

Positive Effects

The bactericidal (bacteria-killing) effect of ionizing radiation is due to damage of the biomolecules of bacterial cells. The free radicals produced during irradiation may destroy or change the structure of cellular membranes. In addition, radiation causes irreversible changes to the nucleic acid molecules (i.e., DNA and RNA) of bacterial cells, inhibiting their ability to grow. Pathogenic bacteria that are unable to produce resistant endospores in foods such as poultry, meats, and seafood can be eliminated by radiation doses of 3 to 10 kilograys. If the dose of radiation is too low, then the damaged DNA can be repaired by specialized enzymes. If oxygen is present during irradiation, the bacteria are more readily damaged. Doses in the range of 0.2 to 0.36 kilograys are required to stop the reproduction of Trichinella spiralis (the parasitic worm that causes trichinosis) in pork, although much higher doses are necessary to eliminate it from the meat.

The dose of radiation used on food products is divided into three levels. Radappertization is a dose in the range of 20 to 30 kilograys, necessary to sterilize a food product. Radurization is a dose of 1 to 10 kilograys, that, like pasteurization, is useful for targeting specific pathogens. Radicidation involves doses of less than 1 kilogray for extending shelf life and inhibiting sprouting.

Negative Effects

In the absence of oxygen, radiolysis of lipids leads to cleavage of the interatomic bonds in the fat molecules, producing compounds such as carbon dioxide, alkanes, alkenes, and aldehydes. In addition, lipids are highly vulnerable to oxidation by free radicals, a process that yields peroxides, carbonyl compounds, alcohols, and lactones. The consequent rancidity, resulting from the irradiation of high-fat foods, is highly destructive to their sensory quality. To minimize such harmful effects, fatty foods must be vacuum-packaged and held at subfreezing temperatures during irradiation.

Proteins are not significantly degraded at the low doses of radiation employed in the food industry. For this reason irradiation does not inactivate enzymes involved in food spoilage, as most enzymes survive doses of up to 10 kilograys. On the other hand, the large carbohydrate molecules that provide structure to foods are depolymerized (broken down) by irradiation. This depolymerization reduces the gelling power of the long chains of structural carbohydrates. However, in most foods some protection against these deleterious effects is provided by other food constituents. Vitamins A, E, and B1 (thiamine) are also sensitive to irradiation. The nutritional losses of a food product are high if air is not excluded during irradiation.

Safety Concerns

Based on the beneficial effects of irradiation on certain foods, several countries have permitted its use for specific purposes, such as the inhibition of sprouting of potatoes, onions, and garlic; the extension of shelf life of strawberries, mangoes, pears, grapes, cherries, red currants, and cod and haddock fillets; and the insect disinfestation of pulses, peanuts, dried fruits, papayas, wheat, and ground-wheat products.

The processing room used for irradiation of foods is lined with lead or thick concrete walls to prevent radiation from escaping. The energy source, such as a radioactive element or a machine source of electrons, is located inside the room. (Radioactive elements such as ^{60}Co are contained in stainless steel tubes. Because an isotope cannot be switched on or off, when not in use it is lowered into a large reservoir of water.) Prior to the irradiation treatment, personnel vacate the room. The food to be irradiated is then conveyed by remote means into the room and exposed to the radiation source for a predetermined time. The time of exposure and the distance between the radiation source and the food material determine the irradiation treatment. After treatment, the irradiated food is conveyed out of the room, and the radioactive element is again lowered into the water reservoir.

Packaging

Because packaging helps to control the immediate environment of a food product, it is useful in creating conditions that extend the storage life of a food. Packaging materials commonly used for foods may be classified as flexible (paper, thin laminates, and plastic film), semi-rigid (aluminum foil, laminates, paperboard, and thermoformed plastic), and rigid (metal, glass, and thick plastic). Plastic materials are widely used in food packaging because they are relatively cheap, lightweight, and easy to form into desired shapes.

The selective permeability of polymer-based materials to gases, such as carbon dioxide and oxygen, as well as light and moisture, has led to the development of modified-atmosphere packaging. If the barrier properties are carefully selected, a packaging material can maintain a modified atmosphere inside the package and thus extend the shelf life of the food product.

Dehydrated foods must be protected from moisture during storage. Packaging materials such as polyvinyl chloride, polyvinylidene chloride, and polypropylene offer low moisture permeability. Similarly, packaging materials with low gas permeability are used for fatty foods in order to minimize oxidation reactions. Because fresh fruits and vegetables respire, they require packaging materials, such as polyethylene, that have high permeability to gases.

Smart packages offer properties that meet the special needs of certain foods. For example, packages made with oxygen-absorbing materials remove oxygen from the inside of the package, thus protecting oxygen-sensitive products from oxidation. Temperature-sensitive films exhibit an abrupt change in gas permeability when they are subjected to a temperature above or below a set constant. These films change from a crystalline structure to an amorphous structure at a set temperature, causing the gas permeability to change substantially.

Storage

Food storage is an important component of food preservation. Many reactions that may deteriorate the quality of a food product occur during storage. The nutrient content of foods may be adversely affected by improper storage. For example, a significant amount of vitamin C and thiamine may be lost from foods during storage. Other undesirable quality changes that may occur during storage include changes in colour, development of off-flavours, and loss of texture. A properly designed food storage system allows fresh or processed foods to be stored for extended duration while maintaining quality.

The most important storage parameter is temperature. Most foods benefit from storage at a constant, low temperature where the rates of most reactions decrease and quality losses are minimized. In addition, foods containing high concentrations of water must be stored in high-humidity environments in order to prevent the excessive loss of moisture.

Food Preservatives

Food is so important for the survival, so food preservation is one of the oldest technologies used by human beings to avoid its spoilage. Different ways and means have been found and improved for the purpose. Boiling, freezing & refrigeration, pasteurizing, dehydrating, pickling are the traditional few. Sugar, mineral salt and salt are also often used as preservatives food. Nuclear radiation is also being used now as food preservatives. Modified packaging techniques like vacuum packing and hypobaric packing also work as food preservatives.

Food preservation is basically done for three reasons:

- To preserve the natural characteristics of food.
- To preserve the appearance of food.
- To increase the shelf value of food for storage.

Natural Food Preservatives

In the category of natural food preservatives comes the salt, sugar, alcohol, vinegar etc. These are the traditional preservatives in food that are also used at home while making pickles, jams and juices etc. Also the freezing, boiling, smoking, salting are considered to be the natural ways of preserving food. Coffee powder and soup are dehydrated and freeze-dried for preservation. In this section the citrus food preservatives like citrus acid and ascorbic acid work on enzymes and disrupt their metabolism leading to the preservation.

Sugar and salt are the earliest natural food preservatives that very efficiently drops the growth of bacteria in food. To preserve meat and fish, salt is still used as a natural food preservative.

