



Extraction and Industrial Applications of Macro Molecules: A Review

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Abstract

Macromolecules, large molecules composed of repeating subunits called monomers, play a crucial role in living organisms, performing diverse functions such as energy storage, structural support, information storage and transfer, and catalysis of chemical reactions. Carbohydrates, lipids, proteins, and nucleic acids are the four major classes of biological macromolecules. Extracting macromolecules from natural resources is critical in developing analytical processes and subsequent fortified products. Public awareness has grown due to using natural resources for environmental preservation and sustainable development. Extraction might be the first step in developing analytical methods and give room to product development. However, conventional techniques use organic solvents like acetone, ethanol, methanol, and ethyl acetate along with heated or boiling water. As a result, high temperatures and lengthy extraction times are produced when procedures like maceration, percolation, and solvent extraction are utilized. Due to these drawbacks, other extraction techniques have recently started to replace these conventional methods. These conventional procedures frequently entail using an energy source to hasten the transfer of the macromolecules compounds for further processing. This paper explores emerging techniques, such as pulse electric field-assisted extraction, Ionic liquid-based extraction, Subcritical water extraction, pressurized liquid extraction, Enzyme-assisted extraction, supercritical fluid extraction, ultrasonication-assisted extraction,



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and microwave-assisted extraction. The extraction of macromolecules for fortification purposes offers significant health and commercial benefits, addressing nutritional deficits and malnutrition. By understanding each macronutrient's specific benefits and purposes, effective fortification strategies can be developed to maintain a healthy body.

Introduction

Macromolecules

A macromolecule is a large molecule composed of repeating subunits known as monomers. These subunits are typically covalently bonded to form a polymer, a long chain of repeating units. It can be natural materials like sugars and natural fibers (like cotton) or synthetic materials like plastics, synthetic fibers, and adhesives.¹ Large molecules called macromolecules fill a cell with essential life-sustaining tasks. For instance, macromolecules provide structural support, act as a storage space for electrical energy, enable the storage and retrieval of genetic information, and hasten biological processes. Giant molecules that are biologically necessary for life can be found in key nutrients. These organic macromolecules (polymers) are combinations of monomers, which are smaller organic molecules. In Table 1 below, everyone of the four major biological macromolecules—carbohydrates, lipids, nucleic acids, and proteins,—is briefly reviewed about their sources, structure and composition, and their commercial applications. The human diet's primary caloric components are macromolecules present in food systems. The main source of carbohydrates in our daily diets is starch, which accounts for about 50% of our daily energy needs. Among other useful

properties of food, starch may affect the texture, mouthfeel, moisture, viscosity, and shelf life of food products. Proteins are also necessary for the human body to operate. The amino acid chain that makes up proteins can be found in both plant and animal sources. The three kinds of amino acids are essential, nonessential, and conditional.² Protein has the desirable abilities to thicken, gelatinize, emulsify, foam, and have a high capacity to hold water. Our everyday diet must contain lipids, such as fats and oils because they are crucial to nutrition and food preparation. While vital fatty acids and fat-soluble elements are essential in generating the particular flavour of cooked foods, the lipid is ingested in daily meals as a crucial energy source.³ It is crucial to remember that while lipids are advantageous in moderation, consuming too many bad fats can lead to health issues like obesity and cardiovascular illnesses. For maintaining life, nucleic acids are the most crucial macromolecules. Nucleic acids are vital biopolymers that are found in all living organisms and are utilized to transmit, encode, and express genes. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two major forms of nucleic acids.¹ This study aims to show macromolecule extraction and present some cutting-edge technologies for food fortification, health, and commercial applications.

Table 1: Known Macromolecules as Recommended by Food and Drug Administration

Macromolecule	Carbohydrates	Proteins	Fats	Nucleic Acids	References	
Sources	Fruits and Vegetables; Apples, Oranges, Carrots, and Bananas. Legumes; Kidney beans, Chickpeas, and Lentils. Cereals and Grains; Rice, Corn, Wheat, and Barley. Tubers and Root Vegetables; Cassava,	Meat; Poultry, Pork, Beef, and Lamb. Seafood; Fish and Shellfish. Dairy products; Cheese, Milk, Yoghurt, and Eggs. Legumes; Chickpeas, lentils, Beans, and Peas. Nuts and Seeds;	Animal sources: Red meat; Beef, Pork, and Lamb. Poultry; Turkey and Chicken. Dairy products; Cream, Butter, Whole milk, and Cheese. Plant-Based fat Sour-		Nucleic acids are found in cells, particularly in the nucleus; sources include animal tissues and organs; Kidney, Liver, and Muscle. Plant tissues	1,2,4-9

	Potatoes, and sweet potatoes. Milk and Dairy products; Yoghurt, Lactose, and Cheese. Shellfish and Seafood; Chitin and Glycogen.	Walnuts, Hemp seeds, Almond, and Chia seeds. Barley, Rice, and Wheat. Soy Products; Tempeh, Soy milk, and Tofu.	ces: Nuts and Seeds; Flax-seeds, Chia seeds, Walnuts, and Almonds. Oils; Olive oil, coconut oil, Avocado oil, and Flaxseed oil.	and organs; Seeds, Roots, and Leaves. Microbial sources; Bacteria and Yeast.
Structure & Composition	Carbohydrates are classified into four types: Monosaccharides; Glucose, Fructose, and Galactose. Disaccharides; Sucrose, Lactose, and Maltose. Oligosaccharides; Raffinose and Stachyose. Polysaccharides; Starch, Cellulose, and Glycogen.	Classification of Proteins: Complete Protein; Animal-based, and Plant-based sources. Incomplete Protein; Plant-based sources. There are four levels of protein structure; primary, secondary, tertiary, and quaternary. Proteins are water-soluble polymers of small building blocks known as amino acids. Twenty distinct amino acids; Nine are essential, five are nonessential while six are conditional.	Classification of Dietary fats: Saturated Fatty Acids; Plant-based, and Animal-based sources. Monounsaturated fatty acids; Plant-based sources. Polyunsaturated Fatty Acids; Omega-3 fatty acids, and Omega-6 fatty acids. Trans Fats; Natural sources, and Artificial sources.	Classification of Nucleic acids: Deoxyribonucleic Acid (DNA); Animal-based sources and Plant-based sources. Ribonucleic Acid (RNA): Messenger RNA (mRNA), Ribosomal RNA (rRNA), and Transfer RNA (tRNA). ^{1,7,10-15}
Commercial Applications	Food and Beverage Industry; Sweeteners, Fiber, Starch, Bakery and Confectionery. Healthcare and Pharmaceutical Industry; Stabilizers and Fillers, Injectable Carbohydrates, Wound healing and Tissue Engineering, and Excipients. Textile Industry; Starch and Dextrins. Biofuel Production; Ethanol. Personal Care and Cosmetics Industry; Emulsifiers	Food and Beverage Industry; Dairy and Meat Alternatives, Protein Fortification, Bakery and Confectionery. Healthcare and Pharmaceutical Industry; Vaccines, Therapeutic Proteins, Diagnostic Assays. Personal Care and Cosmetics Industry; Anti-aging and Anti-wrinkle, Hair and Skin Care. Animal Feed; Animal Protein Supplement. Sports	Food Industry; Cooking and Frying, Shelf-Life Extension, Flavor and Texture Enhancers, Emulsifiers and Stabilizers, Fat-based Spreads and margarines. Personal Care and Cosmetics Industry; Lipsticks and Lip balms, Moisturizers and Emollients. Health care and	Agriculture and Food Industry; Genetically Modified Organisms (GMOs), Food Safety Testing. Genetic Testing and Personalized Medicine; DNA Sequencing, Pharmacogenomics. Disease Detection and Diagnostics; Nucleic Acid Probes, Polymerase Chain Reaction, Gene Expression Analysis. Forensic Science; DNA Profiling. ¹⁶⁻²⁵

and Thickeners, and Humectants. Bioplastics and Biomaterials; Polysaccharides like cellulose, starch, and chitosan.	Nutrition and Supplements; Protein drinks, Protein Powders and Bars. Bioplastics and Biomaterials; Protein-based materials such as silk and collagen.	Pharmaceutical Industry; Excipients, Controlled -Release System. Industrial Application; Biofuels and Lubricants. Animal Feed; Energy Source. Industrial Chemicals; Surfactants.	Biotechnology and Biopharmaceuticals; Gene Therapy, Recombinant DNA Technology, RNA Interference (RNAi). Environmental Monitoring; Biodiversity Assessment.
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Benefits of Macromolecules

Macromolecules, as essential components of cells, perform a variety of crucial functions necessary for sustaining life. These macromolecules, which include proteins, carbohydrates, lipids, and nucleic acids, offer several advantages and benefits. Carbohydrates, a type of biomolecule, are divided as four categories depending on their structure and chemistry: Monosaccharides: Monosaccharides are the simplest form of carbohydrates and cannot be further hydrolyzed into smaller sugar units. Common monosaccharides include glucose, fructose, and galactose. Glucose is the primary source of energy for many organisms, while fructose is found in fruits and is often used as a sweetener. Galactose is a component of lactose, a disaccharide found in milk.⁴ Disaccharides: Disaccharides are formed by the condensation of two monosaccharide units. They can be hydrolyzed into their constituent monosaccharides by enzymatic or chemical reactions. Common disaccharides include sucrose, lactose, and maltose. Sucrose, popularly known as white sugar, is made up of two sugars: fructose and glucose. Lactose, found in milk, consists of glucose and galactose. Maltose is a product of starch digestion and consists of two glucose units.¹ Oligosaccharides: Oligosaccharides are composed of a small number of monosaccharide units, typically between 3 to 10 sugar residues. They are often found in plant-based foods. Examples of oligosaccharides include raffinose and stachyose. These compounds are not readily hydrolyzed by human digestive enzymes but can be fermented by gut bacteria, providing prebiotic benefits. Polysaccharides: Polysaccharides are large, complex carbohydrates composed of many monosaccharide units joined together by glycosidic linkages. They serve as storage forms of carbohydrates in plants and

animals and can be classified as either structural or storage polysaccharides. Starch, found in plants, is a storage polysaccharide composed of glucose units. Cellulose, also found in plants, is a structural polysaccharide that forms the cell wall. Glycogen is the storage polysaccharide in animals, including humans, and is structurally similar to starch but has more extensive branching. These different types of carbohydrates play vital roles as energy sources, structural components, and functional molecules in various biological processes.⁴ According to Kaur,²⁶ rye is a good source of carbohydrates, proteins, fiber, minerals and antioxidants. Starch takes up a sizable amount of the carbohydrates in rye and other cereal grains (55-70%). Starch is a major human diet's energy source. It also improves the utility of food products. Dietary fibers are just as essential as carbohydrates because they support healthy weight management and digestive health. Rye's grain nutritional fiber composition indicates that lignins, arabinoxylan, beta-glucan, and fructan are all present in varying degrees. Cereal grains are a rich source of carbohydrates in general. By far the most common constituent group, carbohydrates comprise 66 to 80% of rye grains. Starch (56-75%) makes up the majority of the carbohydrates found in rye grains, followed by fiber (15-22%) and sugars (0.61%, 0.18%, and 2.58%) at 3%.

