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Tanmay Sarkar · Siddhartha Pati *Editors*

Bioactive Extraction and Application in Food and Nutraceutical Industries

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Methods and Protocols in Food Science

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Methods and Protocols in Food Science series is devoted to the publication of research protocols and methodologies in all fields of food science.

Volumes and chapters will be organized by field and presented in such way that the readers will be able to reproduce the experiments in a step-by-step style. Each protocol will be characterized by a brief introductory section, followed by a short aims section, in which the precise purpose of the protocol will be clarified.

Bioactive Extraction and Application in Food and Nutraceutical Industries

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Preface to the Series

Methods and Protocols in Food Science series is devoted to the publication of research protocols and methodologies in all fields of food science. The series is unique as it includes protocols developed, validated and used by food and related scientists as well as theoretical basis are provided for each protocol. Aspects related to improvements in the protocols, adaptations and further developments in the protocols may also be approached.

Methods and Protocols in Food Science series aims to bring the most recent developments in research protocols in the field as well as very well established methods. As such the series targets undergraduate, graduate and researchers in the field of food science and correlated areas. The protocols documented in the series will be highly useful for scientific inquiries in the field of food sciences, presented in such way that the readers will be able to reproduce the experiments in a step-by-step style.

Each protocol will be characterized by a brief introductory section, followed by a short aims section, in which the precise purpose of the protocol is clarified. Then, an in-depth list of materials and reagents required for employing the protocol is presented, followed by a comprehensive and step-by-step procedures on how to perform that experiment. The next section brings the dos and don'ts when carrying out the protocol, followed by the main pitfalls faced and how to troubleshoot them. Finally, template results will be presented and their meaning/conclusions addressed.

The Methods and Protocols in Food Science series will fill an important gap, addressing a common complain of food scientists, regarding the difficulties in repeating experiments detailed in scientific papers. With this, the series has a potential to become a reference material in food science laboratories of research centers and universities throughout the world.

University of Campinas
Campinas, Brazil

Anderson S. Sant'Ana

Preface

The field of bioactive extraction and its application in the food and nutraceutical industries has witnessed significant growth and innovation in recent years. Bioactive compounds, which possess beneficial properties for human health, have garnered attention for their potential in improving dietary quality and promoting overall well-being. This book, titled *Bioactive Extraction and Application in Food and Nutraceutical Industries*, aims to provide a comprehensive overview of the various aspects of bioactive compound extraction and its industrial applications.

The chapters in this book cover a wide range of topics, encompassing both traditional and novel extraction techniques, as well as exploring diverse sources of bioactive compounds. The primary objective is to present a holistic view of the field, catering to the needs of researchers, industry professionals, and students who are interested in this rapidly evolving area.

Chapter 1 delves into traditional extraction techniques commonly employed in the food, nutraceutical, and biotechnology industries. It provides a solid foundation by discussing the technological advancements and applications of these techniques in extracting bioactive compounds, enabling readers to grasp the fundamentals of the field.

Chapters 2 and 3 specifically focus on the extraction of bioactive compounds and nutraceuticals from plants and marine sources, respectively. These chapters shed light on the wide array of bioresources available and explore the applications of plant-based and marine-derived compounds in the food and nutraceutical sectors. Readers will gain insights into the diverse range of bioactive compounds that can be extracted from these sources and their potential benefits.

The subsequent chapters delve into the application of specific extraction techniques. Chapter 4 discusses the utilization of microwave-assisted extraction for bioactive compounds, highlighting its efficiency and effectiveness. Chapter 5 explores ultrasound-assisted extraction, another powerful technique that has gained popularity in the food, pharmacy, and biotech industries. Both of these chapters present practical insights into these innovative extraction methods.

Chapters 6 and 7 introduce supercritical and subcritical fluid extraction and novel solvent-based extraction, respectively. These advanced techniques offer unique advantages in terms of efficiency, selectivity, and sustainability. The chapters provide comprehensive coverage of their principles, applications, and potential in the extraction of bioactive compounds.

Chapter 8 focuses on enzyme-assisted extraction, a promising method that harnesses the power of enzymes to enhance extraction efficiency. This chapter delves into the enzymatic hydrolysis of plant and microbial sources and its potential for extracting valuable bioactive compounds.

Chapter 9 introduces pulsed electric fields as a green technology for the extraction of bioactive compounds. This emerging technique utilizes electrical pulses to disrupt cell membranes and facilitate the release of bioactive compounds, offering a sustainable alternative to traditional extraction methods.

Chapter 10 explores pressurized liquid extraction, which utilizes high-pressure solvents to extract bioactive compounds. This chapter discusses its applications and advantages, providing valuable insights for researchers and industry professionals.

Chapter 11 presents case studies and applications of different novel extraction methods discussed throughout the book. It showcases real-world examples to demonstrate the practical implementation and effectiveness of these techniques. Chapter 12 explores pressurized liquid extraction for the isolation of bioactive compounds and the application of this high-pressure solvent extraction technique in obtaining valuable bioactive compounds with efficiency and precision.

The subsequent chapters continue to broaden the scope by exploring the extraction of bioactive compounds from fruit waste (Chapter 13) and plant seeds (Chapter 14), providing innovative approaches to utilize these underutilized bioresources. Chapter 15 focuses on essential oils, discussing their sources, extraction techniques, and nutraceutical perspectives.

Chapter 16 addresses green and clean technologies for the production of novel nutraceuticals, emphasizing sustainable practices and environmentally friendly approaches.

Chapters 17 and 18 delve into the optimization of nutraceutical extraction processes and the computational approaches used in this field, respectively. These chapters provide valuable insights into improving extraction efficiency and understanding the behavior of bioactive compounds through advanced modeling and simulation techniques.

In conclusion, *Bioactive Extraction and Application in Food and Nutraceutical Industries* aims to serve as a comprehensive guide for researchers, industry professionals, and students involved in the extraction and application of bioactive compounds. The diverse range of topics covered in the chapters will provide readers with a solid foundation, practical knowledge, and insights into the latest advancements in the field. It is our hope that this book will contribute to the continued growth and development of this exciting area of research and application.

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Odisha, India ***Siddhartha Pati***

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Chapter 1

Technologies for Extraction of Bioactive Compounds and Its Applications

Rinku Sudarshan Agrawal and Nilesh Prakash Nirmal

Abstract

The successively growing consumer interest in natural bioactive compounds associated with human health promotion and disease prevention has received huge attention in the food market toward effective extraction techniques. Bioactive compounds can be extracted from natural sources and commercially utilized in the development of nutraceuticals and functional foods. Various novel extraction technologies, such as supercritical fluid extraction, ultrasound and microwave-assisted extraction, and accelerated solvent extraction, have been considered effective for large-scale recovery, less extraction time, and superior extract quality. The choice of an appropriate extraction technique could be based on the final applications or the process optimization of bioactive compounds. This chapter aims to present conventional and emerging techniques suitable for extraction of bioactive compounds from natural sources and its potential utilization in food and nutraceutical industries.

Key words Bioactive, Health promotion, Extraction, Application

1 Introduction

Consumers' attitude toward a healthy and well-balanced diet is encouraging the utilization of natural products. Plant-derived natural products have gained extensive attention as a significant source of active compounds with functional efficacy. Plants are found to be an effective reservoir of numerous bioactive compounds and thus have been hugely exploitated in various commercial sectors, especially in the food and pharmaceutical industries. These substances are chemical structures that perform specialized functions at the biological level. Bioactive compounds derived from plants, also referred as phytonutrients, are produced as secondary metabolites eliciting pharmacological effects in human health management [1].

Natural bioactive compounds influence the health of living organisms as they are associated with some extra nutritional constituents that typically occur in low quantities in foods. Different

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fruits, vegetables, whole grains, millets, etc. provide health benefits beyond the basic nutritive function. A number of bioactive compounds, such as polyphenols, phytosterols, dietary fiber, flavonoids, phytoestrogens, carotenoids, and vitamins, are known to exert beneficial physiological effects. The quest for bioactive compounds from natural sources has been driven by scientific research of these targeted molecules against a vast array of diseases, besides their use in food science and technology. Bioactive compounds are thus recognized as a vital ingredient in human health owing to their multiple biological effects, such as reduction in risk factors of various cardiovascular diseases, and they also act as antioxidant, antifungal, antiviral, anti-carcinogenic, antiallergenic, anti-inflammatory, and antimicrobial agents [2]. Phytochemicals being a natural source of health-promoting compounds find greater opportunity in the development of fortified foods with these functional ingredients.

Extraction is one of the most important steps to analyze the bioactive compounds present in plant material. Bioactive compounds from green plants are currently the subject of considerable research interest, but their extraction as part of phytochemical and/or biological investigations presents a key challenge. The use of bioactive compounds in different commercial sectors, such as food and pharmaceutical industries, signifies the need for the most appropriate and standard technique for the extraction of these active components from plant materials. The main conventional techniques related to extraction of bioactive compounds are maceration, percolation, hydro distillation, and Soxhlet extraction. Along with conventional methods, numerous advanced techniques have emerged as green or clean extraction techniques due to less consumption of solvent and energy, faster extraction rate, high-quality products, better yield, and eco-friendliness [3]. No single method is recommended as a standard method for extracting bioactive compounds from plant materials as individual methods are associated with some strengthes and weaknesses. Biological activities of the extract show significant variation depending on the extraction method, and this also opens a gateway for selecting a suitable extraction method.

Thus, the extraction of active compounds from plants needs an appropriate extraction technique that can provide bioactive ingredients–rich extracts and fractions. The extraction procedures, therefore, play a crucial role in the yield and nature of the phytochemical content. Hence, this chapter aims to provide an overview of bioactive extraction from natural sources with various conventional and emerging technologies and their potential application in the food and pharmaceutical industries (Fig. 1).

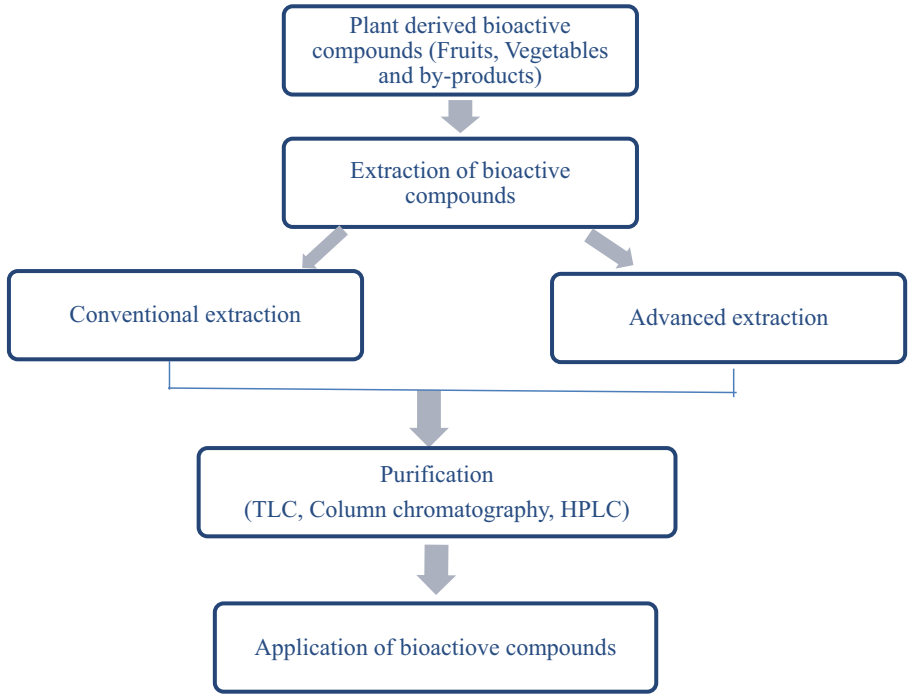

Fig. 1 Schematic representation of bioactive compounds and their applications

2 Extraction of Bioactive Compounds

Extraction is one of the most important unit operations followed in the food industry that involves separation of medicinally active compounds from plant materials using selective solvents through standard procedures. Extraction of bioactive constituents has always been a great challenge for scientists as the target compounds may be non-polar to polar, thermally labile to resistant, and the suitability of the extraction techniques must be considered. The extraction of these active compounds requires a suitable extraction technology that considers the plant parts used as starting material, extraction time, nature of the solvent, particle size, and the stirring during extraction [4]. The bioactive compounds can be extracted by conventional and advanced technologies, which have their own advantages and disadvantages (Table 1).

Conventional methods such as pressing, hydro-distillation, steam distillation, and solvent extraction have been used since ancient times in food processing industries (Fig. 2). The major challenges of conventional extraction are longer extraction time, requirement of costly and high-purity solvent, more quantity of solvents, loss of volatile compounds, degradation of thermolabile compounds, the possibility of leaving toxic solvent residues in the extract, low yield and extraction efficiency. Consequently, there is a

Table 1
Advantages and disadvantages of extraction techniques

Conventional extraction techniques	Advanced extraction techniques
Longer extraction time	Shorter extraction time
Requirement of costly and high-purity solvent	Reduced solvent usage and environmental friendly
Extraction efficiency is limited	High extraction efficiency
Extraction yield is less	Improved extraction yield
Labor intensive	Less labor is required
Loss of volatile compounds	Effective for volatile compounds
Degradation of thermolabile compounds	Suitable for thermolabile compounds
Less initial capital investment	Initial capital investment is more

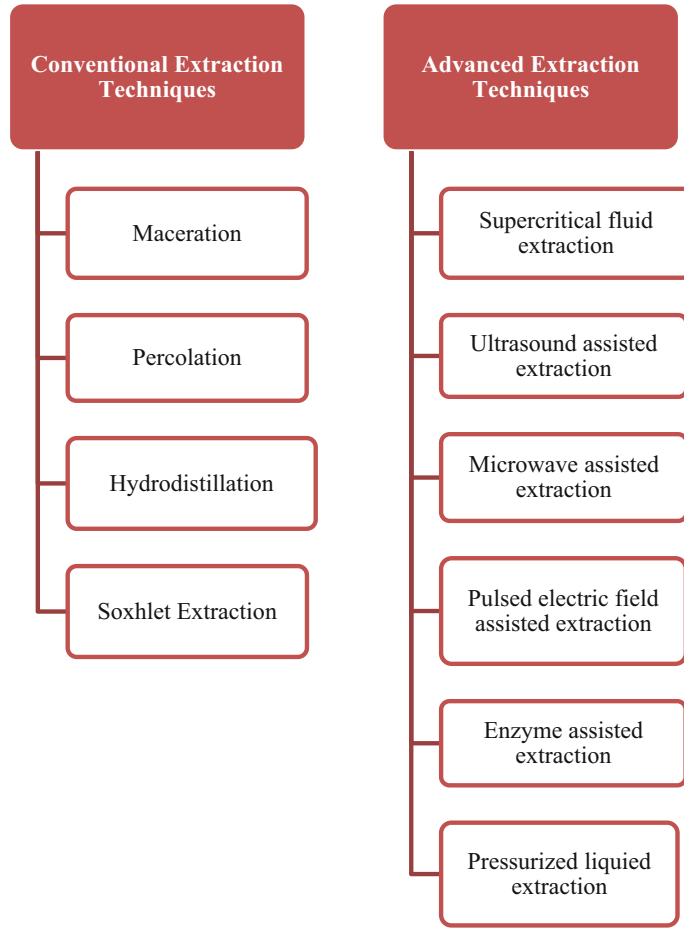

Fig. 2 Conventional and advanced extraction methods

growing demand for emerging extraction techniques that have several advantages, such as less energy and solvent consumption and reduced extraction times and can replace conventional solvents with eco-friendly substitutes. The qualitative and quantitative studies of bioactive compounds from natural sources mostly rely on the selection of proper extraction methods. The selection of extraction technology is based on their advantages, disadvantages, operation parameters, required degree of purity of the extract, physical and chemical properties of the compound of interest, cost-effectiveness, and value of the extracted product [5].

3 Conventional Extraction Techniques

Conventional or classical techniques such as maceration, Soxhlet extraction, and hydro distillation are the most widely used methods for extraction of bioactive compounds from natural sources. These methods are primarily based on liquid–solid extraction [6]. The efficiency of conventional extraction methods depends on the choice of solvent and the polarity of the compound.

3.1 Maceration

Maceration, since long, is one of the simplest and inexpensive techniques widely used for the extraction of essential oil and bioactive compounds from plant material. The whole or coarsely powdered plant material is soaked with a solvent, such as ethanol, acetone, or hexane, in a closed vessel. This is allowed to stand at room temperature for 2–3 days with frequent stirring, which facilitates extraction. The process is intended to rupture the cell structure and help in the removal of different plant components. The mixture is then pressed or strained by filtration or decantation after a specific time [7]. The extraction efficiency was lowest in the extracts of the maceration method, and it is a time-consuming method. But it could be used for the extraction of thermolabile components.

3.2 Percolation

Percolation is a more efficient extraction method than maceration as it is conducted by passing the boiled solvent through the plant material at a controlled and moderate rate because it is a continuous process in which the saturated solvent is constantly being replaced by fresh solvent [8].

3.3 Hydro Distillation

Hydro distillation is another conventional method that uses water or steam for the extraction of bioactive compounds, especially essential oils from plants. Hydro distillation is often carried out using an equipment known as Clevenger apparatus or simple steam distillation. In the Clevenger apparatus, sample mixed water is boiled to evaporate volatile components, while in the steam distillation approach, the steam is passed through a bed of the sample. In

both methods, two layers (aqueous and oil-rich) are obtained and oil can be further separated via separating funnels [9]. Hydro distillation consumes more time, high levels of energy distillation rates may vary if the heat source is not controlled, and the direct heat source may cause charring of plant material at the base of the chamber [10].

3.4 Soxhlet Extraction

Soxhlet extraction is one of the most popular conventional techniques for extracting valuable bioactive compounds from various natural sources. This is an automatic continuous extraction technique having high extraction efficiency that requires less time and solvent consumption than maceration or percolation. However, it is widely applied to compounds with high thermal stability [8].

The finely ground material is placed in a thimble-holder made from cellulose or filter paper and kept in a Soxhlet apparatus. Extraction solvents are heated in a round bottom flask, vaporized into the sample thimble, condensed in the condenser, and dripped back. When the liquid reaches an overflow level, a siphon aspirates the whole content of the thimble-holder and unloads it back into the distillation flask, carrying the extracted analytes in the bulk liquid. This process is continued until complete extraction is achieved [6]. The efficiency of this process depends on parameters such as solubility, mass transfer, and solid material characteristics. The selection of a suitable solvent is one of the most important factors for the extraction of bioactive compounds since it must be based on its ability to extract the target compound [11]. This technique has also been combined with microwave-assisted extraction and ultrasonic extraction in an attempt to improve extraction efficiencies.

4 Advanced Extraction Techniques

The attention toward novel approaches for the extraction and isolation of bioactive compounds from plant-based materials is increasing in the field of research and development. Various new and promising extraction techniques have become the recent area of interest for extraction of bioactive compounds as they are able to overcome the limitations of conventional methods. These advanced extraction methods are being explored as clean, green, and environmentally sustainable technologies that act as efficient alternatives to conventional extraction technologies. Green extraction aims to reduce the usage of solvent and energy, generates less waste, and prevents environmental pollution while obtaining the highest product yield with good quality [12].

Advanced techniques are also referred to as “assisted” extraction techniques where an additional physical phenomenon (ultra-high pressure, ultrasound, electric fields, use of enzymes) is used for

identification of the process [13]. Owing to the high yield, reduced processing time, high-quality products, and less generation of waste, these emerging technologies have replaced the conventional extraction methods. The effectiveness of these techniques varies with the properties of the source matrix, its chemical structure, and process parameters such as solvent, pressure, time, and temperature. The food industry in the extraction sector should select a suitable extraction method. The main objectives of advanced extraction processes are related to achieving high yield, increased heat and mass transfer, more effective use of energy with less time consumption, reduction in the number of processing steps, low environmental impact, and ensuring a desirable balance between product quality and process efficiency [14].

4.1 Supercritical Fluid Extraction (SFE)

The supercritical fluid extraction (SFE) technique offers numerous operational advantages over conventional methods as it uses supercritical solvents with various physico-chemical properties. The SFE technology is extensively adapted for the extraction of thermolabile biomolecules without any degradation of compounds. It involves modulation of physical features, such as increasing the temperature and pressure of a substance or solvent above its critical values. The changes in fluid density in its supercritical state allow for variation in solvency power, which results in selective extractions of compounds of interest [15].

This technique makes use of supercritical fluids such as CO_2, ethanol, and water, which are generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA). Particularly, supercritical carbon dioxide (SC-CO_2) is extensively used as a solvent as it is inert, nontoxic, economical, and easily separable from the final product [8]. SC-CO_2 is characterized by low viscosity, high density, and diffusivity than conventional solvents, which helps to have enhanced transport properties than liquids, diffuses very easily through solid materials, and thus increases the extraction rate of compounds [16]. SC-CO_2 has unique solvent properties that make it a desirable compound for separating antioxidants, essential oils, pigments, flavors, fragrances, and fatty acids from plant and animal materials. The productivity and profitability of a supercritical fluid extraction (SFE) process largely depend on the selection of process parameters.

4.2 Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) is extensively used in the food industry as a viable alternative to the conventional extraction techniques that have been inclusively reported for achieving high extraction rates of plant-derived bioactive compounds [17]. UAE is also referred to as ultrasonic extraction or sonication, which uses ultrasonic wave energy in the extraction. This technology involves the use of acoustic vibrations or mechanical waves with ultrasound to produce cavitation in which microbubbles form in the liquid

phase. Ultrasonic treatment causes mechanical impact on the cell wall. The plant cell wall can get disrupted, which enhances solvent penetration into the cell and thus helps to improve the mass transfer. Ultrasound in the solvent-producing cavitation accelerates not only dissolution but also diffusion of the solute and heat transfer, which improves the extraction efficiency [18].

The factors affecting the efficiency of UAE are extraction time, power, solvent, liquid/solid (L/S) ratio, plant material, frequency, amplitude, and intensity. Ultrasound power directly affects the cavitation and shear forces in the extraction medium. The frequency used for the extraction of bioactive compounds from natural products usually ranges between 20 and 120 kHz [19].

UAE is considered a more promising choice than conventional extraction methods due to lower consumption of solvent and energy. It is explored as a more sustainable process as it is associated with several advantages, such as: rapid heat and mass transfer efficiency, greater solvent penetration into solid material, reduction of extraction temperature and time, and lowest carbon emission [6]. UAE also provides faster extractions, with high reproducibility and higher purity of the final product when compared to conventional extraction methods. UAE is widely applicable for the extraction of thermolabile and unstable compounds from natural sources [8].

4.3 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction has been accepted as an effective alternative technique to recover bioactive compounds from plant materials using microwave energy. Microwaves are electromagnetic radiation that occur in frequencies ranging from 300 MHz to 300 GHz and wavelengths between 1 mm and 1 m. These waves are composed of two mutually perpendicular oscillating fields, that is, an electrical field and a magnetic field, which are used as energy and information carriers [6].

The principle of microwave heating depends on its direct impact on polar materials. Microwaves can penetrate biomaterials and interact with polar molecules such as water to generate heat. The heat is generated by ionic conduction and dipole rotation mechanisms [20]. During microwave treatment, polar compounds align themselves in the direction of electric field and rotate at high speed which leads to disruption of the cell membrane. The cell wall breakage enhances the release of the extract from the source materials.

The important parameters that influence the extraction efficiency of MAE techniques are microwave power and temperature, microwave energy density, plant sample characteristics and its water content, solvent-to-feed ratio, and irradiation time [21]. The combination of ultra-sonication with microwave amplifies the efficiency of the extraction process. MAE is a promising novel extraction technique that offers many advantages, such as rapid heating

rates, increased extract yield, short processing time, energy saving, and no further generation of secondary waste. Additionally, due to less consumption of organic solvent, this technique gets a broad recognition as a green extraction technique [22].

4.4 Pulsed Electric Field (PEF)-Assisted Extraction

Pulsed electric field involves the application of high voltage pulses for a brief time period (nanoseconds/milliseconds) through the sample placed between two conducting electrodes [23]. Pulsed electric fields (PEF)-assisted extraction is attracting great attention as a nonthermal extraction technology due to being a cost-effective technique that is energy efficient, time-saving, and eco-friendly [24]. PEF has proven to be a promising technique to enhance the extraction of valuable bioactive compounds such as anthocyanin, polyphenols, and plant oil from plant tissues, as well as their byproducts and soluble intracellular matter from microorganisms.

The targeted material is placed between the electrodes, and a high voltage electric field of 10–60 kV is applied through electrodes. The applied high voltage pulse induces pores in the cell membranes (electroporation), which enhances the permeability of the cell membrane. The cell membrane loses its structural functionality, leading to cell disintegration, which accounts for the leakage of intracellular content, and the plant material is extracted [25]. PEF has the ability to electroporate the cell membranes and is thus commonly used as pretreatment to facilitate the extraction of bioactive compounds, followed by subsequent conventional or advanced extraction techniques [11]. This technique is usually preferred for liquid foods as electrical current flows into the liquid food more fast and effectively and the transfer of pulses from one point to another in liquids is quite easy due to the presence of charged molecules [26].

Numerous studies concluded PEF as a promising tool to recover phytonutrients used in the food and pharmaceutical industries. PEF-assisted extraction leads to cost efficiency, higher extract yield of bioactive compounds, lower energy consumption, and less treatment time, providing the optimum process parameters. The extraction efficiency of bio ingredients with PEF treatment depends on electrical parameters such as electric field strength, energy input, pulse polarity, and delay time between pulses of opposite polarity.

4.5 Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction is a novel approach for extracting bio ingredients from plant materials. The application of enzymes for extraction of bioactive compounds is a recent and emerging approach from small-scale, laboratory optimization studies to large-scale, industrial applications. Enzymes are ideal catalysts that can assist in the efficient extraction of bio ingredients from natural sources. The EAE technique has been applied for extraction of macromolecules such as proteins, polysaccharides, and low

molecular compounds such as phenols and polyphenols, oils and fatty acids, essential oil, sugars, di- and triterpenes, and vitamins [27].

Enzyme-assisted extraction (EAE) technique makes use of specific enzymes to hydrolyze cell wall components. This disintegrates the structural integrity of plant cell wall and increases the permeability of the intracellular material for extraction thereby releasing bioactive compounds. In EAE, the plant material is pretreated with desirable enzymes such as protease, pectinase, pectin esterase, cellulase, hemicellulase, cellobiase, and α-amylase to hydrolyze the cell walls and release the phytochemicals bound to lipid and carbohydrate chains inside the cell [28].

Enzymes are applicable to extract many phenolic compounds, including flavonoids and anthocyanidins. EAE can also be applied for the additional recovery of compounds such as pectins from agricultural wastes and byproducts by increasing the permeability of plant cell wall. During enzymatic treatment, the highest enzyme efficiency mainly depends on the type, dosage, and required condition of the enzymes, treatment time and temperature combinations, substrate ratio, and particle size [5]. This technique has also been used in combination with other extraction techniques such as UAE, SFE, and MAE to improve the overall recovery of bioactive compounds from source materials [29]. The combination of ultrasonic waves with enzymes can improve the capability of enzymes. Many research studies have shown highly positive effects of both ultrasonic and enzymes in the improvement of extraction yield with superior product quality for nutraceutical and pharmaceutical applications.

EAE has been regarded as a valuable substitute for conventional techniques to isolate various biologically active compounds more rapidly and with better recovery. EAE has emerged as an eco-friendly alternative to recover bioactive compounds with several advantages, such as increased yield and quality of product, reduction in extraction time, lower solvent consumption, in addition to the increased transparency of the system. However, EAE has probable commercial and technical limitations, such as worldwide regulations of enzyme usage and relatively high cost of enzymes for large industrial production. An important area of research is investigating the stability of enzymes and their interaction with other food and plant ingredients during processing and storage.

4.6 Pressurized Liquid Extraction (PLE)

Pressurized liquids are able to recover phytonutrients faster than conventional low pressure methods. PLE can be viewed as an extension of supercritical fluid extraction, utilizing organic solvents instead of carbon dioxide. PLE uses liquid solvents below their critical point with controlled temperature and pressure [30]. PLE was first introduced as accelerated solvent extraction (ASE) technology in 1995 by Dionex Corporation as an alternative to other

extraction techniques. It is also referred as pressurized solvent extraction (PSE), enhanced solvent extraction (ESE) or pressurized fluid extraction (PFE) [31]. Pressurized liquid extraction (PLE) technology has gained remarkable research interest to achieve fast and efficient extraction of the bioactive components from the food matrix in short time using organic solvents at elevated temperature and pressure.

The sample is placed in the extraction cell with an organic solvent such as toluene or hexane/acetone at an elevated temperature (up to 200 °C) and relatively high pressure (500–3000 psi) to increase the efficiency of the extraction process. The use of a closed system allows extraction at elevated temperatures since the boiling point of the solvent increases. The rise in temperature usually above their boiling points causes dramatic changes in the physical and chemical properties of water such as increase in the diffusion rate and decrease in the viscosity of solvents. The PLE conditions provides better solubility of target analytes with the solvent. The elevated temperature can also break down analyte-matrix interactions and increases mass transfer of the essential compounds present in the plant matrix to the solvent, as well as the stability of the process [32].

PLE has been consolidated as a high-throughput and green extraction technique for sustainable extraction of bioactive compounds from various natural sources. This technique requires less amounts of solvents due to the combination of high pressure and temperature, which dramatically decreases the time consumption of extraction. It is a solid-liquid extraction technique that offers improved extraction efficiency with less time and cost, is eco-friendly, automates minimum production of waste, and eliminates post-extraction steps such as filtration and centrifugation [33]. The main limitations of the process are high equipment cost and the need for a thorough optimization of variables to avoid a matrix-dependent efficiency [34].

4.7 Combination of Modern Techniques for Effective Extraction of Bioactive Compounds

The combination of different extraction techniques is a desirable approach that presents advantages to overcome the limitations of an individual extraction technique. Many research studies have shown that a combination of these novel extraction strategies can be effective for rapid and efficient extraction with the aim of enhancing the amount of the target molecules and reducing the waste of solvents.

Ultrasound is a promising choice, particularly when it is combined with other substitutes among the different available combinations. Ultrasound can be used for rapid heat and mass transfer in the extraction field when combined with other technologies [35]. Ultrasound-assisted extraction technique combined with SFE has been recently estimated to improve the flexibility in the extraction process [36]. Different combinations, such as

ultrasound-assisted enzymatic extraction (UAEE), microwave-assisted enzymatic extraction (MAEE), and ultrasonic microwave-assisted extraction (UMAE), can provide a synergistic effect and exhibit higher potential extraction ability [37].

5 Purification of the Bioactive Compounds

Plant materials are a multi-component mixture that contains numerous types of bioactive compounds having different polarities; however, their separation still remains a big challenge. The isolation and purification of bioactive compounds is very important to evaluate its biological activity by in vitro and in vivo assays [38]. Chromatography is a separation technique immensely used for identification and/or purification purposes in different research and industrial sectors. The chromatographic technique is a widely adopted technique that has a vital role in the chemistry of plant-derived natural products, as well as a significant contribution to the discovery of innovative bioactive compounds of pharmaceutical and biomedical importance. Chromatographic techniques such as paper chromatography, thin-layer chromatography, high-performance liquid chromatography (HPLC), gas chromatography, column chromatography, or combination of several chromatographic techniques can be potentially used to obtain pure compounds [39].

Various scientific studies support that silica gel column chromatography and thin-layer chromatography (TLC) are still mostly used due to their convenience, economy, and availability in various stationary phases [40]. High-performance liquid chromatography (HPLC) is an accurate and selective chromatographic technique potentially used in pharmaceutical industries for the isolation and purification of bioactive compounds from medicinal plants. Various nonchromatographic techniques, such as immunoassay, phytochemical screening assay, and Fourier-transform infrared spectroscopy (FTIR), are recognized as valuable tools for the characterization and identification of bioactive compounds present in an unknown mixture of plant extracts [41].

6 Extraction of Bioactive Compounds from Agro-industrial Waste

The huge quantity of waste produced from agricultural and food production remains a great challenge as well as an opportunity for the food industry. Agro-industrial waste has a great potential to generate food additives that can be beneficial in ensuringe global food sustainability [42]. Agro-industrial waste is composed mainly of seed, skin, rind, and pomace and is a promising source of potentially valuable bioactive compound components such as

proteins, polysaccharides, fibers, lipids, carbohydrates, peptides, carotenoids, phenolic and other compounds, which can be reused as nutraceuticals and functional ingredients [43].

Due to their complex chemical composition, animal and vegetable byproducts can be utilized as a low-cost raw material to obtain bioactive compounds using suitable extraction technology [44]. The recovery of these bioactive compounds present in agricultural residues such as fruits, vegetables, and plants involves the use of various conventional and modern extraction technologies. The availability and suitability of extraction techniques provides an opportunity for optimal use of any of these for recovery of specific active compounds. The utilization of bioactive compounds isolated from agricultural wastes can reduce the risk and cost of waste treatment as well as potentially add more value to agricultural and food production. These recovered biomolecules help to enhance consumer health by developing nutraceuticals, functional foods, dietary supplements, and active or smart agents for biodegradable materials and packaging. Thus, valorization of bioactive compounds from the byproducts of agricultural industry reduces the waste disposal burden and mitigates the environmental problem with sustainable use of natural resources [45].

Bioactive phytochemicals extracted from tomato byproducts such as tocopherols, carotenes, polyphenols, and terpenes have showed significant amounts of antioxidant activities. Therefore, these value-adding components isolated from such waste can be potentially utilized as a natural antioxidant source in the formulation of functional foods or can serve as additives in food products to extend their shelf-life [46]. Bioactive compounds recovered from various fruit and vegetable processing waste with their bioactivity are summarized in Table 2.

7 Nano Emulsion as Potential Delivery Systems for Bioactive Compounds

Nanotechnology has found enormous applications in different sectors, and nano-emulsion of bioactive compounds and functional food ingredients have a promising potential for application in food industries. Nano-emulsions are small, droplet-sized in the range of 100 nm and are kinetically stable colloidal systems. As compared to conventional emulsions, a nano-emulsion-based delivery system plays an important role in improving the solubility, functionality, and textural properties of bioactive compounds, especially lipophilic active food ingredients. Nano-emulsions include encapsulation of poorly soluble compounds (natural preserving agents, nutraceuticals, colorants, flavors), texture modification, and enhancement in bioavailability, digestibility, solubility, etc. [55].

Table 2
Bioactive compounds from fruit and vegetable processing waste and by-products

Source	Residue	Bioactive components	Bioactivity	References
Tomato	Skin, pomace	Lycopene, carotenoids, Flavonones	Antioxidant, antimicrobial activity	[47]
Mango	Peel, kernel, pomace, seed	Phenolic compounds, carotenoids, flavonoids, vitamin C, and dietary fiber	Lower the risk of cancer, cataracts, Alzheimer's disease, anti-oxidant	[48]
Banana	Peel	Flavonols, phenolic acids, catechins	Antioxidant, antibacterial, reducing blood sugar & cholesterol	[49]
Citrus fruits	Peel, pulp, seeds	Pectin, hesperidin	Immunomodulatory, anti-oxidant, anti-cancer	[50]
Apple	Pomace	Phenolic acids, flavonoids, anthocyanins	Antioxidant, antimicrobial, anti-inflammatory, Cardioprotective	[51]
Carrots	Peel	Phenols, Beta-carotene	Provitamin A, dietary fiber, antioxidant,	[52]
Cauliflower	Stem and leaves	Bioactive peptides, flavonoids, quercetin, Kaempferol	Antioxidant, anti-obesity, Chemopreventive	[53]
Beetroot	Pomace	Betalins, flavonoids, phenolic compounds	Antioxidant, Hepatoprotective activity	[54]

Lipophilic bioactive compounds such as carotenoids, omega-3 fatty acids, polyphenols, flavonoids, phytosterols, and tocopherols are susceptible to being incorporated in food products. The incorporation of highly lipophilic bioactive compounds in food is a great challenge due to their poor water solubility, fast oxidation, and instability in food formulations. Nano-sized structures such as nano-emulsions of oil-in-water are regarded as useful tools with great potential in the food sector to incorporate food ingredients. The reduction in the size of bioactive compounds incorporated within a solution would help to increase the surface area per mass unit of nano-emulsions, thus enhancing the solubility and stability in foods. The smaller droplet size of nano-emulsions will enable higher bioaccessibility of bioactive compounds encapsulated [56].

The nano-emulsion formulations of active ingredients can be used to develop biodegradable coating and packaging films to enhance the quality, functional properties, nutritional value, and shelf life of foods. However, food grade nano-emulsions can find widespread application only if their production cost is commercially feasible and meets the safety standards of the food industry [57].

8 Application of Bioactive Compounds

8.1 Functional Foods

The successively growing demand for foods with beneficial effects on human health, while contributing to the sustainable use of natural resources, enhances the research interest in potential utilization of bioactive compounds. Bioactive compounds are receiving more popularity due to their diverse biological activities and huge exploitation in various commercial sectors, such as food, pharmaceutical, and cosmetic industries. These compounds exhibit beneficial effects such as antioxidant activity, anti-diabetic, anticancerous, antidiuretic, anti-atherosclerotic, and so on for human beings. Bioactive compounds have multiple applications in food, acting as antimicrobials, antioxidants, natural dyes, fortifying ingredients, texture modifiers, and others [58]. Bioactive ingredients such as anthocyanins, curcumins, tannins, and carotenoids are commonly applied as natural colorings in food product preparations [59]. Moreover, they have also been used for the development of active and smart biodegradable food packaging materials [60].

The technological advancements made possible the extraction of bioactive compounds not only from natural sources but also from byproducts and their reintroduction into foods. Bioactive compounds can be used to improve the quality of conventional foods with respect to nutritional, sensorial, and technological properties (e.g., water and oil holding capacities, foaming, emulsion, and gelatinization) [61]. Bioactive compounds are key factors in the development of nutraceuticals and functional foods. The possibility of applying bioactive components in food products and in new technologies to enhance food product quality and safety is enormous. Due to the diversity of compounds, their possible interactions, and various physiological activities, each component must be properly evaluated for the production of food, beverages, and active and smart packaging applied to food to guarantee maximum potential in the applications [45].

8.2 Food Preservatives

Phenolic compounds are well known for their health benefits related to antioxidant activity and thus have potential use as biopreservatives. These compounds are extensively studied for their potential application in the food sector for improving the shelf life of perishable food products. The biological activity of phenolic compounds delays or inhibits the oxidation and growth of microorganisms; these compunds are thus considered as biopreservatives for safe extension of perishable products [62].

8.3 Pharmaceuticals

Phytochemicals are biologically active, naturally occurring chemical compounds in plants with various therapeutic benefits beyond those attributed to macronutrients and micronutrients. Nature-derived bioactive components have been investigated to elucidate

their biological activity for conventional medicines in the prevention and treatment of several chronic diseases. There is currently a growing interest in the study of bioactive compounds, extracts, and new ingredients from natural sources to produce pharmaceuticals, nutraceuticals, and dietary supplements. In recent years, the demand for herbal medicines and several natural products is consistently increasing for a healthy and sustainable life.

Bioactive food ingredients have emerged as a key component related to health-promoting and disease-preventing functions. Plant-derived bioactive compounds can help to suppress inflammation by inhibiting oxidative damage and communicating with the immune system. These bioactive ingredients control diet-related medical conditions such as obesity, cancer, cardiovascular diseases, osteoporosis, and other metabolic diseases [63]. Isoflavones can decrease dietary cholesterol absorption and low-density plasma lipoproteins via binding cholesterol in the intestinal tract.

Bioactive compounds are considered promising ingredients used to meet the human body's requirement and are usually consumed in the form of pharmaceutical preparations, such as pills, tablets, capsules, and powders [64]. The most commonly marketed and used nutraceuticals are amino acids, vitamins (C and E), minerals (copper, selenium, and zinc), carotenoids (β-carotene, lutein, zeaxanthin, and lycopene), fatty acids (omega-3 and omega-6), polyphenols, and several others [65], which can be extracted from agro-industrial commodities and its byproducts as well.

9 Conclusion and Future Perspective

Bioactive compounds are recognized due to their pharmacological and nutraceutical value and can be potentially utilized in the development of functional foods. The ever-growing demand for the extraction of plant-derived bioactive compounds continuously encourages the search for convenient extraction methods. Researchers have been seeking to use these techniques alone or in association with conventional techniques that result in better yield with less extraction time, extraction of heat-labile compounds, and less generation of toxic waste. The selection of these techniques depends on factors such as time, yield, and cost of extraction. Technological advancement and environmental awareness are two important factors for the development of different advanced extraction techniques. Many studies are still being carried out to improve these new green extraction techniques further, with the intention of reducing the cost of extraction, the time consumed, the quality of the extract, health, and environmental safety. These technologies could provide an innovative approach in the upcoming years to increase the production of specific compounds for use as nutraceuticals or as ingredients in the design of functional foods.