Chemical Food Preservative

Chemical food preservatives are also being used for quite some time now. They seem to be the best and the most effective for a longer shelf life and are generally fool proof for the preservation purpose. Examples of chemical food preservatives are:

- Benzoates (such as sodium benzoate, benzoic acid),
- Nitrites (such as sodium nitrite),
- Sulphites (such as sulphur dioxide),
- Sorbates (such as sodium sorbate, potassium sorbate.

Antioxidants are also the chemical food preservatives that act as free radical scavengers. In this category of preservatives in food comes the vitamin C, BHA (butylated hydroxyanisole), bacterial growth inhibitors like sodium nitrite, sulfur dioxide and benzoic acid.

Then there is ethanol that is a one of the chemical preservatives in food, wine and food stored in brandy. Unlike natural food preservatives some of the chemical food preservatives are harmful. Sulfur dioxide and nitrites are the examples. Sulfur dioxide causes irritation in bronchial tubes and nitrites are carcinogenic.

Artificial Preservatives

Artificial preservatives are the chemical substances that stops of delayed the growth of bacteria, spoilage and its discoloration. These artificial preservatives can be added to the food or sprayed on the food.

Types of Artificial Preservatives Food

- Antimicrobial agents.
- Antioxidants.
- Chelating agent.

1. In antimicrobial comes the Benzoates, Sodium benzoate, Sorbates and Nitrites.
2. Antioxidants include the Sulfites, Vitamin E, Vitamin C and Butylated hydroxytoluene (BHT) .
3. Chelating agent has the Disodium ethylenediaminetetraacetic acid (EDTA), Polyphosphates and Citric acid.

Harmful Food Preservatives

Although preservatives food additives are used to keep the food fresh and to stop the bacterial growth. But still there are certain preservatives in food that are harmful if taken in more than the prescribed limits.

Certain Harmful Food Preservatives are Benzoates

This group of chemical food preservative has been banned in Russia because of its role in triggering allergies, asthma and skin rashes. It is also considered to cause the brain damage. This food preservative is used in fruit juices, tea, coffee etc.

Butylates

This chemical food preservative is expected to cause high blood pressure and cholestrol level. This can affect the kidney and live function. It is found in butter, vegetable oils and margarine.

BHA (Butylated Hydroxyanisole)

BHA is expected to cause the live diseases and cancer. This food preservative is used to preserve the fresh pork and pork sausages, potato chips, instant teas, cake mixes and many more.

Caramel

Caramel is the coloring agent that causes the vitamin B6 deficiencies, genetic effects and cancer. It is found in candies, bread, brown colored food and frozen pizza.

In addition to this there are many other harmful food preservatives. These are Bromates, Caffeine, Carrageenan, Chlorines, Coal Tar AZO Dies, Gallates, Glutamates, Mono-and Di-glycerides, Nitrates/Nitrites, Saccharin, Sodium Erythrobate, Sulphites and Tannin.

Preservatives Food Additives

All of these chemicals act as either antimicrobials or antioxidants or both. They either inhibit the activity of or kill the bacteria, molds, insects and other microorganisms.

Antimicrobials, prevent the growth of molds, yeasts and bacteria and antioxidants keep foods from becoming rancid or developing black spots. They suppress the reaction when foods comes in contact with oxygen, heat, and some metals. They also prevent the loss of some essential amino acids some vitamins.

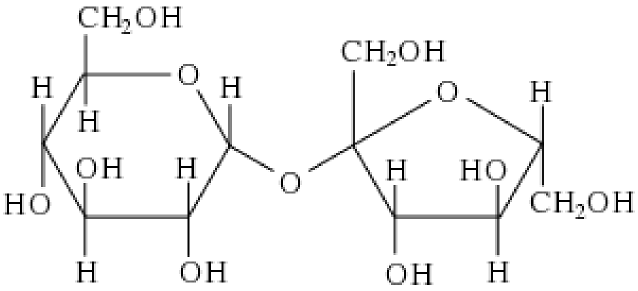
Some common preservatives and their primary activity.

Chemical Affected	Organism(s)	Action	Use in Foods
Sulfites	Insects & Microorganisms	Antioxidant	Dried Fruits, Wine, Juice
Sodium Nitrite	Clostridia	Antimicrobial	Cured Meats
Propionic Acid	Molds	Antimicrobial	Bread, Cakes, Cheeses
Sorbic Acid	Molds	Antimicrobial	Cheeses, Cakes, Salad Dressing
Benzoic Acid	Yeasts & Molds	Antimicrobial	Soft Drinks, Ketchup, Salad Dressings

There are other antioxidants like Sodium Erythorbate, Erythorbic Acid, Sodium Diacetate, Sodium Succinate, Grape Seed Extract, Pine Bark Extract, Apple Extract Tea Proplyphenols, Succinic Acid and Ascorbic Acid and food preservatives like Parabens and Sodium Dehydro Acetate used frequently for preservation.

Food Chemistry

Food chemistry is the study of chemical processes and interactions of the biological and non-biological components of foods. It overlaps with biochemistry in that it deals with the components of food such as carbohydrates, lipids, proteins, water, vitamins, and dietary minerals. In addition, it involves the study and development of food additives that can be used to preserve the quality of food or to modify its color, flavor, and taste. It is, thus, closely linked to food processing and preparation techniques. There is, however, an ongoing debate about the health effects of a number of food additives.



The molecular structure of sucrose, or ordinary table sugar.
This sugar is probably the most familiar carbohydrate.

Water

A major component of food is water, which can range in content from 50 percent in meat products to 95 percent in lettuce, cabbage, and tomato products. It also provides a place for bacterial growth and food spoilage if it is not properly processed. One way of measuring this in food is by water activity, which is very important in the shelf life of many foods during processing. One of the keys to food preservation is to reduce the amount of water or alter the water's characteristics to enhance shelf-life. Such methods include dehydration, freezing, and refrigeration.

Carbohydrates

Carbohydrates form the largest group of substances in food consumed by humans. A common carbohydrate is starch.

The simplest version of a carbohydrate is a monosaccharide, made up of molecules in which carbon, hydrogen, and oxygen atoms are in the ratio 1:2:1. Thus, the general formula of a monosaccharide is $C_nH_{2n}O_n$, where n is a minimum of 3. Glucose and fructose are examples of monosaccharides. The familiar table sugar is sucrose, a disaccharide. Each molecule of sucrose is made up of a combination of one glucose and one fructose molecule.

A chain of monosaccharides forms a polysaccharide. Such polysaccharides include pectin, dextran, agar, and xanthan.

Sugar content is commonly measured in degrees brix.

Lipids

The term lipid encompasses a diverse range of molecules and to some extent is a catch-all for relatively water-insoluble (nonpolar) compounds of biological origin. Examples of lipids are waxes, fatty acids, fatty-acid derived phospholipids, sphingolipids, glycolipids, and terpenoids, such as retinoids and steroids. Some lipids are linear aliphatic molecules, while others have ring structures. Some are aromatic. Some are flexible, and others are rigid.