The majority of organic molecules in biological systems are proteins, which also play the greatest range of functions of any macromolecule. In general, proteins are enzymes that serve as structural, regulatory, contractile, or protective components for membranes, transport, and storage.²⁷ Each cell in a living organism may have thousands of proteins, each with a distinct function. Protein activities and structures are very different from one another.

However, they are all amino acid polymers that are linearly organized. Proteins are made up of amino acid polymers. Each amino acid has a core carbon that is joined to a R group, also referred to as a side chain, an amino group, a carboxyl group, and a hydrogen atom. There are 20 commonly occurring amino acids, each with a unique R group. Each amino acid is joined to its immediate neighbours by a peptide bond. A polypeptide is an extended chain of amino acids. Proteins are categorized at the primary, secondary, tertiary, and (optionally) quaternary levels of the organization.¹ The specific arrangement of amino acids determines the primary structure. The secondary structure is produced when the polypeptide locally folds to produce structures like the helix and pleated sheet. The total three-dimensional structure is known as the tertiary structure. When two or more polypeptides combine to form the full protein structure, the configuration is known as the quaternary structure of a protein. Protein form and function are closely connected; any alteration brought on by variations in temperature or pH may cause denaturation of the protein and a loss of function.²⁸ The protein that is most supported among all other cereal proteins in terms of amino acid makeup is rice protein. The most popular type of rice is refined, which has only the endosperm of the grain left after being milled to remove the husk and bran. According to Li,¹⁴ it has been found that the husk and bran layer contains a sizable amount of rice protein—between 11.3% and 14.9%. The limiting amino acid in grains, lysine, is also present in second-highest concentration. Compared to other plant proteins, rice protein is considered the best source of protein for functional foods since it has a protein efficiency ratio similar to milk casein and is hypoallergenic. Whole grains are far healthier to eat than pearled rice because they contain endosperm, germ, and bran. Not only does eating whole grains improve basic nutrition, but it also reduces the risk of various chronic diseases.

Lipids, a class of macromolecules, are non-polar and hydrophobic by nature. This is because most of the bonds in hydrocarbons are non-polar carbon-carbon or carbon-hydrogen bonds. They are hydrophobic, or "hating water," non-polar chemicals that do not dissolve in water.¹ Lipids have several functions within a cell. Cell membranes, which preserve the consistency and order of cells, primarily consist of lipids. Particularly significant for the cell membrane's

structural integrity are phospholipids. Lipids are a more effective energy source than carbohydrates since they may be stored as energy reserves in the body. Cell signaling pathways involve lipids like phospholipids and sphingolipids.

They can serve as signal relayers between cells and are also important in controlling cellular reactions to environmental cues. All biological membranes depend on lipids, which are also the building elements of many hormones. The generation of hormones that control numerous physiological processes, including growth and development, metabolism, and immunological function, is aided by certain lipids, notably cholesterol and steroid hormones. Additionally, lipids can serve as a barrier around some organs, such as the kidneys, preventing harm from physical trauma or injury. Animals and plants are protected from their surroundings by lipids. For example, they help keep aquatic birds and mammals dry by producing a protective layer over fur or feathers due to their water-repellent hydrophobic nature.^{15,29}

Triglycerides, phospholipids, and steroids are the three main types of lipids. Although waxes are a less popular category, they are nonetheless regarded as lipids. Triglycerides: The most prevalent lipids are triglycerides, also referred to as fats and oils. They comprise three fatty acids and glycerol, which the body uses to store energy and provide insulation and cushioning.³⁰ Phospholipids: The lipids that make up the cell membrane are called phospholipids. They include two fatty acids, a phosphate group, and a glycerol molecule. While the fatty acid tails are hydrophobic (fearful of water), the phosphate group makes one end of the molecule hydrophilic (loving water). Because of their special structure, phospholipids can form a bilayer in cell membranes, helping to regulate what enters and leaves the cell.¹³ Steroids: A four-ring structure is a characteristic of steroids, a category of lipid. They are crucial for a number of physiological activities, such as the control of the immune system, metabolism, and reproductive procedures. In addition to being a component of cell membranes, cholesterol is a sort of steroid that serves as a precursor for the creation of other steroids, including hormones like testosterone.³¹ A long-chain fatty acid and a long-chain alcohol make up the lipid known as waxes. They frequently serve as a protective layer in both

plants and animals. Although waxes are a form of lipid, their discussion is less frequent than that of the other three groups indicated above. Lipids are important and serve a purpose in food because they improve flavour, satiety, and nutrition. As a result, lipid quality is a crucial consumer concern and may be connected to a variety of medical issues.³⁰ Each of these categories has a certain purpose. Olives, avocados, almonds, pecans, pumpkin, and sesame seeds are high in monounsaturated fats. Canola oil, corn, fish, walnuts, linseed oils, and flaxseed are some foods containing polyunsaturated fats. Saturated fats can be present in some plant-based foods, such as coconut and palm oils, although they are mostly found in animal products, such as beef or cheese. In terms of overall structure, all macromolecules—aside from lipids—are thought of as polymers. A polymer is a chain of covalently connected homologous monomers, or subunits. Proteins, carbohydrates, and nucleic acids all have monomers that are called sugars, amino acids, and nucleotides, respectively. This diverse collection of molecules is composed of a large variety of nonpolymeric compounds known as lipids.³²

Nucleic acids are molecules made up of nucleotides that regulate biological processes including cell division and protein production. Each nucleotide consists of a pentose sugar, a nitrogenous base, and a phosphate group. The two different types of nucleic acids are DNA and RNA. Parents pass DNA on to their children (in the form of chromosomes), contains the genetic code for every cell. It has a double-helical structure, in which the two strands run counterclockwise, complement one another, and are connected by hydrogen bonds. Single-stranded RNA is made up of the pentose sugar ribose, a nitrogenous base, and a phosphate group. RNA is involved in both the regulation and production of proteins. The messenger RNA (mRNA), which is exported from the nucleus to the cytoplasm, contains the instructions for constructing proteins. Ribosomal RNA (rRNA), on the other hand, is a component of the ribosomes at the site of protein synthesis, whereas transfer RNA (tRNA) delivers the amino acid there. How mRNA is used to produce proteins is regulated by microRNA.³³

Commercial Applications of Macromolecules

Essential macromolecules in the food industry include proteins, carbohydrates, and lipids, which

are added to and supplement foods to enhance their diverse nutritional qualities. They are necessary parts of human nutrition. They are also employed in manufacturing a number of culinary items, including cheese, yoghurt, and bread. Proteins are frequently employed in foods as texturizing agents to enhance their consistency and texture. While gelatin is used to create desserts and gummy candies, casein enhances the texture of cheese and yoghurt. In products like salad dressings and mayonnaise, proteins can also be employed as emulsifiers to facilitate mixing water and oil.¹⁶ In processed foods, carbohydrates like cellulose, pectin, and carrageenan are frequently employed as thickeners, stabilizers, and gelling agents. Foods like sauces, soups, and desserts can benefit from them by having a better mouthfeel and texture. Gums are one of the few food additives with the proper structural and functional characteristics, and they can be obtained from various sources. Polysaccharide gums, additionally referred to as hydrocolloids, have been used in the food, medicinal, fabric, power, water, the field of biotechnology, surroundings, personal care products, and pharmaceutical sectors for a range of applications. This is due to its desirable attributes like affordability, sustainability, recycling potential, accessibility, compositional variety, high potential for binding to water, emulsion formation stabilization, distinctive rheological features, non-toxicity, and gel-making capability.¹⁷ Lipids, such as vegetable oils and animal fats, are frequently added to processed meals as ingredients to improve flavour and texture. They can also be employed as preservatives to increase the shelf life of food products and as emulsifiers to aid in blending ingredients. Proteins and nucleic acids are used extensively in producing various drugs and vaccines in the pharmaceutical industry. The capacity of proteins to interact with specific targets in the body makes them a common component of therapeutic medicines used in medicine. One such protein hormone is insulin, which is used to treat diabetes and control blood glucose levels. Enzymes, growth factors, and antibodies are examples of additional protein medications.¹⁸ Gene therapy, a possible method of treating genetic diseases, uses nucleic acids like DNA and RNA. Gene therapy includes adding new or altered genes to the body to supplement or replace damaged genes. In vaccines, genetic material instructing cells to generate particular proteins that stimulate an immune response is

delivered through nucleic acids. Macromolecules are essential to the production of many drugs and vaccines, and their diverse properties and functions make them valuable tools in the field of medicine.²⁰ Macromolecules—particularly nucleic acids and proteins—are widely exploited in the study and development of biotechnology. Recombinant DNA technology is one such example where nucleic acids are utilized to manufacture desired proteins. Examples include recombinant DNA technology and protein expression systems like yeast, bacteria, and mammalian cells. Proteins are necessary molecules that provide various bodily activities, including signalling, catalysis, and structure.^{19,20}

Genetic engineering is changing an organism's genetic makeup to add new features or characteristics. This can be accomplished by modifying the organism's DNA or RNA using methods like gene cloning, PCR, and CRISPR-Cas.⁹ The procedure of DNA sequencing is used to establish the nucleotide sequence within a DNA molecule. It is a crucial tool in biotechnology research to examine organisms' genetic makeup, spot genetic diseases, and create novel medications and treatments. Using computational tools and algorithms to analyse and understand biological data is the focus of the interdisciplinary field of bioinformatics. The analysis of DNA and protein sequences, the prediction of the structure and function of proteins, and the development of novel medications and treatments all make substantial use of this technology in the biotechnology research.^{20,34}

Macromolecules, particularly polymers, are used to produce various materials such as plastics, adhesives, and coatings. Polymer-based materials, known as plastics, are employed in various industries, including electronics, building, and packaging. Polystyrene, PVC, polyethylene, and polypropylene are examples of common plastic kinds. Depending on their use, these polymers can be generated as films, fibres, or moulded items.³⁵ Materials are bound together by chemicals called adhesives. Polymers, such as cyanoacrylate, epoxy, and polyurethane, are used to make a variety of adhesives. Metals, plastics, and wood can all be joined together with the help of these adhesives. Materials are put to a surface as coatings to improve or protect it. Acrylic, polyurethane, and epoxy are just a few of the polymers that are used to make many

coatings. These coatings protect against wear and tear, UV rays, and corrosion. Macromolecules are important in producing many different materials, and their unique properties and functions make them valuable in a wide range of applications.²¹

Macromolecules like proteins and lipids are used to make different cosmetic items like creams and lotions. The appearance and texture of the skin can be improved by using proteins like collagen and elastin, which are frequently utilized in cosmetic goods. These proteins may contribute to fewer wrinkles, firmer skin, and better skin moisture.¹⁶ Ceramides and fatty acids, two lipids that can aid in increasing the skin's moisture content, are also frequently found in cosmetic products. The skin can be kept soft and supple by using these lipids to help reinforce the skin barrier and stop moisture loss. Polymers like polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) are frequently employed in cosmetic products as thickeners, emulsifiers, and stabilisers. These polymers can enhance cosmetic items' stability and consistency while making them simpler. Macromolecules are crucial in creating cosmetics due to their wide range of attributes and use as ingredients in creating creams, lotions, and other skincare products.²²

To increase agricultural productivity and quality, fertilizers, insecticides, and herbicides are made from macromolecules like proteins and carbohydrates. For plants to grow and develop, fertilizers are compounds that supply them with vital nutrients like nitrogen, phosphorus, and potassium. A lot of fertilizers are composed of macromolecules, such as proteins and carbohydrates, which soil microbes can break down to release nutrients for plant absorption. Insects, weeds, and fungi that can harm crops and lower production are examples of pests that can be controlled or eliminated by applying pesticides.²³ The metabolic or nervous system of pests can be disturbed by several pesticides, which are often manufactured from macromolecules like proteins and lipids. Herbicides are chemicals that are applied to weeds to prevent or reduce their ability to compete with crops for nutrients, water, and sunlight. Numerous herbicides are composed of macromolecules that might obstruct weed growth or metabolism, such as lipids and carbohydrates.²⁴ The creation of fertilizers, insecticides, and herbicides depends on macromolecules, and their distinctive

characteristics and uses make them valuable for enhancing crop productivity and quality.