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Chapter 2

Extraction of Bioactive and Nutraceuticals from Plants and Their Application

Hadia Hemmami, Bachir Ben Seghir, Soumeia Zeghoud, Ilham Ben Amor, Abdelkrim Rebiai, and Imane Kouadri

Abstract

Natural bioactive compounds are a useful source of molecules for the development of nutraceuticals, food additives, and functional foods because they contain a wide variety of diverse structural and functional properties. Although extracting them for use in biological and/or phytochemical investigations presents some particular challenges, plant-derived bioactive and nutraceutical compounds are now the subject of a lot of studies since they offer a variety of biological properties and therapeutic benefits. In order to cut costs related to their synthesis and separation, the process of extracting active phytochemicals is also carefully taken into account. Despite the fact that natural bioactive compounds and functional foods have been used as traditional medicines to treat chronic diseases for decades, recent scientific studies emphasize the health advantages of functional meals and reveal the underlying processes that underlie their activities. To cure and prevent inflammatory and oxidative diseases, phytochemicals perform essential bioactive roles. Plant-derived bioactive compounds that don't cause oxidative damage and interact with the immune system might lessen inflammation. The capacity to bind to poisons or carcinogens that impact the digestive system exists in many bioactive compounds.

This chapter's goal is to present, summarize, and assess the many approaches utilized to extract bioactive and nutritional components from plants, besides their most recent applications.

Key words Extraction, Bioactive compounds, Nutraceuticals compounds, Plants

1 Introduction

Herbal items made from medicinal plants are useful sources that come from all over the world and can include a wide range of ingredients [1, 2]. Natural remedies have been used to cure both acute and chronic illnesses [3] for as long as human civilization has existed. One of the most significant sources of food and medicine for people is natural products [4]. They often contain a range of physiologically active chemicals, such as phenolic substances, vitamins, sulfur compounds, pigments, terpenoids, and other naturally occurring antioxidants [5], which are efficient in enhancing defense

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and treating cancer and cardiovascular disorders [6]. The amount of active components in natural products has a significant impact on their efficacy. The habitat, age, harvest season, drying techniques, and other factors can all affect the chemical makeup of natural products [7]. Natural products are widely used; however due to their accessibility, there are concerns about their precision, efficacy, and safety. Many products are available over the counter and are made up of a mixture labeled with various herbal ingredients [2, 8].

The choice of the best extraction technique is crucial to both quantitative and qualitative analyses of bioactive chemicals derived from plant materials [9, 10]. Despite the fact that extraction is the fundamental initial step in both quantitative and qualitative analyses of the components of medicinal plants. An extraction technique should ideally be comprehensive in terms of the components to be studied, quick, easy, affordable, and highly automated [11]. In the field of natural products, chromatographic and spectrometric methods have made major contributions, particularly in the identification, separation, and characterization of bioactive chemicals from plant sources [12]. The most frequent variables influencing extraction processes are matrix properties of the plant part, pressure, temperature, time, and solvent. The advancement of bioactive analysis over the past 10 years has been fuelled by a greater understanding of the dynamic chemical nature of the various bioactive molecules. These strategies and techniques produced a significant shift toward this "green Eldorado" between 1990 and 2000 [13]. The usage of natural chemicals from plant origin is attracting a lot of attention from numerous industrial sectors and individuals globally. The cosmetic, culinary, and pharmaceutical sectors are using natural substances derived from plants more often, and they may one day replace synthetic chemicals [14]. Different plant components, including leaves, roots, stems, fruits, seeds, and flowers, contain a variety of nutrients and bioactive substances. The identification and characterization of components and the study of bioactive substances have become simpler because of the development of advanced and sophisticated instrumentation techniques [15].

Different extraction techniques can be used to extract plant components. Over the past 50 years, novel techniques have been created that are more environmentally friendly because they utilize less synthetic and organic chemicals, operate more quickly, and provide extracts of higher yield and quality. Ultrasound, pulsed electric field [16], enzyme digestion [17], extrusion [18], microwave heating [19], ohmic heating [20], supercritical fluids [21–26], and accelerated solvents [19, 27] have all been used to increase the overall yield and selectivity of bioactive components from plant materials. have being researched as alternative techniques. Traditional extraction techniques like Soxhlet are still used as a benchmark for evaluating the efficacy of newly developed methodologies. There are several scientific publications, book chapters, and

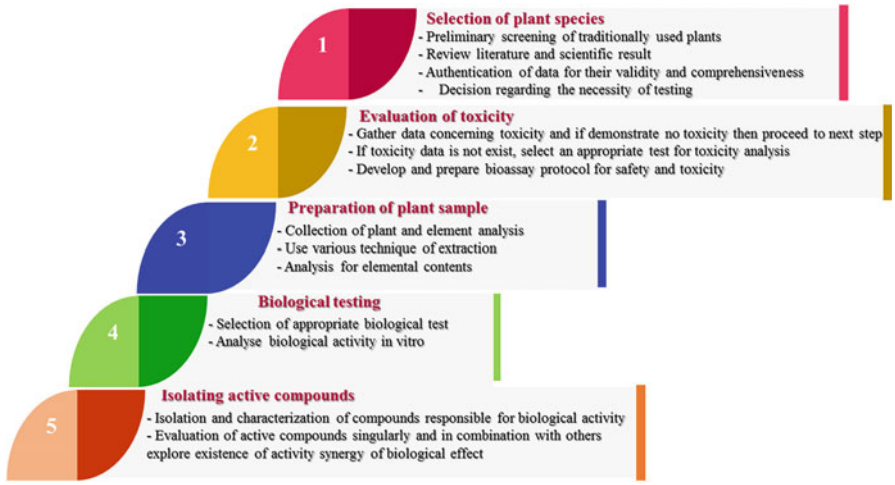

Fig. 1 Chapter summary

monograph where nontraditional methodologies have been thoroughly examined [9, 28–31]. These works emphasize the application of extraction techniques for nutraceuticals, food additives, and many other industries, but they do not discuss the extraction of bioactive chemicals from herbal plants. The goal of this chapter is to give a thorough overview of various techniques. Figure 1 summarizes the main points of the chapter.

2 Primary and Secondary Metabolites

Small molecules called metabolites are intermediary biological processes. Primary metabolites are recognized as crucial or necessary substances and have a direct role in plants' typical growth, reproduction, and development. Cell components such as carbohydrates, polysaccharides, sugars, amino acids, lipids, and proteins and fermentation byproducts such as ethanol, acetic acid, citric acid, and lactic acid are examples of primary metabolites that are primarily used by organisms during their growth and development stages [32–34].

Secondary metabolites typically have a function but are not very vital for the organism because they are not directly involved in those processes (e.g. phenolic, steroids, lignans, etc.). They express the uniqueness of species and can only be found in certain organisms or groups of organisms [10, 32, 34]. In many cases, the purpose of these molecules and how they help the organism are not fully understood, and they are not always generated under all circumstances. Plants contain many primary and secondary metabolites (Fig. 2).

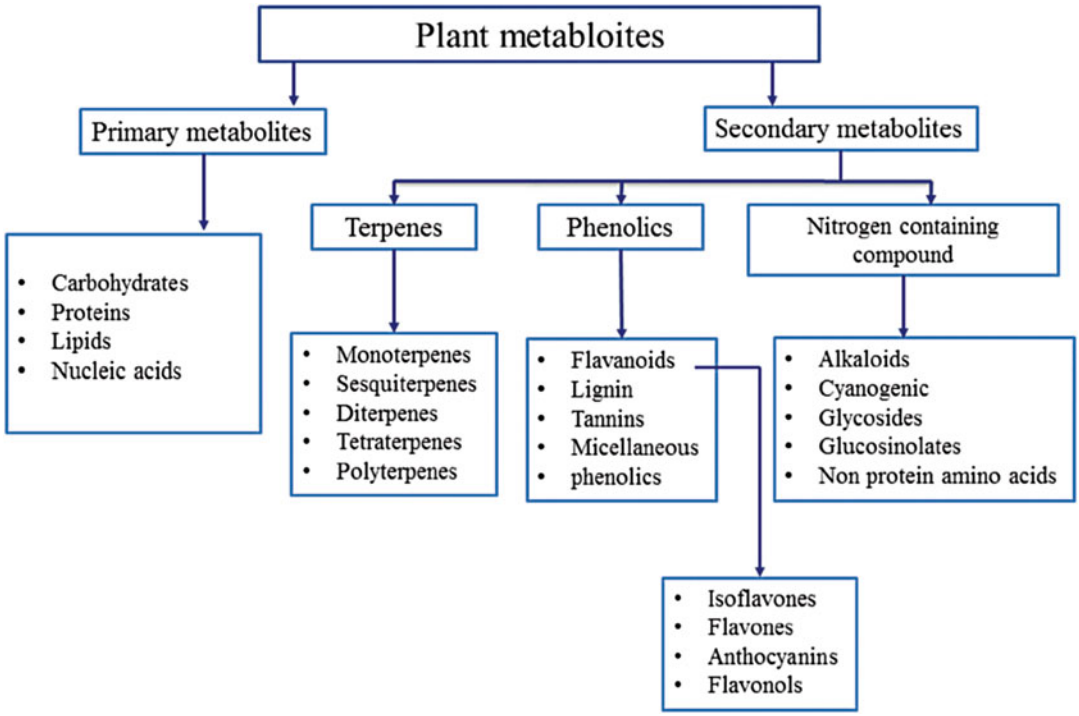

Fig. 2 Primary and secondary metabolites

3 Bioactive Compounds

A plant extract is a material or an active ingredient having useful qualities that have been extracted from the tissues of a plant, typically by subjecting it to a solvent treatment, for use in a specific application. Although not yet recognized as essential nutrients, "bioactive substances" are typically referred to as physiologically significant chemicals [35]. Essential (e.g., vitamins) and nonessential (e.g., alkaloids and polyphenols) substances that naturally occur, are fed to animals, and have the potential to have an impact on human health are known as bioactive compounds [36]. They come from a variety of natural sources, including animals, fungi, plants, and marine organisms (like lichens). Natural sources often only contain small amounts of bioactive substances [37, 38].

Plant composites typically contain plant-active chemicals. All plant organs and parts, including leaves, barks, roots, woods, tubers, gums or oleoresin exudations, figs, fruits, rhizomes, flowers, twigs, berries, as well as the entire plant, are capable of synthesizing active chemicals in minute amounts and at various concentrations. After extraction, additional steps could be needed to separate or purify the target chemicals.

4 Bioactive Compound Types "Natural Phenols"

According to their chemical makeup, bioactive compounds in plants can be classified as antioxidant vitamins, polyphenols, terpene derivatives, phytoestrogens, minerals, polyunsaturated fatty acids, dietary fiber, and phytic acid [39]. Additionally, they can be grouped according to their distribution in diverse plants (specific to vegetable species or widespread), range of concentration found in plant-based diets and the human body, possible sites of action, and efficiency against various [40]. Compounds with an aromatic ring and one or more hydroxyl groups are known as phenols. They are secondary metabolites that originate from the phenyl propanoid, shikimate, or pentose phosphate pathways in plants [41]. They are widely spread across the plant world and range in complexity from simple phenolic acids to tannins. The majority of them are now an essential component of the human diet and may be found in all plant components, including all vegetative organs, as well as in flowers, vegetables, cereals, fruits, seeds, grains, and other foods. Flavonoids make up half of these ubiquitously found phenolic bioactive chemicals [42]. Phenolics are frequently used in a variety of UV radiation protection systems or to fend off attacks from viruses, parasites, predators, etc. Additionally, it incorporates a number of additional defense-related biochemical processes that work as antioxidants, antimutagenic, and anticarcinogens, but most critically, they further alter gene expression [43]. Additionally, it affects the coloring of rare plant species. Primarily divided into various classes, phenolic substances include phenolic acids, tannins, stilbenes, lignins, and flavonoids (Fig. 3).

5 Techniques for the Extraction, Isolation, and Purification of Bioactive Compounds

Phytochemicals are chemical compounds found in plant extracts that have medicinal significance and have a particular physiological effect on humans. These plant compounds have been employed in homeopathic and ayurveda medicine to cure illnesses ever since ancient times. These are nonnutritive compounds with defensive or preventing qualities. Alkaloids, flavonoids, tannins, and phenolic chemicals make up the majority of these bioactive substances [44]. The primary raw materials used to produce new drugs are these molecules in various combinations. Numerous bioactive substances with antibacterial characteristics are found in plants to provide defense against aggressor agents, particularly microbes [10]. For individuals who work on herbal medications, the main issues and major hurdles in the separation, extraction, and

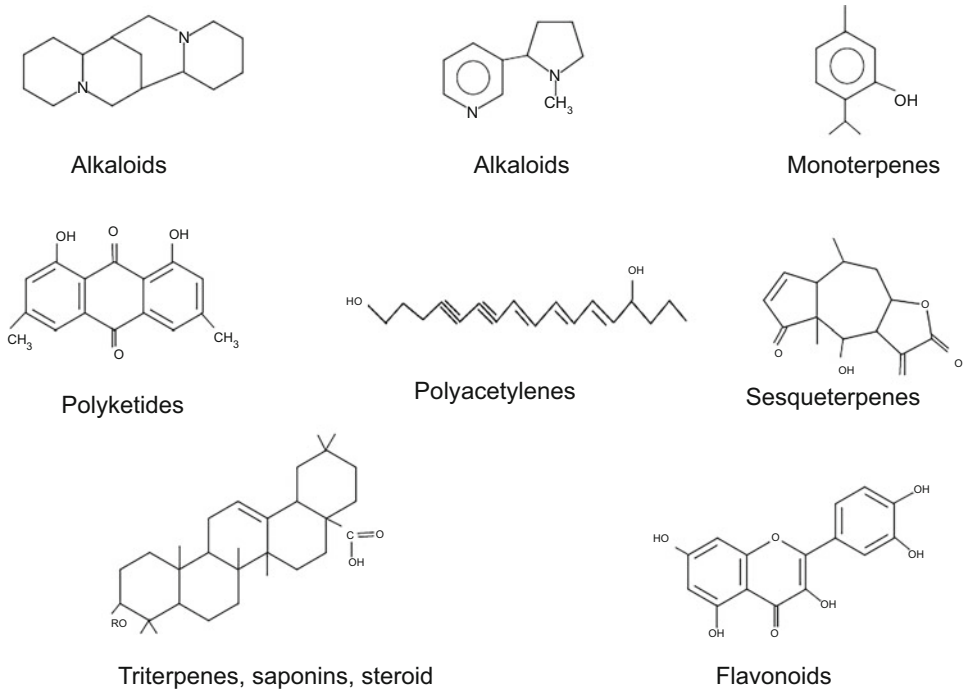

Fig. 3 Bioactive compounds

characterization of bioactive components in botanicals and herbal formulations are covered. The extraction of components from raw herbal preparations is the most crucial stage in the study of constituents; the pros and downsides of various extraction methods are covered in the following. These methods include chromatographic procedures and phytochemical screening tests (high-pressure liquid chromatography [HPLC], immunoassay, thin-layer chromatography [TLC], and Fourier transform infrared [FTIR]).

The WHO estimates that there are over 20,000 medicinal plants in 91 countries with massive biodiversity. Mexico, Peru, Ecuador, Madagascar, India, Zaire, Colombia, Brazil, Australia, Indonesia, Malaysia, and China were among the original 91 nations. Later, the United States, South Africa, the Democratic Republic of the Congo, the Philippines, and Venezuela were joined, bringing the total to 18 nations. The initial steps in employing a bioactive molecule from plant bodies include extraction, pharmacological screening, isolation and characterization of bioactive compounds, toxicological estimation, and clinical assessment.

An overview of the usual methods for extracting, separating, and characterizing bioactive compounds from plant extract is given in Fig. 4.

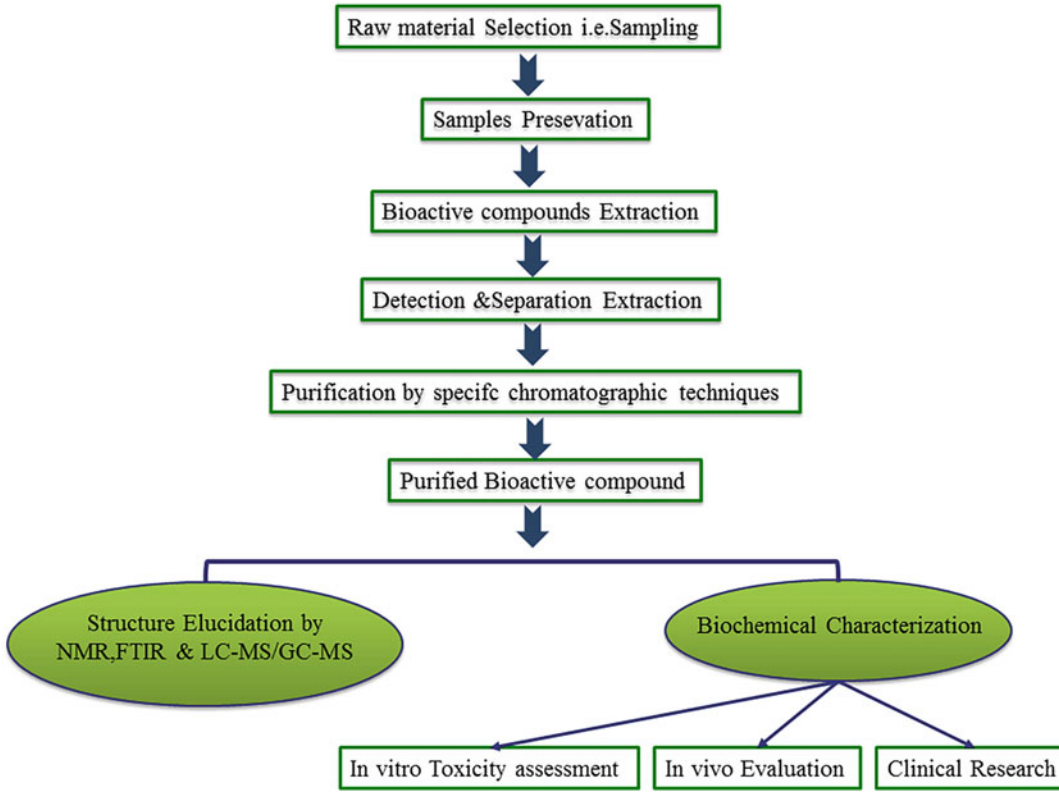

Fig. 4 Steps involved in extraction, isolation, and characterization of bioactive compounds from plant extract

5.1 Extraction Methodology

The primary step in analyzing important plant components for separation and characterization is extraction.

Prewashing, drying plant materials in the air, obtaining a homogeneous sample by grinding, and often enhancing the kinetics of extract by increasing sample contact with the solvent system are the most crucial processes. The proper steps must be followed to prevent possible active extract ingredients from being distorted or destroyed during sample preparation. If the plants are chosen based on their traditional usage [45], plant sample extracts are made according to the traditional healer's instructions in the same sequence to mimic the traditional "herbal" medication as closely as feasible. The clear-cut nature of the bioactive compounds being targeted is a major factor in the solvent system selection. For instance, the hydroalcoholic leaf extract of Aegle marmelos was found to be effective against MNU-induced toxicity, which is responsible for liver inflammation and hepatocarcinogenesis [46]. There are several extraction solvent systems available to extract the bioactive components from natural goods. Polar solvents like methanol, ethanol, or ethyl acetate are used to isolate hydrophilic chemicals, whereas dichloromethane or a 1:1 combination of dichloromethane and methanol is utilized to isolate more hydrophobic compounds.

To get rid of chlorophyll, hexane extraction might be helpful occasionally [47]. For the extraction of plant materials, a variety of techniques, including sonification, heating under reflux, and a few others, are frequently utilized [48]. Additionally, Soxhlet extraction, fresh plants, or dried, crushed plant materials can be macerated or percolated in water and/or organic solvent systems to create plant extracts. The most popular modern extraction methods include solid-phase microextraction, supercritical-fluid extraction, pressurized liquid extraction, solid-phase extraction, surfactant-mediated, and microwave-assisted extraction techniques. These methods have some advantages over chromatographic analysis, including a reduction in the amount of organic solvent used and a reduction in sample degradation.

5.2 Identification and Characterization

Modern methods allow for the concurrent creation of several complex bioassays and their accessibility, while also offering precise methods for isolation, separation, and purification [49]. The objective of searching for bioactive chemicals is to find a method that is suitable for monitoring the source material for bioactivity, such as antibacterial, cytotoxicity, or antioxidant, with simplicity, specificity, and speed (Fig. 5) [50]. Due to the expense, length of time, and potential for ethical problems associated with animal research, in vitro procedures are typically more favorable than in vivo trials. Although diverse plant sections and/or many of them will create different chemicals, in addition to their varying chemical structures and physicochemical qualities, the isolation and characterization techniques for bioactive compounds are not difficult [51]. The

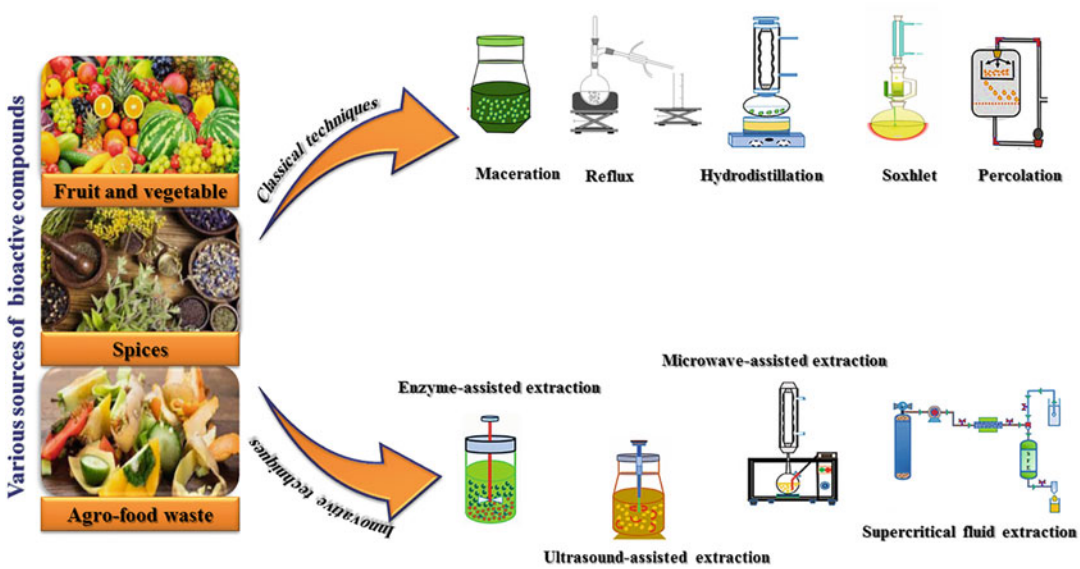

Fig. 5 Methods used for bioactive compound extraction

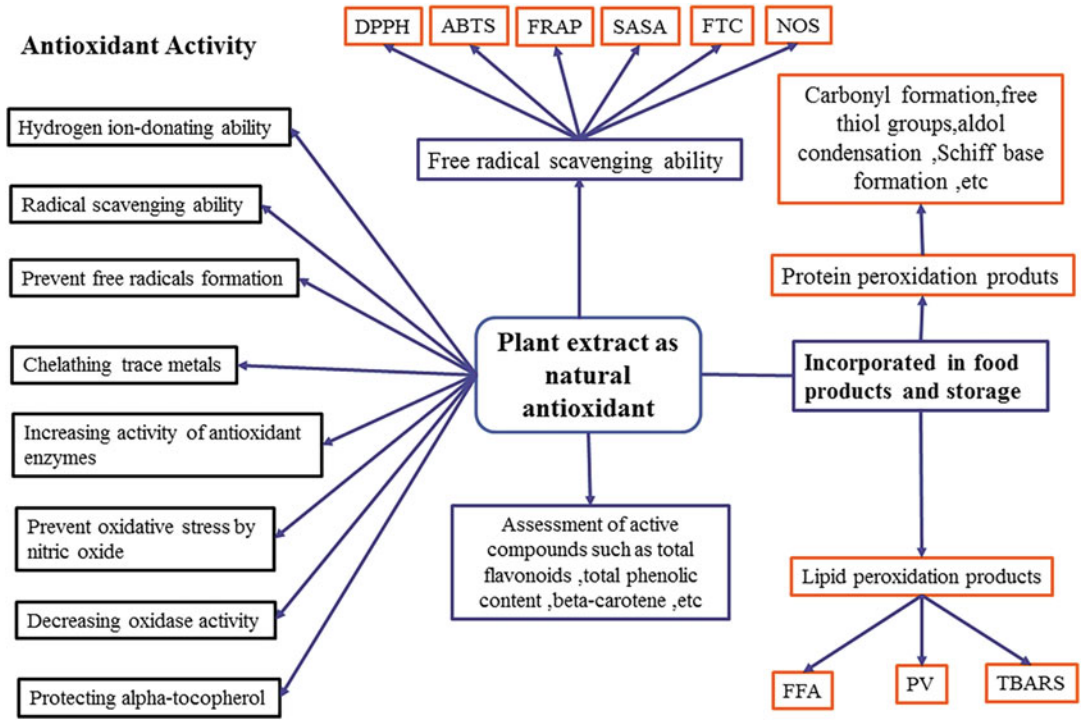

Fig. 6 Plant extract as natural antioxidant

selection and gathering of plant materials are regarded as the first steps in the isolation and characterization of bioactive phytochemicals. The subsequent step entails gathering ethnobotanical data in order to identify potential bioactive compounds.

To separate and purify the active chemicals that are responsible for the bioactivity, extracts can be created using a number of solvents. Based on their characteristics, bioactive substances can be isolated and purified using column chromatographic methods. The purification of the bioactive compounds is sped up by tools like HPLC. The purified chemicals may be identified using a variety of spectroscopic methods, including UV-visible (UV-Vis), mass spectroscopy infrared (IR), and nuclear magnetic resonance (NMR) [52–55] (Fig. 6).

5.3 Purification of the Bioactive Molecules

Paper or TLC and column chromatography have been used to identify and characterize a large number of bioactive chemicals. Due to their accessibility, affordability, and ease in a variety of stationary phases, TLC and column chromatography are still frequently utilized. The most effective methods for separating the phytochemicals may be found in silica, alumina, cellulose, and polyamide.

High concentrations of diverse phytochemicals present in plant materials make effective separation challenging. Therefore, for highly valued separations, increasing the polarity of the various mobile phases is beneficial. Column chromatography has long been used to evaluate the chosen fractions of substances using TLC. Bioactive compounds have been separated using TLC and silica gel column chromatography using a variety of analytical tools [56]. The needed bioactive chemicals' structures are explained and their identities are determined using information from a variety of spectroscopic techniques, including UV-Vis, IR, NMR, and mass spectroscopy. The fundamental principle of spectroscopy is that electromagnetic radiation is transmitted by organic molecules, some of which absorb radiation while others do not. A spectrum may be created by figuring out how much electromagnetic energy is absorbed. The bonds contained in a molecule are peculiar to certain spectra. The organic molecule's structure can be roughly identified or determined based on these spectra. For structural explanation, scientists mostly employ the IR, UV visible, radio frequency, and electron beam spectra generated from three or four areas [52].

5.3.1 UV-Visible Spectroscopy (UV-Vis)

UV-visible spectroscopy may be used for qualitative analysis and the identification of certain types of compounds in both pure and biological mixtures. Quantitative research on aromatic chemicals uses UV-visible spectroscopy because they are potent UV chromophores. Natural chemicals can be identified via UV-visible spectroscopy [56]. Anthocyanidins, tannins, polymer dyes, and phenols are phenolic chemicals that may be easily identified by UV-Vis spectroscopy [57]. It was discovered that UV-Vis methods are less discriminating and provide complicated data on total polyphenol content. The total phenolic extract (280 nm), phenolic acids (360 nm), flavones (320 nm), and anthocyanidins are all quantified using UV-Vis spectra (520 nm). Comparing this procedure to others, it is both less time- and money-consuming [57].

5.3.2 Infrared Spectroscopy (IR)

When infrared light passes through a sample of an organic molecule, some of the frequencies will be absorbed while other frequencies will pass through the sample undetected. When a molecule is exposed to infrared light, it undergoes changes in vibration that are related to infrared absorption. As a result, it is possible to think of infrared spectroscopy as a type of vibrational spectroscopy. The vibrational frequencies of various bonds (C−C, C=C, CC, C−O, C=O, O−H, and N−H) vary [56]. By identifying the distinctive frequency absorption band in the IR spectra, it is possible to determine if such bonds are present in an organic molecule [10]. A high-resolution analytical technology called Fourier Transform Infrared Spectroscopy (FTIR) is used to pinpoint the chemical components and clarify the structural compounds. Herbal extracts or powders can be quickly and nondestructively fingerprinted using FTIR.

5.3.3 Fourier Transforms Infrared Spectroscopy (FTIR)

Infrared spectroscopy using the Fourier transform is a useful technique for locating functional groups in plant extracts. It aids in molecular identification and structural determination. It is a high-resolution analytical instrument for deciphering structural compounds and identifying chemical ingredients. Herbal extracts or powders may be quickly and nondestructively fingerprinted using FTIR [58]. The spectrum of an unknown molecule can be identified by comparison with a known compound spectra for the majority of typical plant compounds. There are several ways to prepare samples for this method. The easiest method for liquid samples is to sandwich one drop of the sample between two plates of sodium chloride. Between the plates, the drop creates a thin layer. Potassium bromide (KBr) may be used to crush solid materials, and the resulting thin pellet can subsequently be examined. Another method for solid samples is to first dissolve the preparation in a solvent system, such as methylene chloride, and then transfer the resulting solution to a single salt plate. A thin coating of the original material is then formed on the plate once the solvent has evaporated. FTIR is a high-resolution analytical method used to reveal the structure of compounds and identify the chemical constituents. Herbal extracts or powders can be quickly and nondestructively analyzed using FTIR [59].

5.3.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

The magnetic properties of atomic nuclei, notably those of the hydrogen atom, proton, carbon, and an isotope of carbon, are the primary focus of NMR. By comparing the differences between a variety of magnetic nuclei, which gives a clear image of where these nuclei are positioned in the molecule, NMR spectroscopy has enabled numerous researchers to investigate molecules. It also shows which atoms are present in the clusters that are close by. Finally, it can be argued that a lot of atoms are present in each of these settings [57, 60].

5.3.5 Identification of Chemical Compounds Using Mass Spectrometry

In mass spectrometry (MS), molecules are attacked with a mixture of electrons or lasers before being changed into charged ions, which have a high energy. A mass spectrum is a graph that shows how many fragmented ions there are in relation to their mass/charge ratio. By using MS, using information about the areas where the molecule has been split apart, it is feasible to calculate the relative molecular mass (molecular weight) with high precision and construct a precise molecular formula [61]. The most significant and popular advanced technology is tandem mass spectrometry (MS/MS). In addition to increasing selectivity, MS/MS offers a plethora of structural data, enabling the identification and measurement of even co-eluting molecules [62]. For the first time, the phenolic compounds quercetin, crysin, quinic acid, chlorogenic acid, and kaempferol were successfully screened and identified in a

hydromethanolic extract of A. aspera by UPLCPDA (ultra-performance liquid chromatography ephotodiode array) and MALDI-TOF-MS (matrix-assisted laser desorption/ionizationetime-of-flight mass spectrometry) [63]. Nowadays, MALDI-LC/MS is often utilized for phenolic compound chemical analysis. However, because of its high ionization efficacy, particularly for phenolic compounds, the electrospray ionization (ESI) approach is a favored technique.

5.3.6 Nonchromatographic Techniques

Research on receptor binding analysis, enzyme assay, and quantitative and/or qualitative analytical procedures in animals or plants has made monoclonal antibody (MAb) against drugs and tiny molecular weight bioactive chemicals an indispensable tool. The immunoblotting approach is based on the western blotting method, which makes use of the antigen-antibody binding capabilities to identify larger molecule analytes like peptides and proteins in a precise and sensitive manner. ELISA, a highly sensitive, precise, and easy-to-use technology, was created for individual competitive testing [64]. Since their introduction, monoclonal antibodies (MAbs), which have a wide range of applications, have grown in significance as a tool in contemporary bioscience research. Recently, several researchers have concentrated on the creation of MAbs against the secondary metabolites, or natural compounds, obtained from medicinal plants [65].

The procedures used to create monoclonal antibodies using hybridoma technology against plant-based medications are as follows:

(i) Adequate purification and characterization of the required antigen.

(ii) Giving mice an immunity boost using the purified antigen.

(iii) The cultivation of myeloma cells that are unable to produce the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) enzyme required for the nucleic acid salvage pathway.

(iv) The removal of mouse spleen cells and their fusion with myeloma cells.

(v) The hybridomas were raised in hypoxanthine aminopterin thymidine (HAT) medium after fusion [66].

5.3.7 Phytochemical Screening Assay

Phytochemicals are a diverse group of bioactive substances (secondary metabolic substances) derived from plants that have a strong potential to improve human health [67].

Three fundamental stages are needed to undertake a natural product analysis: an effective means to separate the different phytochemicals in the extracts, a way to identify and quantify the

phytochemicals, and a mechanism to extract the phytochemicals of interest from the raw plant material. Pure standards are necessary for this.

In only a few minutes, analytical tools are now available that can quickly isolate, clearly identify, and reliably quantify phytochemicals from plant materials [68].

6 Bioactive Compounds: Their Role in the Prevention and Treatment of Diseases

Many bioactive substances seem to have positive impacts on health. Before we can start making dietary recommendations that are supported by science, there is still a lot of scientific study to be done. Despite this, there is enough data to suggest ingesting foods that are high in bioactive substances. This translates into advocating a diet high in a range of vegetables, fruits, oils, nuts, legumes, whole and grains from a practical standpoint [69].

6.1 Use of Natural Bioactive Compounds in the Food and Pharmaceutical Industries

Bioactive food components are essential in both the prevention and treatment of diseases since various chemicals are involved in the pathophysiology of many disease processes. Several bioactive chemicals actively control the inflammatory process, which is the underlying cause of diabetes, cancer, and other inflammatory diseases. Dietary practices, food components, and bioactive substances with anti-inflammatory characteristics have all been found to be protective. Utilizing bioactive food substances with antioxidant and anti-inflammatory characteristics that are found in spices and herbs may thus help avoid inflammation that can lead to carcinogenesis or cardiovascular disorders [70]. For instance, the two most successful nonpharmaceutical therapies for inflammatory bowel disease are dietary changes and functional diets (IBD). IBD can be treated with probiotics and nonstarchy polysaccharide dietary supplements. Omega-3 fatty acids, vitamins, phytochemicals, and plant extracts are a few examples of bioactive compounds. These dietary peptides and functional foods have potent anti-inflammatory effects in both human and animal studies [71]. In order to reduce inflammation, functional foods can alter inflammatory cytokines and work with the immune system. The manufacture of nutraceuticals for inflammatory-related disorders is made possible by the anti-oxidative and anti-inflammatory action of cotoneaster's polyphenolic components [72]. Angiotensin-converting enzyme (ACE) inhibitors, which are bioactive components of Coriandrum sativum, are thought to have anti-hypertensive effects [73].

Plant-derived phytochemicals offer a promising new route for the creation of diabetic mellitus therapies. The more significant alkaloids include flavonoids, glycosides, terpenoids, and steroids [74]. Numerous phytochemicals with possible antidiabetic

properties may be found in many fruits, vegetables, oils, legumes, and nuts. Aloe vera, mango, banana, avocado, coffee, blueberries, bitter gourd, cinnamon, black tea, ginger, garlic, guava, grape, pomegranate, pumpkin, olive oil, jackfruit, papaya, onion, and others are some of them [75]. Dietary flavonoids regulate insulin sensitivity, beta-cell function, glucose metabolism, and the functional availability of antioxidants [76].

The South Indian fruit crop known as jackfruit (Artocarpus heterophyllus Lam) is prized for its therapeutic qualities. It includes a variety of antioxidants that aid in the prevention of numerous chronic illnesses, including diabetes and heart disease. The glycemic index (GI) of jackfruit is said to be quite low, limiting a sharp rise in blood sugar levels [77]. It also has plenty of vitamin C, carotenoids, and flavones, all of which contribute to its anti-inflammatory properties and lower the risk of chronic heart disease, cancer, hypertension, and type 2 diabetes [78, 79]. Antioxidant, antiulcer, antihypertensive, anticancer, and anti-aging activities may be found in jack fruit phytonutrients [80, 81]. Banana inflorescence (Musa paradisiaca) supplementation has also been shown to reduce inflammation, hyperglycemia, and oxidative stress in diabetic rats produced by streptozotocin. Contrarily, the bioflavonoid morin exerts its antidiabetic effects via a mechanism that mimics the actions of insulin [82].

Dietary phytochemicals have also been shown to include a number of anticancer substances. Yang et al. examined the structural characteristics and anticancer activity [83] of water-soluble polysaccharides from Kaempferia galanga (aromatic ginger). By consuming a polyphenol-rich herbal congee with a combined extract of Morus alba and *Polygonum odoratum* leaves, menopausal women's bone mineral density was enhanced, according to Wattanathorn et al. [84].

There are also claims that certain bioactive substances have neuroprotective effects. Several conditions that can affect the brain include Parkinson's disease (PD), Prion disease, experimental autoimmune encephalomyelitis (EAE), Alzheimer's disease (AD), multiple sclerosis (MS), ischemic stroke, and neuropathic pain. Numerous studies have demonstrated that altering lifestyle variables, such as adopting a suitable diet, might postpone or prevent the onset of Alzheimer's disease, an age-related form of dementia. Promising substances include phenolic compounds, fat-soluble vitamins, isothiocyanates, omega-3 fatty acids, and carotenoids. These bioactive substances have antioxidant and anti-inflammatory properties, and they actively contribute to the formation of tau tangles and amyloid plaques [85]. A member of the Caryocaraceae family commonly referred to as "pequi," *Caryocar Brasiliense* (Camb), is a potential neuroprotective phytomedicine with antioxidant and anti-cholinesterase properties as well as neuroprotective benefits [86].

Bioactive substances such as omega-3 fatty acids, plant sterol esters, and phenolic compounds may lower the risk of atherosclerosis and cardiovascular diseases by decreasing inflammation, LDL cholesterol levels, and oxidative stress [87]. Therefore, nutrition and functional foods will be key in treating and preventing illnesses.

6.2 Use of Bio-Based Compounds as Food Additives

The Product and Drug Administration (FDA) defines "natural" as a food that "does not include anything artificial or synthetic, including additives," despite the fact that the phrase has no legal definition. Due to studies indicating negative impacts of the usage of synthetic ingredients, there has been an increase in research and demand for natural foods during the past few years. Additionally, the phrase "natural" adds value to the product because it is now fashionable to consume goods made entirely of natural substances [88, 89].

Because they include chemicals that are good for health, plants, fruits, and spices are well known. The biologically active compounds found in plants that are used as food additives can be unofficially categorized as antioxidants, antimicrobials, flavorings, colorants, and others. More research has been done as a result of increased public awareness of the benefits of ingesting natural goods, leading to potential sources of natural additives [90].

There are a ton of raw materials with a high concentration of bioactive compounds in the by-products and biowaste from the food sector. For instance, orange peels may be used as flavorings, sweeteners, and antioxidants since they include essential oils, cellulose, pectin, hemicellulose, and soluble sugars (galactose, sucrose, fructose, and glucose) [91].

The utilization of vegetable byproducts and bio-residues is an alternative to synthetic additives since consumers are more interested in additives derived from natural sources and have a sustainable mentality [92].

6.3 Neuroprotective Effects of Biological Activity and Toxicity of Plant Nutraceuticals

A host defensive process known as neuroinflammation is linked to the neutralization of an injury and the restoration of the brain's normal structure and function. All significant CNS illnesses have the characteristic of neuroinflammation [93].

In the majority of neurological disorders, including Prion disease, Alzheimer's disease (AD), multiple sclerosis (MS), Parkinson's disease (PD), experimental autoimmune encephalomyelitis (EAE), neuropathic pain, and ischemic stroke, neuroinflammation is the primary mediator of secondary brain damage. Both aging-dependent circumstances and aging-independent pathogenic events can cause neuroinflammation because they both involve related inflammatory cascades [69].