Most lipids have some polar character, in addition to being largely nonpolar. In other words, the bulk of the structure of a lipid molecule is nonpolar or hydrophobic, meaning that it does not interact well with polar solvents like water. Another part of the molecular structure is polar or hydrophilic and will tend to associate with polar solvents like water. Thus lipid molecules are amphiphilic, having both hydrophobic and hydrophilic portions. In the case of cholesterol, the polar group is a mere -OH (hydroxyl or alcohol).

In food, lipids are present in the oils of grains such as corn and soybean, and they are also found in meat, milk, and dairy products. They act as vitamin carriers as well.

Proteins

Proteins make up over 50 percent of the dry weight of an average living cell and are very complex macromolecules. They play a fundamental role in the structure and function of cells. Protein molecules are constructed mainly of carbon, hydrogen, oxygen, and some sulfur, they may also contain iron, copper, phosphorus, or zinc.

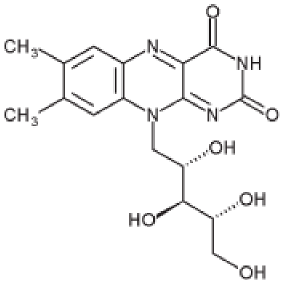
In food, proteins are essential for growth and survival, but the amount of protein needed by an individual varies, based on the person's age and physiology (such as during pregnancy). Proteins in food are commonly found in peanuts, meat, poultry, and seafood.

Enzymes

Many proteins are enzymes that catalyze biochemical reactions. They reduce the amount of time and energy required to complete the reactions. Many areas of the food industry use enzyme catalysts, including baking, brewing, and dairy, to make bread, beer, and cheese.

Vitamins

Vitamins are nutrients required in small amounts for essential metabolic reactions in the body. They are subdivided as either water-soluble (such as Vitamin C) or fat-soluble (such as Vitamin E). An adequate supply of vitamins can prevent such diseases as beriberi, anemia, and scurvy, but an overdose of vitamins can produce nausea and vomiting or even death.



Riboflavin (Vitamin B2) is a water-soluble vitamin.

Minerals

Dietary minerals in foods are large and diverse, with many required for health and survival. Some minerals, however, can be hazardous if consumed in excessive amounts. Bulk minerals with a Reference Daily Intake (RDI; formerly, Recommended Daily Allowance (RDA)) of more than 200 mg/day include calcium, magnesium, and potassium. Important trace minerals, with RDI less than 200 mg/day, include copper, iron, and zinc. They are found in many foods but can also be taken in dietary supplements.

Food Additives

Food additives are substances added to food for such purposes as preserving its quality, adding to or enhancing its flavors, improving its taste, or modifying its appearance. Additives used today can be placed in a wide range of groups, such as food acids, anticaking agents, antioxidants, bulking agents, food coloring, flavoring agents, humectants, preservatives, stabilizers, and thickeners.

Coloring

Food coloring is added to change or enhance the color of any food, mainly to make it look more appealing. It can be used to simulate the natural color of a product as perceived by the customer, such as adding the red dye to ketchup. Alternatively, unnatural colors may be added to a product like Kellogg's Fruit Loops. Caramel is a natural food dye; the industrial form, caramel coloring, is the most widely used food coloring and is found in foods ranging from soft drinks to soy sauce, bread, and pickles.

Flavors

Flavor in food is important in determining how food smells and tastes to the consumer, especially in sensory analysis. Some of these products occur naturally, such as salt and sugar, but flavor chemists (called "flavorists") develop many flavors for food products. Such artificial flavors include methyl salicylate, which produces the wintergreen odor, and lactic acid, which gives milk a tart taste.

Food Biotechnology

Food biotechnology is the use of technology to modify the genes of our food sources. Our food sources are animals, plants, and microorganisms. With food biotechnology, we create new species of animals and plants, for example, specifically animals and plants that we eat. These new species have desired nutritional, production, and marketing properties.

With food biotechnology, we use what we know about science and genetics to improve the food we eat. We also use it to improve how we produce food.

By improvement, we mean either making the food cheaper to produce, longer lasting, more disease resistant, or more nutritional.

Benefits of Food Biotechnology

Food biotechnology offers many benefits for farmers, food companies, consumers, and the environment. Below are some of these benefits.

The Environment

Researchers have made some foods, such as potatoes and papayas, more disease resistant.

If a crop is more resistant to disease that means it needs less chemical spray to protect it. Less spray subsequently means less air, land, and water pollution. Water pollution is a serious global problem.

This is good for animals, plants, etc., i.e., the environment.

Greater Yields

Thanks to biotechnology, plants can ward off insects and have a better tolerance to herbicides. Put simply; science can help plants survive better so that we subsequently get better crop yields.

More Food with Less Land

By the middle of this century, there will be about nine billion people on Earth. Thanks to biotechnology, we will be able to produce more food using the land we are already using.

Food Safety

Scientists are getting better at accurately detecting undesirable bacteria and viruses in our food. Thanks to their technology, there will be a lower risk of food-borne illnesses. A foodborne illness is an illness we get because of something we ate.

Applications of Food Biotechnology

Firstly, the application of biotechnology in the food industry is in the field of genetic engineering. Food raw materials or food microorganisms can be improved by the genetic transfer of animals, plants and microorganisms or the DNA recombination by means of DNA recombination technology or cloning technology, such as the use of genetic engineering to improve the raw materials of food processing and the performance of food microorganisms, and to produce enzyme preparations and active ingredients of health-care food.

Secondly, cell engineering is also an important application field of biotechnology in food industry. The health-care food ingredients, new food and food additives can be produced by cell engineering including cell fusion technology and animal and plant cell culture.

Thirdly, enzymes can be applied to the transformation of substances in food production process. Amylase and cellulase have been successfully developed and used in food industry. For example, cellulase has a wide application in the production of fruit juice, fruit and vegetable, instant tea, and in soy sauce brewing and wine making. Finally, the industrially pre-designed food or food functional ingredients can be easily accessed by means of the modern fermentation equipment to make the preferred cells or the modified strains to undergo the scaled-up culture and controlled fermentation.

As a new technology with great potential and development space, the biotechnology in the food industry will show a trend of developing new varieties of food additives greatly. At present, the international requirement for food additive quality is to make food more natural and fresh, pursue low-fat, low-cholesterol and low-calorie food, and enhance the quality stability of food storage process.

Therefore, two aspects should be paid attention:

1) Using biologically- produced food additives to replace chemically-synthesized ones.

2) Developing the functional food additives with immune regulation, anti-aging, anti-fatigue, anti-hypoxia, antiradiation, blood lipid regulation and gastrointestinal functional components adjustment, etc.