In bioremediation procedures, contaminants are broken down, and contaminated places are cleaned using macromolecules like enzymes and bacteria. Using natural biological processes to remove or degrade contaminants is known as bioremediation, and it is a cost- and environmentally-friendly method of cleaning up contaminated locations. In bioremediation, proteins called enzymes are frequently utilized to break down contaminants into less harmful chemicals and accelerate chemical reactions.²⁵ For instance, certain enzymes can degrade chlorinated solvents like trichloroethylene (TCE), which is frequently discovered in contaminated areas. In bioremediation, microorganisms like bacteria and fungi are frequently utilized because they can break down contaminants and turn them into less toxic compounds. For instance, certain bacteria may break down petroleum hydrocarbons, which are frequent contaminants in soil and groundwater and include gasoline and diesel.³⁶ Macromolecules like enzymes and microorganisms play an important role in bioremediation processes, and their unique properties and functions make them valuable tools in cleaning up contaminated sites and reducing environmental pollution. Macromolecules can be extracted using various cutting edge technologies as discussed below.

Extraction Methods for Macro Molecules

The extraction process is a crucial step in the food industry for obtaining macromolecules and bioactive compounds, such as flavourings, pigments, and antioxidants, from natural sources. The elements that make up our food, whether they come from plants or animals, are all combinations of these macromolecules. Over the past few decades, there has been a lot of research on the most effective ways to extract valuable molecules from natural resources. The use of natural resources to promote sustainable development and environmental protection has raised public awareness. Extraction can be thought of as the initial stage in the development of analytical methods and ensuing products. The use of organic solvents or high temperatures in conventional extraction techniques, such as solvent extraction, maceration, percolation, and steam distillation, harms the environment and causes the extracted molecules to degrade. However, conventional

techniques use organic solvents like acetone, ethanol, methanol, and ethyl acetate along with heated or boiling water. High temperatures and extended extraction times are the results of this. Due to these drawbacks, other extraction techniques have recently started to replace these conventional methods. These procedures frequently entail using an energy source to hasten the transfer of the macromolecule compounds to the solvent. Additionally, techniques that are more eco-friendly and require less solvents have been sought for. Over the past 10 years, new methods and emerging techniques have been developed and used to decrease the amount of sample that needs to be handled.

Green extraction technologies (GETs) have emerged as a sustainable alternative to conventional extraction methods. GETs are characterized by their eco-friendliness, high efficiency, and selectivity. Supercritical fluid extraction, microwave-assisted extraction, ultrasonication-assisted extraction, pressurized liquid extraction, pulse electric field-assisted extraction, subcritical water extraction, ionic liquid-based extraction, and enzyme-assisted extraction are some of the most cutting-edge extraction methods. This paper reviews the recent advances in GETs and their applications in the food industry.

Methods of Green Extraction Technologies

Green extraction technologies prioritize efficiency, sustainability, and safety when extracting macromolecules from various sources. Here are a few of the most popular ways to extract green materials that would be discussed in this article: Supercritical fluid extraction (SFE), Microwave-assisted extraction (MAE), Ultrasound-assisted extraction (UAE), Pressurized liquid extraction (PLE), Pulse-Electric-Field Assisted Extraction (PEF), Subcritical water extraction (SWE), Ionic liquid-based extraction (ILE), Enzyme-assisted extraction (EAE) 79,80,89 for better green extraction of bioactive compounds have developed over the past few decades. The methods, applications, advantages, and disadvantages of these techniques are presented in Table 2.

Supercritical fluid extraction (SFE)

SFE uses supercritical carbon dioxide as a solvent to extract compounds at high pressure and low

temperatures. Supercritical fluid (SF) serves as the extraction solvent in supercritical fluid extraction. Due to its similar liquid solubility and gas diffusivity properties, SF can dissolve various natural substances. Near their critical points, slight variations in pressure and temperature resulted in substantial modifications in their solvating properties. Supercritical fluids having lower surface tension, lower viscosity, and higher diffusion coefficients than normal solvents. In addition, they have characteristics with both gases and liquids. Supercritical fluids can be used to selectively extract from both liquid and solid matrices, and the ability to select of the extraction can be modified merely adjusting the degree of heat. Because supercritical fluids cannot dissolve the extracted matrices, the extraction pressure also depends on the density of the extracted matrices. Highly widely utilized solvent in SFE is carbon dioxide (CO₂) since that it's accessible, safe, non-toxic, recyclable, and can

reach supercritical conditions quickly. Additionally, CO₂ supercritical extraction produces cleaner results than traditional extraction techniques.⁹⁰ To execute SFE, the fluid is pressurized and heated to the necessary temperature and pressure before entering the extractor, bringing it to its supercritical condition (Figure 1). The solvent is then injected into the extractor and uniformly disseminated throughout the fixed bed by solid matter. During the extraction, the solvent passes across the fixed bed and dissolves the soluble components. In the separator, the solutes are separated from the supercritical fluid by rapidly lowering the pressure, increasing the temperature, or both. As a result, the supercritical fluid will become gaseous; the solutes will no longer be solubilized in the supercritical fluid and will be separated by gravity. Extracts are gathered at the separator's bottom. Depending on the equipment, the solvent is cooled and compressed before being returned to the extractor or discharged into the atmosphere.⁸⁰

Table 2: Methods, principles, applications, advantages, and disadvantages of GETs

Method	Principle	Applications	Advantages	Disadvantages	References
Supercritical fluid extraction (SFE)	Utilizes supercritical fluids, such as carbon dioxide, to extract compounds	Extraction of essential oils, pigments, flavours, antioxidants, and other bioactive compounds	High selectivity, minimal degradation of compounds, eco-friendly, low energy consumption	High equipment cost, unsuitable for high-molecular-weight compounds	8,79,80
Microwave-assisted extraction (MAE)	Uses microwave energy to heat the solvent and increase solubility of target compounds	Extraction of polar and non-polar compounds, essential oils, and other bio-active compounds	Fast, energy-efficient, reduced solvent usage	Potential for degradation of some compounds, high temperatures	8,79,80
Ultrasound-assisted extraction (UAE)	Uses ultrasound waves to create cavitation bubbles that disrupt the cell walls and increase solubility of target compounds	Extraction of polysaccharides, flavonoids, essential oils, and other bioactive compounds	Simple, low-cost method, high extraction yield	Potential for degradation of some compounds, low selectivity	8,79,81

Pressurized liquid extraction (PLE)	Uses pressurized solvent to extract compounds	Extraction of polyphenols, flavonoids, and other bioactive compounds	High selectivity, high extraction efficiency, low solvent usage	Limited availability of equipment, relatively new technology	80, c
Enzyme-assisted extraction (EAE)	Uses enzymes to breakdown the cell wall and release target compounds	Extraction of proteins, polysaccharides, and other bioactive compounds	High selectivity, mild extraction conditions	Limited substrate specificity, potential for degradation of compounds	8,79,82
Ionic liquid-based extraction (ILE)	Uses ionic liquids as solvents to extract compounds	Extraction of natural products, polymers, and other bioactive compounds	Eco-friendly, high selectivity	High cost of ionic liquids, limited availability of equipment	83,84
Subcritical water extraction (SWE)	Uses hot water under high pressure to extract compounds	Extraction of polar compounds, essential oils, and other bioactive compounds	Eco-friendly, minimal solvent usage, high selectivity	Limited selectivity, high equipment cost	85-87
Pulse electric Field (PEF)	Uses short bursts of high-voltage electrical pulses to rupture cell walls and release intracellular compounds	Extraction of protein, enzymes, and other bio active compounds	Higher extraction yield, preserves nutritional value, green technology, improved product quality	High initial investment, complex process, limited application, limited scale-up	8,81,88

According to Montañés,⁹¹ who looked into and examined the supercritical fluid extraction conditions, for instance, the kind and concentration of the polar co-solvent utilized, to selectively extract the prebiotic keto sugar lactulose from challenging commercial solid carbohydrate combinations. A general conclusion from the extraction of binary ketose-aldose mixtures (tagatose-galactose and lactulose-lactose) is that supercritical conditions (temperature, pressure, and amount of polar co-solvent dissolved in the SC-CO₂ solvent) affect recoveries but not selectivity, which is primarily influenced by co-solvent composition.

Under optimum conditions, excellent recoveries and high purity of the ketosugars, tagatose or lactulose,

were attained. Based on analytical findings on the remaining protein after supercritical fluid processing, Favati⁹² asserts that treatment with dense carbon dioxide did not affect the nutritional value of this protein supplement. These two factors work together to encourage greater use of Supercritical fluid extraction by processing value-added items and producing a commodity addition for meal use. Zhang⁸⁹ described the extraction of Rosemary (*Rosmarinus officinalis*) essential oil using supercritical carbon dioxide (S-CO₂) extraction, hydro distillation, and steam distillation. He noted that the Supercritical fluid chromatography (SFC) extract exhibited higher essential oil yields and antioxidant activity compared to the other two methods. Due to its low polarity, s-CO₂ helps extract

non-polar natural substances, such as lipids and volatile oils. Using S-CO₂ modified with 2% ethanol at 300 bar and 40 °C, vinblastine (an antineoplastic drug) was extracted from *Catharanthus roseus* more selectively and with 92% greater efficiency than with conventional techniques for extraction. Saini 8 claims that supercritical CO₂ extraction (SCE),

which employs supercritical CO₂ as the extracting solvent, is a method for removing lipids from biological matrix. SCE offers selective extractions of metabolites, including lipids, because pressure and temperature changes can alter the properties of CO₂. The extraction yield (ethanol) can also be increased by using co-solvent.