It makes sense to explore natural treatments for transient cerebral ischemia-reperfusion injury (TCI-RI), as well as to research their mechanisms of action. AFF, an extract of the total flavonoids

from A. esculentus flowers, was studied by Y. Luo et al. [94] for its potential protective effects on TCI-RI. The researchers demonstrated that AFF had protective effects against TCI-RI by scavenging free radicals and indirectly boosting the neuronal Nrf2-ARE pathway to reduce oxidative stress damage.

Alzheimer's disease (AD) is a neurodegenerative illness of the central nervous system that gradually impairs cognition and memory. The disease's molecular characteristics include extracellular amyloid peptide (A) deposition in senile plaques, the emergence of intracellular neurofibrillary tangles (NFT), cholinergic deficit, significant neuronal loss, and synaptic alterations in the cerebral cortex, hippocampus, and other brain regions crucial for cognitive and memory functions. A deposition kills neurons by a number of different possible mechanisms, including oxidative stress, excitotoxicity, energy depletion, inflammation, and apoptosis [95].

A member of the Caryocaraceae family known as "pequi," Caryocar brasiliense (Camb), is one of the promising neuroprotective phytomedicines. In their article, "Neuroprotective Effect of Caryocar brasiliense Camb.," T. S. de Oliveira et al. [86] investigated the antioxidant and anticholinesterase activities as well as the neuroprotective effects of C. brasiliense leaf extracts to provide new information on the potential use of this plant against neurodegenerative disorders.

Memory loss that worsens over time, along with other cognitive impairments, are common AD symptoms. The amyloid hypothesis states that synaptic malfunction and consequent neurodegeneration in AD are primarily caused by amyloid- (A-) associated toxicity and imbalance. A has been proposed as a possible therapeutic target for the treatment of AD as a result. Procyanidins extracted from Lotus seedpod ameliorate amyloid—induced toxicity in rat Pheochromocytoma cells [96] study by H. Huang et al. confirms the anti-A activities and protective mechanisms as a potential natural product for AD therapy. The authors assessed the LSPC's ability to mitigate the harm caused by A-25-35 to rat pheochromocytoma (PC12) cells.

In the case of infection, inflammation, trauma, ischemia, and neurodegeneration in the central nervous system (CNS), microglia cells act as scavenger cells and play a crucial function as resident immunocompetent and phagocytic cells [97]. While prolonged activation of microglia and astrocytes causes neuroinflammation, which can start or accelerate dementia, it is a brain defense system to combat dangerous infections and damaged tissues [98].

Numerous herbal plants and their active ingredients have surfaced in recent years and have been the focus of in-depth study. When opposed to contemporary trendy supplements, these medicines have been time-tested and verified by traditional usage. Recent research has shown that traditional herbal remedies with reliable ethnopharmacological qualities have neurotrophic and

neuroprotective effects. These features can be helpful in avoiding different types of neuronal cell death in neurodegenerative and neuroinflammatory illnesses. Over the last 20 years, a number of natural compounds have been investigated for their potential to reduce neuroinflammation, treat neurodegenerative disorders, or have positive effects on the central nervous system. Many plants have been found to contain anti-inflammatory and antioxidant properties, which may shield the brain from the harm caused by inflammation. The evidence for the neuroprotective effects of traditional herbal extracts, particularly their anti-inflammatory properties, is growing [99–104]. Natural substances that are especially designed to prevent microglial activation may be more effective in treating neurodegenerative and neuroinflammatory illnesses that are linked to microglia. In the parts that follow, we'll concentrate on well-known and significant natural products, their active ingredients, and their anti-inflammatory properties based on suppressing microglial activation.

7 Future Perspectives

Due to the fact that there are still 500,000 plants in the world that need to be found, examined, and investigated by the scientific community for their potential therapeutic capabilities to treat a variety of ailments, bioactive chemicals and herbal medications have a bright future. Worldwide demand for items made from plants has expanded, yet traditional indigenous medical methods are still in their infancy. For the treatment and healing of a wide range of ailments, herbal compositions and diverse formulations have been used for many generations with careful selection and application [105]. For their medical requirements, almost 85% of the population in Asia, Africa, Latin America, and the Middle East largely trust traditional herbal remedies. Skin conditions, jaundice, cancer, TB, hypertension, diabetes, and many other infectious disorders are successfully treated using bioactive chemicals and their analogs at both the chronic and acute levels [106]. These bioactive compounds do, however, have some disadvantages, including changes in composition with climate, the concurrent occurrence of synergistic effects of compounds, their mode of administration, and stability in active form, which may have unfavorable or unexpectedly positive effects on bioactivity. These problems can be resolved by utilizing state-of-the-art techniques for the isolation of pure bioactive compounds, their synthesis as herbal nanoparticles, and convenient examination of their therapeutic effects in addition to toxicity analysis prior to medication after keeping the extract for longer periods of time [107]. A. aspera leaf extract was characterized using MALDI-TOF-MS, which reveals the presence of chlorogenic acid (CGA). The extract was also utilized to create herbal gold nanoparticles, and fresh splenocyte cell culture was

employed to test the extract's toxicity [108, 109]. The anticancerous effects of CGA were later observed separately in vitro, in vivo, and in silico [110]. liquid chromatography mass spectrometry (LC/MS), the development of HPLC/MS, magnetic field, and nuclear magnetic resonance (NMR) has thus significantly lessened the problem of identifying the structures of these compounds from extracts, despite the fact that active compounds are present in plants in very small concentrations [111].

Herbal remedies are becoming more and more popular because of their inherent safety, low cost, and accessibility. However, when compared to traditional allopathic medications, concerns are raised about their pharmacognosy, clinical validation, and standardization. Despite these concerns, attempts to scientifically fix them have increased over the past 25 years in both wealthy and developing nations. Additionally, this prompts us to consider the necessity of further research in creating herbal medicines as cutting-edge therapeutic agents.

8 Conclusions

The world's health systems' greatest sources of therapeutic pharmaceuticals and currently used natural remedies will be bioactive chemicals produced from medicinal plants. These natural remedies may be the most significant and necessary source of medications for both illness treatment and health maintenance. Although these traditional remedies have just recently gained attention, they have been mentioned in ancient literature for thousands of years, and local people from many civilizations have extensive knowledge and firsthand experience with them. However, in-depth scientific investigation and the identification of bioactive chemicals are required to develop the subsequent generation of medicines based on natural formulations. Prioritizing specific aspects, such as the assessment of the quality of raw extracts and their combinations, will enable future therapies to compete with existing ones. New and improved methods for their purification, efficient animal research, and palatable clinical studies are also required for the justified use of these medicinal plant extracts with safety and efficacy.

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Chapter 3

Extraction of Bioactive and Nutraceuticals from Marine Sources and Their Application

Nikheel Rathod, Vijay Reddy, Martina Čagalj, Vida Šimat, Merina Dahal, Nilesh Prakash Nirmal, and Siddhnath Kumar

Abstract

The marine resource hosts wide biodiversity, regarded as a rich source of diverse bioactive compounds, which are widely investigated for their bioactive composition and nutraceutical applications having several health benefits. Several in vitro and in vivo studies have reported the impacts of marine bioactive compounds in controlling several lifestyle disorders. Based on their high bioactive compounds and nutraceutical value, their extraction techniques are known to influence their bioactivity widely. The present chapter summarizes the knowledge on novel extraction technologies of bioactive compounds from marine sources; further, based on their bioactivity and nutraceutical property, their ability to modulate development of several disorders is addressed. Besides, the chapter covers food application of derived bioactive compounds to develop nutraceutical foods.

Key words Bioactive, Nutraceuticals, Extraction, Functional food

1 Introduction

Oceans are habitat to the most diverse plants and creatures on the planet. Marine organisms (planktons, seaweeds, microalgae, microbes, invertebrates, fish, and so on) have been discovered to be a potential source of numerous bioactive and nutraceutical compounds [1, 2]. The compounds exhibit a wide range of functional characteristics, including antioxidant, anti-inflammatory, anti-microbial, anti-hypertensive, anti-cancer, neuroprotective, and so on. In an animal model, consumption of seaweed potentially ameliorates conditions associated with chronic diseases such as cardiovascular diseases, hyperlipidaemia, diabetes, obesity, and hypertension [3, 4] through modulation of various signaling pathways. The major marine-sourced phytochemicals with health-protective effects are phenolics, flavonoids, terpenoids, phytosterols, and alkaloids [5]. In addition to phytochemicals,

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carbohydrate-based bioactive compounds from marine sources such as laminarin, alginic acid, fucoidan, carrageenan, sulfated polymannuronate, heparin, dermatan sulfate, fucosylated chondroitin sulfate, glycolipids, and glycoproteins possess potent health benefits [2]. The peptides from marine animals, on the other hand, have shown promising pharmaceutical and healing properties. Despite the fact that marine sources are a rich source of bioactive and nutraceutical compounds with expanded functional characteristics, extracting these bioactive compounds with preserved bioactivity remains a challenge.

Extraction is an operation where plant or animal tissues are treated through a specific procedure to obtain targeted compounds. For the separation, characterization, and significant health applications of the bioactive and functional compounds, their optimal extraction with excellent quality is most important. However, no universal process has yet been developed for ideal extraction. Maceration, Soxhlet extraction, and hydrodistillation are some conventional extractions that are still in practice for extraction of phytochemicals from marine weeds. These methods do, however, have certain limitations. For example, conventional extraction approach has a prolonged extraction time, high operating cost, and poor compound selectivity. In addition to this, these processes have high solvent consumption, low solvent recovery, and eventually different environmental hazards. Moreover, the heat-sensitive bioactive compounds are thermally decomposed by high temperatures during processing. Researchers and investigators are exploring the best possible methods for optimal quantification of the bioactive compounds [6]. The selection of the extraction process and medium of extraction, along with other factors, is usually based on the targeted compound, so one should consider the possible influencing factors during extraction. Thus, considering the drawbacks of conventional methods and limiting factors, a lot of novel procedures have been explored and applied to the extraction of bioactive compounds from marine resources. Ultrasonication, microwave-assisted extraction (MAE), pulse electric field (PEF), eutectic solvent-based extraction, and sub- and supercritical fluid-based extraction are some of the major green extraction technologies developed in the last half-century [7]. These procedures are claimed to be more environmentally friendly since fewer solvents and other chemical substances are utilized during these processes. Furthermore, these methods can significantly improve extraction yields with better quality extracts, and the operational time is noticeably less than conventional methods. Through this chapter, we have attempted to provide insight into the current trend of novel technology applications in the extraction of bioactive compounds from marine sources. Also, the chapter has articulated the bioactivity and nutraceutical properties of compounds from marine-based sources, along with their commercial applications.

2 Novel Extraction Technologies

2.1 Ultrasound

Ultrasound-assisted extraction employs application of high power and low frequency (20 kHz to 100 MHz and 10 to 1000 W/cm^2) ultrasound waves that generate acoustic cavitation (production of bubbles), which in turn expands and compress in size and finally collapse due to the cavitation cycle (compression and expansion) affecting the cellular structure responsible for improved extraction of target compounds [8, 9]. The collapse of bubbles generates extreme local conditions (high pressure, temperature), causing destruction of cell walls; reducing particle size, resulting in higher contact between solvent and medium; and causing improved extraction of target compounds by diffusion from the cell. Ultrasound results in increased extraction of target compounds, lower solvent requirements, shorter time requirements, extraction of heat-labile compounds, and lesser energy requirement, which make it an environment-friendly technique.

Ultrasound extraction is influenced by several parameters, such as application of power and frequency, extraction time and temperature, viscosity, solubility and stability of sample, and optimized solvent-to-substrate ratio [8–11]. Several recent studies have focused on the ability of ultrasound to extract bioactive and nutraceutical compounds from marine sources [12–16].

A recent study by [15] optimized the ultrasonic-based extraction of astaxanthin from shrimp shell. Application of ultrasound yielded significantly higher quantity of pigment. The astaxanthin yield increased based on polarity of solvent (petroleum ether/acetone/water: 15:75:10) used and extraction time for 6 h achieved highest yield. Based on optimized conditions, extraction using 23.6% ultrasound amplitude for 13.9 min resulted in the highest astaxanthin yield (51.5%), exhibiting the highest antioxidant (1705 μmol of Fe^{2+}/g and 73.9% of radical scavenging) activity. Similarly, [17] reported the impacts of ultrasound-assisted extraction on qualities of oil from fish (*Labeo rohita*) head. The oil extracted by using ultrasound had higher proportion of unsaturated fatty acids and exhibited high thermal and oxidative stability. The higher extraction was obtained using ultrasound treatment due to disintegration of cell structures releasing lipid.

2.2 PEF

Pulsed electric field is based on the principle of electroporation of cell membranes enhancing mass transfer. However, for electroporation (permanent), the electric field applied should be over the threshold value (membrane potential) [8, 18–20]. Under these conditions, the electric field compromises the membrane permeability responsible for extraction of compounds. The nature of electroporation varies based on strength of the field, exposure time, pulse number, pulse frequency, properties of cell, and

treatment medium [21]. Application of PEF helps in extraction of bioactive compounds without inactivation [22]. The low temperature or no external heating requires less solvent operation, which gives it advantages for the recovery of bioactive compounds.

For valorizing fish discards from sea bream and sea bass (gills, bones, and heads), pulsed electric field with water as medium was evaluated by Franco et al. [23]. Discards were found to be rich sources of minerals and amino acids. Furthermore, application of PEF resulted in breaking the membrane and releasing the contents, which increased the antioxidant activity. Similarly, valorizing fish bones were evaluated for extraction of nutraceutical ingredients [24]. Electric filed application at 15 kV/cm resulted in the highest recovery of calcium (10.140 mg/mL), chondroitin sulfate (5.899 mg/mL), and collagen (0.156 mg/mL). Further, the optimization study suggested PEF application at 22.79 kV/cm at pulse number 9 and liquid solid ratio of 11 resulted in higher yield of calcium, chondroitin sulfate, and collagen as 19.8, 39.268, and 3.875 mg/mL, respectively. The higher extraction yield was related to the PEF ability to rupture cell wall.

PEF for extraction of lipids from shrimp cephalothorax was reported by Gulzar and Benjakul [25]. Higher level of cellular disintegration by applying increase pulses was reported, which directly resulted in increase of lipid extraction. Lipid extracted by PEF followed by solvent extraction exhibited higher stability (peroxide value, TBARS, and free fatty acid content). Application of PEF was found to exhibit positive impacts on lipids extracted form cephalothorax containing higher unsaturated fatty acids and pigments in monoester and diester forms.

High-intensity PEF (10–35 kV/cm) application for extraction of protein from mussels was evaluated [26]. Optimized operating conditions (20 kV/cm, pulse number of 8.12, and enzymolysis for 2 h) resulted in the highest protein yield of 77.08%. Hence, PEF could be successfully used for speed and cleaner recovery of proteins from mussels.

2.3 MAE

Microwave-assisted extraction works on the principle of fast heating by electromagnetic radiations (300 MHz to 300 GHz; 915 and 2450 MHz) at high power for a short duration. The exposure to microwaves increases the heat penetration in the matrix, increasing temperature and pressure and resulting in improved mass transfer by penetration of solvent in the matrix. The application of direct heating results in rapid heating, lowering the time and solvent required for extraction. Several factors such as microwave power, frequency, solvent-to-matrix ratio, and exposure time affect the extraction efficiency. Also, the characteristics (dielectric constant) of the solvent used are regarded as important factors determining the solvating capacity.

Microwave-assisted (600 W, 70 °C for 10 min) extraction of lipids from fish (*Upeneus moluccensis* and *Saurida undosquamis*) using ethanol solvent was reported by Ozogul and team [27]. Application of microwave yielded highest lipids in comparison to other methods (bligh and dryer, Soxhlet extraction, and ultrasound). Lipids extracted from *Saurida undosquamis* contained higher proportion of unsaturated fatty acids, suggesting the method of extraction (microwave-assisted extraction) had impacts on the fatty acid profile of fish and lipid quality. Similarly, Afolabi and others [28] reported microwave-assisted extraction (800 W) yielded high quantity (16.13% w/w) and good quality lipid (free fatty acid—1.35% and acid value- 2.69 mg KOH/g) using ethanol solvent. The extracted lipid was rich in bioactive lipid constituents (MUFA-11.11%, PUFA-55.56%, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid). Additionally, the FTIR spectra predicted the presence of groups primarily responsible for antimicrobial activities. Recently, [29] extracted lipids from salmon processing waste using microwave-assisted extraction and compared with results obtained from Soxhlet extraction. Microwave extraction helped in the extraction of 69% of lipids from heads (<11 min at 50 W using 80 g/L) and backbones (<15 min at 300 W using 80.1 g/L), while 92% recovery was obtained from viscera (<15 min at 960.6 W using 99.5 g/L) using *n*-hexane solvent. The lipid extracted using microwave technology was superior in terms of fatty acid profile and bioactivity in comparison to Soxhlet extraction.

The ability of the microwave field to disrupt the cell wall was demonstrated by Magnusson and others [30] for improving extraction of polyphenol (phloroglucinol) from brown seaweed in comparison to solid-liquid extraction. Results suggested water was the best solvent for extraction of polyphenols, the biomass-to-water ratio increased the extraction, and lower temperature conditions exhibited higher phenolic extraction. Furthermore, the conditions were optimized to increase (70%) the yield of bioactive phenolic compounds using 1:30 water-to-solvent ratio, at 160 °C for 3 min.

2.4 Supercritical CO_2 Extraction

It uses solvents at supercritical temperature and pressure conditions, exhibiting properties of between a liquid and a gas, helping in improved extraction of bioactive compounds [31]. Considering the low critical conditions and higher diffusivity of carbon dioxide (30 °C and 7.38 MPa), it is used for recovery of target compounds. The proper relation between the pressure and temperature can be so managed to ensure extraction of heat-labile compounds [32]. The major advantage of using this technique is reduced usage of toxic organic solvents. The low polarity of carbon dioxide limits the extraction of nonpolar compounds. Hence, compounds are used to modify the polarity ensuring improved salvation capacity.

Considering the high bioactivity possessed by lipids from marine origin, several novel techniques for extracting marine lipids have gained importance [33, 34]. Atlantic salmon, widely consumed globally due to their health benefits, while processing discards, accounts a large proportion of waste; hence, [35] evaluated supercritical carbon dioxide method for extraction of phospholipids from frame bone. This method employed ethanol as co-solvent at 45 °C and 27.5 Mpa; the conditions yielded 6.9% lipids of 80% purity and 5.6% of phospholipids. The extracted lipids were rich in bioactive astaxanthin (27.6 μg/g), unsaturated fatty acids, and phospholipids compared to extraction with organic solvents (ethanol). Furthermore, the lipids extracted at lower pressures exhibited higher stability over extraction at higher pressures. Furthermore, the extracted lipids exhibited high antioxidant activity, which increased in dose dependent manner. Similarly, Mexxomo and others [36] evaluated the hypolipidemic effects of extracts derived from pink shrimp processing residue using supercritical fluid extraction method. Significant increase (more than 3 times) in PUFA contents of fatty acid profile of residue extracts from pink shrimp extract by supercritical carbon dioxide extraction (30 MPa using CO_2) was observed. Based on bioactive composition, the extracts exhibited hypolipidemic (reducing cholesterol and triglyceride content) and anti-obesity effects (weight reduction). The abilities were attributed to synergistic effects due to the presence of phenolic, pigments, and fatty acid contents.

Similarly, phospholipids extracted from the viscera of Pacific saury by supercritical carbon dioxide method employing ethanol solvent were evaluated for their neuroprotective activity [37]. PUFA was regarded as the main bioactive constituent present in lipid fractions derived. The extract exhibited inhibition of amyloid beat $(A\beta)_{1-42}$ by 69% responsible for neurodegenerative disorders. Authors suggested the bioactivity possessed by omega-3 fatty acids plays a vital role in imparting neuroprotective capacity to lipids from marine origin.

3 Bioactivities and Nutraceutical Application of Bioactive Compounds from Marine Sources

3.1 Bioactivity

3.1.1 Antioxidant Property

Antioxidants are used to lessen oxidative damage to the human body and to extend the shelf life of lipid-containing foods and maintain their nutritious content. They also work to prevent damage caused by free radicals. In contrast to naturally occurring antioxidants such tocopherol, ascorbate, and carotenoids, synthetic antioxidants like butylated hydroxy anisole (BHA), tertiary-butylhydroquinone (TBHQ), and butylated hydroxytoluene (BHT) are also available. Natural antioxidants from marine sources, such as protein hydrolysates, peptides, and amino acids, are increasingly

being investigated due to worries about the potential for carcinogenic effects. Table 1 represents the bioactive compounds isolated from different marine sources and their bioactivities. Generally speaking, a peptide's ability to stabilize free radicals depends on its power to provide or take electrons away from the radical in order to reduce its reactivity. The hydrophobicity, chain length of peptides, size (0.5–3 kDa), and amino acid composition and sequence (hydrophobic and aromatic) have shown advantages over proteins in terms of antioxidative activity. When compared to other natural antioxidants like tocopherol, high antioxidant peptides isolated from the visceral organs of horse mackerel utilizing gastrointestinal digestion demonstrated superior antioxidative effect [38].

By-catch byproducts of crabs and shrimp can be inexpensive sources of natural antioxidant raw materials [40]. Antioxidant peptides of marine origin act in the scavenging of free radicals or in preventing oxidative damage by interrupting the radical chain reaction of lipid peroxidation, which compromises the cell's viability [59]. As a functional dietary ingredient or supplement, cephalothorax hydrolysates of Pacific white shrimp were found to have antioxidant effects [39]. Shrimp hydrolysates from *Acetes chinensis* demonstrated antioxidant peptides [40]. The crayfish *Procambarusclarkii* hydrolysates showed significant antioxidant activity [41]. Seaweeds contain significant amounts of polyphenols or phenolic chemicals, especially those with strong antioxidants. Phlorotannins, a class of polyphenols, have drawn a lot of attention as functional food additives. Phlorotannins are tannin compounds created when phloroglucinol (1,3,5-trihydroxybenzene) units are polymerized through the acetate-malonate route. They are primarily found in brown algae [42] and are secreted in the cell wall, where they combine with other compounds like alginic acid to form complexes.

Significant levels of phenolic compounds with possible antioxidant action have also been found in sea cucumbers. It's possible that the considerable hepatoprotective effect was facilitated by the active phenolic compounds found in the extract of the body wall of *Holothuria atra*. In comparison to solvent extracts, which only contained slightly higher total phenolic contents (1.53–2.90 mg GAE/g DW), aqueous extracts of the *Holothurialeucospilota*, *Holothuriascabra*, and *Stichopuschlorontus* had significantly higher total phenolic contents (4.85–9.70 mg GAE/g DW) and demonstrated strong antioxidant properties [43].

Phycobilins (PC and PE), a type of protein covalently connected to chromophores, make up phycobiliproteins. These water-soluble proteins can be utilized as a natural food colorant and are effective antioxidants. The cyanobacteria *Lyngbya* spp. and *Arthrospira* spp. both generate PE, a pink-colored protein pigment, and PC, a blue-colored phycobiliprotein [60]. *Spirulina*, *Botryococcus*, *Chlorella*, *Dunaliella*, *Haematococcus*, and *Nostoc* are

Table 1
Bioactive compounds from marine sources and their activities

Active compound	Activity	Source	References
Peptides	Antioxidant activity	Visceral organs of horse mackerel	[38]
Cephalothorax hydrolysates	Antioxidant activity	Pacific white shrimp	[39]
Hydrolysates and peptides	Antioxidant activity	Shrimp (*Aceteschinensis*)	[40]
Hydrolysates	Antioxidant activity	Crayfish (*Procambarus clarkia*)	[41]
Phlorotannins	Antioxidant activity	Brown algae	[42]
Phenolic compounds	Antioxidant activity	Sea cucumber (*Holothurialeucospilota*, *Holothuriascabra*, and *Stichopuschlorontus*)	[43]
Porphyrans	Immunoregulatory, antioxidant, and anticancer properties	*Porphyra*spp	[44]
Fucoidans	Antioxidant activity	Seaweed (*Sargassum thunbergi*, *Ascophyllum nodosum*, Viz *fucusvesiculosus*, *Laminaria japonica*, *Fucusevanescens*, and *Laminaria cichorioides*)	[44]
Ethyl acetate extract	Antimicrobial activity	European abalone (*Haliotistuberculata coccinea*)	[45]
Peptide fractions	Antimicrobial activity	*Chlorella vulgaris*	[46]
Hydrolyzed secondary raw material	Antimicrobial activity	Atlantic mackerel *(Scomberscombrus)* and half-fin anchovy *(Setipinnataty)*	[47]
Short-chain fatty acids and unsaturated chain fatty acids	Antimicrobial activity	Microalgae. *Isochrysisgalbana*, *Scenedesmus* sp., and *Chlorella* sp.	[48]
Peptides	Antihypertensive activity	Tuna frame protein hydrolysate	[49]
Tri-peptides	Antihypertensive activity	Northern shrimp (*Pandalus borealis*)	[50]
Peptides	Antihypertensive activity	Sardine muscle	[51]
Triterpene glycosides	Anti-cancer properties	Sea cucumber	[43]
Fucoidans	Anticoagulant properties	Seaweed	[52]
Fucoidans	Anticoagulant properties	Seaweed (*Saccharina japonica*, *Ochrophyta*, *Phaeophyceae*)	[53]

(continued)

Table 1
(continued)

Active compound	Activity	Source	References
Polysaccharide fractions	Anticoagulant properties	Marine alga (*Dictyopterisdelicatula*, Ochrophyta, Phaeophyceae)	[54]
Laminarin	Would healing property	*Cystoseirabarbata*	[55]
Alginates	Would healing property	Cell wall of brown macroalgae	[56]
Fucoxanthin	Neuroprotective property	Brown algae	[57]
Carotenoids	Neuro protective property	Marine fungi	[58]

just a few of the algae that have been identified as excellent sources of phycobiliproteins. These pigments have been found to have antioxidant capabilities, according to a recent study by Menaa and others [44]. Carrageenans derived from *Hypnea* spp. exhibit antioxidant characteristics and hypocholesterolemic effects by lowering cholesterol and sodium absorption while increasing potassium absorption [60]. The complex sulfated polysaccharide, known as porphyran, which is derived from red *Porphyra* spp., possesses immunoregulatory, antioxidant, and anticancer properties [44]. *Sargassum thunbergi, Ascophyllum nodosum,* Viz *fucusvesiculosus, Laminaria japonica, Fucusevanescens,* and *Laminaria cichorioides* are a few examples of brown algae that contain fucoidans, which have antioxidant properties [44].

The antioxidant properties of sulfated polysaccharides isolated from *Monostromaangicava* are correlated with the degree of sulfation and a moderate molecular weight [43]. A powerful antioxidant, iodine is present in most edible seaweeds [44]. The water-soluble vitamins thiamine, riboflavin, niacin, pantothenic acid, and biotin, as well as the fat-soluble vitamins retinoic acid and tocopherols with antioxidant properties, are both abundant in seaweeds [44].

3.1.2 Antimicrobial Property

Food antimicrobials are used to stop the development of germs that lead to food spoiling. The most widely used antimicrobial medications are organic acids (sorbic acid, acetic acid, citric acid, etc.), which alter the permeability of cell membranes to substrates and create pH conditions that are unfavorable to bacterial development. Despite being a very effective preservative, organic acids like sorbic acid are known to break down when exposed to water and generate potentially dangerous compounds like acetaldehyde. Ethyl acetate was used as the extraction solvent to produce an extract from the edible abalone species *Haliotistuberculata coccinea*, sometimes

known as "European abalone," by Tortorella et al., 2021. It demonstrated antimicrobial action against *Staphylococcus aureus* ATCC 6538P, the most susceptible strain, the developing multi-drug-resistant *Stenotrophomonas maltophilia* D71, and the methicillin-resistant *Staphylococcus epidermidis* strain RP62A. Additionally, it demonstrated anthelmintic action as measured by its toxicity toward the target model helminth *Caenorhabditis elegans.*

The antibacterial efficacy of *Chlorella vulgaris* peptide fractions that were pepsin-digested and demonstrated antimicrobial activity against *E. coli* CECT 434 was assessed by Sedighi and others [46]. Guzmán et al. [61] reported the discovery of antibacterial peptides from *Tetraselmissuecica*, specifically the AQ-1766 peptide (LWFYTMWH), which exhibited antimicrobial activity against both gram-positive and -negative bacterial strains, including methicillin-resistant *S. aureus, B. cereus, M. luteus*, and *P. aeruginosa*. The majority of antimicrobial peptides have been found to be cationic, meaning they have a net positive charge as a result of positively charged amino acid groups such as lysine and arginine, which also have hydrophobic and amphipathic properties that help them bond with their hosts and become soluble in aqueous (lipid) membranes. Antimicrobial peptides are hypothesized to function by opening pores in the membrane before entering the cell, where they release biological components from the microbes and kill the cell. Peptides with less than 50 amino acids and a low molecular weight of less than 10 kDa are typically found to have antimicrobial activity when released under the right hydrolysis circumstances [62].

Atlantic mackerel (*Scomber scombrus*) and half-fin anchovy (*Setipinnataty*) hydrolyzed secondary raw materials exhibited antibacterial efficacy against gram-negative *Escherichia coli* and gram-positive *Listeria innocua* [47]. Microalgae have tremendous application potential as a natural antibiotic and have the ability to reduce microbial infection in aquaculture [63]. Now, numerous researches have demonstrated the antibacterial action of short-chain fatty acids and unsaturated chain fatty acids isolated from microalgae. *Isochrysisgalbana, Scenedesmus* sp., and *Chlorella* sp. were the three microalgae whose major chemicals were identified by Alsenani et al. [48] as DHA, EPA, linoleic acid, and oleic acid and whose extracts may suppress the growth of gram-positive bacteria

3.1.3 Antihypertensive Property

Different marine fish and fish body parts, such as those from tuna, yellowfin sole, scad, Hoki, Pacific hake, yellow stripe trevally, and conger eel, have been used to make peptides (fish bones, muscles, skin, intestines, etc.). Peptides from fish have been found to have antihypertensive effects [64]. The production of Angiotensin-converting enzyme inhibitory peptides is influenced by the hydrolysis circumstances, substrate protein, and peptidase type.

Angiotensin-converting enzyme-inhibitory protein hydrolysates were produced by the enzymatic digestion of a marine protein substrate, according to studies [65]. Lee et al. [49] extracted the peptide GDLGKTTTVSNWSPPKYKDTP from tuna frame protein hydrolysate. This peptide significantly reduced the systolic blood pressure of spontaneously hypertensive rats, and its antihypertensive efficacy was comparable to that of captopril. Sardine cannery byproducts are another potential source of bioactive peptides. In the hydrolysate produced from Northern shrimp (*Pandalus borealis*), two novel ACE inhibitory tri-peptides, FTY and FSY, were revealed [50]. The two peptides are quite similar since both of the terminal residues are the same and both of the intermediate residues (Serine and Threonine) have R-groups that contain hydroxyl. The sole distinction is that FTY is noticeably bulkier than FSY due to the presence of a free methyl in addition to the hydroxyl in the R-group of Thr. The findings demonstrate that this modest change has a large impact on ACE inhibitory activity since FSY is around 30 times more active than FTY. The peptides KW, RVY, and MY, which are recognized as antihypertensive peptides, have been reported to have ACE-inhibitory activity in sardine muscle hydrolysates with alkaline protease [51].

3.1.4 Anticancer Property

The world's population has been impacted by cancer as a significant cause of mortality in both direct and indirect ways. Although cancer cases are on the rise, some of them may be avoidable or even treatable by using natural substances. The risk of chronic diseases is said to be reduced and overall health is maintained by bioactive peptides that can be found on land and in the water. Fish byproducts are a source of bioactive peptides and may be anticarcinogenic [66]. Anticarcinogenic peptides inhibit the growth of cancer cells in a number of different methods, such as (1) in the cytoplasm, (2) by promoting membrane rupture through micellization, and (3) by interacting with cells during apoptosis via gangliosides on the surface. Anticancer properties were found in peptides made from leftover fish processing raw material [62].

Cytotoxicity is the most frequent biological characteristic of sea cucumber glycosides, making them one of the most researched anticancer drugs. To present, a variety of sea cucumber species have produced more than 300 triterpene glycosides with notable pharmacological characteristics. Argusides A–E, triterpene glycosides derived from *Bohadschiaargus*, have exhibited significant in vitro cytotoxicity against a number of human carcinoma cell lines. Against six different tumor cell lines (P-388, A-549, MCF-7, MKN-28, HCT-116, and U87MG), the triterpene glycosides, pentactasides I–III, as well as philinopsides A–B, isolated from *Pentactaquadrangularis* elicited a remarkable in vitro cytotoxicity effect with an IC50 value ranging from 0.60 to 3.95 M. In

numerous types, including pancreatic ductal adenocarcinoma, colon, prostate, cervical, and bladder cancer cells, frondoside A, a triterpene glycoside derived from the orange-footed sea cucumber *Cucumariafrondosa*, has shown anticancer properties. It has been claimed that this glycoside has anti-cancer properties through a variety of mechanisms of action, including the activation of cellular apoptosis, suppression of cancer cell proliferation, migration, metastases development, invasion, and angiogenesis. On human leukemia HL-60 and human hepatoma BEL-7402 cells, it was found that the triterpene glycosides fuscocinerosides A–C, pervicoside C, and holothurin A, which were isolated from the sea cucumber *Holothuriafuscocinerea*, had a strong cytotoxic effect. Three sulfated triterpene glycosides from *Pseudo colochirus violaceus ides* have demonstrated notable in vitro cytotoxicity action against stomach cancer MKN-45 and CT-116 cells. Sea cucumber may be utilized as a functional diet to prevent cancer, despite the fact that the precise mechanism(s) behind the anticancer effects of numerous triterpene glycosides are still completely unknown [43].

3.1.5 Anticoagulant Property

A series of events take place during blood coagulation that, if unchecked, might result in coronary artery blockage. During platelet activation, a large number of platelets gather. Next, prothrombin is changed into thrombin, a serine protease that turns soluble fibrinogen into its insoluble form, fibrin. As more thrombin is produced, the conversion of fibrinogen to fibrin is enhanced. The combined effects of vasoconstriction and obstruction of coronary arteries by fibrin complex formation within the blood vessel result in myocardial ischemia and heart attacks. Limiting platelet aggregation will thereby reduce vasoconstriction and the risk of myocardial ischemia in these circumstances. The king of physiologically active chemicals, fucoidan is a highly branched, diverse monosaccharide with a high molecular weight (10,000–100,000 Da). The complex chemical structure promotes its anticoagulant potential [52].

The molecular weight of fucoidans and the amount of galactose they contain both affect the seaweeds' ability to prevent clotting. Four of the seven fucoidans isolated from the seaweed Saccharina japonica (Ochrophyta, Phaeophyceae) differ in both average molecular weight and the ratio of fucose to galactose. These fucoidans' results from the activated partial thromboplastin time (APTT) assay demonstrate that larger molecular weight fucoidans have strong anticoagulant activity, which further raises the galactose concentration [53]. Sulfated polysaccharides from the brown seaweed *Sargassum fulvellum* also showed substantial anticoagulant action in the APTT assay, as did several fucans isolated from the brown algae *Padina gymnospora*. The APPT test demonstrated that six families of sulfated polysaccharides from the marine

alga *Dictyopterisdelicatula* (Ochrophyta, Phaeophyceae) inhibit both the intrinsic and common pathways of coagulation and that some polysaccharide fractions have anticoagulant activity comparable to that of clexane (a commercial anticoagulant drug). Three seaweeds, *Laurencia filiformis* (Rhodophyta), *Ulva compressa* (Chlorophyta), and Turbinariaconoides (Ochrophyta, Phaeophyceae), contain polysaccharides that prolonged the coagulation of human plasma tested by the APTT assay [54].

In the APTT assay, an extract of the green seaweed *Udotea flabellum*, which is rich in sulfated polysaccharides, revealed a plasma coagulation time that was twice as long, comparable to the outcomes from 1 g of heparin [53]. The use of ulvans as an anticoagulant is based on the polar interaction of sulfated polysaccharide with proteins, such as heparin cofactor II, which is thought to be the cause of the anticoagulant activity [67]. The ulvan's anticoagulant effect was the most anticipated biological characteristic because of its molecular similarity to mammalian heparinoid substances. There have been reports of substantial antithrombotic and anticoagulant activity in sulfated polysaccharides isolated from *Ulvales* spp. [68].

As a naturally occurring, highly sulfated mucopolysaccharide, algal sulfated polysaccharide has an anticoagulant effect similar to heparin. Due to the sulphate group's anionic nature, sulfated polysaccharides are good candidates for bio-applications as antioxidant and anticoagulant medicines. A fascinating prospect for use in medicine is microalgal sulfated polysaccharide, a glycosaminoglycan that contains sulfate ester in its different sugar units [69]. While some seaweed polysaccharides directly decrease fibrin polymerization and thrombin activity without interacting with antithrombin III (AT III) and heparin cofactor II (HC II), others exhibit anticoagulant effect via influencing AT III and boosting HC II, a key endogenous inhibitor [53].

The anticoagulant properties of Chlorella sorokiniana could be attributed to a variety of elements, such as the sulfate concentration and their binding site, monosaccharide residues, and glycoside bonds that are important for the bioactivity of polysaccharides [70].

The anticoagulant and antiplatelet effects of bioactive peptides made from fish muscle are also established [62]. Anticoagulants from the hydrolysate of yellowfin sole protein were recognized by Phadke and team [62] in 2021. It formed an inactive compound that prevented activated coagulation factor XII regardless of Zn2+. Peptides comprising the amino acids His-Cys-Phe, Cys-Leu-Arg, Leu-Cys-Agr-Agr, and Leu-Cys-Arg have higher anticoagulant activity. Protein hydrolysates from the adductor muscle of the sea bivalve mollusk *Mytilus edulis* showed better anticoagulant ability at 1.49 mg/mL. Similar to this, blood coagulation factor was

suppressed by the hydrolysate of an anticoagulant peptide from an oyster (*Crassostrea gigas*). The inhibition of thrombin time was observed to be prolonged and partial thromboplastin time was activated by oyster (*Crassostrea gigas*) hydrolysate and *Scapharcabroughtonii* protein (26 kDa) [71].

3.1.6 Wound Healing Property

The process of healing a wound involves a number of steps, including cell migration and proliferation as well as the production of new extracellular matrix. Brown algal polysaccharide laminarin has a small molecular weight (MW; 5 kDa). *Laminaria* and *Saccharina* species as well as some *Ascophyllum* and *Fucus* species contain it. Laminarin is made up of sβ-(1,6)-intrachain linkages and (1,3)-β-D-glucan. Laminarin from Cystoseirabarbata (5% cream) greatly accelerated reepithelization, increased wound contraction, and permitted restitution of mice skin tissue during the in vivo healing process [55]. Alginate is a linear polysaccharide made up of consecutive block structures of (1–4-)linked β-D-mannuronic acid (M) and α -L-guluronic acid (G) monomers. The cell wall of brown macroalgae contains it naturally. Alginates have use in materials for dental impressions and alginate fiber wound dressings [56].

Carrageenan is a sulfated polysaccharide with a high molecular weight that is a structural component of the cell membranes of red macroalgae. It is made up of alternating linear chains of α-1,3-galactose and β-1,4,3,6-anhydrogalactose with ester sulphates (15–40%). Carrageenan is used in the transport of drugs, the regeneration of bone and cartilage tissue, and wound healing due to its physiochemical characteristics and gelling mechanism [72]. Additionally, gelatin is produced into sterile sponges for use in medical and dental operations, as well as a material for treating wounds [73]. Oral administration of peptides from different fish species and their byproducts, like collagen hydrolysates, shows moisture retention across the face in addition to improved viscoelastic characteristics and decreased sebum levels. Enzymatic protein hydrolysates made from the silver carp's bones and isolated peptides were more effective at promoting keratinocyte metabolism and wound healing processes, highlighting the potential of bone peptides for treating wounds in the cutaneous region [74].

3.1.7 Neuroprotective Property

There has been a significant increase in research on marine microbial pigments in recent years. Pigments are molecular structures that can absorb specific wavelengths of light and reflect the rest of the visible spectrum. Pigment production by marine bacteria is thought to be mediated by a quorum-sensing mechanism [75]. Carotenes, which are pure hydrocarbon carotenoids without any substituents in their structures, and xanthophylls, which are molecules containing oxygen, are the two main categories of carotenoids

[76]. Astaxanthin, a xanthophyll carotenoid compound, has demonstrated its ability to provide neuroprotection through the inhibition of lipopolysaccharide-induced neuroinflammation, amyloidogenesis, and oxidative activity in mouse models [77, 78]; the prevention of hippocampal insulin resistance and complications of Alzheimer's disease in Wistar rats [79]; and the prevention of brain damage in offspring exposed to prenatal epilepsy seizures [80]. Furthermore, the neuroprotective potential of astaxanthin and fucoxanthin against amyloid-mediated toxicity in pheochromocytoma neuronal cells has also been studied. Results showed many neuroprotective effects but also pointed to fucoxanthin's greater potential as a potential treatment approach [57].