The development of microbial health-care food has a long history, and soy sauce, vinegar, beverage wine and mushrooms belong to this area. Like bifidobacteria drinks, yeast tablets and dairy products, they have a huge development potential.

Microbial production of food has some unique characteristics:

- Fast breeding process of microorganisms: the large-scale production of food can be achieved in a certain equipment conditions.
- Simple requirements of nutrients.
- Higher input and output ratio of edible fungi than other economic crops.
- Easy to achieve industrialization, etc.

Transgenic biotechnology has brought new impetus to the agricultural, medical and food industry, which has directly accelerated the cultivation of new varieties of agriculture, the prevention and treatment of various diseases, the improvement of food nutrition and the management of ecological environment. The development of genetically modified technologies can accelerate the development of agriculture, forestry and fisheries, and increase crop yields.

Food Fortification

Fortification is the addition of key vitamins and minerals such as Iron, Iodine, Zinc, Vitamins A & D to staple foods such as rice, wheat, oil, milk and salt to improve their nutritional content. These nutrients may or may not have been originally present in the food before processing or may have been lost during processing.

Need of Fortification of Food

Deficiency of micronutrients or micronutrient malnutrition, also known as? hidden hunger?, is a serious health risk. Access to safe and nutritious food is a must and sometimes due to lack of consumption of a balanced diet, lack variety in the diet or unavailability of food one does not get adequate micronutrients. Often, there is considerable loss of nutrients during the processing of food as well. 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.

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.

Food Microbiology

Food microbiology encompasses the study of microorganisms, which have both beneficial and deleterious effects on the quality, and safety of raw and processed meat, poultry, and egg products. Food microbiology focuses on the general biology of the microorganisms that are found in foods including: their growth characteristics, identification, and pathogenesis.

Bacteria are the most abundant of all organisms, they are unicellular, are relatively small ranging in size from 0.5- to 5.0 μm, and for the most part they reproduce asexually.

Although there are bacterial species capable of causing human illness (pathogens) and food spoilage, there are also beneficial species that are essential to good health and the environment (examples: synthesize vitamins, digest plant cellulose, fixing nitrogen in plant roots, etc.). Every bacterial species have specific nutritional requirements, temperature, humidity, etc for energy generation and cellular biosynthesis. The bacterial cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation and under favorable conditions, a growing bacterial population doubles at regular intervals ranging from about 15 minutes to 1 hour. This means that if we start with 1,000 cells with a generation time of 30 min. then after an hour we end with 4,000 cells.

Bacteria are also known as prokaryotes because they do not possess nuclei; i.e., their chromosome is composed of a single closed double-stranded DNA circle. Structurally, a prokaryotic cell has three architectural regions: appendages (attachments to the cell surface) in the form of flagella and pili (or fimbriae); a cell envelope consisting of a capsule, cell wall and plasma or inner membrane; and a cytoplasmic region that contains the cell genome (DNA), ribosomes and various sorts of inclusions. Following is a brief discussion of some of these structural components.

- Cell envelope- is made of three layers: cytoplasmic membrane (inner layer), the cell wall (relatively rigid outer layer called peptidoglycan), and – in some bacterial species- an outer capsule. The role of the bacterial capsule is to keep the bacterium from drying, can serve as a virulence factor and as an antigen for identification, mediate adherence of cells to surface (crucial in biofilm formation), and confer protection against engulfment and attack by antimicrobial agents of plants, animals, and the environment. Bacteria can be placed into two basic groups, Gram-positive or Gram-negative, based on the profiles of the bacterial cell wall.

- Chromosome- where the bacterium’s genetic information is contained. It is a crucial tool for genetic fingerprinting.

- Cytoplasm- is where the function for cell growth, metabolism, and replication are carried out. It is composed of water, enzymes, nutrients, metabolic wastes, and gases; it also contains the ribosomes, chromosomes, and plasmids. As mentioned before, the cell envelope encases the cytoplasm and all its components.

- Flagella- are hair-like structures that serve as propellers to help bacterium move toward nutrients and away from toxic chemicals. This structure can be found at either or both ends or all over the bacterium surface and serve as antigen (Hantigen) for serotyping. In addition, this organelle is a contributor for biofilm formation.

- Pili and fimbriae- many species of bacteria have these small hair-like projections emerging from the outside cell surface. Its function is to assist in attaching to other cells and surfaces. Specialized pili are used for passing nuclear material between bacterial cells (conjugation).

- Plasmid- short length of extra-chromosomal genetic structure (circles or loops) which are carried by many strains of bacteria. They are not involved in reproduction but replicate independently of the chromosome and are instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal and human hosts. Plasmids are extremely useful tools in the area of genetic engineering.
- Ribosomes- these are organelles that translate the genetic code DNA to amino acids which are the building blocks of proteins. They are also an important tool in the fields of molecular biology and genetics.
- Spores- produced by some species and they are resistant to hostile conditions such as heat and drying. They serve as survival mechanisms when environmental conditions are not suitable for growth and replication.

The cell wall of bacteria is dynamic and extremely important for several reasons:

1. They are an essential structure for viability; protects the cell protoplast from mechanical damage and from osmotic rupture or lysis.
2. They are composed of unique components found nowhere else in nature.
3. They are one of the most important sites for attack by antibiotics.
4. They provide ligands for adherence and receptor sites for drugs or viruses.
5. They cause symptoms of disease in humans and animals.
6. They provide for immunological distinction and immunological variation among strains of bacteria.
7. They can be modified to protect the cell against harsh environmental conditions like heat, pH, antimicrobials, etc.

Cell wall composition varies widely amongst bacteria and is an important factor in bacterial species analyses and differentiation. The main functions are to give the cell its shape (rod, sphere, helix, or comma) and surround the cytoplasmic membrane, protecting it from the environment. As mentioned above, the profiles of the cell walls of bacteria, as seen with the electron microscope, make it possible to distinguish two basic types of bacteria as follows:

- Gram-positive bacteria (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure) - the cell wall adjoining the inner or cytoplasmic membrane is thick (15-80 nanometers), consisting of several layers of peptidoglycan, also known as murein. Intertwine within the cell wall are polymers composed of glycerol, phosphates, and ribitol, which are known as teichoic acids. In general, Gram-positive bacteria produce extra cellular substances that typically account for most of the virulence factors and this is illustrated by Staphylococcus aureus.

- Gram-negative bacteria (which do not retain the crystal violet) - the cell wall adjoining the inner membrane is relatively thin (10 nanometers) and is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS or endotoxin), which is toxic to animals. This outer membrane is usually thought of as part of the cell wall. The pathogenesis and virulence properties of Gram-negative bacteria are far more complex including outer membrane components as well as the production of extra cellular substances, which can be illustrated by.