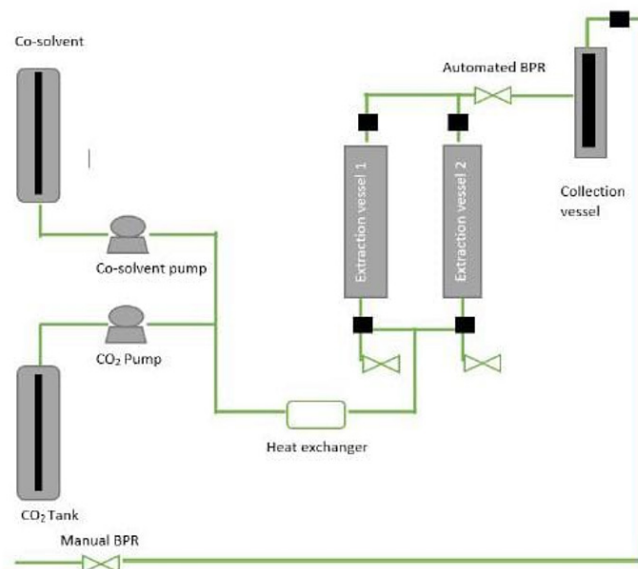


Fig. 1: A supercritical CO₂ extraction (SFE) system is shown schematically. Adapted from⁸⁰

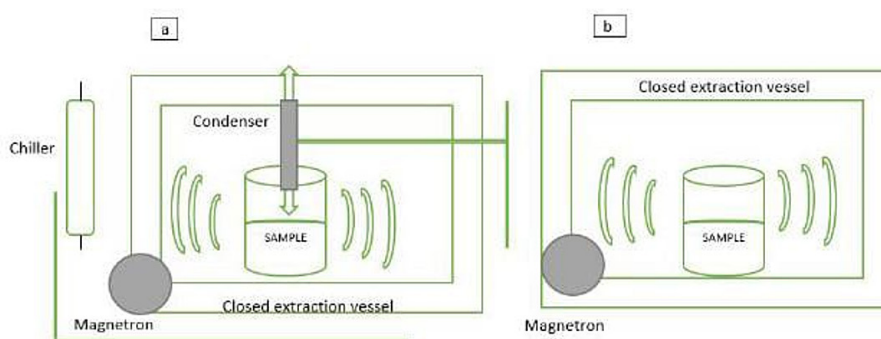


Fig. 2: A microwave extractor is shown schematically (MAE). Inside the beaker is a mixture of the sample and the solvents. A) closed system with condenser B) Simple closed system. Adapted from.⁸⁰

Microwave-Assisted Extraction (MAE)

MAE utilizes microwave energy to heat the solvent, increasing the target compounds' solubility. Ionic conduction and dipole rotation are the two mechanisms by which microwaves interact to produce heat with polar substances such as water and some components of the organic plant

matrix. Mass and heat transfers occur in the same direction during microwave-aided extraction (MAE), accelerating extraction and boosting extraction yield. The microwave approach can take several distinct routes, employing various solvents or none at all (solvent-free microwave extraction, SFME), reflux or no-reflux, time and temperature, and atmosphere

(nitrogen, argon, helium, etc.) or pressure (Figure 2). MAE usage has many advantages, such as better process monitoring, increased extract yield, decreased solvent volume, extraction time, energy use, and cost.

Additionally, it reduces thermal deterioration and targeted plant heating. The MAE process is considered green because it utilizes less organic solvent. MAE⁹³ ability to extract materials depends on its liquid-to-solid ratio, power, irradiation time, and particle size. This study by Lopes⁹⁴ shows that MAE can produce extracts with different carbohydrates, caffeine, chlorogenic acids, and coloured compounds, approaching the composition of instant coffee at high extraction temperatures (180 °C). The extracts are still comparable to home brewing techniques at milder conditions, such as 120 °C and 150 °C.

Microwave-aided extraction was the best method for increasing the extraction yield, protein content, and protein characteristics of soymilk. The extraction yield and protein content of soymilk produced using this approach both significantly ($p < 0.05$) improved by 24% and 44.44%, respectively.⁹³ Coelho⁹⁵ most recent study has advised MAE to extract grape seed oil. The authors describe advantages such as yields greater than those of conventional techniques (soxhlet) and advantages like shorter extraction durations and lower temperatures that slow down the decomposition of oily components. The extraction yield from *P. vera* leaves was enhanced in this study Elakremi⁹⁶ employing an MAE technique. The

extracts generated under the optimal MAE conditions displayed higher amounts of TPC, TFC, antioxidant activities, and antibacterial activity than maceration extracts. MAE is superior to the conventional extraction approach for recovering bioactive compounds. MAE offers additional advantages such as a quicker extraction process and fewer solvent use in addition to having a greater extraction yield and being richer in phenolic compounds.

Ultrasound-Assisted Extraction (UAE)

UAE uses ultrasound waves to create cavitation bubbles that disrupt plant materials' cell walls, increasing the bioactive compounds' solubility. Ultrasonic wave energy, or ultrasonic extraction or sonication, is used in ultrasonic-assisted extraction (UAE). The cavitation that ultrasound causes in the solvent boosts extraction efficiency while also accelerating heat transfer, solute dissolution, and diffusion (Figure 3). Two other advantages of the UAE are low energy and solvent use and a reduction in extraction temperature and time. Compounds that are unstable and thermolabile can be extracted using UAE. UAE routinely extracts a variety of organic substances.⁹⁹ Monroy⁹⁷ claims that after being treated with ultrasound, cassava starch exhibited structural disarray and changes to its techno-functional properties. Physically changing the starch caused microstructural changes that were mostly apparent in the granules' morphological characteristics and level of crystallinity. Having a bimodal distribution cassava starch was established that larger granules responded to ultrasonic therapy with a more pronounced effect.

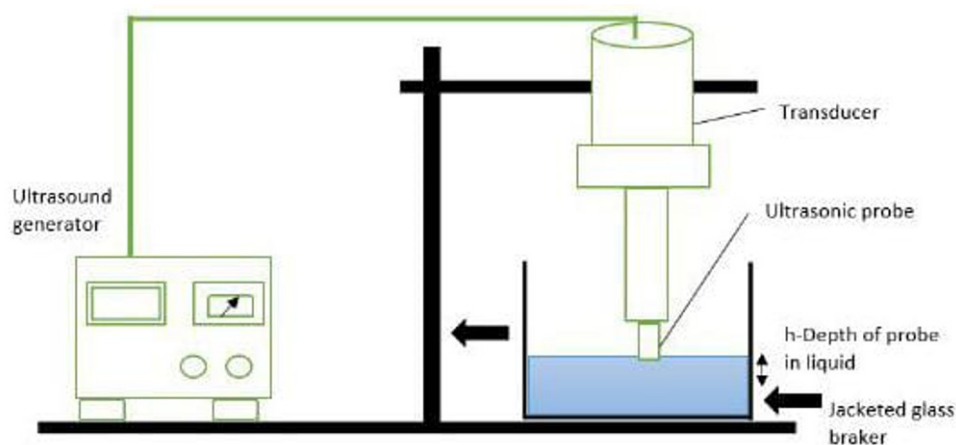


Fig. 3: An ultrasound-assisted extraction (UAE) system is shown schematically. Adapted from⁹⁸

The ability of ultrasound to increase the output of plant-based proteins extracted and their ensuing modifications is acknowledged by researchers. Greater extraction yields can be achieved without the use of an organic solvent or an enzymatic procedure. Proteins can be physically and structurally altered simultaneously to alter their functions and nutritional properties. A highly specific substrate is required for ultrasonic-assisted protein extraction since larger particles are more sensitive to ultrasound than tiny ones.⁹⁹

Rapeseed protein can be extracted using ultrasonic-assisted extraction and ultrafiltration, proven significantly more effective than the conventional approach.¹⁰⁰ With the pH at 11.5, the ultrasonic temperature set at 35 °C, the ultrasonic power set at 450 W, and the ultrasonic treatment time set at 80 min, the impact of the solid/liquid ratio was evaluated to improve the settings for ultrasonic-assisted extraction. The efficiency of protein extraction increased as the solid/liquid ratio increased. These tests showed that several parameters, including ultrasonic power, ultrasonic treatment time, and solid/liquid ratio, which are typically thought to be the most important factors, play important roles in the experimental conditions required for optimising the extraction efficiency of rapeseed protein.

In the current work by Thilakarathna,¹⁰¹ mahua seed oil was successfully extracted utilizing ultrasound

for the first time. The UAE obtained more than 99% of oil recovery and a yield of 56.97% oil utilizing a binary mixture of acetone and isopropanol (1:1 v/v), according to the results, with the least amount of energy consumption, shortest extraction time (35 min), and lowest temperature (35 °C). Despite the fact that the main components of the oil—fatty acids (46% oleic, 22% palmitic, 21% stearic, and 10% linoleic), melting (complete 38 °C), and triacylglycerides (23% POO, 17% POS, 16% SOO, 14% POP, and 9% SOS), appeared unaffected by UAE mechanism, bioactive compound, and antioxidant capacity elution was significantly enhanced.

Pressurized Liquid Extraction (PLE)

PLE are the most commonly used GETs in the food industry. PLE involves using a pressurized solvent to extract compounds, similar to SFE. When compared to methods carried out at close to room temperature and atmospheric pressure, pressurized liquid extraction improves extraction performance by extracting liquid solvents at higher temperatures and pressures. Improved solubility and mass transfer properties come from permitting solvents to be employed at temperatures above their ambient boiling point. The kind of sample matrix, the analyte to be extracted, and the location of the analyte within the matrix all influence extraction efficiency.⁸⁰

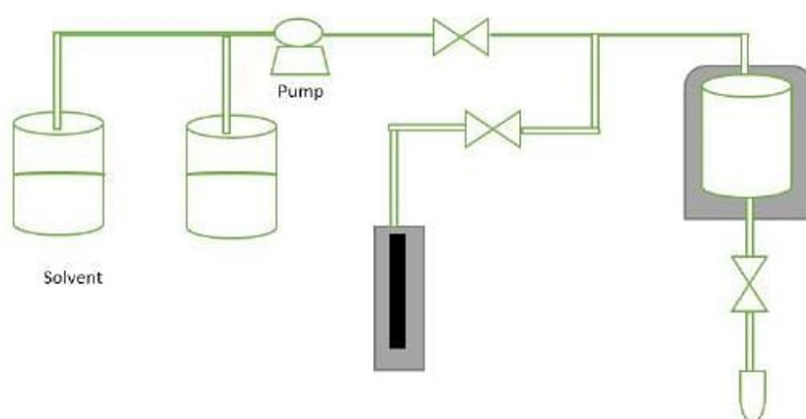


Fig. 4: An example of a pressurized liquid extractor (PLE) in schematic form. A sand column can be found inside the pressured chamber. Adapted from⁸⁰

Before using the equipment, the sample must first be prepared (homogenization, sieving, and drying).