Furthermore, research into the potential of β-carotene for the treatment of acute spinal cord injury revealed that it could slow the advancement of secondary damage events by blocking the nuclear factor-κB pathway [58]. Due to its advantageous effects on the central nervous system, lycopene is a biocompound that has been extensively studied. According to one study, it has the ability to lessen oxidative stress and the cell apoptosis caused by tert-butyl hydroperoxide, two important elements in the pathogenesis of Alzheimer's disease. Following lycopene injection, cell survival and neuronal morphology were enhanced, mitochondrial membrane potential was restored, and reactive oxygen species were reduced [81]. Additionally, giving lycopene to rats with hippocampal lesions caused by aluminum chloride has improved cognition impairment and reduced oxidative stress by lowering levels of malondialdehyde and 8-hydroxy-2′-deoxyguanosine and raising levels of glutathione and superoxide dismutase activity. In turn, these mechanisms have demonstrated that they can stop neuroinflammation and apoptosis [82]. Moreover, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated Parkinson's disease mouse models showed that lycopene had neuroprotective effects by raising dopamine levels and lowering oxidative stress levels [83]. In rat models of spinal cord ischemia/reperfusion injury, lycopene has also shown promise for improving neurological function recovery and inhibiting neuronal death and neuroinflammation [84], including prevention of cerebral vessel injury caused by hyperlipidemia by reducing astrocyte activation and inflammatory cytokine production [85].

Through several research studies, marine biocompounds have demonstrated their neuroprotective benefits, primarily targeting the prevention of neurodegeneration and the decrease of oxidative stress in the central nervous system. However, the study of chemicals with neuroprotective properties derived from marine sources is still in its early stages, necessitating additional research.

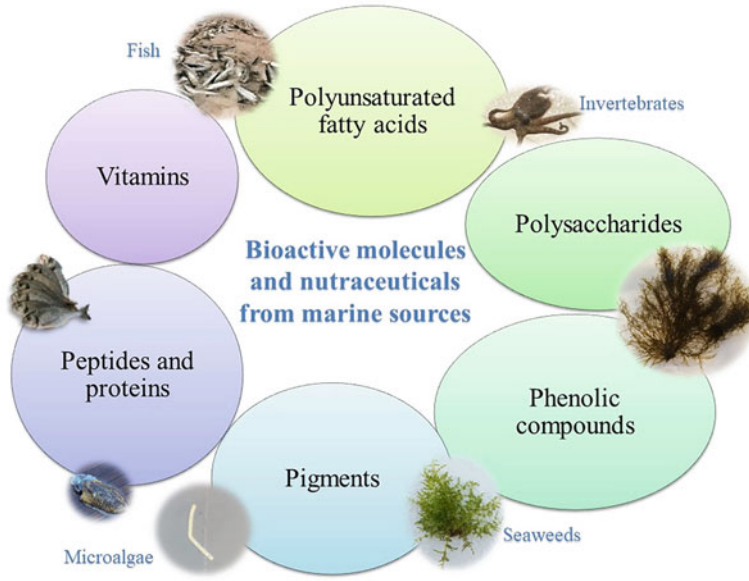

Fig. 1 Various bioactive compounds obtained from different marine sources

3.2 Nutraceutical Property

The marine ecosystem is so diverse that it presents a great pool for obtaining new compounds that are beneficial for health improvement and can potentially be used in food, supplement, or therapeutic industries [86]. Thus, newly identified or isolated compounds from marine sources that carry nutraceutical properties have been intensively researched in recent years. Marine-origin nutraceuticals include polysaccharides, peptides and proteins, polyunsaturated fatty acids (PUFAs), and other lipids, pigments, enzymes, phenolic compounds, minerals, and vitamins (Fig. 1) [86–88]. The sources from which these compounds have been isolated include seaweeds [89], microalgae [90], fish, and a large group of marine invertebrates (arthropods, echinoderms, sponges, mollusks, cnidarians, lophophorates, marine worms, and the hemichordates) [91, 92].

Sulfated polysaccharides found in seaweeds are the most studied group due to their biological activities. They have shown anticarcinogenic, anti-inflammatory, antioxidant, antiviral, and anticoagulant properties [89, 93]. Polysaccharides make up more than 80% of the seaweed weight; they serve as structural compounds and energy reserves. The main sulfated polysaccharides isolated from brown seaweeds are fucoidan, laminarin, and alginate; from red seaweeds is carrageenan; and from green seaweeds ulvan [86]. Microalgal polysaccharides also exhibit various biological activities; also they are components of the microalgal cell wall, energy reservoirs and serve as cell protection. They are mainly composed of pentose and hexose monosaccharides with glycosidic linkage [94]. Marine invertebrates, such as sea cucumbers, ascidians, sea urchins, and nudibranchs, are also the source of

polysaccharides, mainly belonging to the group of glycosaminoglycans. These can be used as nutraceuticals as they are known to have anti-inflammatory effects, improve cartilage structure and function, and relieve the osteoarthritis pain. Invertebrates that do not possess glycosaminoglycan synthesize sulfated polysaccharides, which have the structure and biological functions similar to glycosaminoglycans [95]. Sulfated polysaccharides that carry antimicrobial activity and can be used as nutraceuticals can also be isolated from fish skins [96].

Marine algae, fish, and invertebrates are considered a great source of proteins and bioactive peptides. Seaweeds are natural reservoirs of bioactive peptides with numerous health benefits, and they are considered an alternative source of protein. However, extraction of proteins and bioactive peptides from seaweeds is challenging and yields are low. Further improvements in extraction are needed to break down the covalently bounded polysaccharides and protein complex structures present in seaweeds [97]. Further, more than 50% of microalgal dry weight consists of proteins. Some species belonging to the genera *Dunaliella* and *Arthrospira* are currently marketed as nutraceuticals [98]. The amino acid profile of fish proteins makes them a high-quality nutritive component since they contain all essential amino acids that are easily digestible and have high bioavailability. Besides, they are a rich source of bioactive peptides, biomolecules derived from fish protein hydrolysates that have shown numerous biological activities (antioxidant, antimicrobial, antihypertensive, antiproliferative, immunomodulatory, antidiabetic, etc.). For this reason, they are considered biomolecules with positive health effects and potential therapeutic applications [91]. Proteins and peptides derived from marine invertebrates, squids, snails, shellfish, and jellyfish have shown antioxidant, anti-inflammatory, anticancer, antiviral, antibacterial, immunomodulatory, and antihypertensive activities [99].

Marine sources have unique lipid composition containing large amounts of PUFAs. The long-chain $n-3$ PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have numerous health benefits. They reduce the risk of cardiovascular diseases, arthritis, and Alzheimer's and Parkinson's diseases. Besides, they have neuroprotective effect, and they stimulate neurodevelopment and neurotransmission in humans. Fatty fish, microalgae, seaweeds, krill, and fungi are significant sources of these fatty acids [100–103]. Like humans, fish cannot synthesize PUFAs, but they obtain them from feed (krill or algae that they consume) [91]. Lipids, including PUFAs, are mostly extracted from fish and marketed as fish oil. Fish oil is conventionally extracted with solvents or by wet pressing, while recently supercritical fluids and fish silage have been used [104]. Fish oil can also be extracted from fish byproducts and discarded species [105–109]; byproduct fish oil can be produced and refined to maintain high-quality PUFAs [110]. Microalgae

have a lower lipid content than fish, but they have a high content of PUFAs and could therefore be a potential alternative source for their extraction [111, 112].

The main natural pigments isolated from marine sources are chlorophylls, carotenoids, and phycobiliproteins, which have health benefits due to their antioxidant, anti-inflammatory, anti-obesity, antiangiogenic, anticancer, and wound healing properties [101, 113]. Chlorophylls are synthesized by cyanobacteria and algae and are mainly responsible for photosynthesis. Apart from their potential use as substitutes for synthetic pigments in the food industry, chlorophylls exert antioxidant, antibacterial, anti-inflammatory, and antimutagenic activities, which make them potential nutraceuticals [86, 101]. Promising marine sources for the extraction of chlorophylls are microalgae [114]. The most abundant carotenoids found in marine sources are fucoxanthin, astaxanthin, lutein, zeaxanthin, neoxanthin, violaxanthin, and canthaxanthin [101]. Astaxanthin and fucoxanthin are the most abundant carotenoids in seaweeds and microalgae [115]. Some fish species, such as families of Salmonidae and Mullidae, are a good source of astaxanthin, β-carotene, zeaxanthin, and canthaxanthin [91], while crustaceans and their byproduct are a valuable source of natural astaxanthin [78]. Carotenoids found in sponges, jellyfish, mollusks, crustaceans, sea urchins, and tunicates come from their diet, and in the case of sponges are also associated with their symbionts [116]. Carotenoids exhibit various biological activities, such as antioxidant, anti-inflammatory, anticancer, anti-obesity, anti-diabetic, wound healing, and photoprotective activities, which are the foundation for their beneficial effects on health through the reduction of cardiovascular diseases and cancer risks, atherosclerosis, non-communicable diseases, and macular degeneration [86, 101]. Phycobiliproteins are found in red seaweeds and cyanobacteria and exhibit antioxidant properties. Research on their extraction and bioactivity is increasing [117, 118].

Phenolic compounds are a large group of phytochemicals that have recently gained attention because of their bioactivities and health-promoting benefits, including antioxidant, antimicrobial, anti-inflammatory, anti-tumor, anti-allergic, anti-hypertensive, anti-cholesterol, antithrombotic, anti-diabetic, immunomodulatory, wound healing, neuroprotector, photoprotector, and algicidal properties [119, 120]. This group of compounds can be divided into simple phenols, benzoic acid derivatives, flavonoids, tannins, stilbenes, lignins, and lignans. Marine sources of phenolic compounds are microalgae and seaweeds. These organisms produce them for protection against oxidative stress, biofouling, predators, pathogens, and other external factors [101, 119, 121]. Among the three groups of seaweeds, brown algae have been known to yield more phenolic compounds [119, 122, 123].

Microalgae, seaweeds, and fish are rich sources of all vitamins (water and lipid soluble) and minerals. They are essential for humans as they maintain health through the coordination of physiological functions. Besides, vitamins have a strong antioxidant effect. Their deficiency leads to numerous diseases [91, 100, 114, 124]. Commonly found minerals in marine sources are iron, manganese, zinc, selenium, sodium, potassium, calcium, phosphorus, sulfur, copper, magnesium, and iodine. Their contribution to human health and well-being is exceptional, especially for immunity, transmission of nerve impulses, oxygen transport, maintaining electrolyte balance, thyroid health, and formation of bones and teeth [91, 125].

4 Food Application of Bioactive and Nutraceuticals Derived from Marine

With an increasing world population, a large share of elderly people, and increasing incidence of chronic diseases, the global market of functional foods and nutraceuticals is constantly increasing and is expected to grow in the next decade. In the food and nutraceutical industry, marine organisms are well recognized as a source of valuable nutritive components that can be consumed as whole foods or be used to extract valuable components. For example, fish and marine algae are a well-known source of valuable nutraceuticals such as PUFAs, proteins, and minerals. On the other hand, these compounds can be utilized for enrichment of other foods either to increase their nutritional value, to enhance their oxidative stability, or even give them functional properties.

Table 2 provides an overview of laboratory trials based on the applications of bioactive compounds and nutraceuticals from marine sources in food models. Pigments isolated from microalgae, seaweeds, and shrimps have been used in various meat, dairy, and bakery products to improve their oxidative stability. Protein hydrolysates and peptides from fish, shrimp, and seaweeds were successfully used in meat and bakery products, improving their nutritional value and sensory properties while retarding lipid oxidation. Oils from marine sources (fish and shrimp) improved the nutritional value of meat, dairy, and bakery products, without affecting their sensory properties, and in some cases reduced lipid oxidation. Powdered seaweed and microalgae were used to increase the nutritional value of products such as pasta, bread, snacks, cheese, tomato puree, and tuna jerky.

The effect of the seaweed extracts application in food models [126–128] or in edible films [129, 130] has also been investigated; however, in most cases, the chemical identification of the compounds from the extracts was not reported. Interestingly, the phenolic compounds purified from marine algae have not yet been used in food models, although they are the most studied secondary

Table 2
Food application of bioactive and nutraceuticals derived from marine sources

Bioactive molecules or nutraceuticals	Marine source	Food application	Main findings	References
Astaxanthin	Pink deep-water shrimp (*Parapenaeuslongirostris*) by-products (heads, cephalothorax and appendices)	Marinated chicken steaks	Natural astaxanthin was effective as vitamin C in preventing lipid oxidation of marinated chicken steaks Texture of samples was improved Microbiological stability was improved	[133]
Astaxanthin	Microalga *Haematococcus pluvialis*	Raw and cooked lamb patties	The oxysterol levels decreased The amount of volatiles was reduced Oxidative stability of raw and cooked lamb patties was improved	[134]
Astaxanthin	Microalga *H. pluvialis*	Cookies	The oxidative stability of cookies during storage was improved The addition of astaxanthin did not affect the cookies' taste acceptability	[135]
Astaxanthin	White shrimp (*Litopeneusvannamei*) shells	Yogurt	Astaxanthin was encapsulated with alginate and chitosan and added to yogurt Yogurt maintained desirable sensory attributes	[136]
Extract rich in astaxanthin	Microalga *H. pluvialis*	Raw ground pork meat	Application of extract delayed lipid oxidation and improved color stability of raw ground pork meat during 7 days of refrigerated storage	[137]
Fucoxanthin	Microalga *Phaeodactylumtricornutum*	Whole and skimmed milk	In terms of stability and bioavailability, skimmed milk was an excellent food matrix for application of fucoxanthin	[138]

Fucoxanthin	*Laminariales* sp.	Goat whole and skim milk	Pasteurization did not affect fucoxanthin stability Addition of fucoxanthin did not affect the physicochemical properties of milk Milk color was influenced Goat milk can be a suitable matrix for fucoxanthin supplementation	[139]
Extract containing fucoxanthin	*Sargassum polycystum*	Snack bar	When used in combination with cacao butter and 1% stevia, the extract maintained its stability and antioxidant properties; it was the combination that panelists preferred in organoleptic evaluation	[140]
Biomass and phycocyanin extracts	Spirulina (*Arthrospiraplantensis*)	Biscuits	Biscuits had a high oxidative stability during 30 days of storage Biscuits had good nutritional and sensory profiles	[141]
Protein-procyanidin hybrid nanoparticles	Spanish mackerel *(Scomberomorus maculatus)*	Surimi	Protein-procyanidin hybrid nanoparticles were used for stabilization of high internal phase pickering emulsions that were applied in surimi; surimi showed good cooking stability	[142]
Peptides and peptide-loaded nanoliposomes	Tuna (*Thunnusobesus*) skin	Pork patties	Inhibition of lipid oxidation in pork patties was observed during 14 days of refrigerated storage	[143]
Protein hydrolysate	Pacific white shrimp (*L.vannamei*) cephalothorax	Biscuits	Biscuits fortified with shrimp protein hydrolysate powder had higher nutritional value and increased sensory properties	[144]
Protein hydrolysate	*Palmariapalmata*	Bread	The texture and sensory properties of wheat bread were not affected by the addition of *P. palmata* protein hydrolysate Renin inhibitory bioactivity of hydrolysate was retained after the baking process	[145]

(continued)

Table 2
(continued)

Bioactive molecules or nutraceuticals	Marine source	Food application	Main findings	References
Protein hydrolysate	Skipjack tuna (*Katsuwonuspelamis*) roe	Emulsion sausage from broadhead catfish (*Clarias macrocephalus*)	Lipid oxidation-reduction of sausages during 12 days of storage was observed Skipjack roe protein hydrolysate did not affect sausages' organoleptic properties	[146]
Fish concentrate	Sea bass (*Dicentrachuslabrax*) filleting by-products (trimmings)	Fresh pasta	Nutritional value of pasta was improved; $n-3$ fatty acids remained in satisfactory quantities during the shelf-life Enriched pasta had acceptable sensory properties	[147]
$n-3$-rich oil	Sardine (*Sardinapilchardus*) gill and viscera	Wheat flour chips	Lipid oxidation of chips was prevented and antioxidant enzymes were activated Chips' lipid profile was improved	[148]
Fish oil	Cod liver	Cooked and dry-cured sausages	Fish oil was microencapsulated Nutritional value of sausages ($n-3$ content) was improved without influence on oxidative stability, physicochemical characteristics, and acceptability	[149]
Fish oil	Cod liver	Chicken nuggets	Bulk fish oil and microencapsulated fish oil were added to nuggets Microencapsulated fish oil nuggets had lower levels of lipid and protein oxidation, and volatile compounds No change in sensory quality was observed for microencapsulated fish oil nuggets when compared to control	[150]

Fish oil	Not specified	Yogurt	Yogurt was fortified with fish oil and nano-encapsulated fish oil Higher $n-3$ contents and better sensory characteristics were found for yogurt fortified with nanoencapsulated fish oil	[151]
Shrimp oil	Pacificwhite shrimp (*L. vannamei*) cephalothorax	Skim milk	Shrimp oil was encapsulated in nanoliposomes β-glucan was added to mask bitterness caused by shrimp oil In vitro digestion showed that $n-3$ fatty acids were bioaccessible for absorption in the gut after digestion Oxidative stability was enhanced throughout the storage	[152]
Shrimp oil	Pacific white shrimp (*L.vannamei*) hepatopancreas	Biscuits	Shrimp oil was encapsulated with sodium caseinate, fish gelatin, and glucose syrup before addition to biscuits Nutritive value of biscuits was improved No adverse effect on biscuit quality and sensorial properties was observed after addition of micro-encapsulated shrimp oil	[153]
Powder and sulfated polysaccharide	*Ulva intestinalis*	Fish fingers	Lipid oxidation was retarded over 6 months of storage when compared to control Products were acceptable organoleptically Sulphated polysaccharides had better impact on products' texture preservation	[154]
Powder	Spirulina (*A. plantensis*)	Pasta	Nutritional value of pasta was significantly improved Pasta with spirulina had high acceptability scores	[155]

(continued)

Table 2
(continued)

Bioactive molecules or nutraceuticals	Marine source	Food application	Main findings	References
Powder	Spirulina (*A. plantensis*)	Cheese	When cheese was fortified with *A. platensis* powder, the amount of protein and iron increased The positive effects on the survival of probiotic bacteria were observed during the storage	[156]
Powder	*Ascophyllum nodosum*	Gluten-free bread	Bread with seaweed powder showed favorable changes in the texture Antioxidant activity of bread was increased	[157]
Powder	*Fucusvesiculosus*	Bread	Bread density and bread crumb firmness were enhanced	[158]
Biomass	*Nannochloropsis*sp.	Tomato puree	Higher oxidative stability of tomato puree with *Nannochloropsis* sp. biomass was observed when compared to control (fish oil)	[159]
Powder	*Spirulina* sp.	Snacks	Nutritional value of snacks was improved without impacting physical parameters significantly Snacks had high sensory acceptance index	[160]
Powder	*Sargassum wightii*	Tuna (*Thunnus albacores*) jerky	Tuna jerky had improved antioxidant quality Organoleptic quality was not affected	[161]

metabolites with many proven bioactive properties. On the other hand, seaweed polysaccharides (agar, alginate, carrageenan) are widely used in technological processes as emulsifiers, stabilizers, and thickeners, but not as nutraceuticals. They are used in the production of various foods, dairy products, wine, jam, jelly, and bakery products [131]. In addition, seaweed polysaccharides are used for the development of biodegradable packaging (e.g., edible films) as natural compounds to replace synthetic polymers [132].

Overall, it is evident that the application of marine bioactive components and nutraceuticals will be investigated in the future. Their application is sometimes limited due to the marine or fishy odor they produce; however, formulation and adaptation of the recipes are needed as well as industrial trials that will fabricate new functional products.

5 Conclusion

The extraction of bioactive and nutraceuticals from marine sources is currently being pursued due to the potential resources of several compounds with well-known functional and biological activities. Ample research has proven the undeniable health benefits of sea product extracts, thus attracting food industry and pharmaceutical interest. The marine-extracted chemical compounds have a wide range of functional characteristics, including neuroprotection, wound healing, antioxidative properties, and many more. The intensive demand for marine product-derived bioactive compounds in the food industry has compelled researchers and industries to employ various methods for extracting these compounds with high efficiency. In the application of emerging extraction technology, the extraction yield of functional compounds from marine products is higher with the minimization of byproducts. The aforementioned methods are environmentally friendly extraction techniques due to the minimal use of synthetic and organic chemicals, reduced extraction time, better yield, and excellent quality of the extract. Thus, these extraction methods have gained significant popularity and are commercialized at an industrial scale. Many standards and protocols have been used for the optimal extraction of the bioactive from sea resources. However, the exploration of the optimization and quantification of the bioactive from marine sources is still limited. Moreover, the combined application of assisting technology and green solvents in the extraction of those bioactive compounds needs to be explored.

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Chapter 4

Microwave-Assisted Extraction of Bioactive and Nutraceuticals

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Abstract

The increasing consumer awareness about the link between nutrition and health has led the food industry to produce fortified food with bioactive compounds. Considering that not all bioactive compounds are freely available and in the light of increasing attention to preserve environmental resources, the new trend consisted of waste recovery of industrial food processing residues with active potential. Currently, clean label and eco-friendly extraction methods have realized reputation accounts for the removal of solvent usage and reduction in energy consumption. In this context, microwave-assisted extraction (MAE) evolved as a novel procedure for the extraction of bioactives and nutraceuticals. With higher extraction efficiency, this process was noted to consume less time and energy, and interestingly, the bioactive compound's functionality has not degraded. In this chapter, MAE's potential as an eco-friendly technique was explored. To improve its efficiency, microwave-assisted extraction has been coupled with conventional techniques. Accessible data stress the significance of various hybrid techniques: microwave/conventional ones for the extraction of bioactive compounds. Information about this topic could help students and scientific researchers who are engaged in chemical engineering, chemistry, and meat technology communities to approach the complex theme of microwave-assisted extraction.

Key words Microwave-assisted extraction, Bioactive compounds, Nutraceuticals, Eco-friendly, Hybrid technique extraction

1 Introduction

Nowadays, extraction and production of various bioactive compounds have gained momentum as there is increased demand for herbal products globally. This is because they are safe, possess various biological activities as compared to synthetic formulations, and are cost-effective. To provide higher recoveries and greater reproducibility, the chief tendencies in analyte extraction was to reduce solvent and energy consumption and to provide higher

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recoveries and greater reproducibility. The implementation of traditional extraction methods may be more time-consuming, requiring large volumes of solvents, and are principally related with the degradation of heat-sensitive compounds [1]. In this sense, to overcome the drawbacks of these extraction methods, it is decisive to explore contemporary techniques. Green solvent extraction methods have been developed, including microwave-assisted extraction (MAE), which has gained a wide attention due to its various advantages, namely, a reduced solvent consumption, a shorter operation time, and an enhanced recovery yield [2]. Numerous comparative studies have shown that MAE allowed better performance in terms of compound recoveries [3–7]. By applying MAE, plentiful kinds of compounds, comprising essential oils, antioxidants, pigments, and other organic compounds have been successfully isolated from various natural plant resources [5, 8–10]. These previous studies displayed that MAE could be a promising alternative to conventional extraction of plant pigments (carotenoids and anthocyanins) [9, 11], polyphenols, polysaccharides [12–14], essential oils [5, 15, 16], and proteins and lipids [17]. Compared to maceration and Soxhlet extraction, it was established that MAE approach was more effective [9]. It was found that the obtained extracts using MAE had a greater concentration of volatile terpenoids (α- and β-pinene) [18]. Microwaves have electric and magnetic fields since they are electromagnetic devices [19]. MAE employs microwave radiation to heat solvents and facilitates the transfer of target compounds from the sample matrix to the extractant by inducing polar molecules, ions, and dipoles movement and rotation [20]. Microwaves can penetrate the sample and incite cell molecules to absorb their energy, resulting in an increase in temperature and pressure. Then, it facilitates the cell's rupture and the reachability of the components into the solvent solution [21]. In fact, there are several categories of MAE such as solvent-free MAE (SFM), focused-MAE (FMAE), ionic liquid-based MAE (ILMAE), ultrasonic MAE (UMAE), microwave hydro-distillation (MHD), microwave hydro-diffusion and gravity (MHG), and microwave-assisted subcritical extraction (MASE) [12, 22–27]. The extraction techniques employed for this purpose are highly dependent on the following factors: microwave power, time, solvent, sample-to-solvent ratio, temperature, and matrix characteristics [28]. The aim of this chapter is to spotlight the versatility of MAE in the recovery of bioactive compounds and nutraceuticals from various types of vegetal materials using different techniques of MAE with a special focus on the factors that could influence its processing and efficiency.

2 Mechanism of MAE Process

Microwaves have electric and magnetic fields since they are electromagnetic devices. These fields lead to a heating effect via two mechanisms, dipolar rotation and ionic conduction [19].

(i) Dipolar rotation refers to the phenomenon that occurs when molecules with uneven distribution of charge, known as a dipole moment, attempt to align themselves with the alternating electric field produced by microwaves. The oscillation of these dipolar molecules results in collisions with other molecules in the surrounding medium, which then generates heat. This process happens quickly and repeatedly, making it an efficient way to convert electromagnetic energy to thermal energy [29].

On the other hand, (ii) *ionic conduction* is defined as a process that occurs when charged particles, such as ions and electrons, move through a medium in response to an electric field produced by microwaves. This movement or migration generates friction between the ions and the medium, which results in the generation of heat. The degree of heat generated by this process depends on factors such as the strength of the electric field and the conductivity of the medium [20].

The relative contribution of these two mechanisms to the overall heating of the sample is largely dictated by temperature. Specifically, as the contribution of dipole rotation decreases, the temperature of the sample increases, while the contribution of ionic conduction increases. It means that if a sample contains both polar molecules and ions, then as it is heated by microwave energy, the heating will initially be dominated by dipole rotation. The relative contribution of these two mechanisms also depends on the mobility and concentration of the ions within the sample [20]. Consequently, these mechanisms induced the destruction of hydrogen bonds in organic molecules, which increased solvent penetration into the plant matrix [30] and thereby dissolution of extractable molecules. In fact, microwave-assisted extraction (MAE) may be summarized in two main steps as Chemat et al. [21] and Vinatoru et al. [20] mentioned:

1. Penetration of the solvent into the plant cell by diffusion: Initially, in the equilibrium phase, solubilization and partitioning phenomena come into play, which leads to the detachment of the substrate from the particle's outer surface at a relatively consistent rate. This step is then followed by an intermediate phase of transition to diffusion, where resistance to mass transfer begins to appear at the interface between the solid and liquid phases. During this period, a mass transfer occurs through convection and diffusion.

2. Cell rupture and leaching out of cell components into the solvent solution: When the dielectric loss tangent of the plant cell is higher than that of the solvent, the vegetal material could absorb more electrical energy, which can lead to an increase in the temperature of the plant material and subsequently an increase in cell pressure. As the extract is removed mainly through diffusion, it is typically regarded as the limiting step of the process.

Throughout the extraction process, a variety of forces and relationships can be observed, including dispersion forces, interstitial diffusion, driving forces, and chemical interactions, with the persistence and strength of these phenomena often closely linked to the solvent's properties, such as solubilization power, solubility in water, purity, and polarity [21].

3 Factors Affecting MAE

Numerous types of compounds, including essential oils, antioxidants, pigments, and other organic compounds, have been effectively isolated from various natural plant resources using MAE [5, 8–10]. The extraction techniques employed for this purpose are highly dependent on the following factors (Fig. 1).

3.1 Microwave Power

In a reaction medium, the amount of electromagnetic energy that is transformed into heat depends, in a practical sense, on the permittivity and permeability of the chemical compounds or mixture, as well as the intensity of the electromagnetic field [21].

Microwaves belong to the electromagnetic spectrum, and their frequency range spans from 300 MHz (classified as radio radiation) up to 300 GHz. In scientific research, two specific frequencies are usually utilized: 2.45 GHz, which is commonly used in laboratory equipment, and 915 MHz, which is mostly used in industrial equipment [20, 31]. It has been shown that the range of power delivered was between 60 and 960 W (Table 1). Increasing

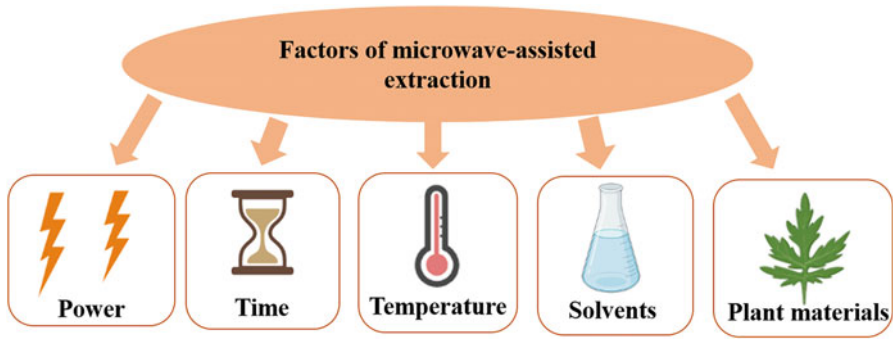

Fig. 1 Factors affecting MAE

Table 1
MAE extraction of different analytes from various vegetal materials

Materials	Solvent	Ratio	Temperature	Time	Power	Analyte	References
Mango peel	Diluted acidic solution (distilled H_2O, with 2 M HCl)	2 g/600 mL	–	3 min	700 W	Pectin: 1485.78 mg/mol	[58]
Sapindus mukorossi	40% Ethanol	1 g/19 mL	–	13 min	425 W	Saponins yield: 280.55 mg/g	[59]
Red cabbage	50% Ethanol	1 g/20 mL	–	10 min	600 W	Total monomeric anthocyanin content: 220 mg cyanidin-3-glycoside/L	[10]
Melissa officinalis L	25.9% Ethanol	300 mg/10 mL	–	29 min	400 W	Rosmarinic acid: 49.5 mg RA/g of DW	[60]
Melastoma sanguineum fruit	31.33% Ethanol	0.5 g/30 mL	52.24 °C	45 min	500 W	TPC: 39.02 mg GAE/g of DW	[55]
Chaya (*Cnidoscolus aconitifolius* Mill.) leaves	99.8% Ethanol	1 g/20 mL	140 °C	10 min	850 W	TPC: 57 mg GAE/g	[61]
Tomato pericarps	100% Ethanol	45 g/L	180 °C	20 min	200 W	TPC: 66.8 mg GAE1/g TFC: 3.89 mg CE/g	[62]
Hibiscus sabdariffa	60% Ethanol	3 g/30 mL	164 °C	22 min	850 W	Flavonoids yield: 55%	[63]
Banana peel	Water	2 g/100 mL	–	6 min	960 W	TPC: 50.55 mg GAE/g DM	[64]
Apple dust by-product	40% Ethanol		–	15 min	600 W	TPC: 36.99 mg GAE/g DW	[65]
Grape juice waste	Water	1 g/18.43 mL	–	2.23 min	428 W	Total monomeric anthocyanin yield: 1.32 mg/g	[66]
Onion peels	Choline chloride: urea: water (1:2:4)	1 g/54.97 mL	–	15.03 min	100 W	TPC: 80.45 mg GAE/g DW	[67]

(continued)

Table 1
(continued)

Materials	Solvent	Ratio	Temperature	Time	Power	Analyte	References
Pomelo peels	10 mmol/L [HO3S (CH2)4 mim] HSO4 aqueous solution	1 g/26 mL	–	15 min	331 W	Yield of pectin: 291.60 mg/g Yield of naringin: 8.38 mg/g	[68]
Spruce (*Picea abies*) bark	50% Ethanol	–	–	4 min	300 W	Catechin: 1.91 mg/g of VM Epicatechin: 6.72 mg/g of VM α-pinene: 2198.33 μg/g of VM β-pinene: 2997.66 μg/g of VM Camphene: 71.6 μg/g of VM Myrcene: 105.46 μg/g of VM Limonene: 101.07 μg/g of VM	[18]
Quercus cerris bark extracts	Water	10 g/ 200 mL	–	30 min	850 W	TPC: 382.26 mg GAE/g of DW Total tannin content: 49.14%	[4]
Quercus cerris bark extracts	70% Ethanol	10 g/ 200 mL	–	18 min	650 W	TPC: 403.73 mg GAE/g of DW Total tannin content: 45.68%	[4]
Avocado *(Persea americana Mill.)* seeds	58% Ethanol	1 g/20 mL	–	5 min	400 W	TPC: 82.36 mg GAE/g TFC: 19.93 mg QE/g	[69]
Olive tree leaves	Solvent-free	5 g	–	2 min	250 W	TPC: 2.480 ppm Oleuropein yield: 0.060 ppm	[70]
Coleus aromaticus leaves	Solvent-free	500 g	–	30 min	500 W	Essential oil yield: 0.54%	[16]
Lagenaria siceraria fruit	Solvent-free	20 g	–	60 s	480 W	TPC: 288.9 mg GAE/g DW TFC: 214.1 mg rutin equivalent/g DW	[22]
Pegagan (*Centella Asiatica* L.) leaves	Solvent-free	20 g	–	60 min	450 W	Yield: 4.5474%	[1]

Cinnamomum camphora leaves	Solvent-free	200 g	–	23 min	580 W	Essential oil yield: 3.51%	[5]
Black carrot pomace	19.8% Ethanol	1 g/ 9.3 mL	–	9.8 min	348.07 W	Total anthocyanin content: 753.4 mg/L TPC: 264.9 mg GAE/100 mL	[47]
Black currant	60% Ethanol	1 g/28.3 mL	–	3 min	551 W	Flavonols: 2323.3 μg/g Anthocyanins: 473.7 μg/g	[71]
Patchouli (*Pogostemon cablin*)	Water	0.15 g/mL	–	51.61 min	634.024 W	Patchouli oil yield: 2.8%	[72]
Ananas comosus peel	H_2SO_4 (0.5 N, pH 1.83)	1 g/10 mL	80 °C	2.5 min	600 W	Yield of pectin: 2.43% Anhydrouronic acid content: 54.61%	[73]
Brown seaweeds	0.1 M HCl containing 2 M $CaCl_2$	1 g/25 mL	–	1 min	560 W	Sugar content: 0.47 mg glucose equivalent/mg extract	[74]
Eucalyptus globulus bark	Water	1 g/48.5 mL	141 °C	15 s	500 W	TPC: 350 mg of GAE/g of extract	[75]
Curcuma longa	Choline chloride-citric acid (1:1)	1 g/20ML	–	6 min	60 W	Curcuminoids yield: 89.87 mg/g	[53]
Pineapple peel waste	50% Ethanol	1 g/20 mL	–	40 min	600 W	TPC: 14.188 mg GAE/g DW TFC: 12.925 mg QE/g DW Total Tannin Content: 371.25 mg TAE/g DW Protein content: 59.49 mg BSAE/g DW	[76]

Db dry basis, *DM* dry matter, *DW* dry weight, *VM* vegetal material, *TPC* total phenolic content, *TFC* total flavonoids content

microwave power from 180 to 300 W gave rise to high trans-lycopene and β-carotene contents [32]. Additionally, Vu et al. [33] reported that high phenolic compounds were acquired when the power was raised from 240 to 960 W. These radiations led to the disruption of cell wall and then cell membrane followed by the release of bioactive compounds [2]. Hence, it permitted the gradual efflux of plant exudates. Consequently, it affected the yield of bioactive compounds [33]. However, increasing microwave power beyond 300 W decreased the contents of trans-lycopene and β-carotene [32]. In fact, there is a risk of losing/degrading plant bioactive components caused by the usage of higher power with extended exposure [34]. Thus, microwave power and irradiation time are completely opposed [2].

Nisca et al. [4] found that the TPC of *Quercus cerris* bark extracts was improved when the microwave power was increased. The variation of microwave power from 200 to 850 W had a significant influence on the content of the total phenolics and tannins.

Microwaves could be influenced by different types of materials, which can be categorized as follows [35]:

Opaque materials: Conductive materials that possess free electrons, like metals, have a tendency to reflect electromagnetic waves, preventing them from passing through. These materials are utilized in constructing microwave applicants [36].

Transparent materials: Materials that have a low dielectric loss or insulating properties, such as ceramics and glass, only absorb and reflect electromagnetic waves to a minimal extent, thus enabling microwaves to pass through with minimal attenuation [37]. These materials are typically used in reactors that are placed within microwave applicants.

3.2 Extraction Time

Heating time is an essential factor that affects the extraction mechanism [19]. Furthermore, increasing time augmented extraction efficiency and quantity of analytes [2]. However, it also increases the possibility of the degradation of thermolabile compounds. For the extraction of different kinds of plant matrices, various time scales are required [38]. Sometimes, 60 s−60 min are required for the maximum production of analytes in MAE (Table 1). Several findings agreed that extended exposure to microwave irradiation resulted in a greater release of phenolic compounds from *Hibiscus sabdariffa*, *Aegle marmelos*, and *Myrtus communis* leaves [39–41]. The amount of polyphenols extracted increased by over 30% when the extraction time was changed from 5 to 29.5 min [42]. Whereas, Belwal et al. [8] reported that 2 min was suitable for MAE of alkaloids, berberine and palmatine with concentrations of 46.38 mg/g DW and 20.54 mg/g DW, respectively. In the investigation of Kadi et al. [9], it was noticed that the total

carotenoid content from *Citrus clementine* peel reached its maximum (186.55 μg/g DM) at 7.64 min; then the compounds of interest easily decomposed as a result of the long exposure. According to Samanta et al. [3], MAE technique has exhibited an increase in yield of 70% compared to other traditional methods, resulting in higher TFC and TPC levels, which was accomplished in a shorter time frame.

3.3 Extraction Solvent and Sample-to-Solvent Ratio

The proper selection of extraction solvent is one of the key elements that have a significant impact on MAE's total output. The properties of the solvent (nature, polarity, solubilization, purity, etc.) are other variables that affect the process of extraction [2]. Several forces, such as the physicochemical interactions, may be strongly associated with the properties of the solvent [21]. Typically, when a solvent has a high dielectric constant and dielectric loss, it tends to have a greater ability to absorb microwave energy. Essentially, the capacity of the solvent to absorb this energy increases as the dielectric constant and dielectric loss increase, resulting in a faster heating rate for the solvent relative to the plant material [43]. In order to measure relative solubility, the Hildebrand solubility parameter scale is commonly used. This parameter is the measure of the cohesive energy between the solvent and the matrix in a solution [20]. In fact, δ is linked to the hydrogen-bonding capacity, the polarity, and the dispersion coefficient. As a result, there is a significant correlation between the polarity and the Hildebrand solubility parameter (δ) [20]. The solvent volume is also a crucial component to take into account since it needs to be sufficient to ensure that the entire sample is submerged in the solvent during the whole irradiation process [21]. In addition, the selected solvent must be more selective toward the target analyte than the other matrix constituents [44]. MAE can use water as a solvent for both polar and nonpolar compounds, making it an attractive option for more environmentally friendly extraction processes [43]. By blending different solvents, it is possible to alter the properties of the solvent, resulting in differing selectivity for various compounds [43]. In fact, the use of an ethanol-water mixture as an extraction solvent facilitated the recovery of TPC due to its high dielectric constant and dissipation factor, which enables the effective absorption of microwave energy. Furthermore, this solvent mixture increased the penetration of the solvent into the sample matrix, thereby enhancing heating efficiency. These results are in line with the findings of Nisca et al. [4], who carried out an optimization of extraction parameters for aqueous and hydroalcoholic extractions, and the total polyphenolic and tannin contents were determined. The results indicated that the optimal extraction conditions for aqueous (30 min at 850 W) and hydroalcoholic (18 min at 650 W) extracts were different. The hydroalcoholic bark extract exhibited a higher yield of total polyphenols (403.73 mg GAE/g

dried weight) compared to the aqueous extract that had a lower level of tannins. Hence, MAE may yield higher levels of polyphenols when mixtures of solvents are used due to the increased solubility of target compounds and better penetration into the plant material [4].