It may be advantageous for epidemiological purposes to identify a particular bacterial strain by serotyping, which is a useful tool to accomplish this goal. There are components in the cell envelope that serves as antigens for serotyping, therefore, serotyping is based on the ability of the bacteria to agglutinate antibodies specific for those antigens. Following is a brief description regarding to the serotyping of those pathogens of public health concern.

Serotyping of Gram-negative bacteria (examples: E. coli and Salmonella spp.) consist of the immunoreactivity of three classes of antigens: the O-antigen (somatic), H-antigen (flagellar), and the K-antigen (capsular) surface profiles. The O-antigen is a polysaccharide which is a polymer of O-subunits, composed of 4-6 sugar residues, attached to the lipid A-core polysaccharide portion of the LPS molecule. Differences in the immunoreactivity of antibodies (O antiserum) with the O-antigen result from the variation in the sugar components and covalent linkages between the O-subunit. On the other hand, the H-antigen is the filamentous portion of the flagella, which is composed of protein subunits called flagellin. The antigenically variable portion of flagellin determines the H serotype as determined by H antiserum. Finally, the K- antigens are the somatic or surface antigens that occur as envelopes, sheaths, or capsules. They act as masking antigens for the O-antigen, inhibiting agglutination of living cell suspensions in O antiserum (for the purpose of the scope of this module this antigen will not be further discussed). A specific combination of O- and H-antigens defines what is known as the serotype and serogroups of a bacterial isolate. The serotype and serogroups in particular species provide identifiable chromosomal markers that correlate with specific bacterial virulent clones.

In E. coli, a total of 170 different O-antigens and 55 H-antigens, defining the isolate serotype, have been identified; a well-known example is E. coli O157:H7 serotype, which is part of the enterohemorrhagic (EHEC) serogroup. More than 2,500 Salmonella serotypes have been described and reported. Serotyping regarding to this species is complex due to the multiple composition of the O-antigen and these are divided into serogroups or O groups, designated by the primary O factor(s) that are associated with the group. In addition, Salmonella is unique among enteric bacteria in that it can express two different flagellin antigens, referred to as Phase 1 and Phase 2. Examples are S. Enteritidis and S. Newport, which belong to Serogroup D and B, respectively.

Likewise, the serotyping of Gram-positive bacteria (an example is Listeria monocytogenes) is based on the combination of somatic (O; teichoic acids) and flagellar (H) antigens. Although serological confirmation is not necessary for regulatory identification of L. monocytogenes, it is useful for determining the prevalence of specific serotypes in epidemiological studies and for environmental recontamination tracking. Strains of L. monocytogenes can be assigned to 13 different serotypes, based on their combination of O- and H-antigens. While all of them are considered to be potentially pathogenic, most (>95%) human clinical isolates belong to three serotypes 1/2a, 1/2b, and 4b.

It is evident that bacteria are a complex system with the capability to adapt and survive to adverse environmental conditions. This explains, in part, why some microorganisms are very difficult to eliminate (biofilm formation), why other becomes pathogenic, and why other develops resistance toward antibiotics or antimicrobial interventions. In slaughter as well as in the processing establishments there are bacterial species associated with particular meat and poultry products, including the environment.

Parameters Affecting the Growth of Microorganisms

There are two parameters affecting the growth of microorganisms in food products: extrinsic and intrinsic. Extrinsic parameters are those properties of the environment (processing and storage) that exist outside of the food product, which affect both the foods and their microorganisms. In the other hand, intrinsic parameters, are properties that exist as part of the food product itself, for example, tissues are an inherent part of the animal that, under a set of conditions, may promote microbiological growth.

Following is a list of these parameters that may result in multiplication or inhibition of microbial growth in meat, poultry, or egg product.

Examples of intrinsic parameters are:

- pH: It has been well established that most microorganisms grow best at pH values around 7.0 (6.6 – 7.5), whereas few grow below a pH of 4.0. Bacteria tend to be more fastidious (complex nutritional or cultural requirements for growth) in their relationships to pH than molds and yeasts, with the pathogenic bacteria being the most fastidious. Most of the meats have a final pH of about 5.6 and above; this makes these products susceptible to bacteria as well as to mold and yeast spoilage.
- Moisture content (water activity [aw]): One of the oldest methods of preserving foods is drying or desiccation. The preservation of foods by drying is a direct consequence of removal or binding of moisture, without which microorganisms

do not grow. It is now generally accepted that the water requirements of microorganisms should be described in terms of water activity (aw) in the environment. Water molecules are loosely oriented in pure liquid water and can easily rearrange. When a solute is added (like salt) to water, the water molecules orient themselves on the surface of the solute, in this case the Na^+ and Cl- ions, and the properties of the solution change dramatically. Therefore, the microbial cell must compete with solute molecules for free water molecules. The water activity of pure water is 1.00; the addition of solute decreases aw to less than 1.00. Most food borne pathogenic bacteria require aw greater than 0.9, however, Staphylococcus aureus may grow in a_w as low as 0.86.

- Oxidation-reduction potential: Microorganisms display varying degrees of sensitivity to the oxidation-reduction potential (O/R or EH) of their growth medium or environment. Aerobic microorganisms require more oxidized environments (more oxygen) versus anaerobic organisms which require more reduced environments (lacking oxygen).
- Nutrient content: In order to grow and function normally, the microorganisms of concern in the food industry require the following: water, source of energy, source of nitrogen, vitamins and related growth factors, and minerals.
- Antimicrobial constituents: The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances that have been shown to have antimicrobial activity. Nisin and other bacteriocins are good examples.
- Biological structures: The natural covering of some food sources provides excellent protection against the entry and subsequent damage by spoilage organisms. Examples of such protective structure are the hide, skin and feathers of animals.

Examples of extrinsic parameters are:

- Storage temperature: Microorganisms, individually and as group, grow over a wide range of temperatures. It is important to know the temperature growth ranges for organisms of importance in foods as an aid in selecting the proper temperature for product storage.
- Relative humidity: The relative humidity of the storage environment is important from both the standpoint of water activity (aw) within foods and the growth of microorganisms at the surfaces. Humidity can also be an important factor to consider when producing some types of product.
- Presence/concentration of gases: Carbon dioxide (CO_2) is the single most important atmospheric gas that is used to control microorganisms in foods. It has been shown to be effective against a variety of microorganisms. Because of its effectiveness, CO_2 is used as one of the methods for modified-atmosphere packaging.

- Presence/activities of other microorganisms: The inhibitory effect of some members of the food microbiota on other microorganisms is well established. Some food borne organisms produce substances that are either inhibitory or lethal to others. These include antibiotics, bacteriocins, hydrogen peroxide, and organic acids (such as lactic acid). General microbial interference is a phenomenon that refers to general nonspecific inhibition or destruction of one microorganism by other members of the same habitat or environment; the mechanism for this interference is not very clear. Some of the possibilities are competition for nutrients; competition for attachment/adhesion sites; unfavorable alteration of the environment and combinations of these.