The extractor cell is then filled with the material and placed inside the oven, where a pump introduces

the extracting solvent. Thirdly, the cell is heated and compressed. After passing through the extraction cell, the solvent extraction is collected in a tube at the end of the flow channel. After extraction, the extraction cell is purged with nitrogen gas (for 1-2 min) to remove any remaining residues from the sample (Figure 4). Different kinds of green algae were the subject of one of the first experiments that demonstrated the use of PLE in the extraction of carotenoids. This study demonstrated that PLE, which uses smaller quantities of standard solvents and shorter extraction times than conventional organic extraction techniques, has a similar extraction efficiency.¹⁰² This study by Berrada¹⁰³ is the first to demonstrate how to simultaneously and successfully determine seven macrolide antibiotics from meat and fish filets using LC-(ESI)MS and PLE. Low quantification limits and good recovery characterized the accuracy of the methodology. Samples were lyophilized before analysis, resulting in clean extracts and eliminating the need for additional clean-ups. The limits of quantification were lower than the MRL, and the effectiveness of PLE was similar to that of conventional methods. For the first time, PLE was successfully applied in the current study by Fuente¹⁰⁴ to get protein extracts with antioxidant activity from gilthead sea bream, processing side streams sustainably. Viscera recovered proteins at the highest rate (78%), whereas residual muscle had the best antioxidant

capacity. Using the Pressurized Liquid Extraction (PLE) technique, Fuente¹⁰⁵ obtained protein extracts from the side streams of Atlantic salmon processing for the first time. Since the SDS-PAGE patterns of the extracts from the heads, viscera, skin, and tailfins differed, PLE-assisted extraction probably affected the size of the protein fragments gathered in the extracts.

Pulse-Electric-Field Assisted Extraction (PEF)

PEF-aided extraction is a non-thermal method that slows the degradation of thermolabile components. PEF therapy's success depends on several variables, including field strength, particular energy input, pulse count, and treatment temperature (Figure 5). Because it can facilitate mass transfer by altering membrane structures during extraction, pulsed electric field extraction significantly increases extraction yield while reducing extraction time. Compared to conventional heat treatments, PEF technology is advantageous since it eliminates microorganisms while retaining the unprocessed food's nutritional value and original colour, flavour, and texture. Additionally, it prevents or significantly minimizes detrimental modifications to food's nutritional and physical properties. Numerous food firms have used this method to speed up the process of extraction and the prior-treatment of food materials.¹⁰⁶

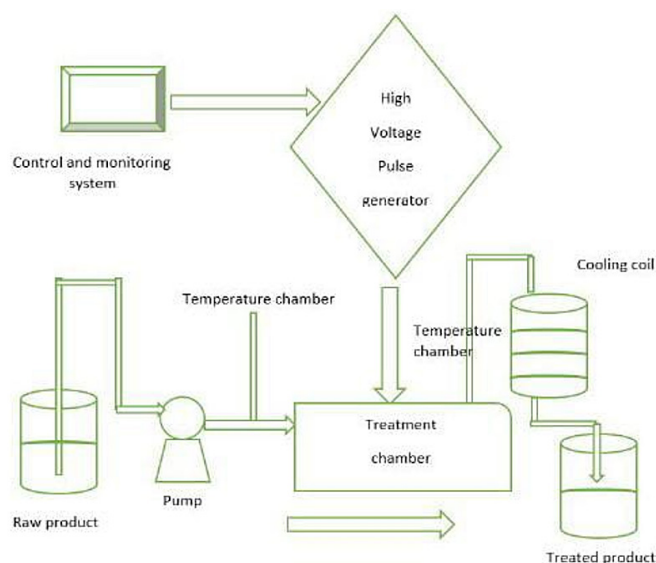


Fig. 5: Flowchart for a PEF food processing system showing the fundamental parts. Adapted from⁸⁸

The main variables affecting PEF extraction, according to the results of the Nowosad 106 experiment, are temperature, electric field strength, and treatment time. A review of the PEF-extracted extracts' polysaccharide, protein contents and total polyphenol. The white button mushroom extracts had higher polysaccharides, polyphenols, and protein as the electric field intensity, temperature, and treatment time were increased. When polysaccharides, polyphenols, and proteins extracted from white button mushrooms using conventional and PEF methods are compared, it is clear that the latter method greatly increases extraction yield. The results also unequivocally show how temperature and electric field interact. According to the data acquired, PEF extraction enables high cell membrane permeabilization from white button mushroom solution in a comparatively brief amount of time. Food research has several difficulties, two of which are obtaining high-production proteins and improving the biological activity of peptides. PEF processing has emerged as a possible alternative treatment for conventional thermal processing in protein-based meals due to its non-thermal nature, low energy consumption, and short processing timeframes.¹⁰⁷ An industrial-scale pulsed electric field (PEF) pre-treatment has been created, according to Guo,¹⁰⁸ who disclosed that proteins and juice might be extracted from grass and clover mixtures. After

PEF pre-treatment, the release of crude protein increases by around 31% while the output of juice mix increases by about 25%. At pH 5 and $T = 60\text{ }^{\circ}\text{C}$, a disk separator successfully separated continuously squeezed raw juice and gradually precipitated crude protein.

PEF application as a pre-treatment for the extraction of essential oils (EO) from three different plants—rosemary, eucalyptus leaves, and dry thyme leaves—was examined by Barros.¹⁰⁹ EO was extracted from eucalyptus and rosemary leaves under industry-representative circumstances, accounting for the tdest of 30 and 60 minutes. Comparisons were made between conventional and PEF pre-treatment extraction methods with $E = 2\text{ kV/cm}$ and $W_s 10\text{ kJ/Kg}$. PEF treatment increased the extraction yields for both plants. The extraction yield can rise by up to 17% and 11% for eucalyptus and rosemary, respectively, when less time is needed to extract the same amount of EO as with the conventional approach, which requires 60 minutes. The third option was dry thyme leaves; the best PEF settings were found to supply the least amount of energy ($E = 1\text{ kV/cm}$ and $W_s \sim 0.4\text{ kJ/Kg}$), with an extraction period of 30 minutes, managing to produce an extraction yield 17% greater than an extraction of 2 hours with the conventional approach.

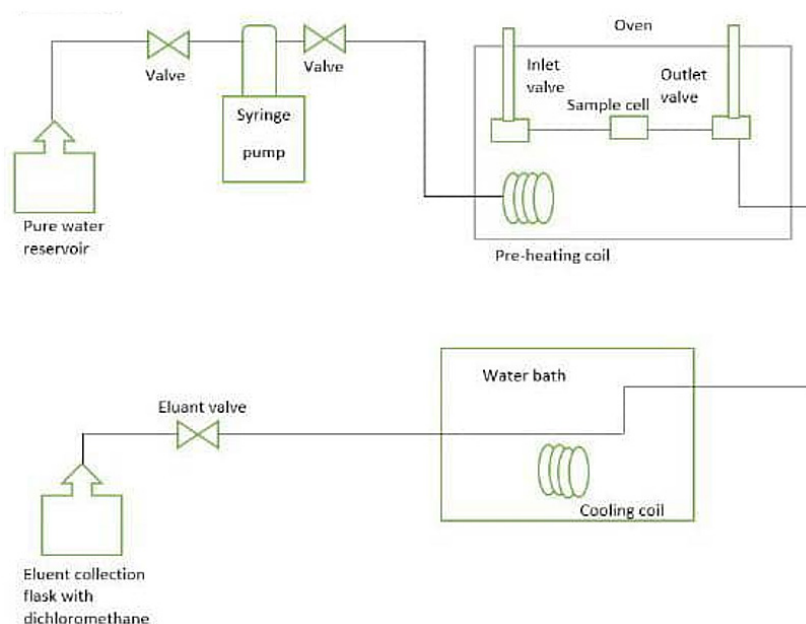


Fig. 6: The system for extracting subcritical water is shown schematically. Adapted from⁸⁶

Subcritical Water Extraction (SWE)

Subcritical water extraction (SWE) is a green extraction procedure that uses water to extract compounds from various sources, including plant matter and food waste, at temperatures and pressures below the critical point. It has lately come to light as a practical and eco-friendly technology to replace traditional extraction techniques. It water may dissolve and extract various substances due to its intermediate qualities between liquid water and steam.⁶⁰ The apparatus comprises an extraction cell, a container for water connected to a elevated pump to deliver solvent, and a clamp or regulator to regulate pressure (Figure 6). After the extraction system, the resulting substances are gathered in a glass container. Many vegetable matrices, including rosemary, have been subject to subcritical water extraction, which has been utilized to extract various compounds from them.^{66 65}

SWE offers several advantages over conventional extraction methods: It can extract a wide range of compounds with high efficiency in a relatively short time. SWE does not require organic solvents, making it safer and more environmentally friendly. It can be optimized to selectively extract specific compounds from complex matrices, such as plant materials. SWE requires less energy than conventional extraction methods since it operates at lower temperatures and pressures. It has been successfully used to extract various compounds, including essential oils, bioactive compounds, and flavour compounds from multiple sources. However, SWE also has some limitations, including the need for specialized equipment, the limited solubility of some compounds in subcritical water,

and the potential for degradation of thermally labile compounds at high temperatures.⁸⁵

Rosemary (*Rosmarinus officinalis* L.) has been one of SWE's most thoroughly researched materials. Herrero⁸⁶ demonstrated the purification of antioxidant components from rosemary via SWE throughout a broad temperature range in his review. Heat degrees ranging from 25 to 200 °C were examined to investigate the extraction preference for antioxidant compounds. The water temperature strongly affected the quantity extracted, which rose as the extraction temperature increased. The researchers confirmed that rosmanol, the strongest polarized molecule, has been shown the primary component extracted at below room temperatures (25 °C). After the process of extraction was carried out at 200 °C, water's ability to disintegrate the highly polar molecules became reduced, while other high concentration of different substance, such as carnosic acid, was produced. SWE could provide antioxidant extracts comparable to those obtained with supercritical carbon dioxide extraction. Aside from antioxidants, the SWE extraction of aroma compounds from rosemary, savoury, and peppermint has been explored.⁸⁶ *Thymbra spicata* essential oil extraction was investigated by Ozel⁸⁷ using subcritical water extraction. Several parameters, including pressure (20, 60, and 90 bar), flow rate (1, 2 and 3 ml/min), and temperature (100, 125, 150, and 175 °C) were investigated. The highest extraction yields (3.7%) were obtained at 150 °C and 60 bar, with a flow rate of 2 ml/min for 30 minutes. The essential oils of *Timbra spicata* were discovered to suppress the mycelial growth of numerous fungus species.⁸⁷

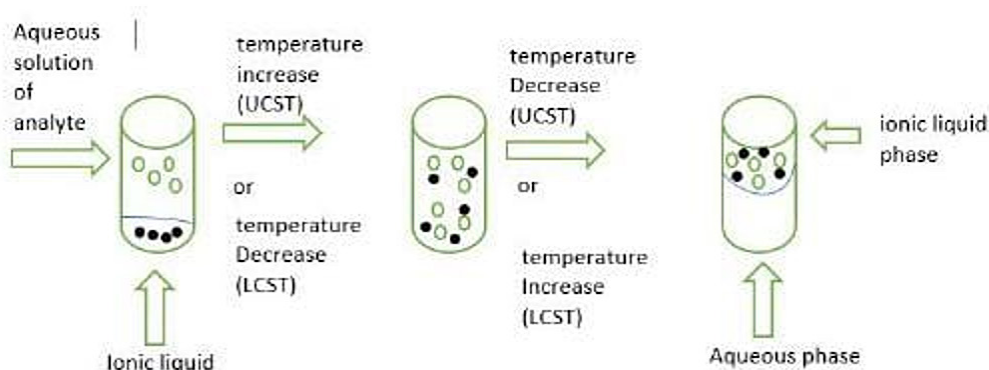


Fig. 7: In the separation process, ionic liquids serve as solvents. Adapted from⁸⁴