In addition, optimizing the solvent-to-solid ratio (S/S) is a crucial parameter. It is necessary to ensure that the solvent volume is adequate to fully immerse the sample during the entire irradiation process, particularly when dealing with a matrix that may expand during the extraction process [45]. As the ratio of sample to solvent increased from 2:100 g/mL to 8:100 g/mL, the TPC decreased by nearly 50% [33]. The reason coming behind these results is that when a smaller sample ratio is utilized, the plant material swells, which leads to an increase in the contact area between the plant matrix and the solvent [46]. While Kumar et al. [47] stated that the retrieval of phenolic content showed a notable upsurge when the ratio of solvent to solid (S/S) increased, achieving the maximum at 20:1 and declining afterward at higher levels. Hence, 20:1 was considered the best ratio for subsequent process parameters. The range of 10–30 (v/w) was then used to refine the process parameters through response surface methodology (RSM) optimization. Choosing the appropriate ratios can have significant difficulty in MAE. This decision is typically influenced by various factors, including the solvent's selectivity toward the target analyte, its ability to absorb microwaves, its interaction with the sample matrix, and its compatibility with the analytical methods used downstream [48].

3.4 Matrix Characteristics

MAE depends on the type of plant utilized as a raw material, which can produce a variety of valuable compounds as well as the composition of the chosen plant tissue/cell or part of the plant that incorporates different kinds of components. Moreover, bioactive and nutraceutical compounds are typically bound to other compounds within plant structures, such as polyphenols, which are uncommonly found in their unbound form. Instead, they are often covalently linked to the plant cell wall, may exist in waxes or on the exterior surfaces of plant organs, and are linked via glycosides [49]. For example, plants' leaves contain high content of phenols [50]. Moreover, Rahmawatii et al. [1] reported that the yield of extraction from Pegagan (*Centella Asiatica* L.) leaves is significantly impacted by the quantity of material present. Also, the particle size of the plant matrix is an important factor [51]. Several studies reported that the extraction yield improved when matrix particle size decreased [51–53]. According to Poureini et al. [52], the apigenin extraction yield was enhanced by decreasing the particle size from 0.75 to 0.10 mm. A similar trend was depicted by Patil et al. [53]. These authors detected an optimal range of particle size

between 0.150 and 0.212 μm for curcuminoids extraction. Thus, fine matrix particles promote the deeper penetration of the microwave [38]. This improvement can be explained by the raise of the contact area between the solvent and the plant matrix. In fact, reducing the size of the particles decreased the distance that the solvent needs to diffuse, which in turn accelerated the rate of mass transfer between the solute and the solvent [54].

The level of moisture in the sample matrix affects the extraction efficiency. The presence of water can increase the microwave-absorbing ability of the sample and facilitate heating by making the extractant more polar [40]. By utilizing, the RSM combined with a Box–Behnken design to extract essential oil from *Cinnamomum camphora* leaves by MAE, Liu et al. [5] showed that the optimal moisture content was found to be 60%.

3.5 Temperature

Increasing temperature until a certain level increases the extraction yield of some bioactive compounds. In fact, Zhao et al. [55] mentioned that the impact of extraction temperatures was examined while holding other variables constant (30% ethanol, 30 mL/g, 30 min, 500 W). As the temperature increased (20–50 °C), there was a significant increase in TPC value from 23.88 to 34.46 mg GAE/g DW. Elevated temperatures have the potential to accelerate intermolecular interactions and molecular movement, which may lead to increased solubility of solutes in the solvent [43]. Therefore, the TPC value depicted a marked improvement as the extraction temperature increased from 40 to 50 °C, but subsequently decreased as the temperature continued to rise. Kapoore et al. [48] noticed that an increase in temperature resulted in decreased yields of phycoerythrin, which confirmed that thermal damage can occur over 40 °C, while carotenoids degrade at temperatures over 60 °C. The extraction of phenolic acids from green tea was found to be more effective at a temperature of 100 °C, whereas the flavanols and flavonols, which are sensitive to high temperatures, displayed better extraction yield at a lower temperature of 80 °C [56]. In addition, according to these authors, the extraction of quercetin glycosides is more efficient at 80 °C compared to 100 °C. This finding could be explained by the fact that quercetin glycosides have an oxidizable catechol ring (B-ring), making them more susceptible to thermal degradation than kaempferol glycosides, which have a mono-phenolic B-ring. Moreover, when extracted using MAE at a temperature of 90 °C, the sulfated polysaccharides obtained from *Ulva prolifera* using an acidic solvent (0.05 M HCl) exhibited superior water- and oil-holding capacities. Conversely, the polysaccharides extracted at a higher temperature of 150 °C demonstrated the best foaming properties as well as the highest antioxidant and pancreatic lipase inhibition activities [57].

4 Some Techniques of MAE

Coupling MAE with other extraction methods was proved to have potential applications due to the popular effectiveness of MAE (Fig. 2).

4.1 Solvent-Free MAE (SFM)

This method involves using microwaves to perform a dry distillation on a fresh matrix, without adding any water or organic solvent. The process involves heating of the raw material with water to release the essential oil from glands, which is then carried away by steam produced from the matrix water. The distillate, made up of water and essential oil, is continuously condensed using a cooling system placed outside the microwave oven. Any excess water is returned inside the balloon to maintain the appropriate humidity level of the matrix [21]. This straightforward approach allows the efficient extraction of essential oils without the use of additional solvents. The findings of Iftikhar et al. [22] revealed that the SFM technique, which does not require solvents and utilizes a power setting of 480 W and a duration of 60 s, is an efficient approach for extracting antioxidant compounds from gourd fruit. Likewise, Wei et al. [15] mentioned that the combination of SFM and moisture regulation was a potent approach to extract essential oil from deciduous leaves of *C. longepaniculatum*. In addition, compared to conventional hydro-distillation, Liu et al. [5] depicted that SFM exhibited better performance in terms of various parameters such as extraction efficiency (3.51% in 23 min vs. 3.35% in 240 min), initial extraction rate (3.3772 vs. 0.1868), extraction rate constant (0.3002 vs. 0.0152), extraction capacity (3.67% vs. 3.51%), oxygenated compound content (83.93% vs. 74.81%), energy

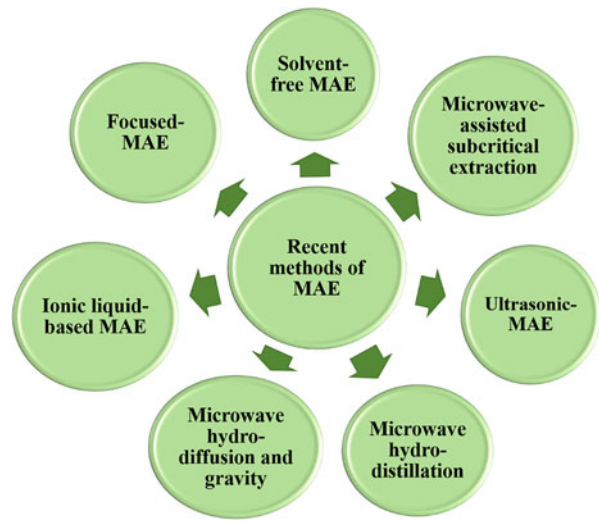

Fig. 2 Recent methods of MAE

consumption (0.22 kW h vs. 4 kW h), and environmental impact (177.87 g CO_2 vs. 3200 g CO_2). These findings demonstrated that SFM is a time-efficient, energy-saving, and eco-friendly method that has great potential as a preferable alternative to traditional methods for the extraction of essential oil from *C. camphora* leaves. Hence, SFM was suggested as it showed higher yield and volumetric mass transfer coefficient, greater proportions of oxygen compounds, lower electricity consumption, and less CO_2 emission and water waste compared to conventional hydro-distillation [77].

4.2 Focused-MAE (FMAE)

In FMAE, the sample is placed in an opened vessel and a specific area is exposed to microwave radiation. This system functions at atmospheric pressure [78], while the maximum temperature is provided by the boiling point of the extraction solvent utilized [79]. Hence, it can be used for the extraction of thermolabile components. In addition, this system is composed of a condenser that is set on the top of the vessel to avoid the loss of volatile compounds [80]. Therefore, the microwave reactor's configuration influences heat production in the reaction medium [21]. In fact, using a central composite experimental design, the extraction of betulinic acid from *Zizyphus joazeiro* was optimized by employing FMAE technology. This analysis confirms the applicability of FMAE extraction as a speedy, environmentally friendly, and effective extraction method. As per the study, the optimal temperature and duration of extraction are 70 °C and 15 min, respectively [81]. By directing microwave energy to a small region of the sample [82], FMAE attained a more efficient extraction with less energy consumption [81].

4.3 Ionic Liquid-Based MAE (ILMAE)

The merging of microwave irradiation with ionic liquids (ILs) presents an influential approach toward achieving high effectiveness and less harmful procedures. ILs are liquefied salts that retain their liquid form at low temperatures, frequently under 100 °C, and they are comprised of organic cations and organic or inorganic anions [83]. In comparison to conventional organic solvents, ILs exhibit numerous distinguishing characteristics, such as trivial vapor pressure, elevated temperature stability, low volatility, chemical stability, wide electrochemical stability window, and ionic conductivity [84]. Thus, ILs are considered as outstanding microwave absorbers. As stated by Li et al. [85], ILMAE can enhance the extraction efficiency of total biflavonoids in a shorter time and with a reduced amount of solvents, compared to conventional soxhlet extraction. In fact, according to Motlagh et al. [6], when compared to the commonly used conventional Soxhlet method, the protein yield obtained under optimized conditions using choline acetate ([Ch][Ac])-mediated water-based MAE technique (26.35%) is much higher, indicating the superiority of this approach over the Soxhlet extraction method (0.63%). The results indicated that [Ch]

[Ac]-based MAE of proteins from *Nannochloropsis oceanica is* superior to the conventional method of Soxhlet methods, making it a highly recommended innovative approach for protein separation. The findings of this investigation had the potential to aid in identifying and utilizing important biochemical compounds from microalgae through IL-based MAE, leading to the development of new and enhanced bioproduct technologies. Furthermore, the major obstacle in astaxanthin extraction is the effective disruption of the thick and resistant cell walls of *Haematococcus pluvialis*. However, the utilization of biocompatible protic ionic liquids-based microwave-assisted liquid-solid extraction (PILs-MALSE) has resolved this issue in the study of Fan et al. [7]. One of the protic ionic liquids, ethanolammonium caproate (EAC), has the ability to dissolve mannan, which is one of the key components of the cell walls of *Haematococcus pluvialis*. Fan et al. [7] elucidated that compared to traditional extraction techniques, the PILs-MALSE method is more efficient for extracting astaxanthin. In addition, the effectiveness of MAE combined with protic ionic liquids (PILs) in obtaining phycobiliproteins was assessed by Rodrigues et al. [24]. The most efficient solvent was a combination of 2-hydroxyethylammonium acetate (2-HEAA) and 2-hydroxyethylammonium formate (2-HEAF), using a process conducted at 62 W power and a ratio of 10 mL/g. These authors found that MAE using PILs could be effective for the extraction of phycobiliproteins with concentrations of 33 g L^{-1}, 0.84 g L^{-1}, and 0.41 g L^{-1} of allophycocyanin, phycocyanin, and phycoerythrin, respectively. Guo et al. [86] successfully utilized ILMAE to extract 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols from ginger. The highest extraction yields of gingerols and shogaols were obtained using 1-decyl-3-methylimidazolium bromide [C10MIM]Br. The efficiency of extraction of these compounds was greatly influenced by the alkyl chain length and anions of cations. Compared to methanol-based MAE (MMAE), ILMAE not only produced higher extraction yields but also had a shorter extraction time. The same tendency was observed when 1-octyl-3-methylimidazolium acetate [Omim][OAc] was added to the water as the extracting solvent. This IL has been demonstrated to enhance lipid extraction ability when exposed to microwave irradiation. [Omim][OAc] at 2.5% allowed the extraction of 19.2% of lipids [87]. Despite many accomplishments, there is still insufficient knowledge about the precise mechanism linking microwaves, ILs, and nanostructures or polymers [83].

4.4 Ultrasonic MAE (UMAE)

The concurrent utilization of ultrasonic and microwave extraction methods led to a notably greater quantity of bioactive compounds in comparison to the traditional decoction extraction techniques, highlighting the synergistic effects of these novel approaches (Kwansang et al. 2022). As reported by Sun et al. [12], the

UMAE approach, developed for the extraction of polysaccharides from *Camptotheca acuminata* fruit (CAFP), yielded higher amounts in a shorter duration than traditional hot water extraction (HWE) techniques. The CAFP yield obtained via UMAE was 6.81%, which is 1.04 times greater than the yield from HWE. Furthermore, the UMAE approach required a shorter extraction time of 20 min compared to HWE, which necessitated 120 min of extraction. In this line, Zheng et al. [88] indicated that the extraction yield of polysaccharides from *Trametes orientalis* was 7.52%. In addition, the investigation of Zhang et al. [13] revealed that the research results showed that UMAE had a greater degree of damage to the cell wall of *Dictyophora indusiata* polysaccharides (DPs) and better antioxidant capacity. The UMAE method had the highest polysaccharides yield, which was related to the conformational stretching and degradation avoidance of DPs in the higher molecular weight components under the simultaneous action of microwave and ultrasonic. In the same line, according to Shen et al. [89], *Panax notoginseng* polysaccharides (PNPS) were extracted using UMAE, and RSM was utilized to optimize the extraction parameters. The ideal extraction conditions were identified as 10 min ultrasonic duration, 50 W ultrasonic power, 4 min microwave duration, and 540 W microwave power, which led to a PNPS extraction rate of 11.03%. Characterization of PNPS using SEM, FTIR, and UV-Vis indicated that the UMAE method did not cause any degradation to the polysaccharides.

The findings of Xu et al. [90] suggested that UMAE resulted in a greater yield of pectin compared to conventional heating. The ideal parameters for UMAE were identified as an extraction temperature of 86 °C, an extraction time of 29 min, and a solid-liquid ratio of 1:48 (w/v), resulting in a maximum pectin yield of 21.5%. The study of Lu et al. [91] aimed to optimize the extraction of various degrees of polymerized oligosaccharides from lotus seeds using UMAE through RSM. The results demonstrated that the optimal UMAE conditions for lotus seed oligosaccharides were determined to be an extraction time of 325 s, a liquid-solid ratio of 10.00 mL/g, ultrasonic power of 300.46 W, and microwave power of 250 W. These conditions resulted in a 76.59% increase in the yield of total oligosaccharides, with a 17.47% increase in trisaccharides and a 27.21% increase in tetrasaccharides. Additionally, the extraction time was significantly reduced compared to traditional hot water, ultrasonic-assisted, and MAE methods. Regarding oil extraction, Wang et al. [92] found that the utilization of UMAE resulted in higher oil yield and greater superoxide radical scavenging activity of white pepper compared to MAE and UAE, indicating its superior efficiency as an extraction method. In terms of the specific components extracted, UMAE generally yielded more monoterpenes and sesquiterpenes than MAE and UAE. Therefore, UMAE has the potential to become a prominent

eco-friendly method for extracting essential oil from *P. nigrum* due to its maximum extraction yields and short extraction time. Furthermore, Yu et al. [93] employed the UMAE method to extract polyphenols, flavonoids, triterpenoids, and vitamin C from *Clinacanthus nutans*. The optimized conditions for the extraction process included the use of distilled water, a solid-liquid ratio of 1:55 g/mL, an irradiation power of 90 W, and an extraction cycle lasting 75 s. With the previously described conditions, the extraction yields of polyphenols, flavonoids, triterpenoids, and vitamin C were found to be 8.893, 25.936, 16.789, and 0.166 mg/g, respectively. These findings suggest that UMAE is a highly efficient method for the extraction of bioactive substances from *Clinacanthus nutans*. Overall, these findings suggest that UMAE has the potential to be a more efficient and effective technique for bioactive compounds extraction as compared to traditional methods.

4.5 Microwave Hydro-distillation (MHD)

The mechanism of MHD produces heat by absorbing microwave radiation from the plant material, resulting in the evaporation of essential oil components [21]. The condensed vapor is then collected as a liquid that consists of essential oil. Newer research has examined the possibility of MHD for extracting essential oils from diverse aromatic plants [94–97]. Elyemni et al. [97] compared the efficiency of two extraction methods, microwave-assisted hydro-distillation (MHD) and Clevenger hydro-distillation (CH), for obtaining essential oils from *Rosmarinus officinalis* L. MAH only requires 20 min to obtain the same yield of essential oils that takes CH 180 min. Furthermore, the quality of the essential oil was enhanced by an increase of 1.14% in oxygenates. Additionally, as reported by Megawati et al. [95], the utilization of microwave-assisted hydro-distillation (MHD) for the extraction of mace essential oil was proven to be more effective than hydro-distillation (HD). MHD resulted in 8.62% essential oils in just 42 min, whereas HD only produced 7.03% in 73 min. In addition, MHD consumed less energy (756 kJ) compared to HD (1095 kJ). As the power input is increased, a higher yield of essential oil is obtained. At 300, 600, and 800 W within 10 min, yields obtained were 2.68, 4.56, and 5.41%, respectively, while at 20 min, yields obtained were 5.13, 7.39, and 6.83%. The findings of Mollaei et al. [25] devoted that MHD may be a useful technique for extracting essential oil from *F. angulata* due to its ability to decrease the distillation duration, minimize energy usage, and enhance biological properties when compared to the HD method.

4.6 Microwave Hydro-diffusion and Gravity (MHG)

The MHG apparatus is essentially a microwave unit that operates similarly to a standard commercial model. It utilizes a combination of microwave radiation and the force of gravity at ambient pressure to perform extraction from fresh plant material [98]. As described

by De Castro and Peinado et al. [54], this method involved the introducing of a matrix into a reactor inside a microwave oven. Microwaves trigger the heating of the water present in the matrix, leading to the elimination of cells that contain essential oil. The hydro-diffusion process took place, wherein both the essential oil and internal water of the matrix were released from the plant's interior to its exterior. To condense the distillate, a cooling system situated outside the microwave oven is employed and then collected under gravity. In addition, MHG extraction has demonstrated optimal outcomes in diverse applications aimed at extracting active compounds, including antioxidant molecules from aromatic plants such as *Cuminum cyminum*, *Cytisus scoparius*, *Brassica rapa*, *Quercus robur*, and *Pleurotus ostreatus* [17, 26, 99]. As detailed by Benmoussa et al. [99], the use of MHG resulted in a higher yield of essential oil (1.579%) in a shorter time (16 min vs. 150 min required for HD) as compared to conventional hydro-distillation (HD) (1.550%). Additionally, the MHG technique requires less electricity, emits less carbon dioxide, and generates less wastewater. Examination via GC-MS affirmed that the quality of cumin essential oils procured by MHG and HD were comparable. According to this investigation, the effectiveness of MHG and ethanolic solid-liquid extraction methods were compared using various plant sources, that is, *Cytisus scoparius*, *Brassica rapa*, *Quercus robur*, and *Pleurotus ostreatus* [17] (Table 2). The results illustrated that MHG technology is suitable for generating extracts with interesting antioxidant characteristics.

4.7 Microwave-Assisted Subcritical Extraction (MASE)

By combining microwave-assisted and subcritical water extraction (MASE), Moirangthem et al. [11] found that anthocyanins could be extracted from straw with an 85.8% efficiency when exposed to a temperature of 90 °C for 5 min. This combination was also observed to have superior antioxidant activity as compared to a conventional methanol extract. Both the straw and bran's microwave extracts did not exhibit any noticeable cytotoxicity on Jurkat cells in vitro.

In addition, Yang et al. [27] used MASE to extract steviol glycosides from *Stevia rebaudiana* (Bertoni). The results depicted that the yields of major steviol glycoside, including rebaudioside A and stevioside, and rebaudioside C were comparable to those obtained by the conventional extraction method that used 70% ethanol under sonication for 45 min, within just 1 min of reaching subcritical water conditions at 140 °C. This method can be a cost-effective alternative for producing high-purity steviol glycoside sweeteners. Moreover, Cai et al. [28] attempted to enhance oil extraction efficiency by utilizing seed pretreatments, such as microwave assistance, to increase subcritical extraction fluid penetration without affecting the physicochemical properties of the phytochemicals in oilseeds. These authors established that using

Table 2
MAE methods for bioactive compounds extraction

Methods	Compounds	Plants	References
Solvent-free MAE	TPC/TFC Essential oil TPC Essential oil	*Lagenaria siceraria* fruit *Coleus aromaticus* *Centella asiatica L.* *Cinnamomum longepaniculatum* leaves	[22] [16] [1] [15]
FMAE (Focused MAE)	Betulinic acid Sesamol	*Zizyphus joazeiro* bark Sesame seed	[81] [23]
ILMAE (Ionic liquid-based MAE)	Lipids and eicosapentaenoic acid Flavonoids Quercetin Heneicos-1-ene Phycobiliproteins Amentoflavone/hinokiflavone Astaxanthin	*Nannochloropsis oceanica* Passion fruit and mango leaves *Nothopanax scutellarium* leaves Coriander foliage *Arthrospira platensis* *Selaginella sinensis* *Haematococcus pluvialis*	[101] [102] [103] [104] [24] [105] [7]
Ultrasonic MAE	Polysaccharides Caffeic and ferulic acids Polysaccharides α-mangostin Polysaccharides	*Trametes orientalis* *Clinacanthus nutans* *Camptotheca acuminata* fruits *Garcinia mangostana* *Pericarp* *Dictyophora indusiata*	[88] [106] [12] [107] [13]
Microwave hydro-distillation (MHD)	Essential oils	*Rosmarinus officinalis* L. Clove (*Syzgium aromaticum*) stem *Myristicae arillus* *Ferulago angulate* *Pelargonium graveolens*	[97] [96] [95] [25] [94]
Microwave hydro-diffusion and gravity (MHG)	Essential oil Phenolic compounds Carotenoid/phenolic/lipid/protein contents	*Cuminum cyminum* L. Blackberries (*Rubus* spp.) *Cytisus scoparius;Brassica rapa; Quercus robur; Pleurotus ostreatus*	[99] [26] [17]
Microwave-assisted subcritical extraction	Anthocyanins Steviol glycosides Oil Berberine hydrochloride	*Manipur black* rice *Stevia rebaudiana* leaves Tigernut *Berberis aristata* roots	[11] [27] [28] [100]

microwaving as a pretreatment prior to subcritical extraction is an uncomplicated technique to improve oil output while producing high-quality oil. In fact, Cai et al. [28] employed subcritical n-butane extraction with the aid of microwave pretreatment to extract tigernut oil from tigernut meal. Microwaving (560 W, 6 min) substantially increased subcritical extraction efficiency. The most oil was obtained by subcritical extraction of tigernut oil at a temperature of 52 °C for 32 min after three extraction cycles, with a

liquid-solid ratio of 3.62 kg/(kg of tigernut meal), resulting in a maximum yield (24.736%) of tigernut oil. The oil's high-quality attributes, including a ratio of unsaturated to saturated fatty acids of 4.68 UFA/SFA, low acid value (3.30 mg KOH/g oil), low peroxide value (0.28 meq.kg^{-1}), and a predominance of oleic acid, were identified. Additionally, an optimization of the conditions for extracting berberine from *Berberis aristata* roots by microwave-assisted subcritical water extraction (MASCW) was conducted by Manikyam et al. [100]. This method was employed to extract berberine at temperatures ranging from 110 to 170 °C using various combinations of five subcritical parameters. The experimental data concentration of berberine (223.82 μg/mL) was found to be significantly correlated under specific subcritical parameters. This new hybrid extraction technique can be an eco-friendly option for producing high-purity compounds [27].

5 Conclusion

The MAE has minimized energy, time, and solvent consumption, which makes it a sustainable technology. Furthermore, it has been coupled with other extraction techniques such as ultrasonic, hydrodistillation, and subcritical extraction. These combinations had considerably reduced energy and time, and improved bioactive compound yields. The versatility of MAE in the recovery of bioactive and nutraceuticals from various kinds of vegetal materials could be applied in advanced practical applications in food and pharmaceutical fields.

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Chapter 5

Ultrasound-Assisted Extraction for Food, Pharmacy, and Biotech Industries

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Abstract

Extraction is an important step in the route of phytochemical processing for the discovery of bioactive constituents from plant materials. The extraction technique plays a significant role in the yield, chemical structure, and bioactivity of the extracts. Ultrasound-assisted extraction (UAE) has been widely applied as a novel, green, and rapidly developing extraction method suitable for upscaling and improving the extraction efficiency of bioactive compounds. UAE has substantial advantages such as low consumption of solvent and energy, simplification of manipulation and work-up, high extraction yield and purity of the final product, and fewer damages of active compounds, over the conventional Soxhlet extraction and cold maceration. UAE can also provide the opportunity for enhanced extraction of heat-sensitive bioactive and food components at lower processing temperatures. Ultrasound-assisted herbal extracts exhibit higher anticancer, antimicrobial, and antidiabetic activities than extracts prepared through conventional methods. Nowadays most of the industry-based extractions are carried out using UAE as full extraction can be completed in minutes with high reproducibility. UAE of herbal, oil, protein, polysaccharide, bioactive compounds, such as phenolics, flavonoids, and natural colors, which have importance in food, pharmaceutical, and allied industries, is discussed here.

Key words Ultrasound-assisted extraction, Green extraction, Cavitation effect, Bioactive compounds, Industrial application

1 Introduction

Extraction is the first step toward isolating and identifying the desired natural products from the raw materials. Plants are the richest source of bioactive compounds that serve many purposes. Extracts of plant origin play an important role as natural additives or industrial inputs to food, pharmaceutical, cosmetic, perfumery, and other allied industries. Plant extracts have been widely used in traditional medicine, perfumes, food flavor, and preservatives from ancient times. The medicinal properties of the plant extracts result from the synergistic effect of the bioactive compounds present in them. These bioactive compounds, commonly known as secondary

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metabolites, exhibit potent antioxidant, antidiabetic, anti-inflammatory, and anticancer activities [1]. Great interest has been paid to extract these valuable compounds from different parts of the plants using various extraction techniques. According to the extraction principle, extraction methods include the distillation method, solvent extraction, pressing, and sublimation. Solvent extraction is the most extensively used method for the preparation of plant extracts [2]. Preparation of the plant extract using solvent extraction involves: (a) the solvent penetrates into the plant matrix; (b) the solute dissolves in the solvent; (c) the solute diffused out of the solid plant matrix; (d) the extracted solutes are collected and concentrated. Conventional solvent extraction techniques, such as cold maceration, Soxhlet extraction, and percolation, have been used for a long time. The use of only organic solvents, consumption of large quantities of solvents, and longer extraction time are some disadvantages associated with these conventional methods. Some modern extraction techniques, such as ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, and supercritical fluid extraction, have been developed and extensively used for the natural products extraction. These modern extraction methods offer many advantages such as lower consumption of solvent, shorter extraction time, and improved yields of natural extracts over the conventional methods [3].

The use of ultrasound technology has been considered as an innovative and one of the most promising technologies of the twenty-first century. Ultrasonic waves have been extensively used in the fields of pharmaceuticals, chemistry, cosmetics, and nourishment since after the Second World War. Nowadays it is hard to find a production line for food, pharmaceuticals, and biotech-related industries that does not use ultrasonic waves. In general, use of conventional extraction techniques is less efficient for industrial applications. For example, conventional extraction techniques have certain scientific and technological drawbacks to overcome: often demanding up to 50% of investments in a new plant and more than 70% of total process energy used in food industries [4]. The use of efficient, enhanced, and greener extraction techniques such as ultrasonic-assisted extraction (UAE) proved to be more prominent in such industrial extraction processes. Indeed, such modern techniques are ideal to compensate for the increasing energy costs and to reduce greenhouse gas emissions. UAE has substantial advantages such as low consumption of solvent and energy, simplification of manipulation and work-up, high extraction yield and purity of the final product, and fewer damages of active compounds over the conventional methods.

For achieving the objective of green extraction of natural products, ultrasound is recognized as a key technology. "Green extraction" includes the extraction process which reduces energy consumption, allows use of alternative solvents and renewable

natural products. It also refers to use of a minimal quantity of solvent, reducing risk of environmental pollution and waste, while ensuring a safe and high-quality extract [5]. Driven by the green extraction goals, food and pharmaceutical industries have paid special interest in using ultrasound technology for extraction purposes. Higher product yield, low maintenance cost, and shorter processing time are the green impacts of UAE on extraction. The application of UAE as a green technology for the extraction of different classes of food components such as pigments, aromas, antioxidants, and other organic compounds are also found in food and pharma industries. Technologically, integration of ultrasound with already available extraction techniques is quite simple. Due to this fact, the implementation of UAE for improving the extraction efficiency of flavonoids, flavonols, polyphenols, sugars, minerals, and carotenoids in juices is commonly seen for industrial purposes [6, 7].

Using ultrasound technology, the extraction of organic compounds from natural matrices such as plants and seeds can be improved significantly. The intensification of ultrasonic solid-liquid extraction is attributed to the physical and chemical effects caused by high-power ultrasound; more specifically the cavitation phenomenon. The cavitation effects of ultrasound provide better penetration of solvent into cellular materials and thereby results in higher mass transfer than other extraction methods. Cavitation phenomena also lead to high shear forces in the media. When high-power ultrasound is applied to the surface of the materials, the asymmetric collapse of the cavitation bubbles occurs. This results in rapid micro-jetting toward the surface of the solid matrix. This effect causes surface peeling, erosion, breakdown of cell walls, and the exudation of cellular contents. It facilitates the extraction of various compounds from natural products [8]. Ultrasound-induced cavitation bubbles provide hydrophobic surfaces in the extraction liquid and thereby net hydrophobic character of the extraction medium increases. Hence extraction of polar components into a hydrophilic aqueous media becomes possible through UAE. It also reduces the need for normally undesirable hydrophobic and strongly polar solvents [9].

Phytochemicals have less toxicity risk compared to their synthetic counterparts. Plant-based foods, including fruits, vegetables, nuts, grains, seeds, and legumes, may contain hundreds of different phytochemicals. Phytochemicals-rich functional food can provide necessary health benefits when consumed regularly through diets. Therefore, the extraction of bioactive phytochemicals from natural resources is still a hot topic of research [10]. Using UAE, biologically active compounds from *Salvia officinalis* were extracted efficiently with some 60% of the target compounds within 2 h at ambient temperature [11]. UAE of tea solids from dried tea leaves using water as a solvent increased the yield up to 20% at 60 °C in

comparison to the thermal extraction at 100 °C [12]. Due to such benefits, it has been suggested that UAE could be used to prepare a variety of herbal extracts for the phytopharmaceutical industry.

Applications of UAE in biotech industries are very common at present time. For example, Ana et al. reported UAE to be a reliable technology for genipin extraction from *Genipa americana*. Genipin is an excellent natural cross-linker for proteins, gelatin, collagen, and chitosan cross-linking. Due to its low acute toxicity, genipin is used as a regulating agent for drug delivery. Ana et al. reported that ultrasound improves the liberation of genipin and proteins from the plant matrix as well as enhances the formation of polyelectrolyte complexes. They found eight times improved yield of non-cross-linked genipin while using UAE at 10 °C for 15 min [13]. This also explains the effectiveness of UAE at low temperatures. Lutein is the major carotenoid found in the human eye. Lutein improves age-related macular disease that causes blindness and vision impairment. Egg yolk is one of the major sources of lutein in our foods. UAE improved the yield of lutein when saponified solvent was used [14]. Adam et al. reported a solvent-free UAE method for lipid recovery from fresh *Nannochloropsis oculata* microalgae biomass. With a simple and scalable pre-industrial device, they proved the better efficiency of UAE for lipid recovery than conventional (e.g., Bligh and Dyer) methods [15]. Reports of UAE of various bioactive compounds, such as pigments, polysaccharides, lipids, acids, and antioxidants, from different microorganisms, are found in the literature [4].

2 Ultrasonic System for Extraction

Ultrasonic cleaning baths (Fig. 1a) and the more powerful probe systems (Fig. 1b) are the two most common ultrasound equipment used for extraction. For large volumes of fluids, ultrasound baths or continuous or recycled-flow sonoreactors are commonly used. While an ultrasound horn with the tip submerged in the fluid is sufficient for small extraction volumes. Ultrasonic devices for extraction have also been manufactured by several companies on an industrial scale. These devices usually have a volume capacity of 30–1000 L with a power range of 500–16,000 W [14]. Usually, laboratory ultrasonic systems are used in batch mode, whereas flow mode is often used in operating industrial systems.

2.1 Bath Systems

1. The basic component of an ultrasonic bath consists of a tank, an electronic generator, and a transducer. The generator supplies electrical power to the transducer. Generally, multiple transducers are attached to the tank on sides by epoxy resin in today's practice. A thermostatically controlled heater can also be provided for the bath.

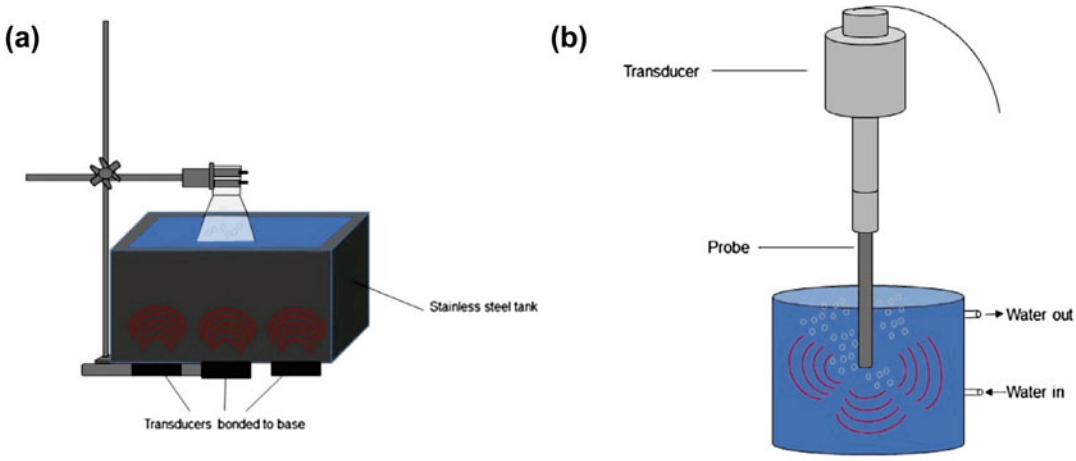

Fig. 1 Commonly used ultrasonic extraction systems (**a**) ultrasound bath (**b**) ultrasound probe. (Reproduced from Chemat et al. [4] with permission from Elsevier)

2. For industrial use, units for stuffing and de-stuffing, rinsing, washing, drying, solvent recovery, transport, etc., can be incorporated with these systems.
3. The vessel with raw matrix and extractant is placed inside the bath for extraction to be carried out. Suitable ultrasonic power must be provided for achieving cavitation within the vessel. The frequency is the key factor for determining the cavitation and the energy released during the extraction process. For commercial purposes, most of the ultrasonic baths operate at a single frequency; such as 40–45 kHz during extraction [16].

2.2 Probe Systems

1. Probe systems comprise a generator, transducer, and horn. The generator supplies alternating electrical frequencies, usually 20 kHz. It allows the regulation of the amplitude and also controls the cooling of the medium. The horn may consist of an upper horn made of titanium and a detachable horn. Although the detachable horn can be made of different materials, titanium alloy is often used.
2. The extraction operation is carried out by immersing the probe into the vessel with raw material and extractant. The total volume of the mixture to be sonicated governs the choice of the detachable horn to be used. Sonochemical effects were found to be stronger near the tip, as most of the energy transmitted to the medium occurs near the tip. Stepped probes are commonly used, whereas the use of spiral probes is also seen [17].
3. Probes for industrial use can be developed with different boosters to adjust the ultrasound energy transmitted to the medium. For continuous sonication, a closed flow cell is used and thereby a homogeneous sonication can be achieved.

2.3 Probes Versus Baths

1. The extraction process in the probe is a type of direct sonication, while the process involving baths is regarded as indirect sonication.
2. The system arrangement in the probe enables amplification and concentration of ultrasonic energy. This in turn enhances the sonication effectiveness compared to bath systems, sometimes up to 100 times greater.
3. In probe systems, higher energy is transmitted to the medium giving rise to better performance. This helps to minimize the extraction time than in bath systems. This shortening of extraction time increases the reproducibility of probe systems.
4. Sonication in a probe leads to the generation of heat and hence this system may not be suitable for the recovery of volatile compounds. So, bath systems are generally preferable for the extraction of materials containing volatile compounds.
5. Cross-contamination is easier with ultrasonic probes than with ultrasonic baths [16].

2.4 Online UAE System

Online UAE is a considerably faster approach for carrying out extraction. It comprises an open system where fresh solvent continuously flows through the sample. It leads to the displacement of mass transfer equilibria toward the solubilization of analyzing substances into the liquid media. The extract is then passed to the continuous manifold for online analysis. The analytical procedure involves preconcentration, derivatization, filtration, and finally detection of the active compounds by various available techniques (e.g., gas chromatography mass spectrum) [18].

Advantages of online UAE

1. Sample contamination, as well as losses of analytes, is minimum in online UAE.
2. Less consumption of reagents is observed in online UAE as compared to offline UAE.
3. Centrifugation or the filtration step is not required in online UAE to separate the liquid phase from the solid particles. Thereby sample preparation can be completed in a short duration of time [19].

3 Extraction Mechanism

3.1 Basic Principle

Ultrasound is sound waves having frequencies greater than 20 kHz, which is higher than the upper audible limit of human hearing. Usually, the output source of ultrasound is a vibrating body that causes vibration of the surrounding medium and then the transfer of energy from the ultrasonic wave to the neighboring particles

occurs. Physical parameters including power, frequency, and amplitude play vital roles in the ultrasonic process. The energy level at which ultrasonic waves propagate through the medium is expressed in terms of ultrasound power (W), ultrasound intensity (W/cm^2), or acoustic energy density (W/cm^3) [20].

3.2 UAE Mechanism

1. In UAE, ultrasound frequency in the range of 20–50 kHz is normally used. In extracting media, ultrasound can produce vibration, cavitation, crushing, mixing, and some comprehensive effect. These effects can break the cell wall and results in successful extraction of natural product components from plant matrices [21, 22].
2. Normally it is believed that cavitation effects, thermal effects, and mechanical effects have a substantial influence on UAE. The combined results of these effects lead to the destruction of the cell wall, reduction in particle size, and enhance the reaction rate through the mass transfer of the cell wall. In general, these effects do not cause any changes in the structure and functions of the extracts [23, 24].

3.3 Cavitation Effect in UAE

1. Cavitation effect in ultrasound is a unique physical phenomenon and was discovered first by Thornycroft [25].
2. This effect arises due to the propagation of strong ultrasonic waves in liquid media.
3. The ultrasound cavitation arises from the negative pressure, which is a distinct critical value when the liquid can be dragged out to form a vapor or gas cavity in the local domain. This critical negative pressure that drags the liquid out is termed as the cavitation threshold.
4. There are two types of cavitation effect—(a) stable cavitation and (b) transient cavitation.
   (a) Ultrasonic wave propagates longitudinally in the liquid phase and its alternating pressure is stretched and compressed periodically in the liquid. Due to the constant compression and decompression cycle, the cavitation bubbles produced have different frequencies of the sound wave pulse. This phenomenon is called "stable cavitation."
   (b) The cavitation bubbles so produced keep on growing and after reaching their critical value (high temperature, 5000 K and high pressure, 100 MPa), a cavitation zone will be generated. This cavitation phenomenon is known as "transient cavitation" [26, 27].
5. Cavitation phenomenon leads to high shear forces in the media. The collapse of cavitation bubbles on the surface of a solid matrix results in micro-jetting and creates several effects

such as surface peeling, erosion, and particle breakdown. Additionally, the crumbling of cavitation bubbles in a liquid media lead to macro-turbulences and micro-mixing.

6. Cavitation effect can alter the chemical processes in the system. In some cases, the cavitation effect causes the formation of various free radicals. For example, hydroxy free radicals are primarily generated when water is used as a solvent. The generated free radicals can modify other molecules such as proteins present in the extract. Thus, it is important to control parameters such as power and external temperature that affect the generation of radicals.
7. Depending on the extraction parameters and nature of the natural products matrix, some physical effects of ultrasound have been identified. These physical effects are fragmentation, erosion, sonocapillary effect, sonoporation, local shear stress, and detexturation as described by Chemat et al. [4]. These influences of ultrasound can be attributed to the cavitation effect (Fig. 2).