Primary Sources of Microorganisms in Food

Bacteria can be found virtually everywhere including humans and can enter food products through different routes. The following list outlines some of the most common ways in which microorganisms enter food products.

Soil, water, and establishment environment: Many bacteria are carried in soil and water, which may contaminate food. In addition, the establishment environment is an important source of contamination because of the daily activities and pest infestation. Listeria, Clostridium, Salmonella, and Escherichia are good examples.

- Animal feeds: This is a source of salmonellae to poultry and other farm animals. It is a known source of Listeria monocytogenes to dairy and meat animals when fed silage. The organisms in dry animal feed are spread throughout the animal environment and may be expected to occur on animal hides, hair, feathers, etc.

- Animal hides: The hide is a source of bacterial contamination of the general environment, hands of establishment employees, and skinned carcasses. this may be a primary source for E. coli O157:H7, Salmonella, and Listeria in cattle.

- Gastrointestinal tract: The intestinal biota consists of many organisms; notable among these are pathogens such as Salmonella, Campylobacter, E. coli O157:H7, and other microorganisms. Any or all of the Enterobacteriaceae may be expected in feces of livestock and poultry.

- Food handlers: The microbiota on the hands and outer garments of handlers generally reflect the environment and habits of individuals (hygiene), and the organisms in question may be those from hides, gastrointestinal tracts, soil, water, dust, and other environmental sources.

- Food Utensils: Saws, cutting boards, knives, grinders, mixers, etc. may become contaminated during slaughter and processing operations and ensure a constant level of contamination of meat-borne organisms.

- Air and dust: A variety of bacteria may be found in air and dust in food-processing operations at any one time. Listeria is an example of a Gram-positive organism that survives in the environment.
- Vegetables (plant) and vegetable products: May be a significant concern in the processing of meat, poultry and egg products. A good example is the processing of frozen entrees, salads, etc. containing meat and poultry components. Many or most soil and water organisms contaminate vegetables and fruits.
- Globalization of food supply: This is a major factor of contamination resulting in transfer of pathogenic agents between countries (import/export) such as Bovine Spongiform Encephalopathy (BSE) infective agent and Salmonella Typhimurium DT104, among others. Also, with the increase in international travel this imposes a risk of introducing pathogens to this country like Foot and Mouth Disease.
- Terrorist attacks: There are growing concern in the food industry that terrorist could use pathogens to contaminate food and water supplies in attempt to disrupt the economy, health, and lifestyle among others.

Food Contamination

There are many opportunities for food to become contaminated as it is produced and prepared. Many food borne microbes are present in healthy animals (usually in their intestines, hides, feathers, etc.) raised for food. Meat and poultry carcasses can become contaminated during slaughter by contact with small amounts of intestinal contents or poor dressing procedures. Also, it has been shown scientifically that some Salmonella serotypes can infect a hen's ovary in such a manner that the internal contents of a normal looking egg can be contaminated with Salmonella even before the shell is formed.

In food processing, food borne microbes can be introduced from infected humans who handle the food, or by cross contamination from some other raw agricultural product and the establishment environment. For example, the unwashed hands of food handlers who are themselves infected can introduce bacteria and viruses.

In the RTE processing environment exposed product that is fully cooked can become cross contaminated if it touches raw meat or poultry that contain pathogens or from food contact surfaces that are contaminated.

In the kitchen, microbes can be transferred from one food to another food by using the same knife, cutting board or other utensil to prepare both without washing the surface or utensil in between.

The way that food is handled after it is contaminated can also make a difference in whether or not an outbreak occurs. Many microorganisms need to multiply to a larger number before enough are present in food to cause disease. Given warm moist conditions and an ample supply of nutrients, one bacterium that reproduces by dividing itself every half hour can produce 17 million progeny in 12 hours. As a result, lightly contaminated food left out overnight can be highly infectious by the next day. If the food were refrigerated promptly, the bacteria would not multiply at all or at a very slow rate.

To inhibit bacterial growth in meat, poultry, or egg products or in food handled by the consumer, it is important to store foods at a reduced temperature. Refrigeration or freezing prevents virtually all bacteria from growing but freezing preserves them in a state of suspended animation.

Foodborne Illness

Microorganisms can cause a variety of effects in food products including spoilage, which primarily affects product quality, and food poisoning, which is generally caused by pathogens. As regulators, we are most concerned with the effects that microorganisms have on food that leads to food borne illness, because this affects public health.

A food borne illness (or disease) is exactly what the term indicates - a disease or illness caused by the consumption of contaminated foods or beverages. It would seem rather obvious that a food borne microbial pathogen, or a preformed microbial toxic product, or another poison such as a poisonous chemical that has somehow contaminated the food and beverage, leads to one of the many different food borne illnesses.

There is no one “syndrome” that is representative of food borne illness/disease. Different diseases have many different symptoms. However, the microbe or toxin enters the body through the gastrointestinal tract, and often causes the first clinical signs such as nausea, vomiting, abdominal cramps and diarrhea, which are common symptoms in many food borne diseases.

More than 250 different food borne diseases have been described. Most of these diseases are infections, caused by a variety of bacteria, viruses, and parasites. Other diseases are poisonings, caused by harmful toxins or chemicals that have contaminated the food, for example, poisonous mushrooms or heavy metal contamination.

To cause illness, the pathogen must overcome several hurdles. A simple summary of these hurdles are as follows:

- Survive the acidic environment of the stomach.
- Attach to/colonize intestinal walls.
- Compete against the natural microbiota of the gut.
- Survive the host defense mechanisms.
- Once attached in the large intestine: elaborate toxins and virulence factors, and cross the epithelial barrier, which then results in the symptoms characteristic to the disease or illness.

Foodborne Pathogens

Following is a list of pathogens and infectious agents of public health concern. This list is not exhaustive; however, it contains most of the food borne pathogens that affect meat, poultry, and egg products.

1. Bacteria
    - Gram Positive:
        - Listeria monocytogenes
        - Staphylococcus aureus
        - Bacillus cereus
        - B. anthracis
        - Clostridium botulinum
        - C. perfringens
    - Gram Negative:
        - Salmonella spp
        - Campylobacter spp
        - Escherichia coli 0157:H7
        - Yersinia enterocolitica
        - Brucella spp
2. Viruses:
    - Hepatitis
    - Rotaviruses
3. Prions:
    - new variant CJD
4. Tapeworms:
    - Taenia spp
5. Roundworms:
    - Trichinella spp

6. Protozoa:

- Toxoplasma spp
- Sarcocystis spp.

Pathogens and infectious agents of concern from the public health regulatory perspective.