Ionic Liquid-Based Extraction (ILE)

Ionic liquid-based extraction (ILE) is a green extraction technique that extracts target compounds from various sources using ionic liquids as solvents. Ionic liquids, molten salts made entirely of ions and with melting temperatures below 100 °C, are the best solvents for extraction.⁸³ The first step is to select an appropriate ionic liquid based on the solubility and selectivity of the bioactive compounds to be extracted. The ionic liquid should also be non-toxic, environmentally friendly, and have low volatility. The plant material is dried, ground, and sieved for homogenous powder. It is mixed with the ionic liquid in a suitable vessel. The mixture is stirred at a specific temperature and time to enhance extraction efficiency. After extraction, the mixture is centrifuged to separate the liquid phase (ionic liquid) and solid phase (plant material) (Figure 7). The bioactive compounds are extracted from the ionic liquid phase using a suitable solvent or method such as evaporation, precipitation, or chromatography. The ionic liquid can be recovered and reused for subsequent extractions, making the process more environmentally friendly and cost-effective.⁸⁴

ILE offers several advantages over conventional extraction methods: Ionic liquids can be designed to have specific properties that enable them to extract the target compounds while leaving other components behind selectively. It has a wide range of solubility and can dissolve many compounds, including polar and nonpolar compounds. Ionic liquids have lower toxicity than conventional solvents, making them safer for the environment and operators. It has a lower environmental impact than conventional solvents as they are not volatile and do not contribute to air pollution. ILE has been successfully used to extract various compounds, including essential oils, pharmaceuticals, and bioactive compounds from natural sources such as plants and algae. However, there are also some limitations to ILE, including the high cost of ionic liquids, the need for specialized equipment, and the limited availability of ionic liquids with specific properties.⁸⁴ According to Kilulya,⁸³ This work devised an ionic liquid-based approach for extracting fatty acids from blue-green algae biomass. Its performance was assessed by contrasting it with a conventional organic solvent extraction method. Ionic liquid solvation capacity was shown to be dependent on anion type and dissolution temperature. Thus,

the former of the two ionic liquids tested, 1-butyl-3-methylimidazolium chloride [BMIM][Cl] and 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM][PF₆], dissolved the algal biomass while the latter could not. Ionic liquid is a solvent to replace volatile organic solvents to reduce environmental pollution, as evidenced by the similarity in qualitative and quantitative composition of fatty acids obtained using ionic liquid-based extraction enhanced by direct transesterification and conventional organic solvent extraction method. According to Dreyer,¹¹⁰ the ionic liquid using AmmoengTM¹¹⁰ has been used to create aqueous two-phase systems based, and this research has looked at the key factors that affect how proteins partition in these systems. Since protein partitioning is a surface-dependent process, studies have concentrated on surface-related protein characteristics such as charge, hydrophobicity, and surface area. Surprisingly, the protein hydrophobicity and the partition coefficient only showed a weak correlation. However, a significant relationship between protein charge and partition behaviour could be seen. According to our results, the primary mechanism governing protein partitioning is the electrostatic interaction between the amino acids on the protein surface and the ionic liquid cation.

Enzyme-Assisted Extraction (EAE)

A green extraction technique (EAE) uses enzymes to help extract substances from natural sources, including plants and microorganisms. When complex substances are broken down into simpler ones by enzymes, they become easier to remove. Enzymes are biological catalysts.⁸² The first step is to select an appropriate enzyme based on the type and nature of the bioactive compounds to be extracted. The enzyme should also be stable under the extraction conditions and have a high affinity for the target compounds. The plant material is cleaned, dried, ground, and sieved for homogenous powder. The plant material is mixed with the selected enzyme in a suitable vessel. The mixture is stirred at a specific temperature and time to enhance extraction efficiency. The enzymes can be added to the mixture before or after the solvent extraction. After enzyme treatment, the mixture is extracted using a suitable solvent such as water, ethanol, or both. Depending on the specific enzyme and bioactive compound, the solvent can be added before or after the enzyme treatment. After extraction, the mixture is centrifuged to separate the liquid phase (solvent)

and solid phase (plant material) (Figure 8). The bioactive compounds are extracted from the solvent phase using a suitable method such as evaporation, precipitation, or chromatography. The enzymes can

be recovered and reused for subsequent extractions, making the process more environmentally friendly and cost-effective.⁸²

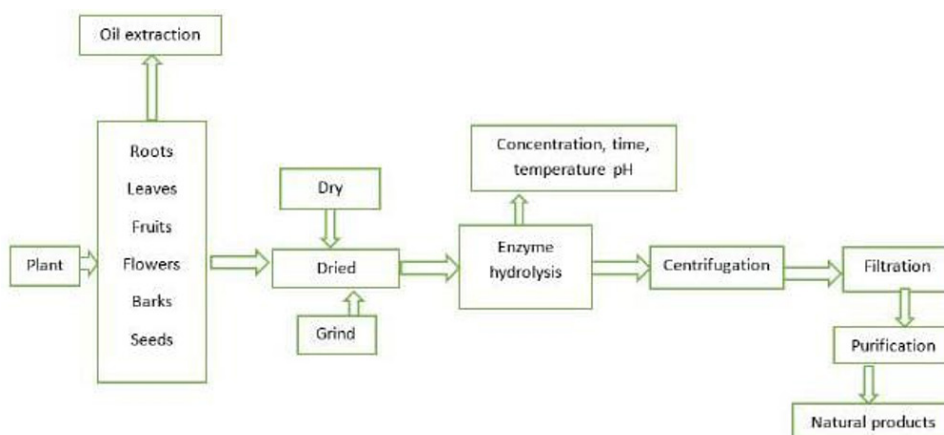


Fig. 8: The method of natural product extraction using enzyme assistance. Adapted from ⁸²

EAE offers several advantages over conventional extraction methods: Enzymes can be selected based on their specificity for specific compounds, allowing for selective extraction of target compounds. EAE requires less energy than conventional extraction methods since it operates at lower temperatures and pressures. It does not require organic solvents, making it safer and more environmentally friendly. EAE operates under mild conditions, preserving the integrity of the extracted compounds and reducing the potential for degradation. It has been successfully used to extract various compounds from various sources, including proteins, polysaccharides, and bioactive compounds. However, there are also some limitations to EAE, including the need for specialized enzymes, the potential for enzyme deactivation during extraction, and the need to optimise extraction conditions, such as pH and temperature.⁸²

Advantages and Disadvantages of Green Extraction Technologies

Green extraction technologies, as shown in (Figure 9) refer to eco-friendly and sustainable extraction methods. The primary advantage of GETs such as SWE, SFE and PLE is their eco-friendliness, as they use non-toxic, renewable, and biodegradable solvents, such as water, carbon dioxide, and ethanol.⁴ This reduces environmental pollution and health hazards for the communities and workers. Additionally, GETs such as UAE, MAE, PLE, SFE,

PEF and ILE are energy-efficient, as they require lower temperatures and shorter extraction times, which decreases the energy costs associated with the process. EAE and ILE also have higher selectivity than conventional methods, enabling the extraction of specific bioactive compounds with minimal degradation.^{87,111,112} Green extraction technologies use environmentally friendly solvents, reducing the amount of toxic waste and pollution generated during extraction. Some green extraction technologies use low-energy techniques such as microwave, ultrasound, and enzyme-assisted extraction, which require less energy than conventional extraction methods. It can help preserve the extracted compounds' natural properties, such as flavour, colour, and aroma. Some green extraction technologies, such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE), can improve extraction efficiency and reduce the time required for extraction, resulting in cost savings.¹¹³

However, GETs can also have disadvantages, such as the need for expensive equipment and a lack of scalability. SFE has high selectivity and can extract compounds without degradation, but it requires expensive equipment and is unsuitable for extracting high-molecular-weight compounds. MAE is fast and energy-efficient, but it can lead to the degradation of some compounds due to high temperatures. UAE is a simple and low-cost method, but it can also

cause the degradation of some compounds due to high-intensity ultrasound waves. PLE is a relatively new technology with high selectivity and efficiency but requires expensive equipment.¹¹⁴

Green extraction technologies may not be suitable for all types of products or compounds. For example, some natural compounds may require harsh solvents to be extracted effectively. Some green extraction technologies, such as SFE, UAE, PEF, MAE, EAE, and ILE, can be expensive, requiring

specialized equipment and trained personnel. There is a lack of standardization in green extraction technologies, making it difficult to compare the efficiency of different methods. Some green extraction methods may result in lower extraction yields compared to conventional methods, resulting in reduced profitability. In essence, green extraction technologies provide more advantages over conventional extraction methods, their suitability depends on the specific product being extracted and the desired result.^{80,111,115,116}



Fig. 9: The benefits of using green technologies to extract bioactive compounds from plantsources. Source ¹¹⁶

Examples of Plant Materials and Bioactive Compounds Extracted by Green Extraction Technologies (GETs)

"Green extraction" focuses on developing extraction methods that require less energy, permit using renewable natural resources and alternative solvents, and produce safe and high-quality extracts and products.¹¹⁷ GETs have been used to extract a wide range of bioactive compounds from plant materials, such as phenolic compounds,¹¹⁸ carotenoids,⁸⁰ essential oils,¹⁰⁹ and polysaccharides,⁴ and antioxidants.⁸⁶ These compounds have diverse applications in the food industry, such as antioxidants, antimicrobials, colourants, and

flavourings. These plant materials and bioactive compounds can be extracted using different GETs, such as those discussed earlier. Therefore, the choice of GET depends on the specific bioactive compound and plant material to be extracted, as well as the desired properties of the final product. It has been determined that there are three main ways to create and execute green extraction on a scientific and commercial dimension in order to achieve an ideal usage of essential components, chemicals, and power: (1) enhancing and maximizing current techniques; (2) utilizing non-dedicated tools; and (3) innovating methods and techniques, and finding substitute chemicals.¹¹⁷

Here are some examples of plant materials and bioactive compounds that can be extracted using GETs: For example, SFE is employed in lipid extraction from grape seeds.⁸ Polyphenols, which have the potential to be exploited as natural preservatives and antioxidants in food items, have been extracted from grape seeds using SFE.⁸⁹ Response surface technology was successfully employed to optimise supercritical CO₂ extraction of grape seed oil. Grape seed oil can be utilized in different industries and as a dietary supplement because it is high in vitamin E and was extracted using supercritical CO₂. Additionally, the defatted grape flour produced by supercritical extraction might be applied to various fields, such as the baking industry, the production of enriched extruded products, etc.¹¹⁵ SFE has been utilized to extract oil from seeds of Indian almonds, Chilean palo verde, apples, raspberries, and carrots.⁹⁰ Supercritical CO₂ extraction (SCE) of lipids entails removing lipids from the biological matrix while using supercritical CO₂ (a green solvent) as the extracting solvent. SCE provides selective extractions of metabolites, including lipids, as CO₂'s characteristics can be changed by adjusting pressure and temperature.⁸ A relatively recent technique called microwave-aided extraction extracts various plant components. It is quick, reasonably cheap, and green in keeping with the dictates of modern environmental concerns. It is quite effective, extracting about 90% of the available seed polyphenols in just a few minutes. According to a recent study by Ghafoor,¹¹² MAE extracts significantly more polyphenols than the UAE, about twice as much. MAE was effective in rapidly removing caffeine and polyphenols from green tea leaves. The food and pharmaceutical industries would benefit from MAE's new technology, which was faster, safer, and more environmentally friendly than current extraction methods.¹¹⁴ Green tea extracts that have been cleaned up and contain high levels of phenolic compounds and catechins were researched in paper.¹¹⁹ Marshmallows produced polysaccharides with the aid of MAE. Additionally, the essential oil was obtained from oregano. To make carotenoids, carrot pomace was extracted. Cluster fig fruits and sweet olive flowers were employed to obtain phenolic compounds with bioactive properties.