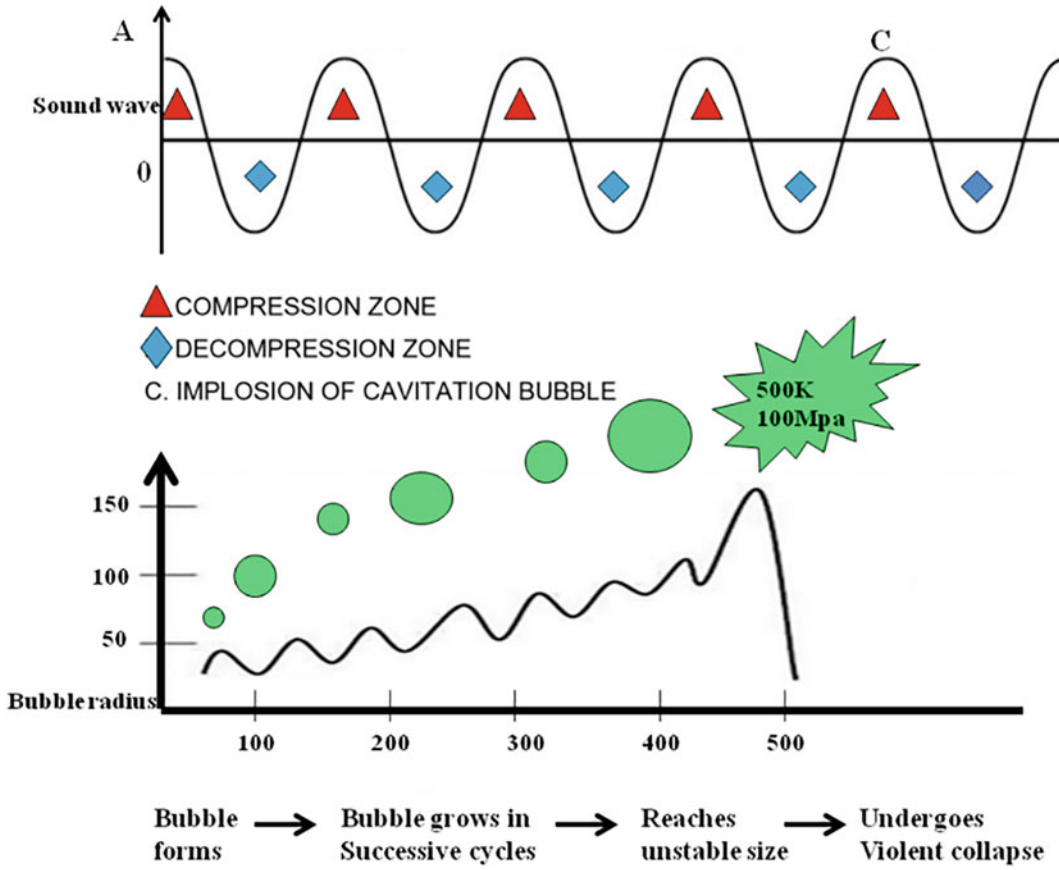

Fig. 2 Scheme of ultrasound cavitation physical process. (Reproduced from Wen et al. [26] with permission from Elsevier)

3.4 Factors Effecting UAE

Several parameters effect the process of UAE. To obtain a high extraction efficacy, the study of these parameters is of great importance. However, it is not always necessary to consider that yield is the only objective of the extraction process. Moreover, minimal use of non-renewable resources along with low energy consumption should be taken into account. The influencing factors in UAE include the shape and size of the ultrasonic reactor device, extraction process parameters, solvent type, temperature, and matrix particle size [26].

3.4.1 Shape and Size of the Ultrasonic Reactor Device

When contacts a solid surface, ultrasonic waves are reflected. In the UAE extraction using an ultrasonic bath, the shape of the reaction vessel is crucial. In order to attain a minimum reflection of waves the use of a flat bottom vessel such as a conical flask would be the best choice. A vessel with minimal thickness should be used to reduce attenuation. It is necessary to compute the optimal reactor dimension and the location of the transducer-related elements to obtain maximum energy transfer to the fluid. In the case of ultrasonic probes, a rapid decrease in intensity is detected both axially and radially. Thus, it is preferred to keep a minimal space between the ultrasonic probe and the wall of the container. The probe should not touch the container to avoid damage to the material [28].

3.4.2 Extraction Process Parameters: Power and Frequency

Several studies reveal that high ultrasonic power induces greater shear forces that cause major alterations in materials. However, in the food industry optimization of this parameter is usually performed. Optimization is done to achieve the best results using minimum ultrasonic power [29]. Generally, improvement in yield and composition of the extract in UAE can be achieved by increasing the ultrasound power.

Ultrasound frequency may also influence the extraction process and have to be optimized. The optimal choice of ultrasound frequency enables to obtain the desired cavitation effect. An increase in the ultrasound frequency or intensity in the extraction process leads to the gradual decrease of the liquid cavitation bubbles. The high frequency suppresses the compression-rarefaction cycles, which is more difficult to induce acoustic cavitation bubbles due to the short period. While the low frequency may reduce the formation of transient cavitation bubbles [30]. The effect of frequency not only influences the cavitation bubble size but is also related to the mass transfer in the extraction process. Ultrasound frequencies in the range of 20–100 kHz are commonly used in UAE. Thus, optimization of the frequency is important for ultrasound extraction [4].

3.4.3 Solvent

Selection of the most appropriate solvent for extracting the analytes from the sample matrix is an important step of UAE. Ultrasound extraction is largely influenced by the amount and type of solvent used, concentration, and the ratio of solvent and solute. These factors contribute to the transmission of ultrasonic energy. The initiation of cavitation is affected by the physical properties of the solvent such as viscosity, surface tension, and vapor pressure [31]. The collapse of the cavitation bubble is more intense in solvents with low vapor pressure compared to that of the solvents with high vapor pressure. So, a solvent with low vapor pressure is generally preferred in UAE [4].

3.4.4 Temperature and Time

Sonochemical effects are favored by low temperatures as the cavitation effect is prominent at lower extraction temperatures. Although high temperature enhances solvent diffusion rates, it could lead to the degradation of thermolabile compounds. At higher temperatures, the collapse of cavitation bubbles may be reduced and consequently, sonochemical effects are less effective. Therefore, the temperature of the solvent should be controlled within a suitable range to obtain the highest yield of the target compounds. Similarly, the extraction time should be optimized. Short time may result in incomplete extraction and very long extraction time may induce undesirable reactions and less selective extractions [32].

3.4.5 Particle Size of the Matrix

UAE yield may also be effected by the size of the matrix particles. Usually, the reduction of particle size increases the surface contact area. When particles of the matrix are small enough, most cell walls are disrupted by ultrasound, thus facilitating better extraction [32].

4 Application of UAE in the Perspectives of Food, Pharmacy, and Biotech Industries

4.1 UAE of Fruits, Vegetables, and Their By-Products

Fruits and vegetables are the sources of a wide range of secondary metabolites present in their pulp, seed, peel, and bark. Various phytochemicals, antioxidants, lipids, pigments, aromas, and other molecules of industrial importance have been extracted from fruits and vegetables. Laboratory-based extraction of these molecules can be integrated further for their applications in the food, pharmaceuticals, biotech, and cosmetics industries. Extensive use of UAE for the extraction of such bioactive components has been observed. UAE has substantial advantages for extracting such compounds from fruit and vegetable matrices over conventional extraction techniques. Using UAE, bioactive component extraction can be performed in very short time, at a relatively low temperature, with optimal energy and solvent requirement. As a non-thermal extraction technique, UAE facilitates the retention of the functionality of bioactive compounds [33].

In the food industry antioxidants are broadly used for the preservation and extension of the shelf life of foodstuffs [34]. Antioxidants are substances that may prevent or delay the oxidation processes taking place in our bodies. Antioxidants can prevent cell damage by reacting with the oxidizing species formed due to different biochemical reactions inside the body. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have some negative side effects on human health. As such, there is a visible interest in the food, pharmaceutical, and allied industries for the isolation and use of natural food grade antioxidants as alternatives for synthetic antioxidants [35]. In this context, UAE serves as an influential tool for the extraction of such antioxidants mainly from plant matrices. The concentration of the extracted antioxidant compounds depends on the studied food matrix and the applied extraction method [36]. Many researchers have reported that the application of UAE significantly increases the antioxidant capacity of the prepared extract in comparison to conventional extraction techniques. Table 1 represents the reports of using UAE, in which improvement of the residual antioxidant capacities of food and by-product extracts have been found.

In the same way, Mayra et al. reported the improved antioxidant capacity of hybrid mandarin peels using ultrasound extraction [44]. Recently, Selahvarzi et al. claimed significant enhancement in the antioxidant activities of ultrasound extracted phenolic compounds from pomegranate and orange peels [45]. The antioxidant capacity of the common bean (*Phaseolus vulgaris* L.) was found to be increased when UAE was used [46].

The extraction of valuable bioactive compounds from fruit and by-products of vegetable processing has been carried out over the last decade. Fruits such as apple, orange, grapefruit, pineapple, and chokeberry, and vegetables such as carrots, potatoes, onions, and asparagus are processed to yield different value-added products. By-products of such fruits and vegetables, namely, skin, seed, rind, and pomace contain valuable phytochemicals and secondary metabolites. In some cases, it was found that these non-consumable by-products contain a higher concentration of bioactive compounds than their edible parts [47, 48]. For example, 50% of the total phenolics in potato are present only on the potato peel [49]. Ultrasound extraction using high-intensity sound waves is found to be effective for the extraction of bioactive components from fruit and vegetable waste and by-products. UAE of different classes of valuable compounds from the viewpoint of their importance in food, pharmaceutical, and biotech industries are discussed here.

4.1.1 Extraction of Pectin

Pectin is an important heteropolysaccharide with multiple applications in food, pharmaceutical, and other industries. Pectin is used in jellies, jams, frozen foods, and edible films. It is also used as a fat and sugar replacement in low-calorie foods. In pharmaceutical

Table 1
Enhancing effect of UAE on antioxidant capacity compared to conventional extraction methods

Plant matrix	Treatment conditions of UAE				Solvent ratio	Increase in antioxidant capacity	Reference
	Power (W)	Frequency (kHz)	Temperature (°C)	Time (min)			
Apple pomace	150	25	10–40	5–55	Water	30%	[37]
Orange peel	50–150	25	10–40	60	Ethanol: water (20–80:80–20)	30% (DPPH) 40% (ORAC)	[38]
Black chokeberry	100	30.8	20–80	250	Ethanol: water (50:50)	85%	[39]
Pomegranate peel	2.4–59.2	20	25	2–90	Ethanol: water (30–70:70–30)	22–24% (per antioxidant yield) 160% (FRAP)	[40]
Rice bran	140	35	40–60	15–45	Ethanol: water (50–90:50–10)	80% (FRAP) 229% (DPPH)	[41]
Grape by-products		35	70	60	Ethanol: water (50:50)	100%	[42]
Origanum majorana L.	1500	20	15–35	5–15	Methanol: water (80:20)	106% (FRAP)	[43]

industries, pectin is mainly used to reduce cholesterol levels in the blood and gastrointestinal disorders [50]. Pectin is present in many fruits, vegetables, and their by-products. In present times, pectin is mostly extracted through UAE. Reports suggest that above 25% yield of pectin was obtained from grape pomace, pomegranate peel, orange peel, grapefruit peel, eggplant peel, and tomato waste using ultrasonic extraction. The quality of ultrasound extracted pectin was found to be better than pectin extracted through heat-based conventional process [51]. UAE of pectin from various fruit and vegetable by-products reported by different research *workers* is presented in Table 2.

4.1.2 Extraction of Polysaccharides and Other Functional Compounds

Polysaccharides are composed of several smaller monosaccharides and are the most abundant carbohydrates found in foods. They are commonly used in food industries as functional ingredients. Polysaccharides possess various bioactivities including immunomodulatory, antioxidant, antitumor, and hypoglycemic activities [33]. Hence, they are equally important materials for the pharmaceutical and biotech industries. In earlier days, conventional heat-based extraction methods were used to extract hemicellulose, cellulose, and xyloglucan components from plant and food by-products. UAE of these components is more convenient than the conventional techniques. Ultrasound extraction of these compounds accelerates the extraction process and preserves their structural and molecular properties. UAE enhanced the extractability of hemicellulose from sugarcane bagasse. Destruction of cell walls and cleavage of links between hemicellulose and lignin by ultrasound improved the extraction [66]. In the ultrasonic aqueous extraction of polysaccharides from edible fungus, glycan-chitin complexes with high average molecular weight are extracted. Whereas, no such compounds were obtained in simple hot water extraction. These compounds are reported to have antitumor activities [67]. UAE of water-soluble polysaccharides from litchi seed and their bioactivity studies were reported by Chen et al. [68]. Raja et al. extracted antioxidant polysaccharides from the stem of *Trapa quadrispinosa* stem ultrasonically. The extracted polysaccharides exhibited higher total antioxidant capacity compared to the hot water extracted polysaccharides in terms of ABTS and DPPH radical scavenging activity [69]. High-pressure UAE of polysaccharides from *Hovenia dulcis* having antioxidant and hypoglycemic properties was reported by Yang et al. [70].

4.1.3 Extraction of Polyphenols

UAE is a promising tool for the extraction of phenolic compounds for laboratory and industrial purposes. Phenolic compounds are widely used in wine industries for providing characteristic color and flavor in wine samples. Most of the plant-derived polyphenolic compounds exhibit antioxidant properties and can serve as natural

Table 2
UAE of pectin from different fruit and vegetable by-products

By-product/ waste	The optimum condition for UAE					Solvent	Yield (%)	References
	Power (W)	Frequency (kHz)	Temperature (°C)	Time (min)	pH			
Grapefruit peel	200	24	70	25	1.5	Acidified water (0.1 N HCl)	17.92	[52]
Grapefruit peel	12.56	20	67	28	1.5	0.5 M hydrochloric acid	27.34	[53]
Grapefruit peel	0.4	20	60	60	1.5	0.5 M hydrochloric acid	18.11	[53]
Grape pomace	140	37	75	60	2	Citric acid solution	32.3	[54]
Pomegranate peel	130	20	61.9	28	1.2	Citric acid solution	23.87	[55]
Banana peel	323	20		27	3.2	Citric acid solution	8.99	[56]
Mango peel	497.4	20	85	10		1 M nitric acid solution	8.6	[57]
Passion fruit peel	664	20	85	10	2	1 M nitric acid solution	12.67	[58]
Orange peel	150	20		10	1.5	Citric acid solution	28.07	[59]
Jackfruit peel	130	20	60	24	1.6	Citric acid solution	14.5	[60]
Eggplant peel	50 W			30	1.5	Acidified water	33.64	[61]
Tomato waste		37	60	15		Ammonium oxalate (16 g/L)	35.7	[62]
Sunflower head	375	20		32	3.2	Citric acid solution	8.89	[63]
Durian rind			85	240	2.3	1 N HCl solution	8.8	[64]
Lemon peel[a]			60–75	15–45		0.5 M nitric acid solution	10.11	[65]
Mandarin peel[a]			60–75	15–45		0.5 M nitric acid solution	11.29	[65]
Kiwi peel[a]			60–75	15–45		0.5 M nitric acid solution	17.3	[65]

[a]Optimum condition is not reported

antioxidants. Phenolic compounds are able to inhibit in vitro oxidation of low-density lipoproteins and hence associated with reduced risk of cardiovascular diseases. Polyphenolics also exhibit anti-inflammatory, anti-ulcer, antimutagenic, and anticarcinogenic properties. Due to the significant benefits of phenolic compounds on human health, pharmaceutical industries have paid particular interest in their use [71]. As UAE is a rapid process, consumes a minimal quantity of solvent, and has a comparable recovery rate, it is useful for industrial- or large-scale extraction of polyphenolic compounds.

Selection of the appropriate solvent systems is a crucial step in UAE to obtain a high yield of phenolic compounds. Methanol was found to be the most appropriate solvent to extract phenolic compounds from the leaves of *Centaurea* species [72]. While in most cases, ethanol was proved to be the best solvent for ultrasonic extraction of phenolic compounds; such as from mango peel [73], lime peel [74], grape seed [75], and olive leaf [76].

4.1.4 Extraction of Flavonoids

Flavonoids are a class of polyphenolic plant secondary metabolites. These are found in a variety of fruits and vegetables. Flavonoids exhibit a broad spectrum of health-promoting effects and are considered an indispensable component in a variety of pharmaceutical, nutraceutical, and medicinal applications. Flavonoids are important for human health because of their antioxidative, antibacterial, anti-inflammatory, anticarcinogenic, and antimutagenic properties. They can modulate key cellular enzyme functions. Applications of flavonoids in the food industry include the preservation of foods and the making of dietary supplements. Flavonoids are also used to provide color and flavor of food products [77].

UAE of valuable flavonoids has been carried out to enhance the extraction yield and their recovery. Some of the UAE-extracted flavonoids having pharmaceutical importance are listed in Table 3.

All the cited flavonoid compounds have different biological activities as reported by the respective authors. It can be concluded that flavonoids are associated with significant medicinal properties and may be used as natural antioxidants in the pharmaceutical and food industries. UAE provides a high yield of flavonoid compounds than the conventional extraction methods.

4.1.5 UAE of Anthocyanins and Carotenoids

Anthocyanins and carotenoids are the natural color pigments present mostly in fruits and vegetables. In present times, natural colors are very demanding in the food industry. Increasing use of anthocyanins as natural colorants mostly in food products and beverages has been observed. Anthocyanins are also used as bioactive compounds in pharmaceutical industries which further boosted their market requirements. Similarly, carotenoids have been used as bioactive compounds in pharmaceutical and allied industries.

Table 3
UAE of bioactive flavonoids

Plant	Extraction parameters			Reported flavonoids	References
	Power (W)	Temperature (°C)	Time (min)		
Olive leaves (*Olea europaea*)	180–270	50	30	Luteolin-4′-O-glucoside Apigenin-7-O-glucoside Rutin	[78]
Ocimum tenuiflorum leaves	50 W	40	30	Apigenin Luteolin Rutin	[79]
Curry leaves (*Murraya koenigii* L.)	80–150	40–80	20	Catechin Myricetin Quercetin	[80]
Moringa oleifera leaves	88	30	20	Catechin Hyperoside Kaempferol-3-O-rutinoside	[81]
Euonymus alatus		90	15	Catechin Dihydromyricetin	[82]
Lycium barbarum L. fruits		50	90	Myricetin Morin Rutin	[83]
Chestnut peels (*Eleocharis dulcis*)	50	35–55	120	Luteolin Eriodictyol Fisetin	[84]

Carotenoids are the colorants found in special food items [85, 86]. Pinela et al. reported the ultrasonic-assisted extraction of anthocyanins from *Hibiscus sabdariffa* calyces. They obtained higher levels of anthocyanin than previously reported literature. They also suggested that extracted anthocyanins may be used as a natural food colorant in industrial applications [87]. Ochoa et al. carried out the extraction of anthocyanin from purple yam (*Dioscorea alata*) by both UAE and conventional methods. According to their findings, UAE proved to be the feasible technique for obtaining anthocyanin rich extracts from purple yam [88]. UAE enhanced the concentration of corn carotenoids as reported by Jun et al. They got a 3.6 times higher concentration of corn carotenoids with UAE than extraction carried out without ultrasound assistance. Their report also determined that UAE did not affect the structure of the corn carotenoids [89].

4.1.6 Extraction of Edible Oils

UAE has been recognized as a valuable extraction method in the edible oil industries to improve yield and reduce the duration of extraction time. Rapid extraction of oil from soybeans using high frequency ultrasound waves was reported in the literature. The yield was significantly improved in UAE [90]. Garcia and Castro reported that ultrasound-assisted Soxhlet extraction is an effective method for the extraction of a higher amount of oil from raw matrices. They used this combined methodology and extracted a higher amount of total fat content from soybean, sunflower, and rape seeds than the Soxhlet extraction performed alone [91]. The benefit of ultrasonic pre-treatment of oleaginous seeds before oil extraction was also reported. Ultrasonic pre-treatment of the almond and apricot seeds prior to oil extraction provided better yield with a reduction in extraction time [92]. Application of UAE on the extraction of oils from different food by-products, such as rice bran, soybean germ, and papaya seed, has also been reported.

4.1.7 Extraction of Proteins

Protein extraction is very much associated with pharmaceutical industries. All the big pharmaceutical industries now develop and sell recombinant protein as a drug. UAE has been becoming the most well-known technology for the extraction of proteins for the last four decades from laboratory-based research to industrial applications. In 1982, Moulton and Wang studied both batch and continuous ultrasonic extraction of soybean protein. They reported that both ultrasonic processes gave a higher yield of protein than the conventional methods. The continuous ultrasonic extraction provided 54% and 23% higher yields of protein than the batch process for aqueous and alkaline extraction, respectively. These findings are very helpful for the industrial use of the UAE at that time. Later on, many researchers reported the ultrasound extraction of protein from various natural sources. Some of them also optimized the extraction process for industrial applications. UAE also influences the functional properties of the protein. In a recent study, Wang et al. carried out the ultrasound-assisted alkaline extraction of pea protein and found significant improvements in the functional properties of extracted protein. They reported that the extracted protein was associated with increased solubility, high water retention, gel formation, and emulsifying capacity and stability. The biological activities of the protein were also found to be enhanced. The hydroxyl radical scavenging capacity of the extracted protein was doubled as reported by them [93]. Improvement in the techno-functional characteristics of bitter melon seed protein was reported by Naik et al. They employed pulsed ultrasound-assisted extraction for this purpose. Their findings are also similar to those described by Wang et al. Pulsed UAE is an innovative green technology used to extract and recover specific active compounds from biological materials [94].

4.2 UAE for Phytopharmaceutical Extraction

In the phytopharmaceutical extraction industry, UAE is considered a key extraction technique for the preparation of a wide range of herbal extracts. Such extracts contain various compounds of interest such as antioxidants, aromas, capsaicinoids, and volatile compounds. Herbal extracts have extensive pharmaceutical applications [4].

4.2.1 Extracts with Anticancer Properties

Anticancer activities of herbal extracts are mainly associated with the polyphenolic compounds they possess. Polyphenols have the ability to prevent cancer by diminishing or hindering the harmful effects of free radicals on cells through their scavenging properties. Their diverse chemical structures allow them in neutralizing free radicals produced in the body. Polyphenolic compounds can prevent oxidative stress to a level that does not harm cellular DNA and regulatory protein synthesis metabolism. The importance of UAE in cancer studies is mainly due to its capability in preserving the anticarcinogenic properties of polyphenols in plant extracts. Activities of ultrasound extracted polyphenols have been studied extensively in both in vitro and in vivo systems [95]. Polyphenols extracted with ultrasound assistance from *Thelephora ganbajun* exhibited superior antiproliferative activities toward human breast (MCF-7), liver (HepG2), lung (A549), and colon (HT-29) cancer cells compared to Soxhlet and maceration extraction methods [96]. Polyphenolic compounds extracted from *Trapa quadrispinosa* Roxb. showed effective antitumor action against Hela, HepG2, and U251 tumor cells [97]. Berkani et al. used UAE to prepare the extract of the herbal plant *Zizyphus lotus*. The extract was found to contain a high amount of total phenolic and flavonoid contents. The herbal extract significantly inhibited cell proliferation on the MCF-7 and HepG2 tumor cell lines with IC_{50} values of <0.05 and 3 ± 0.55 mg/mL, respectively [98]. Extracts of *Ocimum basilicum* and *Ocimum canum* were found to reduce the proliferation of human breast cancer cells MCF-7, when extracts were prepared ultrasonically as reported by Koolamchal et al. [99]. Thus, UAE can preserve and enhance the anticarcinogenic and antitumor activities of polyphenol extracts.

4.2.2 Extracts with Antimicrobial Properties

Many ultrasound-assisted herbal extracts containing polyphenolic compounds reported to have antimicrobial properties. As the use of toxic organic solvents is less common in UAE, many extraction protocols have accepted the use of UAE as a treatment method in this regard. It has been observed that UAE not only improve the extraction efficiency of the extracts but also improves their biological activities [100]. UAE was used by Hu et al. to extract flavonoids from *Cyclocarya paliurus* after initial enzymolysis. Extracted flavonoids exhibited higher antimicrobial properties against *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* compared to that of the extracts prepared by conventional extraction techniques [101]. Hydroethanolic extract of *Erodium*

glaucophyllum obtained through UAE and Soxhlet extraction was analyzed for its antibacterial effects. The effect was studied on *Salmonella aureus*, *Salmonella enterica*, *Lactobacillus casei*, *Listeria innocua*, and *Bifidobacterium lactis*. The ultrasound extracted hydroethanolic extract possessed higher amount of phenolic contents and showed the highest antimicrobial activities. The antiviral effect of the extract was also studied on hepatitis A virus and murine norovirus. Antiviral activity of the UAE extract was found to be higher than that of the Soxhlet extract [102].

Hashemi et al. carried out both continuous and pulsed ultrasound-assisted extraction to extract essential oils from *Aloysia citriodora* Palau leaves. They reported that the antibacterial and antioxidant properties of the extracts increased in both the ultrasonic extraction processes compared to non-sonicated extraction [103]. Four different extraction techniques, namely, UAE, maceration, assisted solvent extraction (ASE), and supercritical fluid extraction (SFE) were used to prepare different extracts from *Lepidium sativum*. When the antimicrobial activities of the extracts were analyzed, it was found that SFE extract exhibited showed the highest antimicrobial activity for freeze-dried and air-dried sprouts, followed by UAE, maceration, and ASE [104]. Ricardo et al. evaluated the antibacterial activities of *Annona cherimola* Mill leave extracts prepared with ultrasound assistance, Soxhlet, and maceration extraction methods. They obtained the best results against gram-positive bacteria using UAE water extract [105].

4.2.3 Extracts with Antidiabetic Properties

Reports suggest that UAE improves the antidiabetic properties of herbal extracts. Sunita et al. investigated the in vivo antidiabetic properties of *Gymnema sylvestre* leave extracts prepared through UAE and Soxhlet extraction. It was found that insulin released from rat pancreatic RINm-5 F β cells was affected by the extracts prepared by both the methods and the amount of extract used. The ultrasound-assisted extract at a concentration of 100 μg/mL showed up to about four times more insulin production from RINm-5 F β cells than extracts obtained from Soxhlet extraction [106]. Hypoglycaemic properties of ultrasonically extracted aqueous crude extracts of *Azadirachta indica*, *Bryophyllum pinnatum*, *Carica papaya*, and *Mikania cordata* were measured by Sadat et al. They have carried out in vivo studies on artificially developed diabetic mice. According to their report, *A. indica*, *B. pinnatum*, and *C. papaya* significantly reduced the plasma glucose level below 126 mg/dL. The effect was observed almost similar to the standard antidiabetic drug glibenclamide, which reduced the plasma glucose level to 100.35 ± 12.32 mg/dL [107].

These observations accomplished that UAE significantly enhances the anticancer, anti-inflammatory, and antidiabetic properties of herbal extracts. This explains the importance of the pharmaceutical application perspectives of the UAE.

5 Hybridization of UAE for Industrial Application

UAE has been combined with several other technologies to improve extraction efficiency. In the food and beverage industries, ultrasonic technology has already been expanded into various processing technologies [4].

5.1 Combination of UAE with Microwave Assisted Extraction

For fast and efficient extraction, the Combination of UAE with Microwave Assisted Extraction utilizing simultaneous irradiation is one of the most promising hybrid techniques. It is commonly used for the extraction of oils from vegetable sources (Fig. 3a).

Fig. 3 Hybrid extraction techniques (**a**) ultrasound-microwave extraction, (**b**) ultrasound-supercritical fluid extraction, and (**c**) ultrasound-DIC extraction. (Reproduced from Chemat et al. [4] with permission from Elsevier)

5.2 Combination of UAE with Supercritical Fluid Extraction

Supercritical fluid extraction is based on the improved solvent power of fluids above their critical point. When UAE is combined with supercritical fluid extraction, ultrasound enhances the mass transfer of the targeting species from the solid phase to the solvent for extraction (Fig. 3b).

5.3 Combination of Ultrasound and Extrusion Extraction

Applications of this hybrid technique have been seen in vegetable oil industries and the production of sugar, wine, and fruit juices.

5.4 Combination of UAE and Instantaneous Controlled Pressure Drop Process (DIC)

Application of this combined technology was seen in the sequential extraction of oil and antioxidants. Using this hybrid technology, it was possible to improve the kinetics and yields of antioxidant extraction [108] (Fig. 3c).

6 Conclusion

Ultrasound-assisted extraction is the most valuable and promising extraction technique widely used in laboratory-based research and industrial applications. It has substantial advantages over conventional extraction techniques in terms of yield, time of extraction, selectivity, extract quality, and safety. Using UAE, bioactive component extraction can be performed in very less time, at a relatively low temperature, with optimal energy and solvent requirement. As a non-thermal extraction technique, UAE facilitates the retention of the functionality of bioactive compounds. UAE can be considered as a green extraction technology that may produce green extract in concentrate form. UAE can preserve the anticarcinogenic properties of polyphenolic extracts. Ultrasound-assisted herbal extracts exhibit higher anticancer, antimicrobial, and antidiabetic activities. The use of toxic organic solvents was less common in UAE. Volatile compounds can be retained in ultrasound-assisted extract as UAE operates at a lower temperature. The importance of UAE in the extraction of various components for food industries and bioactive compounds having significant pharmacological activities is discussed in this chapter. It may be concluded that UAE is one of the most powerful tools that can be used as a versatile extraction technique in the food, pharmaceutical, and biotech industries.

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Chapter 6

Super- and Subcritical Fluid Extraction of Nutraceuticals and Novel Phytocompound

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Abstract

Alternative methods are currently being investigated to reduce the overuse of organic solvents, which have serious environmental and health consequences. Certain promising green technology-based alternative extractions are employed in a wide range of bioactive and nutraceutical extractions, including ultrasonication, microwave-assisted extraction, pressurized liquid extraction, and enzyme-assisted extraction. Super- and subcritical fluid extractions, on the other hand, are sufficiently sophisticated green technologies that have superior efficacy and selectivity for the extraction of nonpolar and low-polar constituents. In this regard, this chapter gives an overview of the process, the theory of super- and subcritical extraction, and the role of pivotal variables for optimal extraction. Additionally, recent findings of the principal phytochemical and bioactive compounds extracted by this process with their nature, biological activities, and stability during and after processing are discussed.

Key words Supercritical fluid extraction, Subcritical fluid extraction, Natural products, Pharmaceutical, By-products

1 Introduction

Naturally available bioactive compounds, mainly from plants, algae, microorganisms, and other by-products, have potent health-promoting compounds. These compounds include essential oils, peptides, phytosterols, phenols, flavonoids, alkaloids, and terpenoids, which have excellent antioxidant, antimicrobial, anticancerous, and anti-inflammatory properties. The extraction of these bioactive constituents from plants is still a topic of interest due to its potential in the research and production of functional ingredients and nutraceuticals. Yet challenges to maintaining structural stability and functionality after extraction exist because of its sensitivity to environmental condition.

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The optimal extraction of these bioactive with preserved functional characteristics is crucial. The recovery of functional compounds is dependent on parameters such as thermal exposure, type of solvent, time, matrix properties, pressure, and ratio of solvent to matrix [1]. Traditionally employed extraction techniques (maceration, distillation, and Soxhlet extraction with organic or inorganic solvents) has been reported to have several drawbacks, such as low efficiency, higher organic solvent utilization, prolonged extraction time, and targeted compounds degradation. To overcome these drawbacks, methods such as sub- and supercritical fluid extraction are being explored. Supercritical fluids are extensively explored because their low or absent surface tension results in no boundary between the liquid and gas phases. In this state, the compound is dispersed like a gas and dissolves bioactive compounds as in liquid. Supercritical fluid-based extraction improves the extraction speed, density-dependent selectivity, and eventually providing high-quality extracts with higher extraction yield when compared to traditional extraction. Several fluids under supercritical conditions, including carbon dioxide, ethane, ethanol, propane, water, ammonia, and methanol, are used for the recovery of bioactive compounds. For instance, supercritical carbon dioxide (SC-CO_2) based extraction of essential oils allows for a higher quality oil collection with preserved functional properties such as antioxidants, antimicrobials, and other properties of essential oils [2].

Besides supercritical fluid extraction, subcritical fluid extraction of bioactive molecules has also gained interest. Certain solvents, such as propane, ethanol, water, and carbon dioxide, are commonly utilized in subcritical form for nutraceutical recovery from plants because they are promptly recycled, minimally or nontoxic, eco-friendly, and mostly nonflammable. Subcritical waters are most abundantly used for extraction because they improve the extraction yield and reduce the extraction time compared to traditional solvents [1, 3]. Under subcritical conditions, the fluid has low dielectric constant, surface tension, and viscosity, which affect polarity, whereas it has increased diffusivity, mass transfer, and dissociation constant, which have high affinity for nonpolar solutes. Subcritical water, especially, shows the required properties under the specified conditions as mentioned above and is most abundantly utilized for the optimal extraction of phenols and other phytochemicals [1]. At specific conditions, the polarity of subcritical fluids varies, which can dissolve a wide range of compounds with medium- to low-polarity. Both methods can be applied for the extraction of compounds such as proteins, polysaccharides, antioxidants, essential oils, and other phytochemicals, depending on the polarity of the intended compound. The polarity of the both sub- and supercritical fluids can be adjusted by the addition of different co-modifiers, so optimal extraction can be achieved without modifying functionality [4].

2 Principle of Super- and Subcritical Fluid Extraction

A pressure-volume-temperature (PVT) diagram provides an overview of the different states of a pure substance. The known phases (solid, liquid, and gas) are separated by boundaries called "phase boundaries," where the two phases co-exist under specific temperature-pressure combinations. Herein, for pure substances, it has only a degree of freedom as in the two phases (liquid-vapor, solid-liquid, or solid-vapor), thus the equilibrium pressure is a function of the temperature in each case. Under the phase diagram, the three states co-exist at a point called the "triple point," whereby the liquid-vapor boundary ceases to exist at a point called the "critical point," and the temperature and pressure at this point are called the "critical temperature" and "critical pressure," respectively. At this point (the critical point), the liquid and gas phases have the same density, and further increasing temperature or pressure causes the liquid-gas phase boundary to disappear. Beyond the critical point, separation of the fluid and gas phases is no longer possible, and the state is called the "supercritical fluid state," where the characteristics of the fluids are drastically changed. In a supercritical fluid, solubilities vary to a great extent with a minor change in density, just as different solvents have different solubilities under the same circumstances. Solubility of any fluid is directly proportional to the fluid density at constant pressure. Based on the targeted phytochemicals, the solvent and its parameter selection should be made wisely, thereby allowing selective bioactive to be extracted via supercritical fluid extraction. In general, these fluids are broadly classified into two groups: (1) low critical temperature—CO_2, ethane, and propane and (2) high-critical temperature—alkanes, methanol, and water. Solvent power of high critical temperature is much higher than low critical temperature.

Subcritical fluid, on the other hand, is a pressurized hot fluid whose temperature falls between the critical point temperature and the atmospheric boiling point. Under such conditions, the fluid has a low dielectric constant and an increased dissolution property due to the weakening of the hydrogen bonds by high temperature and pressure, and this makes subcritical fluid more like less-polar organic solvents. Subcritical water (SWE), for example, uses liquid water as extractant at temperatures ranged in between 100 °C/273 K, 0.1 MPa and 374 °C/647 K, 22.1 MPa with pH 6 and extraction time 30 min has excellent yield in comparison to the ethanol extraction [5]. Any fluid can be kept in a liquid state by applying enough pressure at high temperatures. Changes in temperature and pressure have a large effect on the dielectric constant. Additionally, through the processes of diffusion and convection, subcritical fluids are noted to promote mass transfer. Disruption in

the solute-solute or solute-solvent interaction is usually seen in the application of subcritical fluid as it lowers the activation energy for the desorption process. Also, elevated pressure in subcritical fluid can help with extraction by forcing water into the matrix via pores, which is impossible at normal pressure [1]. Extraction principle of the subcritical fluid can be summarized as it is governed by solute-solvent interaction in sample matrix and mass transfer principle (diffusion, convection, and partitioning equilibrium) [3]. Although numerous fluids are experimented with for nutraceutical extraction, supercritical CO_2 and subcritical water are the most common fluids used for the extraction of the bioactive. This is due to the fact that supercritical CO_2 has fast diffusivity and nearly zero surface tension, leading to extremely efficient extraction, while water is nontoxic and eco-friendly by nature.

3 Properties of Sub- and Supercritical Fluids

The properties of both sub- and supercritical fluids depend on the types of solvent, such as ethanol, methanol, water, carbon dioxide, ammonia, or n-hexane, used. Thus, to determine the solute specificity and high solvation power of sub- and supercritical fluids, understanding of the solvent-dependent properties of subcritical and supercritical fluids under the aforementioned conditions is required.

Viscosity For both sub- and supercritical fluids, with increasing temperature, viscosity decreases by diffusion and partitioning equilibrium. The viscosity of the supercritical fluid is similar to that of gas, which means it is approximately 1/10 that of liquid.

Dielectric Constant (ε) In general, the dielectric constant of subcritical and supercritical fluids varied widely with temperature and pressure. For example, water has a high dielectric constant (80) at room temperature because of its extensive hydrogen bonding structure, while subcritical water at the same time has a lower dielectric constant, which is 25 at 250 °C and 25 bar pressure, similar to methanol ($\varepsilon = 33$) and ethanol ($\varepsilon = 24$), that can dissolve bioactive compounds with low to medium polarity [1]. This indicates that the dielectric constant of the fluids is reduced to that of organic solvents.

Fluid Surface Tension and Diffusivity Since the vapor-liquid boundary is terminated in supercritical fluids, there is no surface tension. Such fluids have high diffusivity that allows easy diffusion as gas through the solid matrix. In regard to subcritical fluid, with the increase of temperature, surface tension of fluid will decrease

steadily. Moreover, the diffusivity of the solvent increases as a result of lower surface tension with temperature, and the solvating capacity thus rises proportionally with increasing diffusivity.

Density The density of a supercritical fluid is usually between that of a liquid and that of a gas and is closer to that of a liquid. Factors such as pressure and temperature fluctuate the fluid's density of both (sub- and supercritical fluids). Herein, density of fluid decreases with increasing temperature under constant pressure. While, in the case of temperature, increasing pressure increases fluid density when the odd factor remains constant, and vice versa.

Solvating Power The solubility of any fluid is directly proportional to the fluid density at constant pressure. In supercritical fluid, solvation power of fluid increases with density and reaches maximum in the critical pressure. Solvent power of high critical temperature solvents is much higher than low critical temperature solvent. Likewise, investigation in subcritical fluid showed that the solvating power of subcritical fluid is similar to that of organic solvents. Solubility and mass transfer of subcritical fluids, especially water, are dependent on temperature; at higher temperatures, the dielectric constant is lower, allowing better solubility of moderately polar compounds [6].

Polarity Polar solutes are most soluble in polar solvents; however, nonpolar fluids can be effective solvents for many moderately polar molecules. Especially for supercritical CO_2, several cosolvents (nonpolar organic solvents) are typically added to increase polarity since polar compounds have low selectivity [7]. In terms of subcritical fluids, their properties are comparable to those of organic solvents, resulting in an increased solvating power to dissolve compounds with varying polarity, that is, medium- and low polarity. The polarity of the fluid decreases with increasing pressure, while it increases with increasing temperature, and low- or nonpolar compounds are easily dissolved.

4 Factors Affecting Extraction Yields

4.1 Sample and Its Preparation

4.1.1 Sample Matrix and Size

In both subcritical and supercritical fluid extraction, a sample with a smaller particle size has an increased interfacial area and minimal diffusion paths of raw material, which improves the solute extraction rate. An investigation by Peng et al. [8] on the extraction of tocopherol from roselle seed oil by SC-CO_2 extraction under the given conditions (temperature: 40 °C and pressure: 30 MPa) demonstrated that the yield was lowered with increasing particle

size. The size of the solid matrix governs the mass transfer kinetics, as small molecules diffuse easily and access the supercritical fluid throughout the sample matrix. However, extraction from the small sample matrix has the drawbacks of compound re-absorption and clumping, which can decrease fluidized bed velocity, clog the filters, and eventually decrease extraction efficiency. Thus, the optimal particle size of the sample is recommended to maximize extraction yield while minimizing the possibility of particle agglomeration [3, 9].

4.1.2 Moisture and Equilibrium Time

The extraction rate and yield are significantly influenced by the moisture content of the sample. The extraction yield is generally unaffected by a small amount of moisture, but yield steadily decreases as the moisture percentage increases in the sample. Kostrzewa et al. [10] investigated the effect of moisture content on extraction yield and found that moisture had no effect on carotenoid extraction and extraction efficiency until the moisture content was less than 7.5 g/100 g. However, the higher moisture in the sample showed lower carotenoid recovery and also necessitated carotenoid concentration after separation.

4.2 Cosolvent/ Modifier

Cosolvents, when dissolved in the fluid in different proportions, aids the solvation power of sub- and supercritical fluids toward targeted compounds by modifying the fluid density and polarity. The cosolvent, either polar or nonpolar, helps to change the polarity of the supercritical fluid. Also, the cosolvent percentage in the extractant has an impact on the yield of selected compounds because it affects the solubility of the targeted compounds [11]. Herein, supercritical CO_2 has low polarity and limits the extraction of nonpolar and lipophilic compounds. Therefore, a cosolvent (ethanol) can be added to enhance the affinity and solubility of polyphenolic compounds, which eventually increases extraction yield [12]. In the case of subcritical fluid extraction, the addition of solvent modifiers, such as natural deep eutectic solvents, increased the phenolic compound recovery as compared to the exclusive use of subcritical water [13]. The cosolvent is most important for maximum extraction, but it also reduces the specificity of the intended molecules, so careful consideration should be given to cosolvent selection.