Salmonella spp

Salmonella is a rod-shaped, motile bacterium (non-motile exceptions are S. Gallinarum and S. Pullorum), non-spore forming and Gram negative. This microorganism grows at 6.5- 47 ˚C (43.7-116 ˚F), pH as low as 4.5, with or without air, and aw of >0.95 (may vary, e.g., S. Newport = 0.941 and S. Typhimurium = 0.945). The optimum growth temperature is at the human body temperature but it grows very poorly at refrigerated temperatures. Even though freezing and frozen storage can have some deleterious effect on Salmonella, it is known that this microorganism remains viable for long periods of time in frozen foods. There are specific serotypes that are capable of producing food borne illness (salmonellosis) including S. Enteritidis (eggs and egg products), S. Newport (milk and dairy cows), and S. Typhimurium (cattle) among others.

Salmonella spp. have the ability to cross the mucosal barrier invading and replicating within the host causing chronic infections, long term carriage, and systemic disease. Pathogenic Salmonella possess a myriad of virulence factors including those that promote adhesion to host cells in the intestine, endotoxins, siderophores, invasins, and the production of cytotoxins and diarrheagenic enterotoxins, which act in concert in the pathogenesis of infection. It is believed that the enterotoxins are responsible in causing the acute symptoms of the disease.

Campylobacter Species

Campylobacter species, including C. jejuni, C. coli, and C. lari, can be isolated from the intestinal tract of poultry and poultry products. The two most frequently occurring Campylobacter species of clinical significance for human consumption of food are C. jejuni and C. coli, but C. jejuni causes most of the human infections. These species are the ones most often isolated in poultry products.

Campylobacter jejuni is a Gram-negative slender, curved, and motile rod. It is a microaerophilic organism, which means it has a requirement for reduced levels of oxygen and requires 3 to 5% oxygen and 2 to 10% carbon dioxide for optimal growth. The isolation of this pathogen requires special antibiotic-containing media and a special microaerophilic atmosphere (5% oxygen). Campylobacter jejuni is relatively fragile and sensitive to environmental stresses such as 21% oxygen, drying, heating, disinfectants, and acidic conditions. This microorganism can grow at temperatures between 25-42 ˚C (77-107 ˚F), pH range of 5.5-8, and aw >0.95.

This bacterium is now recognized as an important pathogen. The pathogenic mechanisms of C. jejuni are still not completely understood; however, research has demonstrated that a series of virulence factors come into play for the pathogen to be able to cause disease. These factors include motility, chemotaxis, invasins, and adhesins, among others. Some investigators have shown that C. jejuni firstly colonizes the jejunum and ileum, and then the colon producing a heat-labile toxin (Campylobacter invasion antigens or CIA proteins) that may cause diarrhea.

Although anyone can become ill with campylobacteriosis, children under 5 years and young adults (15-29) are more frequently afflicted than other age groups.

Campylobacter jejuni infection causes diarrhea, which may be watery or sticky and can contain blood (usually occult) and fecal leukocytes. Other symptoms often present are fever, nausea, cramping, abdominal pain, headache, and muscle pain within 2-5 days after exposure to the organism. A very small number of the pathogen (fewer than 500) can cause illness in humans. The illness generally lasts 7-10 days and in individuals with compromised immune systems, the pathogen occasionally spreads to the bloodstream and causes a serious life-threatening infection.

Since C. jejuni is an invasive organism long-term effects of this illness can lead to Guillain-Barré syndrome, a rare disease that affects the nerves of the body beginning several weeks after the diarrheal illness. This disease occurs when a person's immune system is triggered to attack the body's own nerves, and can lead to paralysis that last several weeks and usually require intensive care. It is estimated that approximately one in every 1000 reported Campylobacteriosis cases leads to Guillain-Barré syndrome (40% of the syndrome cases).

Many chicken flocks are asymptomatically infected with Campylobacter, i.e., the chickens are infected with the organism but show no sign of infection and can be easily spread from bird to bird through a common water source or contact with infected feces. When infected chickens are slaughtered, the organism can be transferred from the intestines to the meat. More than half of the raw chicken in the United States market has Campylobacter on it. Campylobacter is also present in the giblets, especially the liver.

Raw milk, raw beef and pork are also sources of infection. The bacteria are often carried by healthy cattle, birds, and by flies on farms. Non-chlorinated water may also be a source of infections.

Escherichia Coli

A minority of E. coli serotypes are capable of causing human illness (colibacillosis) by different mechanisms. Escherichia coli are normal inhabitant of the intestine of all animals, including humans; serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. Based on disease syndromes and other characteristics, there are six classes of diarrheagenic E. coli recognized: enteroaggregative (EAggEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohemorrhagic (EHEC), and diffusely adherent (DAEC). EHEC is the class that is of concern to industry, FSIS, and public health; the more significant serotype is E. coli O157:H7.

Escherichia coli serotype O157:H7 is one of the rare serotype of this genus and, as mentioned above, belongs to the EHEC family that causes severe disease. This pathogen is a rod-shaped, generally motile, non-spore forming and Gram-negative. It generally grows at 2.5-45°C (36.5-113°F), pH between 4.6-9.5, with or without air, and aw of >0.935. There are strains of E. coli O157:H7 that possess an unusual tolerance to environmental stress such as temperature, pH, dryness, and can survive in water; recent research have shown that some strains are capable of forming biofilms.

This pathogen produces several virulence factors that cause severe damage to the lining of the intestine, acute renal failure (children and elderly), hemolysis, thrombocytopenia, and neurological problems (the last three occur mainly in adults). All EHEC, including E. coli O157:H7, produce Shiga toxins (Stx 1 and 2; also known as Vero toxins and Shiga- like toxins) which are closely related to or identical to the toxin produced by Shigella dysenteriae type 1; these toxins targets the human kidney, particularly the cortical region which is rich in Gb3 receptors for the toxin. These toxins are encoded on a bacteriophage that was transferred from Shigella to E. coli O55:H7 (parent strain of serotype O157:H7). Other virulence factors are the pO157 plasmid (90-kb size) which encodes the EHEC hemolysins and serine proteases; LEE pathogenic island which enclose the genes accountable for the A/E histopathology including a type III secretion system responsible for the epithelial cell signal transduction events leading to the attaching/effacing (A/E) lesion, and a bacterial adhesion proteins called intimin and Tir (Translocated intimin receptor); as well as other virulence factors.

This microorganism causes three distinctive clinical manifestation including hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). All people are believed to be susceptible to hemorrhagic colitis, but young children and the elderly appear to progress to more serious symptoms more frequently (HUS and TTP, respectively).

HC is characterized by severe cramping (abdominal pain) and diarrhea, which is initially watery but becomes grossly bloody. Occasionally vomiting occurs and some individuals can exhibit watery diarrhea only. Fever is either low-grade or absent. The infectious dose is as few as 10 bacterial cells with an incubation period of approximately 4 days (median) and clinical manifestations can develop within 24-48 hours with duration of 8 days (average).