UAE has been used to extract essential oils from herbs, which can be used as flavourings

and fragrances. Utilizing UAE, protein isolates from peas have been obtained. Additionally, phenolic components were extracted from mango and pomegranate with UAE. *Rubia sylvatica* Maxim. Fruits in UAE have reaped the benefits of phenol, anthocyanins, and natural food colourant.⁹⁰ In order to develop a successful environmentally friendly extraction method for recovering bioactive components from naturally occurring plants, we looked into the possibility of pressurized liquid extraction (PLE) of ginger (*Zingiber officinale* Roscoe). Reduced time and solvent costs, as well as the extract's unique chemical profile as compared to that of Soxhlet extraction, are advantages of PLE over other extraction techniques.¹¹³ Plant materials like pepper seeds, black garlic, tomatoes, blueberries, and lingonberries have been investigated for the use of PLE to recover substances like oil, melanoidins, polyphenols, anthocyanins, and phenolic compounds, respectively.⁹⁰ PEF has been applied to a variety of plant materials, including oil from sunflower, lipid from the green alga, lycopene from tomato by-products, phenolic compounds from apple peels, naringin and hesperidin from orange peels, and flavonoids, phenols, and antioxidant compounds from thinned peach by-products.⁹⁰ In addition to rosemary's antioxidants, the SWE extraction of aroma compounds from savoury, peppermint, and rosemary has also been investigated.⁸⁶ Flavonoids, limonoids, and essential oils are recovered in citrus peels. SWE has been employed in the extraction of essential oils due to its less expensive extraction agent and environmentally friendly process. Some of the plants used are laurel, oregano, and kava.⁸⁶ SWE has been used to extract the essential oils from *Thymbra spicata*.⁸⁷ To extract phenols, flavonoids, and antioxidants from orange peel, UAE, MAE, and SFE were utilized.⁸¹ *Thymus*, *Salvia rosmarinus*, and *Eucalyptus* essential oils have all been extracted using PEF.¹⁰⁹ ILE extracted fatty acids from biomass derived from blue-green algae.⁸³ Protein from sugar beet leaves, soy grit, and polysaccharide from lotus leaves have all been obtained via EAE. To generate chromoplasts containing carotenoids, tomato extracts were used.⁹⁰ Natural plant resources are being overused due to the rising demand for organic goods and extracts. Because overuse has led to the loss of numerous plant species in the past, protecting biodiversity is essential for the sake of present and future generations. In order to favour entirely renewable

resources, either intense culture or *in vitro* plant cell or organism growth must be used.

Fortification of Food Macronutrients

Fortifying food with macronutrients involves adding additional servings of important nutrients like carbohydrates, fats, and proteins to food products. This improves the food's nutrient density and aids people in consuming the recommended daily amounts of these vital elements. These give your body calories or energy. Every type of macronutrient plays a critical function in maintaining the body's health. People typically need a balance of macronutrients for optimal health. For instance, bread and cereals can be made with whole grains or enriched flour to add carbohydrates. Refined grains are preferred over unrefined ones because they are more tasty, according to an assessment by Seal.⁵³ However, removing bran has resulted in grain-based products supporting a worse diet. The reviewer focused on the role of whole grains in maintaining a healthy and long-lasting diet, focusing on gut flora in particular. There is compelling evidence that switching from whole grain products to those manufactured from refined grains reduces the risk of several noncommunicable diseases by improving food quality and changing the microbiota composition. Consumers should perceive transitioning from refined to whole grain cereals as being straightforward once the cereals have been processed to generate nutrient-dense, wholesome foods.

Different foods can be enhanced with proteins, including energy bars and yoghurt. The aim of this study, according to Aita,⁵⁴ was to assess the sensory characteristics of milk yoghurt produced from the milk of a cow (3.37% protein and 3.6% fat) enriched along with various amounts of dairy protein (0.5, 1.0, or 1.5%) as sodium caseinate (NaCn), dried whey protein concentrate (WPC) or ultrafiltrated (UF), and skim milk powder (SMP) to equivalent protein amounts. The yoghurt by UF that has an additional 1.0% protein received the highest overall taste rating. It was maintained at a steady sensory perception until the 14th day, when it was still acceptable. According to Jabeen,⁵⁵ six protein energy bars (B1–B6), including dates, dried apricots, Cheddar cheese, and whey protein isolate, were produced for Pakistani athletes.

Dates weighed 74, 68 and 65 g, while apricots weighed 8, 14, and 17 g for Bars B1–B3, which also holds 5 g of Cheddar cheese, and 13 g of whey protein isolate. All of the PE-bars (B1–B6), except B6, showed excellent sensory properties. Dates and apricots were added to increase the bioactive components, while cheese and whey protein isolate increased the protein content of PE-bars, improving their nutritional value and textural characteristics. According to the findings, PE-bars can be used by seniors, troops, exercise groups, and kids in school as a meal replacement bar. Fats can be added to foods like margarine and cooking oils. According to claim,⁵⁶ the evolution of oil quality has made unsaturated fatty acids a major topic in terms of nutrition. Compared to animal fat, vegetable oils have more unsaturated fatty acids, particularly linoleic and oleic acids, which can lower blood cholesterol levels.⁵⁰ Plant sterols are a class of compounds that are currently being studied. By decreasing the absorption of cholesterol, they may prevent atherosclerosis. They could also shield against colon cancer. Many food firms work to produce foods with high plant sterol content that are useful.⁴⁶

Two types of polyunsaturated fatty acids (PUFA) exist according to ⁵⁷: omega-6 and omega-3. The primary dietary sources of omega-6 are corn oil, soybean oil, and safflower oil. The diet should include oily seafood like tuna, salmon, and herring and a few plant oils like flaxseed and canola. While both types of PUFA are essential, long-chain omega-3 tends to be lacking in the diet due to a decline in fish consumption and an overabundance of omega-6 in the food supply. Assistance with immune system performance, fetal development, mental health, and heart health are just a few of the many health benefits of omega-3 PUFA. Food fortification with macronutrients is also beneficial in developing countries where people may not have access to a wide variety of nutrient-rich meals and where malnutrition is a prevalent problem. It's crucial to remember that while fortification can help increase vitamin consumption, it shouldn't be depended upon as the only way to satisfy dietary needs. The best strategy to make sure you are getting enough nutrients is to continue to eat a balanced, diverse diet that includes a variety of entire foods.

Fortification is a long-term approach to treating nutrient inadequacies in commonly consumed foods by adding fortificants in calculated amounts during processing. The body must be able to easily absorb these fortificants.^{58, 59} This is crucial for tackling food security, hidden hunger, and malnutrition. The majority of the body's calories and energy are derived from macronutrients. Every type of macronutrient serves a different function and has advantages specific to preserving bodily health. Depending on personal characteristics like weight, age, and underlying medical issues, an individual may require a different quantity of each macronutrient. Many people are affected by macronutrient shortages, particularly women, children, and the elderly, as well as the impoverished and those with low incomes and health issues. The individual's weight, fitness goals, health conditions, and muscle mass are other variables that could affect their macronutrient needs. Carbohydrates (45–65%), lipids (20–35%), and protein (10–35%) are the recommended macronutrient ratios for maximum health and basic nutrition according to the government's Acceptable Macronutrient Distribution Range.⁶⁰ Most food eaten in developing Sub-Saharan Africa, Asia/Pacific, the Caribbean regions, and South/Central America is low in calories, protein, critical vitamins, and minerals, all necessary for daily diets.⁶⁰ Iron, calcium, and vitamin D are nutrients found in meals containing protein, such as meat, beans, milk, fish, or eggs. Fruits and vegetables, which contain carbohydrates, offer a wide variety of vitamins, minerals, and fiber, whereas starchy carbohydrates like brown rice, whole-grain bread, and cereals offer fiber, B vitamins, and magnesium. We get our vitamins A, D, E, and K from fats.

Types of Food Fortification

In order to solve population deficits, food is fortified by adding nutrients, necessary minerals, and vitamins. Here are some examples of popular food fortification methods. While macronutrients comprise carbohydrates, proteins, and fats in considerable amounts, micronutrients are vitamins and minerals needed at minute or trace levels.⁶¹ The most popular food fortification approaches, including mass, targeted, market-driven, and mandatory fortification, are acknowledged to address micro- or macronutrients.⁶² Various forms of malnutrition can be addressed through fortification. Malnutrition can take the form of either undernutrition or overnutrition.

It can also describe an imbalanced consumption of macronutrients (proteins, carbohydrates, and fats) or micronutrients (vitamins and minerals). Mass fortification is putting micronutrients in regularly consumed food such as cereals, milk, oils, sauces, vegetable fats and sugar.⁶³ While adding sufficient micronutrients to meals, such as complementary newborn foods, school-age children's program foods, and emergency foods, targeted fortification is aimed at a specific demographic and is not meant for the entire population.⁶⁴ Market-driven fortification is a method the food industry willfully employs to raise the nutritional value high level of processing a product's added value to draw in new customers and boost sales.⁶⁴ Mandatory fortification is the process by which particular foods are fortified by government decree or law. It is done when there is a manifest public health problem, and illnesses caused by nutritional deficiencies have been shown to exist.⁶⁵ It is crucial to remember that food fortification must be done carefully, considering the food's nutritional content and any possible adverse health effects of excessive intake.