4.3 Extraction Procedure

The extraction yield of the bioactive compounds is proportionate to the extraction process employed. Supercritical and subcritical fluid extraction methods overcome the cons of several traditional extraction methods. However, these methods still have limitations, which could be counteracted by the parallel assistance of several green technologies. An integration of either of the novel technologies—enzyme, ultrasound, microwave, pulse electrified, etc.—into supercritical fluid extraction has proven to be efficacious

in nutraceutical extraction specificity with the structural modification of targeted compounds [14, 15]. Enzyme-assisted supercritical fluid extraction, for instance, produces high-quality bioactive compounds, though the choice of enzyme and operational parameters must still be considered for maximum yield [16].

Similarly, novel technology assistance promotes excessive retention of functional quality and also increases extraction efficiency in sub- and supercritical fluid extraction. For example, microwave-assisted subcritical extraction of anthocyanin increased efficiency to 85.8% with higher antioxidant activity [17].

4.4 Extraction Parameters

4.4.1 Temperature

The crossover behavior of the supercritical fluid in the solubility isotherms is observed by the pressure-temperature relationship and its effect on density and volatility. On increasing the temperature, the density decreases under the isobaric process, while the volatility of the solute increases with temperature, thus reducing the extraction rate. Inversion pressure (crossover pressure) has the critical role in defining temperature selection for optimum extraction. To clarify, low temperature permits density to dominate under the crossover pressure that leads higher extraction yield, while over crossover pressure, higher temperature allows solute vapor pressure to dominate, resulting higher extraction [3].

At the subcritical phase, increasing temperature improves the dissociation constant by decreasing surface tension, where mass transfer is supported by the disruption of intermolecular forces in the sample matrix and increased solubility. The diffusivity of subcritical water, for example, increases with increasing temperature, whereas the dielectric constant, surface tension, and viscosity are significantly reduced. Low dielectric constants facilitate the extraction of low-polar organic compounds due to high solubility in low-polarity fluids [18]. On the adjustment of pressure-temperature, one should be conscious of the maximum temperature for the extraction of selected compounds because high temperatures change the structural composition by causing thermal degradation reactions and the neoformation of Maillard compounds [13, 19].

4.4.2 Pressure

Under the constant temperature, the solubility of the bioactive compounds increases with increasing pressure due to an increment in density and solvation power, which successively increases the extraction kinetics. However, extremely high pressure hinders fluid diffusion [20], which could reduce yield. Thus, optimal pressure based on extract specificity is preferred. The effect of the pressure, which is also dependent on temperature variation, depends on the types of selected compounds. If the pressure is near the critical pressure, solubility increases with decreasing temperature, whereas at higher pressures, solubility increases with increasing temperature.

Regarding subcritical extraction, pressure has a negligible influence as compared to the temperature on the phase and characteristics of some subcritical solvents. Water, in particular, is relatively incompressible below 300 °C, which thereby does not affect the physical properties in the liquid phase [1]. However, pressure is still important for subcritical solvents such as sub-CO_2 and sub-ethanol because the pressure in such fluids has a significant impact on extraction yield [4].

4.4.3 Flow Rate

The flow rate of the sub- and supercritical solvents has a direct impact on the solvent-to-feed ratio, contact time, and mass transfer resistance. Increasing the flow rate improves extraction efficiency by minimizing the mass transfer resistance. With the low flow rate, the solute saturates in the solvent as the result of axial dispersion, while for the very high flow rate, yield is minimal due to insufficient contact time [21], indicating the appropriate flow rate as a crucial factor for maximum yield. However, too high solvent flow rate cause substantial dilution of the extracts, necessitating an additional concentration step following extraction [1].

4.4.4 Time

Initially, the extraction yield in super- and subcritical fluid extraction increases with time, but with prolonged extraction time, this rate drops. An investigation of the tocopherol yield from roselle seed oil by supercritical fluid extraction demonstrated an increment in extraction yield with increasing temperature for a certain time, which was limited afterward [8]. This can be explained by the fact that the process reached the saturation point and/or by thermal degradation of bioactive compounds [22]. Consequentially, the yield tends to decline with increasing extraction time. In contrast, too short extraction time results in minimal solid-solvent interaction, leading to a low yield.

5 Comparison with Conventional Method

The conventional methods of extraction are maceration, hydrodistillation, and Soxhlet extraction [3]. These methods are outdated due to low efficiency, high targeted compound degradation, requires considerable period of time and impact on the environment triggered by wastage of unrecovered solvents is massive [23]. In general, conventional solid-liquid extraction (SLE) and liquid-liquid extraction (LLE) methods utilize organic solvents, such as ethyl acetate, hexane, ethanol, acetone, and methanol, of which the latter reigns as the most toxic. Also, the majority of the solvents used in conventional SLE and LLE methods are nonbiodegradable, volatile, and highly flammable [24]. The solvent recovery rate in conventional extraction is rarely satisfactory, which prompts solvents to be dispensed into the environment [25, 26].

The current advancement of green technology-based extraction has allowed for the selective extraction of phytochemicals with low labor intense, less solvent requirement, and ease of automation. These have overcome the challenges of the nutraceuticals and pharmaceutical industries, where the results of conventional extraction technology adoption were not satisfying [23, 26, 27]. Pharmaceutical industries are now more concerned about the stability and functionality of bioactive compounds during and after extraction as they are susceptible to structural modification due to minor environmental changes. The consideration of the process of deploying solvent in green technology dictates not only the efficiency but also the economic aspect [28]. The neoteric/green solvents, such as, supercritical CO_2, deep eutectic solvent (DES), gas-expanded solvents (GXLs), switchable solvents, liquid polymer solvents (Polyethylene glycol), and bio-based/renewable solvent (γ-valerolactone) are also referred as green solvent, as they have minimum contribution on the organic pollution due to solvent loss [26, 28]. Efficiency, selectivity, and diligency are very important factors in an extraction process for bioactives and nutraceuticals [29]; hence, the industrial inclination toward sub- and supercritical fluids is increasing.

At the supercritical state, CO_2 acts as both a gas and a liquid, rendering diffusion like a gas and solubility like a liquid. The recovery process of the solvent after extraction is convenient as it transforms from its supercritical state into its gaseous state at room temperature, which enables the omission of an energy-intensive recovery process. The interphase change due to the shift in the thermodynamic state of the solvent suitably supports spontaneous separation, resulting in a high degree of extract purity. The crucial reasons for selecting CO_2 for supercritical extraction are its moderate critical temperature and pressure (31 °C and 7.38 MPa) and the preservation of its potential oxidizing component. The selectivity and solubility of the supercritical CO_2 can be modulated by adjusting its temperature, pressure, and cosolvent [29, 30], which is not achievable in a conventional extraction technique. Pereira et al. [31] studied the extraction yield and antioxidant capacity of Portuguese myrtle (*Myrtus communis* L.) leaves and fruits using SFE and liquid phase extract (LPE) with diisopropyl ether. It was found that the average extract yields of leaves and fruits using SFE were around 36 and 70 folds higher, as compared to LPE with diisopropyl ether. The mean extraction yield using SFE was found to be 10.8% and 14.1% for leaves and fruits respectively, whereas only 0.3% and 0.2% for LPE. Also, the extract obtained by SFE contains a significantly higher content of polyphenols as well as possesses a higher antioxidant capacity in contrast to LPE. Naturally, the bioactive compound occurs in a multi-component state, so the prudential selectivity of the extraction process is a decisive attribute [32]. Because CO_2 has a low polarity, it cannot be used to extract

polar compounds [33]; therefore, a supplementary cosolvent is required to boost the extraction, especially when the target compound is polar and has a long molecular structure.

Similarly, water, CO_2, propane, etc., at subcritical state is also eligible for its application as a solvent to extract nutraceutical relevant bioactive compounds. In case of water, it is a polar solvent and has high dielectric constant at an ambient temperature and pressure, hence it is not applicable for the extraction of nonpolar compounds. However, subcritical phase of water has low dielectric constant, which is in par with that of organic solvents. This allows water to behave like conventional organic solvents applicable for low-polar target compound [1]. Additionally, it has perks of being high selective without compromising economic and environmental aspects. The cohesive (solute-solute) and adhesive (solute-matrix) interaction in matrix is interrupted by subcritical water, due to which desorption process is activated on the energy level far less than that of conventional extraction. Ko et al. [34] extracted bioactive compounds from Crassulaceae (*Orostachys japonicus* A. Berger) and came into the conclusion that SWE was efficient on extracting total phenols by 3.7–11.5 folds, flavonoids by 1.8–3.2 folds against methanol, and ethanol extraction done at 25 and 60 °C for 2 h. Thus, apparent superiority was reflected against conventional (methanol, Soxhlet, hot-water) extraction techniques [35]. Moreover, the quality characteristics, bioactive phytochemicals, volatile compounds, and antioxidant capacities of virgin avocado oil extracted using a couple of green methods, namely, subcritical CO_2 extraction and ultrasound-assisted aqueous extraction (UAAE), are compared with the oil extracted using the conventional solvent extraction. Results indicate the quality properties of avocado oil are unaffected by extraction methods. The total phenolic content of avocado oil is in the range of 111.27–130.17 mg GAE/100 g and the major phytosterol is β-sitosterol (1.91–2.47 g/kg). Avocado oil extracted using subcritical CO_2 exhibits two to four times greater levels of α- and γ-tocopherols than solvent extraction and UAAE. The volatile components associated with nutty and grassy flavors are only detected in avocado oil extracted under low-temperature extraction conditions such as subcritical CO_2 and UAAE. Based on the antioxidant capacity tests, avocado oil obtained by subcritical CO_2 exhibits the strongest antioxidant capacity compared with solvent extraction and UAAE [36].

6 Novel Technology Integrated Sub- and Supercritical Fluid Extraction

Supercritical or subcritical fluid solely could not extract the polar solvent, so it is necessary to combine these extraction process with novel technologies such as ultrasonication, pulse electrified, enzyme-assisted extraction, microwave-assisted extraction (MAE)

along with the other cosolvent [37]. In a biological system, the intended bioactive compound exists in complex form. It might be bound to a plethora of undesired compounds that hinder the efficacy of the extraction process [3]. For instance, caffeine in the ground coffee is bound with chlorogenic acid, while during the decaffeination process, only caffeine is a target compound. The challenge of freeing caffeine from chlorogenic acid is overcome by coextracting with water. The moisture moisturizes the ground coffee and facilitates the caffeine extraction process by loosening up the chlorogenic acid-releasing caffeine [29].

Ultrasound assists the extraction process by enhancing mass transfer in the system due to cavitation formation and collapse [3]. Da Porto et al. [38] used a combined method of ultrasound-assisted extraction (UAE) followed by re-extraction by SC-CO_2 to extract polyphenols from grape marc-a solid waste of the winery. A maximum of 2336 mg GAE/100 g (dry matter) of total phenolic compound (TPC) was extracted under optimized parameters of UAE (80 °C and 4 min), and the combination with SC-CO_2 increased the yield to 3493 mg GAE/100 g. Similarly, the total flavonoid yield from *Iberis amara* was 18% higher with ultrasound-assisted supercritical CO_2 extraction than SC-CO_2 extraction solely. In addition to that, the extraction time was also reduced from 100 min for SC-CO_2 to 60 min [39].

Enzyme-assisted (EA) extraction refers to the use of enzymes to compromise the structure of the pecto-cellulosic matrix and release the target bioactive compound through hydrolysis. Phenols and flavonoids are bound by hydrogen and hydrophobic bonds with the polysaccharide of the cell wall. The degradation of polysaccharides frees the phytochemicals, easing the extraction process [3]. Krakowska et al. [40] employed enzymes to assist the SC-CO_2 extraction of polyphenols from *Medicago sativa and* determined that SC-CO_2 extraction yielded 94.17 μg/g, whereas enzyme-assisted SC-CO_2 yielded 142.69 μg/g of phenolic compounds. Enzymatic pretreatment also evidently assisted on the extraction of bioactive compounds from ginger. Nourbakhsh Amiri et al. [41] pretreated ginger powder with α-amylase and extracted bioactive compound via SWE. The enzyme-assisted SWE (EA-SWE) yielded 2.8-fold polyphenols and 2.22-fold more gingerols and shogaol than that of extraction without the assistance of enzyme.

The SC-CO_2 extraction has been paired with a microwave-assisted extraction method to recover bioactive compounds. Sánchez-Camargo et al. [42] assessed the subsequent application of MAE1 for the extraction of phenolic compounds from the SC-CO_2-exhausted mango peel. Prior to the MAE, mango peel was subjected to the SC-CO_2 extraction of phenolic compound, and the subsequent re-extraction was carried out, which yielded 52.08 mg gallic acid equivalent/g (dry weight) of phenolic compound.

7 Current Application of Super- and Subcritical Extraction

There is substantial use of sub- and supercritical fluid extraction for bioactive compound extraction from diverse sources, including microbial cells, macroalgae, and complex plant and animal tissues. Macroalgae (seaweed) are abundant in bioactive compounds comprising of carotenoids, flavonoids, tocopherols, and phytosterols [43]. More than 20% of the plants have been subjected to understand their possible utilization as pharmaceuticals. The prominent application of the sub- and supercritical extractions are found in field of phytochemical extraction.

Till now, approximately 8000 of different polyphenolic compounds from variety of sources has been on the radar of the pharmaceutical study [44]. Green extraction technology is constantly revolving around the process optimization as the different source possess different set of condition which requires to be countered with customized parameters. Statistical tool such as, response surface methodology (RSM) has been employed to optimize the parameters such as type of solvent, type and ratio of cosolvent, duration of extraction, temperature, and pressure. For example, Bobinaitė et al. [45] extracted polyphenols from rowanberry (*Sorbus aucuparia* L.) pomace with the combination of SC-CO_2 and pressurized solvent (ethanol, acetone, and water) extraction techniques. SC-CO_2 extraction was optimized by modulating the parameters, such as pressure (45 MPa), temperature (60 °C), and extraction time (180 min) and achieved highest yield extract of 4.8 g/100 g (dry weight). Furthermore, pressurized solvent (ethanol) extraction was carried out to recover residual polyphenol from exhausted pomace yielding 16.04 g/100 g (dry weight). It evidently showed that the best yield of the target extract was achievable with the integrated approach. Similarly, Saravana et al. [46] carried out SC-CO_2 extraction of carotenoid from the seaweed using sunflower oil as a cosolvent. With RSM-guided optimized parameters, such as temperature, pressure, and cosolvent flow rate, the recovery of the carotenoids was comparatively higher.

Extraction of bioactive lipids has been done using conventional methods and the recent advancement in supercritical fluid extraction technique has fuelled up the exploration of new horizon. The study of dandelion seed's extract for its bioactive compound rendered the result, which has abundant polyunsaturated fatty acid (PUFA), primarily linoleic acid [47]. The lipophilic extract of SC-CO_2 comprises of fatty acid, and the profiling identified linoleic and oleic acid as the major one, followed by palmitic acid and stearic acid. Whereas, other acids, such as myristic, α-Linolenic, behenic, and palmitoleic were each below 1% [45]. Patil et al. [48] extracted bio-oils from algae (*Nannochloropsis salina*) biomass via SC-CO_2 extraction technique. Azeotropic mixture (hexane: ethanol, 1:1)

was added as cosolvent to enhance affinity for neutral and polar lipids. Maximum yield was obtained when the parameters optimized as temperature 80 °C, cosolvent to algal biomass ratio 12: 1, time 60 min and pressure 340 bar. It resulted in highest eicosapentaenoic acid yield. Melloul et al. [49] extracted bioactive oil from *Peganum harmala* by SCF extraction and analyzed the bioactivity retention along with the yield. SC-CO_2 extraction process was operated at the condition of 300 bars of pressure, at the temperature of 55 °C with the sample particle size of 0.3 mm, and the extraction yield was maximum. However, the maximum availability of the total phenols (79.04 mg GAE/g equivalent) with IC_{50}:172.199 μ was achieved at pressure (100 bar), temperature (35 °C), and sample particle size (0.9 mm). Maximum flavonoid (7.10 mg QE/g equivalent) was achieved at the similar condition except pressure being raised to 170.7 bar. This gives an overview of how crucial it is to be prudent about the optimization condition, mainly pressure, to modulate the extraction efficiency.

Pharmaceuticals have been exploring bioactive compounds from novel sources by using SCF extraction technique as it is comparatively posing less severity to the viability of compound's bioactivity, hence, it does not overlook the presence of even trace amount of high value compound.

Phytochemical such as polyphenols are the most studied class of plant derived compound. Phenols and flavonoids are the major secondary metabolites that has high bioactivity, hence has high value. Polyphenol are composed of multiple hydroxy phenols which are ubiquitously available in most of the whole grains, fruits, and vegetables. They are highly bioactive and susceptible to the extremities that they are subjected to. Flavonoids have been extracted from the agricultural produce and its by-product, onion skin [50], jujube leaves: 29.052 mg/g [51], grape seed: 7132 mg GAE/100 g DM [52], tea leaves: 194.6 mg QE/100 mL [53]. Majority of the investigation inclined toward the diversified flavonoids on SCF extraction than that of conventional Soxhlet or even outperformed some of the modern extraction techniques (UAE). The major flavonoids, anthocyanin was extracted from roselle (*Hibiscus sabdariffa* L.) using SC-CO_2 extraction. Experimentally, TPC and TFC extracted were 128.16 mg GAE/100 g and 731 mg QUE/100 g, respectively [54]. Similarly, Rahmana Putra et al. [18] conducted optimization study to maximize the yield of phenolic and flavonoid compound from roselle using subcritical ethanol extraction (SEE). The optimization of the parameter leads to the maximum yield of anthocyanin (921.43 mg/100 g), TPC (40.57 mg/100 g), and TFC (559.14 g/100 g). In terms of extraction efficiency, the major takeaway of phytochemicals extraction is the optimization of pressure, as the most of investigation displayed that excess rise in pressure of supercritical fluid increases mass transfer resistance and reduces the diffusion of

solvent into the feed matrix affecting the yield. Investigation for cost cutting on the extraction technique has unfolded possibilities on other method than SC-CO_2 extraction. Comparative study against SC-CO_2 extraction has presented evidence on subcritical alcohol extraction being less time intensive, lower cost to operate, and more convenience on separating solvent from solute.

It has shown promising application on the valorization process too. Agro-based by-products have massive potential as a bioactive compound source, so they are going under an extensive up cycle assay. Apart from that, industrial waste, such as herbal medicine wastes (HMWs) are also eligible for valorization into high value bioactive compounds [55]. By-products, such as spent coffee grounds, were investigated for the extraction of phenolic compounds by using SWE technique [56]. The extraction time was crucial during the extraction process because the longer extraction time yielded less phenols from the spent coffee grounds. Similar drop in phenolic compound (η-caffeoyl-quinic acid) was observed when extraction time was extended. The extended time favors the oxidation of phenolic compound and extensive extraction temperature also aids oxidation process [57]. The innate requirement of extended time and high temperature for SWE shown detrimental effect on the bioactivity of the extracts, hence to minimize the loss-SWE could be combined with the pretreatment (microwave, ultrasonication) of the raw material [58].

The combined application of SC-CO_2 and sequential subcritical hydrothermal liquefaction (SC-HTL), Mathur et al. [59] extracted high value products such as, PUFAs (eicosapentaenoic acid, docosahexaenoic acid, etc.), aromatics, aldehydes, and alkynes from the microalgal biomass. During the valorization of biomass (*Chlorella* sp. and *Phormidium* sp.), the utilization of all the fraction was achieved, and additionally, compared to conventional (Soxhlet) extraction method—the quality was higher. Green extraction technologies are also enabling the exploration of other plant biomass as a source of bioactive compound. Foliage—otherwise waste of different plants are being assayed as a potential source of bioactive compound [60]. Some of the example are as follows: Goyeneche et al. [61] recovered total phenols from leaves of beetroot (*Beta vulgaris* L.) by using SC-CO_2 extraction technique. Similarly, Essien et al. [62] used subcritical water extraction technique to recover phenolic compounds from Kānuka (*Kunzea ericoides*) leaves. The extraction efficiency was directly proportional to the adjustment of the extraction parameters, especially, extraction time, temperature, and solid-solvent ratio. Assessment displayed that there is untapped possibilities for the cheap source of bioactive compound which could be exploited via optimized green extraction technologies.

8 Characterization of Extracted Bioactive Compounds

Extracted solution includes a variety of bioactive compound mixtures, such as polyphenols, alkaloids, essential oils, vitamins, enzymes containing carotenoids, tocopherols, sterols, fatty acids, including flavonoids, antioxidants, bioactive peptides, a small amount of digestive enzymes, and other compounds in minor amounts [63, 64]. Characterization of these compounds is necessary for obtaining specific compounds in pure form, quantification, and bioactivity determination, which enables the application of those actives in pharmaceutical and therapeutic products development. The general overview of extracts along with in vitro/in vivo bioactivity, toxicity, and stability of bioactive molecules from sub/supercritical fluid extraction is articulated (Fig. 1).

In order to extract phlorotannins from *Cystoseira abies-marina* seaweeds, subcritical (water, ethanol, and ethyl lactate) and supercritical (SC-CO2 and SC-CO2 with varying proportions of ethanol) conditions were used. Theoretically, the best solvent was 100% ethanol at a low temperature (25 °C). However, experimentally, pure ethanol at 100 °C in a subcritical state (10.3 MPa) showed the highest selectivity to extract phlorotannins among the four solvents investigated using a thorough two-dimensional liquid chromatography approach [66].

Typically, research in sub/supercritical fluid extraction includes a thorough characterization of the extracts utilizing cutting-edge methods such as high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled to mass

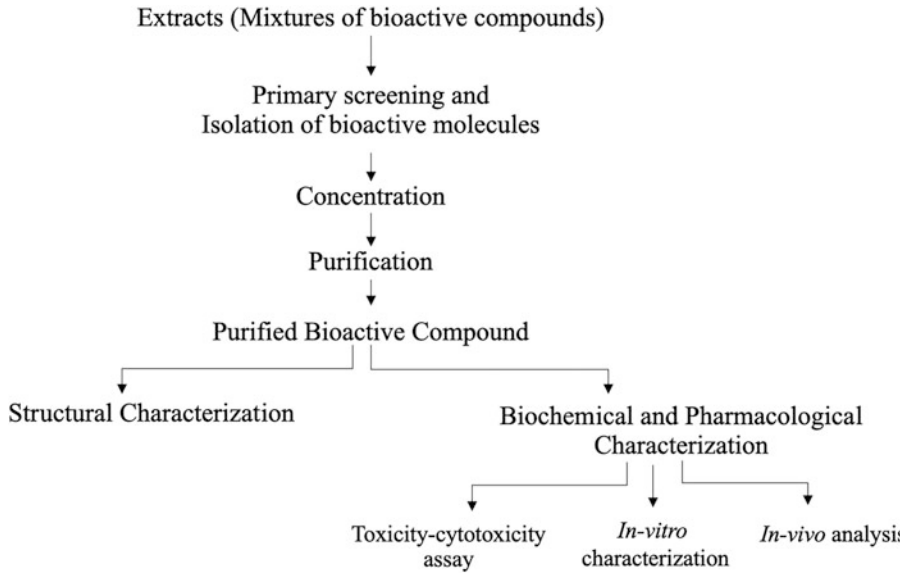

Fig. 1 A general overview of the characterization of extracted bioactive compounds. (Source: Azmir et al. [65])

spectrometry (MS), for identifying and measuring bioactive compounds. But in recent years, novel and more sophisticated tools have developed that enable the simultaneous extraction and characterization of molecules. For instance, [67] designed a SFE-UV/Vis-ELSD apparatus that could quantify total lipids, carotenoids, chlorophyll A, and ergosterol from a microalgae extract prepared by SFE. This strategy not only simplifies the entire extraction-identification-quantification process but also protects extracts from possible damage throughout these processes.

Besides these, the other attributes (appearance, taste, viscosity, and so on) of the supernatant and the residues during sub- and supercritical extraction are desirable in comparison to other extraction methods. The high-pressure supercritical fluid extraction extract exhibited the most desired sensory qualities (spicy, aromatic/herbaceous, pungent, and phenol-like). For example, the optimized parameter for obtaining thymol-rich extracts with high sensory qualities was the SFE at 40 °C and 16.7 MPa [68]. The optimum defatting performance was found for supercritical CO_2 defatting after comparing the effects of two defatting methods, namely, centrifugal defatting and supercritical carbon dioxide defatting, on the color, size distribution, and flow properties of the resulting insect powders. The supercritical CO_2 powders had lighter colors and larger particle sizes. With regard to flow characteristics, supercritical CO_2 powder had considerably higher flowability and floodability indices than other processes [69]. Additionally, compared to native, hexane-extracted, and isopropyl alcohol-extracted flours, supercritical fluid extracted flours displayed a higher peak viscosity. Defatting resulted in an improvement in functional properties, with supercritical fluid extracted flours exhibiting the most significant enhancement. Defatting had an impact on both empirical and fundamental rheological measurements, with supercritical fluid extracted flours exhibiting the greatest change in viscoelasticity [70].

8.1 Extracts Bioactivity

8.1.1 In Vitro Characterization

The most common assessment techniques for in vitro characterization are antioxidant activity using DPPH, FRAP, and ABTS [71], antidiabetic activity using an α-amylase inhibitory assay, anti-inflammatory activity using the protein denaturation inhibition technique [72] and anticancer activity using cell viability determining 50% inhibition concentration (IC_{50}) [73]. The most efficient SC-CO_2 extract had maximum cannabidiol (CBD) and was rich in α-pinene, β-pinene, β-myrcene, and limonene. Under concentrations from 10.42 to 66.03 μg/mL, extract doses exhibited inhibitory effects against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus* [74]. In addition, the observations of antioxidant activity show that the percentage of antioxidant activity decreased in the order of α-tocopherol > SCO_2-extracted oil > solvent-extracted oil >

UAAE-extracted oil. In comparison to solvent- and UAAE-extracted oils, SCO_2-extracted oil had the highest antioxidant activity, indicating a stronger capacity to scavenge free radicals [36].

The bioactivity of SWE extracted phenolics and flavonoids exhibited the significant differences in comparison to Soxhlet methods. However, methanol extracts from *S. variolaris* sea urchin gonads demonstrated comparable antioxidant ability to SWE of gonads [75]. Under specific temperature, α-Amylase inhibition and inhibition of protein denaturation was higher than that of the extracts from Soxhlet extraction, indicating superior antioxidant, antidiabetic, and anti-inflammatory properties. Moreover, the superior bioactivity can be attributed to the higher bioactive compounds (chlorogenic acid, gallic acid, 5-HMF, 5-MF, and ferulic acid) identified on HPLC analysis of phenolic composition. These bioactive compounds were still preserved under high temperature of 190 °C [72]. Beside these, the mean antioxidants activities, DPPH-radical-scavenging ability, ABTS-radical-scavenging ability, and FRAP antioxidant activity are higher in the extracts as compared to liquid phase extraction with methanol and ethanol at both 25 °C and 60 °C, respectively for 2 h. This bioactivity is partly correlated with the flavonoids and phenolics contents, and there was higher content of flavonoid and phenolics in SW-extracted extracts. Being thermal sensitive, several bioactive compounds are unstable and destroyed at a high temperature of 240 °C, resulting low aforementioned activity Ko et al. [34].

8.1.2 *In Vivo Analysis (Clinical Trial/Animal Study)*

For the newly identified bioactive molecules, to support this typical statement, clinical trials are required to show a bioactive compound's efficacy. Clinical trials designed to ascertain the pharmacokinetics, bioavailability, efficacy, safety, and drug interactions of newly created bioactive compounds and their formulations must be considered (extracts). Before the treatment is widely administered to patients, clinical trials are meticulously planned to safeguard the participants' health as well as provide findings to particular research questions by evaluating their results and checking for both short- and long-term negative effects [76]. Raspberry oil extracted by supercritical CO_2 has potent anticarcinogenic properties via suppression potential against several carcinoma cell lines (colon adenocarcinoma, doxorubicin-resistant colon adenocarcinoma, breast cancer, doxorubicin-resistant breast cancer, and lung cancer cell lines). Raspberry oil extraction using supercritical CO_2 significantly increased free radical production and DNA strand damage in cancer cells, particularly doxorubicin-resistant lines, implying effective targets on cancer cell vulnerabilities [77]. Extraction of the bioactive under optimized SC-CO_2 extraction has potentially higher in vivo bioactivity. Animal studies have revealed that compounds such as curcuminoids, oleoresins, and total volatiles have potent bioactive functions [78].

8.1.3 Toxicity Assay

Several toxicity assays are conducted to determine its safety before clinical trials. Cytotoxicity test of the bioactive compounds are generally carried out on VERO and MDCK cells using a MTT assay. A decrease in the cells viability indicates the toxic effect of that test compound. Acute oral toxicity is determined from LD_{50} technique. Other toxicity tests, such as body weight change (drastic gain/loose), hematological and biochemical analysis, and in silico prediction of toxicity, are carried out to evaluate short- and long-term toxicological profiles [79].

It has been discovered that SC-CO_2 is selective in the extraction of desirable chemicals, leaving no harmful residues in the extracts and posing no threat to the processed product's thermal stability. Actually, the use of SC-CO_2 extracts in food products is frequently acknowledged as safe. With potential applications for the extraction of important chemicals from solid plant matrices and seed oil, SC-CO_2 extraction has grown quite mature. While other fluids, such as propane, can be used to extract plant material, CO_2 offers more advantages due to its nontoxicity and favorable thermodynamic properties, which make it easier to employ under supercritical conditions (over 31.1 °C and 7.4 MPa) [80]. Peterson et al. [81] looked at how high temperatures affected the oil during subcritical oil extraction. Peroxide readings were discovered to be less than 5 ppm, which indicates no oxidation when compared to Soxhlet. The FFA results also indicated that using the subcritical approach did not result in any appreciable oxidation deterioration. Only little amounts of ethanol (30 mg/day authorized daily exposure) can be utilized for food extractions because it is a class 3 solvent and Petroleum ether is considered as class 4 because of its less toxicity as compared to class 1 and 2 solvents [82]. Contrarily, water has no such health-based exposure restriction.

8.2 Stability of Bioactive Compounds

Bioactive compounds are mostly heat-liable. Heat treatment has a significantly destructive effect on polyphenols, similarly, other factors such as oxygen, light, and pH tend to accelerate the degradation of bioactive compounds causing a loss in their therapeutic functions. Also, due to the acidic environment in our gastrointestinal tract, that is, in the stomach, most of the bioactive compounds lose their bioactivity [83]. So, for increasing the stability and bioavailability different new techniques are used. Some of them currently used in food and pharmaceutical applications are protecting bioactive compounds from severe exposure to external environments by storing them in moisture, air and light barrier containers, storage at low temperatures, etc. [84]. Furthermore, encapsulation (microencapsulation, nanoencapsulation) and emulsification are the most widely used methods. The most modern techniques involve microencapsulating bioactive and using nonthermal

manufacturing methods for increasing the stability of bioactive compounds [84]. The process of microencapsulation involves encasing an active ingredient, which could be tiny solid particles, liquid drops, or gaseous substances, in an encasing substance that will serve as a protective enclosure [85]. In the research carried out by Šeregelj et al. [86], for the encapsulation of bioactive compounds from sweet potato peel using a whey protein powder as a coating material showed that the encapsulation process enables the carotenoid and phenolic compounds retention and increases the shelf life under different experimental conditions, on under light and dark conditions.

9 Advances and Future Outlook

For the sustainable approach, rather than following one expensive method of extraction, combined methods which can deal with many bioactive compounds should be in more with maximum flexibility. Several emerging extraction techniques can be combined to further shorten the extraction period, boost the extraction yield, and get around the drawbacks of the individual techniques [87]. One of the often-used combination extraction methods is to pretreat sample with a microwave, ultrasonic, or enzyme before undergoing super- and subcritical fluid extraction process to hasten the breakdown of cell walls. Similar to this, a combination of two or more developing extraction methods can also be applied, such as supercritical fluid extraction (SFE) and ultrasonic-assisted extraction (UAE), subcritical fluid extraction, microwave-assisted extraction (MAE) and solvent extraction, enzyme application prior to pulsed electric field (PEF) treatment, and supercritical fluid treatment prior to MAE followed by further solvent extraction [88]. Additionally, these methods can be further developed in terms of efficient extraction of specific bioactive compounds that could be closely linked to the usage of novel food-grade solvents such as ethyl lactate. Another intriguing strategy could emphasize increasing selectivity by choosing the best solvents and cosolvents based on how they interact with the bioactive metabolites. The stability of bioactive compounds is found be shorter in ambient condition, so for increasing the stability of bioactive compounds, more research should be conducted. Research should be focused on increasing the stability, bioavailability, and bio-accessibility of bioactive compounds from the storage to the absorption in the bloodstream.

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Chapter 7

Novel Solvent Based Extraction

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Abstract

Solvent extraction techniques have a wide range of uses in analytical chemistry, including large-scale industrial separations, waste management, the pharmaceutical and biochemical industries, and both inorganic and organic chemistry. For the extraction of nutraceuticals from plants, a number of novel techniques have been developed, including accelerated solvent extraction, supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction. These techniques aim to reduce extraction time, increase extraction yield, and improve the quality of extracts while also consuming less solvent. Solvent extraction methods are currently frequently used in separation technologies. Recovery, concentration, and separation of organic acids and acid mixtures are of great interest to researchers. Both the conventional and traditional precipitation techniques and various more recent efforts to develop extraction-based process technologies. The main problem with current solvent extraction separation is that the majority of methods are empirical, distinctive, and exclusive to particular application domains, requiring a lot of testing. This review offers a succinct look at the most recent developments in new solvent extraction separation techniques for the application of bioactive extraction in the food and nutraceutical industries.

Key words Solvent, Extraction, Bio-active extraction, Organic solvents

1 Introduction

Solvent extraction is an effective separation technique widely used in a variety of applications, ranging from separations in analytical chemistry to industrial processes in hydrometallurgy pharmaceutical, food engineering, and waste treatment [1]. Separate processes of solvent extraction in Fig. 1 have been extensively described using a variety of theoretical chemistry tools. There is still no comprehensive theory and no effective method for combining the extraction phases due to the complexity of solvent extraction systems. Understanding the fundamental solution chemistry of food items and their behaviors in both aqueous and organic phases is necessary for the development of new reagents for bioactive solvent extraction methods. Recent developments have altered the solvent extraction landscape in the food and nutraceutical industries, opening up

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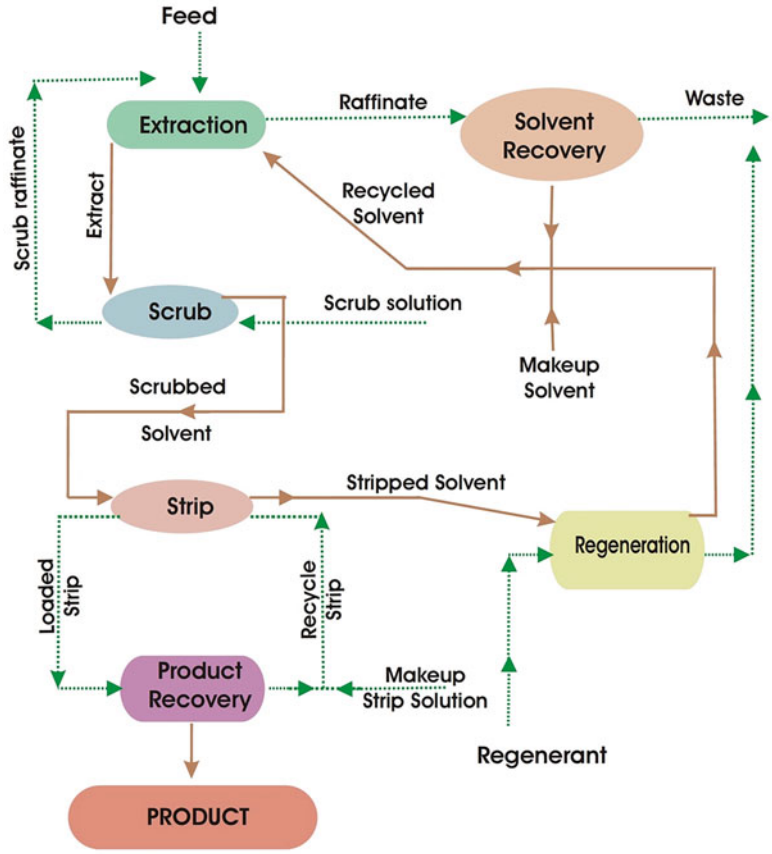

Fig. 1 Basic processes in solvent extraction

new avenues and extending our understanding of how the use of solvents has developed into one of the most effective methods in the study of bioactive extraction. For the separation of solutes from relatively concentrated feeds, such as those used in the industrial synthesis of chemicals and metals by hydrometallurgy, conventional solvent extraction is a tried-and-true process. On the other hand, diluted streams provide a problem. In order to effectively treat these streams using traditional liquid-liquid extraction, the distribution ratio must be quite high; otherwise, the amount of the organic phase would be too great for reasons of safety and the environment. These restrictions are attempted to be overcome by the revolutionary solvent extraction technologies developed in recent years. Hazardous organic solvents can be swapped out with environmentally friendly solvents to make processes greener and more environmentally sustainable. In a recent review, sampling techniques and thorough, modern methodologies for chemical characterization of antibiotic residues in various sample matrices are presented. With applications in antibiotic residue analysis, solvent-based sample preparation methods using green solvents

are explored in particular [2]. Rajapaksha et al. investigated the applicability of semi-continuous subcritical solvent extraction (SSE) for the extraction of polyphenols from wasted black tea at pilot size [3]. SSE improved the phenolics' diffusivity and solubility. When compared to hot water extraction (HWE), the extraction of phenolic content from discarded black tea using a 1:1 ethanol-water solvent at 125 °C and 0.3 MPa yielded considerably more phenol. This chapter analyses their potential to enhance conventional solvent extraction's performance in light of current developments in solvent development and theory.

2 Water as a Solvent

The universality of water as the solvent for living systems is usually justified by arguing that water supports the rich organic chemistry that seeds life. Nevertheless, it has been recently pointed out that alternative chemistries are possible in other organic solvents [4]. Water is undoubtedly the ideal substitute for organic solvents from an environmental standpoint because it is affordable, secure, nontoxic, noninflammable, and recyclable. While polar molecules can be dissolved in water, the majority of the often-examined organic compounds are hydrophobic and have poor to very poor water solubility. This is the fundamental drawback of using water as a solvent. Between the normal boiling point (100 °C) and the critical temperature (374 K), subcritical water, also known as pressurized hot water, delivers liquid water under pressure. Water has a minimal environmental impact because it is harmless to human health and the environment, safe to handle, and can be transported, thanks to existing infrastructure. Water is, therefore, regarded as one of the most environmentally friendly solvents. Additionally, it does not require drying the starting material prior to an extraction process that will be conducted in aqueous conditions [5]. The variation of the dielectric constant with temperature is the most crucial aspect to take into account in this kind of extraction method. With a dielectric constant close to 80, water is a strongly polar solvent when it is at normal temperature. With the right pressure, water can be heated to a temperature of 250 °C while remaining in its liquid condition, significantly lowering this value to numbers near to 27. According to Miller et al. this dielectric constant value is comparable to that of ethanol [6]. Steam needs to be pressurized at high temperatures (above boiling point: 100–374 °C) in order to move through the material effectively. The instrumentation basically consists of an oven where the extraction cell is installed and extraction takes place, a water reservoir connected to a high-pressure pump to inject the solvent into the system, and a restrictor or valve to maintain the pressure. The vial at the conclusion of the extraction mechanism is where the extracts

are collected. The system can also be fitted with a coolant mechanism to allow for the quick cooling of the extracted product. Despite the fact that this method has often been applied as a batch process, research of continuous methods and the online coupling of a subcritical water extraction (SWE) system to an HPLC apparatus via a solid phase trapping have been reported [7].

3 Organic Solvents

In separation techniques such as solvent extraction, sonication, microwave-assisted extraction, and pressurized fluid extraction, organic solvents are frequently utilized. The selection of the solvent is mostly determined by the advised standard procedures. According to the Environmental, Health, and Safety assessment results for 26 regularly used pure organic solvents, formaldehyde, dioxane, formic acid, acetonitrile, and acetic acid all received high marks overall. Dioxane had a high persistency, acetic and formic acids had high ratings for irritation, and formaldehyde had high values for acute and chronic toxicity. Methyl acetate, ethanol, and methanol, which in particular pose modest environmental dangers and relatively low health hazards, on the other hand, received poor total scores.