Listeria Monocytogenes

Listeria species (spp) is a rod-shaped, non-spore forming Gram-positive bacterium.

Within the Listeria genus six species has been identified consisting of L. monocytogenes, L. innocua, L. ivanovii, L. welshimeri, L. seeligeri, and L. grayi.

Specifically, L. monocytogenes is recognized as a human pathogen that causes listeriosis. This pathogen is motile and can grow in cool (temperature range of 0-45 ˚C [32-113 ˚F]) and damp environments, at a pH range of 4.4-9.4, and aw >0.92. Some characteristics that make some strains of L. monocytogenes hearty include growth and/or survival in acidic environment (pH < 5.0), ability to withstand heat treatments, and growth and survival in concentrated salt solutions. The pathogenicity of this microorganism is associated with the virulence factors such as internalin A (allow the pathogen to induce its own uptake by specific host cells), Act A (a surface protein required for intracellular movement and cell-to-cell spread through bacterially induced acting polymerization), listeriolysin O (a toxin that acts as a hemolysin), among others.

Only three L. monocytogenes serotypes (4b, 1/2a, and 1/2b) are pathogenic. One outstanding characteristic of L. monocytogenes is its ability to form biofilm, which serve as a protection shell. This pathogen, as well as other biofilm microorganisms, elicits specific mechanisms for initial attachment (by the production of extra polymeric substance and the bacterial cell surface structures such as flagella, fimbriae, and other proteins) to a surface, development of a community structure and ecosystem (biofilm), and detachment. Biofilm is a heterogeneous structure of microbial cells (can be a mix culture) encased in an extracellular polymeric substance matrix (primarily polysaccharide material) which can entrap non cellular material such as mineral crystals, corrosion particles, blood components, food particles, etc.

Active flow occurs in this niche allowing diffusion of nutrients, water, oxygen, and even antimicrobial agents; there is also exchange of waste metabolic material. Since this ecosystem is dynamic, the community structure changes from a compact to a looser structure over time allowing the dispersion of planktonic cells to other sites, which starts the cycle of biofilm formation at that new site. Biofilm can form as little as a few hours to days depending of the number of bacterial cells, nutrient availability, surface characteristics, temperature, etc. Once formed, they can persist for a long time (years) and they are very difficult to remove, as the biofilm confers protection from the chemicals used to clean and sanitize surfaces.

Food Fermentation

Fermentation is a natural process through which microorganisms like yeast and bacteria convert carbs — such as starch and sugar — into alcohol or acids.

The alcohol or acids act as a natural preservative and give fermented foods a distinct zest and tartness.

Fermentation also promotes the growth of beneficial bacteria, known as probiotics.

Probiotics have been shown to improve immune function as well as digestive and heart health.

Therefore, adding fermented foods to your diet may benefit your overall well-being.

Health Benefits of Fermented Foods

A number of health benefits are associated with fermentation. In fact, fermented foods are often more nutritious than their unfermented form.

Here are the key health benefits of fermented foods.

Improves Digestive Health

The probiotics produced during fermentation can help restore the balance of friendly bacteria in your gut and may alleviate some digestive problems.

Evidence suggests that probiotics can reduce uncomfortable symptoms of irritable bowel syndrome (IBS), a common digestive disorder.

Fermented foods may also lessen the severity of diarrhea, bloating, gas, and constipation.

For these reasons, adding fermented foods to your diet may be useful if you regularly experience gut issues.

Boosts your Immune System

The bacteria that live in your gut have a significant impact on your immune system.

Due to their high probiotic content, fermented foods can give your immune system a boost and reduce your risk of infections like the common cold.

Consuming probiotic-rich foods may also help you recover faster when you’re sick.

Additionally, many fermented foods are rich in vitamin C, iron, and zinc — all of which are proven to contribute to a stronger immune system.

Makes Food Easier to Digest

Fermentation helps break down nutrients in food, making them easier to digest than their unfermented counterparts.

For example, lactose — the natural sugar in milk — is broken down during fermentation into simpler sugars — glucose and galactose.

As a result, those with lactose intolerance are generally fine eating fermented dairy like kefir and yogurt.

Plus, fermentation helps break down and destroy anti-nutrients — such as phytates and lectins — which are compounds found in seeds, nuts, grains, and legumes that interfere with the nutrient absorption.

Therefore, consuming fermented beans or legumes like tempeh increases the absorption of beneficial nutrients, making them more nutritious than unfermented alternatives.

Safety and Side Effects

Fermented foods are considered safe for most people. However, some individuals may experience side effects.

Due to the high probiotic content of fermented foods, the most common side effect is an initial and temporary increase in gas and bloating.

These symptoms may be worse after consuming fiber-rich fermented foods, such as kimchi and sauerkraut.

It's also important to note that not all fermented foods are created equal.

Some products may contain high levels of added sugar, salt, and fat — so it's important to read nutrition labels to make sure you're making a healthy choice.

If fermenting at home, make sure you follow recipes closely for safety purposes. Incorrect temperatures, fermentation times, or unsterile equipment can cause the food to spoil, making it unsafe to eat.

Common Fermented Foods

There are many different types of fermented foods consumed around the world, including:

- Kefir,
- Sauerkraut,
- Tempeh,
- Natto,
- Cheese,
- Kombucha,
- Miso,
- Kimchi,

- Salami,
- Yogurt,
- Sourdough bread,
- Beer,
- Wine,
- Olives.

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We would like to thank the editorial team for lending their expertise to make the book truly unique. They have played a crucial role in the development of this book. Without their invaluable contributions this book wouldn't have been possible. They have made vital efforts to compile up to date information on the varied aspects of this subject to make this book a valuable addition to the collection of many professionals and students.

This book was conceptualized with the vision of imparting up-to-date and integrated information in this field. To ensure the same, a matchless editorial board was set up. Every individual on the board went through rigorous rounds of assessment to prove their worth. After which they invested a large part of their time researching and compiling the most relevant data for our readers.

The editorial board has been involved in producing this book since its inception. They have spent rigorous hours researching and exploring the diverse topics which have resulted in the successful publishing of this book. They have passed on their knowledge of decades through this book. To expedite this challenging task, the publisher supported the team at every step. A small team of assistant editors was also appointed to further simplify the editing procedure and attain best results for the readers.

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The publishing team has been an ardent support to the editorial, designing and production team. Their endless efforts to recruit the best for this project, has resulted in the accomplishment of this book. They are a veteran in the field of academics and their pool of knowledge is as vast as their experience in printing. Their expertise and guidance has proved useful at every step. Their uncompromising quality standards have made this book an exceptional effort. Their encouragement from time to time has been an inspiration for everyone.

The publisher and the editorial board hope that this book will prove to be a valuable piece of knowledge for students, practitioners and scholars across the globe.

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