Health Benefits of Food Fortification

Food fortification lasts a person's entire life and provides several benefits. Food fortification is a valuable strategy for preventing and treating malnutrition, which lowers the condition's occurrence. Populations that do not have access to a varied and nutrient-rich diet can benefit from fortified foods, which can deliver critical nutrients. Malnutrition and nutritional deficiencies are eliminated via fortification. According to reports, fortification has decreased healthcare expenses. Additionally, as some special-purpose foods are processed, nutrients are restored, enhancing the nutrient richness of the meals. Nutrient deficiencies that cause illnesses, including anemia, goiter, and neural tube abnormalities, can be addressed with fortification. As a result, the population's labour force has increased, and kids are now better able to boost their academic standards. Fortification is also a reasonably priced method of addressing micronutrient shortages in people, particularly in developing nations⁶⁶. Without making significant adjustments to processing or storage, fortification can be simply incorporated into current food production systems. Even after fortification, the food's original properties remain unaffected while more nutrients are available equitably. This means that the taste, texture, and look are exactly

as they were initially. People don't need to change their dietary or behavioural patterns, which can be challenging. Fortification is a cost-effective strategy that benefits both the wealthy and the needy. Fortifying staple foods can increase food security for vulnerable populations by increasing their nutritional content. If consumed in moderation, fortified foods are harmless and do not represent a toxicity risk. The widespread manufacturing of fortified foods can help a country's general nutritional problems.⁶⁶

In order to improve food's nutritional content and health benefits, macromolecule fortification entails adding necessary macromolecules to the diet. Protein is a crucial ingredient for the development and upkeep of tissues. People who may not get enough protein from their diets can improve their protein intake by fortifying foods with protein.⁶⁷ Omega-3 fatty acids are crucial for maintaining good heart and brain health and lowering inflammation. Flaxseed and fish oil are two foods that can be fortified with omega-3s to enhance omega-3 intake and improve health outcomes.⁵⁶ Numerous meals that include a lot of macromolecules, including bread and pasta, can be enriched with minerals and vitamins like B vitamins, iron, and folic acid.⁵³ By doing so, nutrient deficiencies can be addressed, and overall health results can be enhanced. In addition to promoting digestive health, fiber lowers the chance of developing chronic illnesses, including heart disease, diabetes, and some types of cancer. Adding fiber to food can help people consume more fiber and experience better health.⁵⁶ Foods fortified with macromolecules can help lower the chance of developing chronic illnesses like diabetes, heart disease, and some types of cancer.⁵⁶ The balance of nutrients and any potential interactions must be considered in order to ensure that fortified foods are effective and safe.

Applications of Fortified Macromolecule Foods

There are many ways to use fortified foods to address vitamin shortages and enhance population nutrition status.⁶⁸ Nutrition for newborns and early children: During crucial stages of growth and development, fortified infant formula and baby meals can give infants and young children the important nutrients they need. Unfortunately, babies and young children are more vulnerable to the shortage of essential materials.⁶⁹ Fortified foods can be used into emergency food aid programs to treat nutrient

deficits in populations affected by disasters, conflicts, or other crises.⁶⁶ Fortified foods can be incorporated into school meal programs to enhance the cognitive function of students of school age and the nutritional status.⁷⁰ Vegetarian and Vegan Diets: Vegetarian and vegan diets, which may be lacking in some essential nutrients, can fill dietary gaps with the help of fortified foods. Public Health Programs: In public health initiatives, fortified foods can treat specific nutritional deficiencies, including anemia and iodine deficiency illnesses. Sport Nutrition: Fortified foods can be used in sports nutrition to improve athletic performance and assist recovery.⁷¹

Fortified foods can be used in community nutrition programs to correct nutrient deficiencies in vulnerable populations, including older adults, expecting and nursing mothers, people with HIV/AIDS, and those with tuberculosis or the TB virus.⁷² Malnourished individuals of all ages must be taken into consideration. Food industry: To give consumers quick and wholesome meal options, the food industry can create and promote fortified foods.⁷³ Although it can also be done at the home or community levels, food fortification is typically done at the industrial level. The expenditures involved in the food fortification process may restrict their use and efficacy. Making food self-sufficient can also be accomplished by fortification. Numerous nutrients' main job is to support metabolism. Food can be fortified with macronutrients to make it biologically self-sufficient and less dependent on other foods to provide the nutrients they lack. Examples of macronutrients include fiber, protein, fat, water, and carbohydrates. Meat, fish, cereals, legumes, nuts, yams, oil seeds, and potatoes are some foods rich in macronutrients.⁷⁴

Protein Fortification

Foods like bread, cereal, and pasta can be fortified with protein to boost the amount of protein and make them a more nutritious energy source. Adding protein, fiber, and other nutrients can make snack foods like granola bars and energy bars more nutritious and a better snack option than traditional ones. Examples of meat replacements that can be enhanced with iron, protein, and other nutrients to increase their nutritional content alternative to meat include tofu,⁷⁵ veggie burgers,⁷⁶ and tempeh.⁷⁷ According to a report from Mohammed,⁶⁷ adding 5%, 10%, or 15% defatted Moringa seed flour to pearl

millet flour increased its protein content and *in vitro* protein digestibility. As a result, the flour's amino acid composition rose, with the limiting amino acid, lysine, seeing a notable rise. The fermented dough made from raw flour had an increase in protein content and digestibility, and greater values were attained when the 16-hour-fermented dough was cooked. Akubor⁷⁸ claims that the high protein content of cowpeas was utilized to improve the nutritional value of snacks like puff-puff, chin-chin, and cake. According to the study, fortifying snack products with cowpea protein was possible without sacrificing their physical or sensory qualities. Therefore, the Nigerian people would benefit from the fortification of cake, chin-chin, and puff-puff with cowpeas.

Fibre Fortification

To aid in digestion and reduce the risk of acquiring chronic diseases, fiber can be added to foods like bread, energy bars, and breakfast cereals. According to Hashemi,⁵⁶ fiber can be added to yoghurt and dairy products because it is found in the cell walls of grains, vegetables, seeds, and fruits. Due to fiber's propensity to enhance structural and textural qualities, water-holding capacity, and reduce lipid retention, dairy products are often supplemented with fiber to increase manufacturing output, lower calorie content by acting as a bulking agent, and improve water-holding capacity. Yoghurt and other dairy products are fortified with fiber to provide nutritious meals and improve functionality. Utilizing high-fibre foods and beverages may help prevent or minimize cancer, diabetes, hypercholesterolemia, gastrointestinal issues, obesity, and other diseases. In addition, according to author,⁵⁶ 1.3% oat fiber improved the body and texture of plain yoghurt while degrading its overall flavour quality.

Healthy Fat Fortification

Foods like salad dressing, margarine, and spreads can be enriched with monounsaturated and polyunsaturated fats to boost heart health and lessen the risk of chronic diseases. Fatty acids are found in every biological tissue. Examples of dietary sources used as functional foods include vegetables and vegetable oils, salmon and fish oils, and eggs. The primary dietary sources of large amounts of fatty acids that might be regarded as having functional food activity are fish oils and those from recently emerging seed oil plants like flaxseed, borage, and evening primrose. Monounsaturated fatty acids are

abundant in olive and rapeseed oil, widely known for their superior health and advantages. Functional meals can be made by including oilseed tissue as the entire seed, as a milled flour, or by adding oil too frequently consumed food products to enrich their fatty acid composition.¹⁵ The human body cannot produce fatty acids that must be obtained from the diet.

For this reason, they are referred to as essential fatty acids. They can be divided into two categories: ω -3 (omega-3 or n-3), found in large quantities in fish oils, and ω -6 (omega-6, or n-6), primarily in plant oils. Omega-3 fatty acids can be added to foods like eggs, milk, and yoghurt to improve heart and brain health. It is believed that polyunsaturated fatty acids are essential for preserving human health. How fatty acids are structured affects how rapidly fats are absorbed, digested, and accessible. The primary seed plant lipids contain a large amount of linoleic acid, also found in fish oils and is often the most abundant di- or polyenoic fatty acid in mammals. Some foods contain relatively high quantities of linoleic and alpha-linolenic acids. The majority of oilseed plants, especially rapeseed, flax, borage, and evening primrose, contain linoleic acid; however, fish oils, particularly those from fatty fish like salmon, trout, mackerel, sardines, and herring, are the most significant sources of alpha-linolenic acid. It has been demonstrated that feeding linoleic and alpha-linolenic acid-rich oils to dairy and beef cattle, pigs, broilers, and layer chickens can increase conjugated linoleic acids in the resulting milk, meat, and eggs. Fish oil and flesh's fatty acid composition, particularly its Ω 3 acid content, can be enhanced similarly to create functional food.⁵⁶ It has been demonstrated that polyunsaturated fatty acids can slow the formation of tumors and can help prevent heart disease, hypertension, type 2 diabetes, arthritis, and autoimmune illnesses.⁵⁶ While eating a diet rich in macromolecules can have many positive health effects, some gaps must be filled for these foods to be effective and accessible to everyone. Here are some of the main flaws in foods that have been enhanced using macromolecules: Cost: The availability of foods containing additional macromolecules to low-income people is constrained by the fact that they can be more expensive than foods without them. Taste and Texture: Fortified macromolecule foods may have a distinct flavour or texture than non-fortified foods,

which could impact customer acceptance as well as their desire to purchase and consume them. Access: Foods enhanced with macromolecules could not be readily available everywhere or might not be regarded favourably by all populations, resulting in unequal access to these foods. Bioavailability: The effectiveness of fortified foods to address nutrient shortfalls may be hampered by some nutrients' lower bioavailability in fortified foods, such as iron and zinc. Overconsumption: Eating fortified macromolecule foods frequently could lead to an excess of particular nutrients, which could have detrimental effects on health. Overall, fixing these gaps in macromolecule-fortified foods will be essential to ensure their effectiveness, accessibility, and safety for all populations. Governmental entities, the food industry, and public health organizations must collaborate to fortify foods with required macromolecules while ensuring they are secure, available, and well-liked by consumers.⁷³

Conclusion

A good way to increase the nutritional value of the food supply and address population nutrient deficits is to fortify foods with necessary macromolecules like protein, carbohydrates, and fats. Foods fortified with macromolecules can offer several health advantages, including better heart and brain function, a lower chance of developing chronic diseases, and improved digestion. It is cost-effective to extract macromolecules for food fortification, which can treat both undernutrition and overnutrition. The prevention and management of macronutrient deficiencies in vulnerable groups, particularly women and children, and favourable social, economic, and environmental effects have all been demonstrated by studies

on food fortification. The best methods to extract valuable compounds from natural resources have been the subject of extensive research over the past few decades. However, conventional techniques lead to high temperatures and lengthy extraction times. Due to these drawbacks, other extraction techniques have recently started to replace these conventional methods. These procedures frequently entail using an energy source to hasten the transfer of the macromolecule molecules to the solvent. Additionally, techniques that are more eco-friendly and require less solvents have been sought for. Over the past ten years, new methods and emerging techniques have been developed and used to decrease the amount of sample that needs to be handled. Since the food companies can reach many consumers through retail, it is a very cost-effective strategy that can successfully apply food fortification. To guarantee that fortified foods offer the most possible health advantages without creating any health hazards, ensuring that they are secure, efficient, and well-balanced in terms of their nutrient content is crucial.

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Conflict of Interest

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