From a life-cycle perspective, the use of tetrahydrofuran, butyl acetate, cyclohexanone, and 1-propanol is not advised because these solvents have significant negative environmental effects throughout petrochemical manufacture. In addition, solvents with a very high environmental impact include formic acid, ethyl acetate, acetonitrile, dioxane, 1-butanol, and dimethylformamide.

On the other hand, solvents that are good for the environment include hexane, heptane, and diethyl ether. These outcomes in relation to alkanes are brought about by energy recovery through incineration along with the comparatively negligible environmental effects of their manufacturing. Following the use of supercritical fluids as substitute solvents, the use of renewable organic or ILs, the use of aqueous solutions of amphiphilic compounds or supramolecules, and the use of water under particular temperature and pressure conditions must all be taken into consideration, as shown in Fig. 2. For their transparency in this spectrum range and for extraction, carbon tetrachloride, chloroform, and dichloromethane were the solvents that had been commonly employed in spectroscopy. However, they are no longer used due to their toxicity, carcinogenic effects, and ozone-depleting properties. Since then, significant research has been put into developing chlorinated solvent substitutes. With volatile organic compounds (VOCs), the issue is the same. Common solvents used in solvent extraction are frequently volatile organic compounds (VOCs), and during the past few decades, they have been linked to a number of direct and indirect environmental and health harms (Table 1).

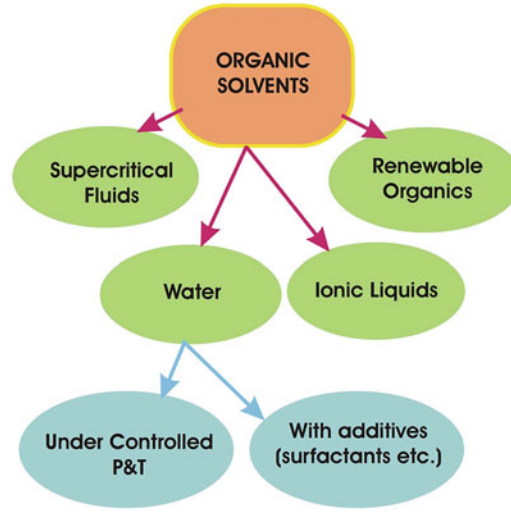

Fig. 2 Greener alternatives to the organic toxic solvents in solvent extraction

The following should be highlighted as direct impacts of VOCs: their toxicity and carcinogenic effects, which depend on the compound, the exposure, and the length of exposure; their flammability and associated fire hazards; and the potential for peroxide generation, particularly in the case of ethers. The following are the three main indirect environmental issues caused by VOCs: (i) their ozone-depletion properties, particularly in the case of chlorofluorocarbons, which are currently being phased out; (ii) the potential for global warming because of the role that VOCs play in the creation of photochemical smog; and (iii) their environmental persistence. The usage of environmentally friendly solvents must be strictly enforced in substitution of regularly used ones for the aforementioned causes. Table 2 demonstrates that the US EPA's list of harmful air pollutants from 2002 includes popular solvents used in solvent extraction.

3.1 Subcritical Hot Water as a Solvent for Extraction

The physicochemical transformations of water from ambient to near-critical conditions, under which subcritical water extraction (SWE, Fig. 3) can be carried out, are one of the key factors that make water an intriguing solvent [5]. Subcritical water extraction or extraction with hot water under pressure at the critical point of water (22.4 MPa and 374 °C) has become a practical instrument to replace the conventional extraction techniques. SWE is a technology that doesn't harm the environment and can increase extraction yields from solid samples [8]. To keep water in the liquid condition, SWE uses hot water (between 100 and 374 °C) under high pressure (often between 10 and 60 bar). At temperatures between 80 and 250 °C and high pressure, the polarity of water reduces significantly, replacing organic modifiers and offering environmentally

Table 1
Common organic solvents present in the list of hazardous air pollutants published in 2002 by the US EPA

Chemical name	Effects on humans and environment
Acetonitrile	Acid rain
Benzene	Recognized human carcinogen (Group 1)
Ethyl benzene	Photochemical smog formation
Toluene	Skin irritation
Xylene	Skin irritation
Phenol	Moderately toxic
Cresol	Moderately toxic
Carbon disulfide	Coronary heart disease
Carbon tetrachloride, Chloroform, Dichloromethane, Trichloroethylene, Tetrachloroethylene, Chloroethane	Ozone-depleting agents/toxic to the liver, heart and kidneys/reasonably anticipated to be a human carcinogen (Group 2B)
Bromoform, Dibromoethane, Bromoethane	Recognized human carcinogen (Group1)
Methanol	Produces formaldehyde, which causes headache, insomnia, gastrointestinal problems and blindness
Methyl ethyl ketone	Potentiates toxicity of haloalkanes and *n*-hexane
Methyl isobutyl ketone	Potentiates toxicity of haloalkanes and *n*-hexane
n-Butyl ketone	Nerve-cell degeneration
Methyl tert-butyl ether	Possible human carcinogen (Group 2B)
Diethanolamine	Negative effects on liver, kidney and blood
Formaldehyde	Allergic contact dermatitis/reasonably anticipated to be a human carcinogen
Triethylamine	Reversible corneal edema
1,3- Butadiene	Recognized human carcinogen (Group1)

beneficial green solutions [9]. It is not a novel idea to employ liquid water at temperatures higher than its boiling point to increase the solubility of organic molecules.

It has long been used as an environmentally friendly substitute for organic solvents in the cleaning process to improve the extraction of oil shale [10], sulfur from ore bodies (Williams et al. 1999), and essential oils from plant materials [11]. Steam needs to be pressurized at high temperatures (above boiling point: 100–374 °C) in order to move through the sample effectively. A potent substitute for the extraction of solid materials is the SWE. It has been used to remove pesticides and polycyclic aromatic hydrocarbons from soil samples as well as contaminants with a variety of polarities from environmental samples.

Table 2
Critical properties of several solvents used in supercritical fluid extraction (SFE)

Solvent	Critical Property			
	Temperature (°C)	Pressure (atm)	Density (g/mL)	Solubility parameter δ_{SFC} ($cal^{-1/2}$ $cm^{-3/2}$)
Ethene	10.1	50.5	0.200	5.8
Water	101.1	217.6	0.322	13.5
Methanol	−34.4	79.9	0.272	8.9
Carbon dioxide	31.2	72.9	0.470	7.5
Ethane	32.4	48.2	0.200	5.8
Nitrous oxide	36.7	71.7	0.460	7.5
Sulphur hexafluoride	45.8	37.7	0.730	5.5
n-Butane	−139.9	36.0	0.221	5.2
n-Pentane	−76.5	33.3	0.237	5.1

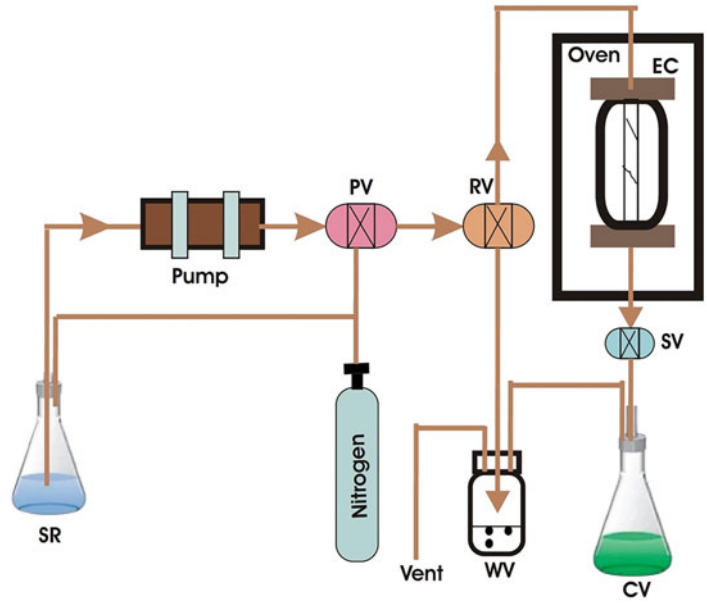

Fig. 3 Diagram of a subcritical water extraction (SWE) system. SR solvent reservoir, PV purge valve, RV pressure relief valve, EC extraction cell, SV static valve, CV collector vial, WV waste vial

Basile et al. suggested using SWE as a very promising substitute for traditional and supercritical CO_2 extraction procedures for the isolation of essential oils [12]. Since that time, the method has demonstrated its applicability in the field of essences in comparison to more traditional methods like solvent extraction and steam distillation, which have a number of well-known drawbacks, including low extraction efficiency, extended extraction times, and significant quantities of toxic solvent waste. Subcritical water, compressed hot water, or pressurized hot water is defined as water that remains liquid in the temperature range of 100–374 °C. Compared to ambient water, this kind of water has special characteristics. A low relative dielectric constant and a high ion product are the two types. These characteristics allow for the extraction of useful substances from natural resources using this water. This chapter reviews the use of subcritical water for extracting chemicals from agricultural waste or products [13].

A large variety of substances can be employed as supercritical fluids (see Table 2 for a list of some of the critical features of various solvents utilized in SFE). Supercritical carbon dioxide solvent is the subject of great interest since it is nontoxic, acceptable to the environment, affordable, and has low and moderate critical pressures (72.9 atm and 31.3 °C, respectively). Carbon dioxide is a relatively nonpolar solvent that mostly dissolves nonpolar solutes.

Advantages and Disadvantages of Supercritical Extraction

SFE's biggest drawback is its high pressure, which necessitates more expensive production equipment. The critical pressures, however, are lower than many of the high-pressure processes now employed in the petrochemical sector. It demonstrates the advantageous mass transport qualities that, when compared to the liquid phase, can be attained in the supercritical region due to low viscosity and high diffusivity. Both the solutes and the solvent of choice affect the separation properties in SFE [14].

4 Renewable Water-Based Solvents

Selective extractants, which can be created, for example, by creating organic compounds specifically with preorganized metal-binding sites, are still in high demand. Such extractants may be developed using computer-based models. The extractants and diluents employed must be either nontoxic, nonvolatile, or recovered during the process due to environmental reasons. There are many new demands placed on the diluents utilized by the increasing combination of extraction and distillation used in biotechnology. It is important to take precautions while extracting biologically active

chemicals to prevent the activity loss that frequently results from contact with organic diluents. As a result, a number of systems have been created with these chemicals in mind.

4.1 Aqueous Two-Phase Systems as Extractants

The first of these employs two-phase separation systems, which combine aqueous solutions with polymers and inorganic salts to form two phases that are primarily composed of water. Using supercritical circumstances, a second system converts the initial two-phase system into a single phase under unique temperature-pressure conditions. Additionally, by encapsulating the active organic compound inside the aqueous center of a micelle of surface-active chemicals, the active organic compound can be protected from the organic diluent. As is explained below, all of these systems are currently the subject of active research.

4.2 Supercritical Fluid Extractants

A fluid becomes a supercritical fluid when it is pushed to pressure and temperature over its critical point. A substance at a temperature and pressure over its critical point is referred to as a supercritical fluid. In these circumstances, the fluid's characteristics are positioned somewhere between those of a gas and a liquid. Although a supercritical fluid's density is comparable to that of a liquid and its viscosity is comparable to that of a gas, its diffusivity is in between those two states. The most effective and efficient method for extracting important component botanicals is supercritical fluid extraction. The king of botanical extraction solvents is CO_2. The critical temperature and critical pressure for supercritical CO_2 extraction are higher than 31 °C and 74 bar, respectively. Supercritical fluids are extremely compressed gases that have intriguingly mixed properties of both gases and liquids. Reactions that are challenging or perhaps impossible to achieve in normal solvents can occur in supercritical fluids. It is a quick process that takes 10–60 min to finish. Simply releasing pressure can separate a supercritical fluid from an analyte, leaving nearly no residue and producing a pure residue [15].

The extraction of polar solutes from supercritical CO_2 is less successful than it is with lipophilic molecules. In order to significantly increase the solubility of amphiphilic molecules in supercritical CO_2, CO_2 must be coupled with a polar cosolvent for the purpose of isolating them. The two cosolvents most frequently utilized are ethanol and methanol. At room temperature, carbon dioxide is a gas; therefore, after the extraction is finished and the system is decompressed, a significant amount of CO_2 is eliminated without leaving any residues, producing a solvent-free extract. When carbon dioxide consumption is high on an industrial scale, the process can be managed to recycle it. Supercritical CO_2, on the other hand, performs less well in the extraction of highly polar chemicals from natural matrices due to its low polarity. Modifiers (also known as cosolvents) are frequently employed to remedy this

issue. Modifiers are highly polar chemicals that can significantly alter the solvent characteristics of pristine supercritical CO_2 when introduced in small amounts. The high investment costs of SFE as compared to conventional atmospheric pressure extraction methods are another disadvantage. The hydroperoxide method used at PAO to extract valuable components (methyl phenyl carbinol, acetophenone, ethylbenzene, phenol, and propylene glycol) from industrial wastewater created during the coproduction of styrene and propylene oxide was used as an example to develop and discuss strategies for improving the efficiency of the extraction process carried out under supercritical fluid conditions beyond the binodal [16].

5 Ionic Liquids as Solvents for Extraction

Organic salts with a melting point less than 100 °C are ionic liquids (ILs). If the salt is liquid at room temperature, they are referred to as room-temperature ILs. They typically consist of an organic or inorganic anion and a bulky, poorly coordinating organic cation. When air- and water-stable ILs were first synthesized in the early 1990s, chemists were interested in ILs [17]. ILs have a wide range of uses in chemistry and are sometimes referred to as a "green alternative." They are frequently combustible and explosive and have low vapor pressures, which suggests they have less of an impact on the environment than VOCs. ILs are helpful in liquid–liquid extraction (LLE), liquid phase microextraction (LPME), and solid phase microextraction (SPME) due to their low vapor pressure and strong solubility for both inorganic and organic chemicals [18]. Studies of the deep eutectic solvent (DES) extraction mechanism, particularly extraction by the creation of a deep eutectic system (DESys), have found similarities between the DES- and ionic liquids (IL)-based extraction systems. Xiao et al. presented new uses for ILs and DES in the extraction of nutritious natural compounds [19]. In the mechanochemical extraction of target chemicals from Moringa oleifera leaves, the extraction behavior of choline chloride (ChCl) and 1-(2-hydroxyethyl)-3-methylimidazolium chloride ([HMIm][Cl]) in DES and IL, respectively, was thoroughly investigated.

5.1 Properties of ILs

ILs have a variety of fascinating and distinctive characteristics, one of which is extremely low vapor pressure. ILs fundamentally do not evaporate under typical process operating conditions. The use of ILs as industrial solvents to replace VOCs and so remove a source of air pollution as well as risks from inhalation and explosion has thus attracted a lot of interest [20]. Additionally, ILs maintain their liquid state throughout a huge temperature range (e.g., 70–400 °C). Both of these characteristics, a negligible vapor pressure and a

wide liquidus range, will make it easier to recover and repurpose ILs in the context of LLE and hence bring financial advantages (such as a make-up required and solvent loss that are incredibly low). Some ILs can remove hydrophobic substances due to their hydrophobic nature [21]. However, using the proper chelators or ligands that could form complexes to improve the hydrophobicity of the metal species, cationic compounds can be successfully recovered from hydrophobic ILs [22]. The following factors are those that determine how well metal ions are extracted: (1) The side chain length and IL structure, which alter the hydrophobicity and increase partition coefficients [23]. (2) Selectivity should be maximized by the ligand utilized. (3) The system's pH [24].

5.2 Toxicology Considerations

The possibilities provided by ILs, with their several million possible structures created by combining various cations and anions, also pose an uncommon issue in terms of defining or identifying the toxicity and adverse impacts of those compounds on the environment. ILs are novel compounds that have not yet gained widespread acceptance. It is challenging for traditional scientists to abandon theories that have sprung out from the fertile ground of molecular solvents over millennia. The number of laboratories that work with ILs has increased significantly in recent years, particularly in China. Of course, it's crucial to have a complete awareness of the potential health risks and environmental effects of ILs.

Unfortunately, research has shown that the initial generation of ILs, which were based on imidazolium or pyridinium cations, were oftentimes much more harmful than conventional solvents. The goal for the elimination of the most harmful IL structures is the development of simple toxicity tests to enable quick and affordable identification of the best IL structures. Selecting only the most suitable candidates for application, the remaining candidate structures can be examined using minimum inhibitory concentrations (MICs) and minimum biocidal concentrations (MBCs) tests, growth rate measurements, and EC50 (medium effective concentration) calculations.

Imidazolium, pyridinium, phosphonium, and ammonium cations were not found to have low freshwater toxicity with EC50 values below 100 mg/L, according to certain researches [25]. No matter the kind of cation, the length of the substituted alkyl chain on the cation had a substantial impact on the toxicity; for example, ILs with eight carbon atoms (C8) were shown to be more toxic than those with six and four. Due to their extensive structural variety, ILs appear to have a particularly promising future in separation approaches. Technologists need thorough research to hasten the introduction of new nontoxic ILs in the created new separation processes because certain ILs are harmful and cannot be considered as general green replacement solvents.

6 Regeneration of Organic Phase

Unwanted components are not always stripped. Then it accumulates in the organic phase to the point where it substantially impedes extraction. In this instance, a more forceful strip is applied to the solvent to remove the impurity and renew its performance. The contaminant may be allowed to accumulate in the extract for some time before the solvent is regenerated in batches, or a tiny side stream may be constantly bled from the recycled organic phase and regenerated. Regeneration of the organic phase can be expensive due to its robust nature, but the contamination can occasionally be highly valuable. Complexes of platinum, gold, and cobalt have occasionally behaved as pollutants, and their recovery has covered the cost of regeneration.

7 Bio-Derived Solvents in Water

Among all bio-solvents, bio-based ethanol is now the most widely manufactured. It is made by biologically transforming sugars. Both edible (sugarcane and maize) and inedible (cellulose) feedstocks are used in these procedures [26]. However, there has been a move towards improving the processes that produce cellulosic ethanol due to worries that edible feedstock is driving up food prices [27], and in fact, Beta Renewable launched the first cellulosic ethanol commercial-scale production plant in the world in 2013. This facility (Biochemtex, Crescentino Biorefinery) in Milan, Italy, uses ProesaTM technology to pre-treat agricultural waste (such as rice straw, giant cane (Arundo donax), and wheat straw) for the generation of ethanol at a rate of 60,000 tons per year.

The most widely used applications for ethanol are as a biofuel and solvent in consumer goods such as perfumes, food coloring and flavoring, alcoholic beverages, and some types of mediation. Both natural and manufactured drugs may contain the latter. A highly effective method of extracting the active antimalarial medication artemisinin from Artemisia annua has been shown [28]. Methanol is currently produced on a large scale from fossil fuels by hydrogenating carbon monoxide in the presence of a catalyst like ZnO/-Cr_2O_3 or $Cu/ZnO/Al_2O_3$ [29]. It can be found in trace concentrations in fermentation broths and when biomass is gasified to create bio-based syngas, which is then converted to methanol.

The latter's economic potential is still being researched [30]. Energy density for n-butanol is 29.2 MJ/L, which is comparable to gasoline's (32.5 MJ/L) and has a low level of toxicity. It is not surprising that this alcohol is occasionally blended with petrol to enhance its qualities because it is likewise miscible with petrol. In addition to being used as a solvent for paints, varnishes, resins, and

Table 3
Solubilities of bio-derived solvents in water

Solvents	Examples	Solubility in Water
Alcohols	Ethanol Glycerol	Soluble Soluble
Glycerol derivatives	Over 60 species	Mostly soluble
Esters	Biodiesel Ethyl Lactate	Insoluble Soluble
Acids	Gluconic acid	Soluble
Terpenes	D-limonene α-pinene	Insoluble Insoluble
p-cymene	*p*-cymene	Insoluble
Furfural family	Furfural Furfural alcohol Levulinic acid Ethyl levulinate Butyl levulinate	Soluble Soluble Soluble Soluble Soluble
γ-valerolactone	γ-valerolactone	Soluble
Furan derivatives	2-Methyltetrahydrofuran 2,5-Dimethylfuram (DMF)	Soluble Insoluble
Dihydrolevoglucosenone	Dihydrolevoglucosenone (Cyrene)	Soluble

waxes, n-butanol is also used to make plasticizers like butyl phthalates, solvents like butyl propanoate, dibutyl ether, and butyl acetate, as well as coatings (varnishes, resins, and waxes). Table 3 lists a variety of bio-derived solvents and their water solubilities; it demonstrates that biodiesels, terpenes, and 2,5-dimethylfuran (DMF) are water insoluble and may, therefore, be employed in solvent extraction procedures [1]. Energy crops like corn, forest goods like wood, aquatic biomass like microalgae, and waste products like urban wastes are all examples of biomass. These bio-derived solvents can be destroyed after usage (Fig. 4).

8 Application of Solvent Extraction in Biotechnological Separations

Traditional biotechnological product recovery strategies have centered on separation techniques like electrophoresis or column liquid chromatography. These techniques are costly for medium- and low-value products and challenging to scale up to production levels. The primary recovery of fermentation cell culture products, such as carboxylic acids, proteins, and amino acids, has thus been recognized as a possible application for liquid extraction. However,

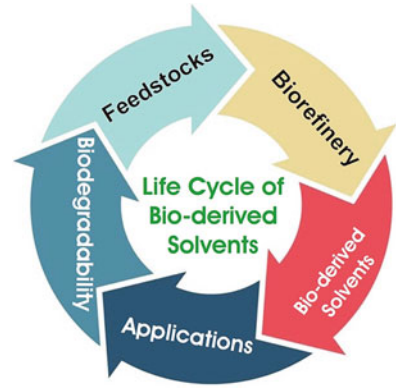

Fig. 4 Life cycle of bio-derived solvents

the separation issue is challenging because the product combinations, which frequently contain cell waste and enzymes, are complicated. Proteins can be handled in aqueous two-phase systems or by extraction in reverse micellar systems but are not appropriate for conventional solvent extraction due to incompatibility with organic solvents. Although it is frequently claimed that biotechnological processes are energy efficient since the reaction temperature is low, it is crucial to understand that the product concentrations are low and that the product recovery step is frequently the one that requires the most energy.

8.1 Carboxylic Acids Separation

For the purpose of separating organic and amino acids from fermentation broth, solvent extraction has been suggested as an alternative [31]. High molecular weight amines and organophosphate solvating agents can both be used to extract citric acid. Important requirements have been defined for the solvent extraction process used to produce citric acid: A distribution coefficient of 10 or below enables simple water stripping. Although the citric acid may be recovered using a base, the production of a citrate salt would require additional processing as in the typical flow sheet, eliminating the benefits of solvent extraction. This is why stripping with water is crucial.

8.2 Amino Acids

Because amino acids include both carboxyl (-COOH) and amino ($-NH_2$) groups, they exhibit cation-like behavior at low pH, anionic behavior at high pH, and zwitterion behavior at intermediate pH levels. Their solubility in nonpolar diluents is limited despite the fact that they have no net charge in this intermediate pH range due to their hydrophilicity. Additionally, its extraction using oxygen donor extractants with carbon bonds is subpar. In order to extract an amino acid, it is typically necessary to change it into one of its ionic forms and then utilize an appropriate ion-pair extractant. Because the amino acid has a net negative charge in high pH

conditions, an anionic extractant, often an alkylammonium salt, such as tri-n-octylamine hydrochloride (R_3NHCl), can be used to remove it. Here, (R_3NH) (RNH_2COO) is the extracted species [32]. Both times, regeneration of the extracting species is simple by the use of diluted alkaline or acidic solutions, respectively.

8.3 Citric Acid

For the purpose of separating organic and amino acids from fermentation broth, solvent extraction has been suggested as an alternative [33]. High molecular weight amines and organophosphate solvating agents can both be used to extract citric acid. Important requirements have been specified for the solvent extraction processing of citric acid: A distribution coefficient of 10 allows for simple water stripping. Although the citric acid may be recovered using a base, the production of a citrate salt would require additional processing as in the typical flow sheet, eliminating the benefits of solvent extraction. This is why stripping with water is crucial. Temperature had an impact on a diluent's extraction, which decreased as temperature rose. So, an effective technique was developed by extraction from the broth at room temperature and water stripping at a higher temperature (60–70 °C). The creation of emulsions and the resulting poor separation made it difficult to extract using long-chain amines.

8.4 Extraction of Oil from Algae Biomass

A difficult task in calculating the total economics of fuel production is the extraction of fuels from microalgae biomass. It has been discovered that the commonly used extraction techniques call for either more sophisticated machinery or challenging processing conditions. The goal of the innovative extraction technique is to remove the oil from the biomass using a combination of solvent extraction and magnetic stirred agitation. When compared to extraction techniques such as supercritical extraction and nano-assisted extraction, which are actively being researched, this technology has been shown to be more cost-effective. If magnetic stirred or electromagnetic-assisted agitation is used on a commercial scale, the use of magnetic stirrer-based extraction for sustainable biofuel production may lead to new dimensions [34]. This method uses natural algae biomass for oil extraction.

8.5 Bioactive from Marine Algae

In a variety of culinary products, compounds from marine algae have been employed as gelling, thickening, and emulsifying agents. Apart from being a rich source of iodine historically, sea algae were not recognized as a source of health-promoting chemicals in the West [35]. Marine algae are a rich source of nutraceuticals with a variety of biological activities, according to a recent study on functional food ingredients [36]. Dietary fiber, sulfated polysaccharides, omega-3 fatty acids, amino acids, bioactive peptides, vitamins, minerals, and carotenoids are all abundant in marine algae. These

algae are also widely distributed in nature. New and improved innovative extraction technologies must be developed in order to fully realize this promise. Traditional extraction methods need a lot of time and employ organic solvents, which are not environmentally friendly [37].

9 Pharmaceutical Separations

For the creation of pharmaceuticals and the isolation of natural compounds, liquid-liquid extraction is widely utilized [38]. Since these compounds are frequently heat-sensitive, techniques like air distillation or evaporation cannot be used to recover them. Due to competition, there is a lack of specific information about ongoing business operations. The purification and concentration of penicillin is a well-known and best-documented example of a process that ran into issues common to medicinal substances [39].

9.1 Production of Penicillin

Before being given to the first extraction step, where it is in touch with substances like butyl acetate, the fermentation broth containing penicillin is first filtered to remove mycelium and pH-adjusted to 2–2.5 to convert it to the mostly undissociated penicillanic acid [40]. The partition coefficient and penicillin stability have been compromised to determine the exact pH that is employed. However, if it becomes necessary to create pure penicillin for pharmaceutical use, it can be refined by reextraction at pH 2–2.5 and further stripping with a phosphate solution at pH 6. The majority of penicillin is utilized as intermediates in the synthesis of, for example, cephalosporin. This penicillin extraction is an illustration of the direct partitioning of a solute using a polar organic molecule. Utilizing organic chemicals that form ion pairs with penicillin is a different method that has been taken into consideration. Here, the authors discovered that in the pH range 5–7, where the product is most stable, the penicillin anion could be extracted effectively with a secondary amine (Amberlite LA-2). This method, which is widely employed in hydrometallurgy, can be utilized to extract either cationic species using organic acid anions or anionic species utilizing cations, as was previously demonstrated. While a hydrocarbon diluent is typically used in hydrometallurgy, more polar diluents are typically needed for medicinal applications. Ion pair creation is used in a number of other biotechnology systems, even though the adoption of such chemically assisted extraction procedures is unlikely to replace the current extraction processes for the commercial extraction of penicillin.

10 Future Trends in the Development of New Solvents

Concern over the effects of chemical activities on the environment will only grow in the future. The liquid effluents must be made environmentally safe. The main efforts focus on enhancing the water's solvent properties at the proper temperature and pressure or using cosolvents or additions that are water-miscible, including surfactants. However, other options, like the creation of new solvents with improved separation capabilities, switching to renewable organic solvents like alcohols from petroleum-derived ones, or the use of supercritical fluids and ILs, can provide less expensive, extremely inventive, and untapped environmentally friendly options.

11 Concluding Remarks

So long as the processes are properly built, solvent extraction is, in theory, an environmentally pleasant process that doesn't pollute the air or the water. Therefore, it might replace a lot of the current polluting operations. The solvent extraction effluents, however, are a specific issue since they may contain biochemically active chemicals that present "new" environmental dangers. These can be processed using a variety of solid sorbents, which can subsequently be burned, although the benefit of solvent extraction may be lost. Therefore, there is a need for environmentally friendly and biodegradable solvent phases. Future research in this area will need more focus. Numerous fields of chemistry have used the principle of solvent extraction, which divides chemical species between two immiscible liquid phases. One such example is liquid partition chromatography, whose widespread application has given rise to a whole field (and industry!) as a result of the principle of solvent extraction providing the most effective separation procedure now available to organic chemistry. Fundamental studies on solvent extraction are anticipated to continue to influence the creation of new, selective analytical methods. Chemical kinetics, final equilibrium distribution of the contents between the two phases, and transit of chemical compounds from one phase into another are all topics covered by solvent extraction. The mechanisms that enable such transport and distribution are what sustain life in biological systems. Fundamental investigations of these "solvent extraction" processes advance our knowledge of all natural processes. Here, only a lack of creativity prevents the development of significant new scientific findings. The focus of future research efforts in this field should be on overcoming the barriers to implementing these cutting-edge technologies on a large-scale basis so that the enormous advantages of better bioactive extraction from food and their use in the nutraceutical sectors may be realized.

Continued research on extraction technologies that are both economically and environmentally viable is driven by the need to extract nutraceuticals from plant-based materials. Traditional solid-liquid extraction techniques take a long time and a lot of solvent. The extensive use of solvent raises operating costs and contributes to more environmental issues. As an alternative to traditional extraction procedures, a number of unique extraction techniques have been developed, giving benefits in terms of extraction time, solvent usage, extraction yields, and reproducibility. However, only a small number of applications have made use of innovative extraction approaches. More study is required to better comprehend the mechanisms of extraction, overcome technological obstacles, enhance the design, and scale up novel extraction systems for use in industrial settings.

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Chapter 8

Enzyme-Assisted Extraction

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Abstract

The extraction of compounds from plant and animal sources that have medicinal properties are known as bioactive compounds, which can either improve health or can be hazardous. As novel natural compounds from various plants are uncovered, we are discovering more biologically active compounds. Enzymes can be employed to extract such bioactive compounds by rupturing plant cells. The extraction of a bioactive compound such as stevioside from a stevia plant with the assistance of enzymes is a better example of processing with potential utility for the food sector. The extraction of bioactive compounds from plant sources with the help of environment-friendly enzymes, especially for food, nutraceutical, or pharmaceutical uses, is a cutting-edge technology used in these industries. This study explains the overall idea about bioactive compounds, their extraction processes, importance, and uses.

Key words Bioactive, Stevioside, Stevia, Enzymes, Enzyme-assisted extraction, Nutraceutical, Biotechnological applications

1 Introduction

The chemical compounds that are produced in the plant, which are not involved in the metabolic activity of the plant, are mostly secondary metabolites. These are biologically active compounds [1]. These are produced by the subsidiary pathways. These bioactive compounds have antioxidant, anti-inflammatory, and antimicrobial properties and greater immunomodulatory potential properties. Detailed information about secondary metabolites and their application is mentioned in Table 1. These compounds possess additional nutritional value to the food that is generally found in less amount, which provides health-related benefits over the product's basic nutritional value [2]. These compounds include pharmaceuticals, flavors, fragrances, cosmetics, food additives, feedstocks, and antimicrobials. These compounds are produced

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Table 1
Type of chemical class, plant source, and enzyme used for the extraction of specific bioactive compounds

Type of bioactive compound	Class of bioactive compound	Plant source	Enzyme used for extraction
Glycosides	Sugar Oligosaccharides Inulin Starch Pectin	Grapefruit peel waste Rice bran Jerusalem artichoke Cassava Pumpkin	Cellulose and pectinase Cellulose Inulinase Pectinase Xylanase Pectinesterase Endopolygalacturonase Beta glucosidase Cellulose
Oil and carotenoids	Oil Carotenoids Lycopene Anthocyanin Capsaicin Carotene	Grape seed Marigold flower Tomato Grape's skin Chilly Carrot pomace	Cellulose Xylase Pectinase Protease Viscozyme Pectin Nutrase Corolase Ht-Proteolytic Pancreatin Cellulose Pectinase Pectin ax BE3-l Cellulose Hemicellulose Pectinase Pectin ultra SP-L
Others	Flavonoids Phenolics Soluble fiber Protein Catechins	Kinnow peel Citrus peel Carrot pomace Lentils and white beans D beverage	Recombinant rhamnosidase CelluzymeMX Cellulose-rich crude preparation Glucoamylase Pepsin

by the plant cell through metabolic pathways such as the malonic acid pathway, shikimic acid pathway, methylerythritol phosphate pathway, and mevalonic acid pathway.

The extraction of bioactive compounds is becoming much easier nowadays, and these extracted compounds are utilized in the drug industries for the production of novel drugs against life-threatening diseases and also utilized in food industries to enhance the quality of the food. Other potential properties of bioactive compounds, for example, antimicrobial, anti-inflammatory, anti-cancer, antimicrobial, and antidiabetic activities are utilized for

various applications. For the extraction of these compounds, the traditional method of extraction gives less yield, and also there is no proper method for extracting the bioactive compounds.

Biotechnologists and other chemists started to find a way to extract bioactive compounds with a greater yield on an industrial scale, which can also be helpful commercially. After so many attempts of research, enzyme-assisted extraction came out to be an efficient way to extract bioactive compounds [3]. The enzymes will disrupt the cell wall and make the cell wall permeable that can be easier to get more yield of bioactive compounds such as flavonoids, terpenoids, and lectins, which are having applications in various types of industries. This method of using specific enzymes for the extraction of compounds seems to be very efficient as compared to the other extraction techniques.

2 Examples of Plant-Based Bioactives

The major classes of bioactive compounds are alkaloids, terpenoids, and phenolic compounds. Other basic examples include bioactive compounds that are flavonoids, terpenes, polyphenols, lycopene, resveratrol, lignan, tannins, and indoles [4]. These types of compounds are mainly used in food industries, for example, marigold flowers are the most significant source of carotenoids. Nevertheless, the efficacy of the extraction depends on the circumstances for collection, drying, and the solvent extraction procedure, which results in 50% losses of the carotenoids. Macerating enzymes have been used before with good results to increase the extraction yield of effective chemicals from natural materials. In this study, a different extraction technique for carotenoids was discovered that involved both a simultaneous enzymatic procedure and solvent extraction [5]. By skipping the ineffective matter and drying procedures, the suggested method uses freshly milled flowers as raw material. A carotenoid recovery yield of 98% was achieved using the described technique at the 80 L scale, under typical testing conditions [6]. Lycopene, a carotenoid found in high concentration in tomatoes and tomato-derived products, is receiving a lot of attention these days because epidemiological evidence suggests that it may protect against cancer and some degenerative diseases that are impacted by free radical reactions. Recent epidemiological studies have shown that consumption of tomatoes and blood lycopene levels are inversely related to the risk of getting cancer at several anatomical sites, including the prostate gland, stomach, and lungs. By successfully delocalizing trapped free radical species, conjugated carbon-carbon double bonds endow lycopene with their antioxidant characteristics. Hence, food industries, pharmaceutical industries, and cosmetic industries all have a significant demand for lycopene [7].

Salvia officinalis, which is communally called sage, is the most commonly known herb used in the kitchen, and it belongs to the family Labiatae. Sage has a long history of helping people stay healthy and alleviate illnesses. Sage essential oil has shown efficiency in the treatment of Alzheimer's disease and efficiency to enhance memory, according to current research and scientific studies. Alzheimer's is a neurological disease. Sage is one of the kitchen plants with an aromatic nature and contains an oil-producing capacity. Sage has been the subject of much research for its phenolic antioxidant components since its early days, roughly 20 years ago. Sage has several powerful antioxidants, according to numerous types of research [8]. Sage is a plant of commercial relevance for scientific research because of its essential oil and antioxidant components. All food, cosmetic, and pharmaceutical products start with sage essential oil and flavorings as their primary ingredient [9]. Sage antioxidants can be utilized as a different approach to the popular rosemary antioxidants to protect and preserve specific foods and nutraceutical items to lengthen their shelf life [8]. Other different types of bioactive compounds and their respective plant source and their uses are mentioned in Table 2.

Bioactive compounds can also be extracted from other sources such as microbes, fungi, and animals. Fishes are one of the sources for bioactive compounds. Different types of bioactives extracted from different fish species and their application are listed in Table 3.

2.1 Extraction Process

The process of extraction involves the separation of our desired natural constituents from the available raw materials. There are different types of extraction methods commonly used in industries for the extraction of compounds from plant and animal sources, for example, distillation extraction, solvent-based extraction, pressing method, and sublimation process, which are categorized based on their working principle. The reason behind the process of extraction of bioactive material is to get the therapeutically valuable molecule and the inert part is eliminated with the help of a selective solvent treatment known as the menstruum. Menstruum is the solvent used in the extraction. The materials that remain after the process of extraction are called "marc." For the extraction process, we need to prepare concentrations of raw materials using various solvents. The solutions such as alcohol and hydro alcohol sols are prepared from raw materials or chemical substances such as belladonna [11].

Using various solvents with different polarities is the key feature of this process. However, to date, there is no single extraction method for extracting all the metabolites at a given time. Some of the normal methods are listed as follows:

1. *Maceration*: In this method of extraction, the plant material is crushed or chopped into small pieces. Sometimes the dried

Table 2
List of bioactive compounds and their plant sources with uses

Product	Plant species	Uses
Digoxin	*Digitalis lanata*	Cardiovascular disorders
Vanillin	*Vanilla* sp.	Vanilla
Jasmine	*Jasminum* sp.	Perfume
Taxol	*Taxus brevifolia*	Anticancer
Baccharine	*Baccharis megapotanica*	Anticancer
Cesaline	*Caesalpinia gillisesii*	Anticancer
Pyrithrins	*Tagetus erecta*	Insecticide
Saffron	*Crocus sativus*	Food color and flavoring agent
Stevioside	*Stevia rebaudiana*	Sweetener
Berberine	*Coptis japonica*	Antibacterial
Quinine	*Cinchona officinalis*	Antimalarial
Atropine	*Atropa belladonna*	Muscle relaxant
Reserpine	*Rauwolfia serpentina*	Hypotensive
Diosgenin	*Dioscorea deltoidea*	Antifertility
Vinblastine	*Catharanthus*	Anticancer
Maytansine	*Maytenus bucchananii*	Anticancer
Thaumatin	*Thaumatococcus danielli*	Sweetener
Capsaicin	*Capsicum frutescens*	Chilli

powder of plants is used to increase the efficiency of extraction. This method is performed in the closed system of the menstruum, which is the solvent used in the extraction. The plant material and this solvent are placed together; then it is incubated for some days with shaking at certain time intervals. After 5–7 days, the fluidics strained off and we can see the two phases in the system, namely, one supernatant and the residue part. Then the residue part is pressed to get the more fluidic content out and further followed by the filtration. Once the filtration is done, the solution is taken and solidified by evaporation method for ease of analysis of the compound followed by concentrating it and sent for further analysis and preparation of drugs in the pharmaceutical industries.

2. *Percolation*: This method is preferable for the production of vegetable drugs in the food industry. In this method, the uniform moistening of drugs with solvent for a period of 3–4 h is done in a separable vessel, which is performed in the

Table 3
List of the bioactive compounds derived from different fish species [10]

Source	Origin	Bioactives
Acipenser schrenckii	Skin	Antioxidant, Cry protective
Navodon	Skin	Antioxidant
Gadus macrocephalus	Gelatin	Antioxidant, ACE inhibitory
Johnius belengerii	Skin	Antioxidant
Theragra chalcogramma	Skin	ACE inhibitory
Oncorhynchus keta	Skin	Neurobehavioral
Priacanthus macracanthus	Skin	Antioxidant
Lutjanus vitta	Skin	Antioxidant
Parastromateus niger	Viscera	Antioxidant
Johnius belengerii	Bone	Calcium binding
Exocoetus volitans	Bone	Antioxidant, antiproliferative
Raja porosa	Cartilage	Antioxidant
Oreochromis niloticus	Skin	Antioxidant
Oncorhynchus keta	Skin	Long bone development
Chanos chanos	Collagen	Iron binding
Oncorhynchus keta	Collagen	Learning and memory
Oreochromis sp.	Collagen	Facial skin quality
Gadus morhua	Bone	Antioxidant
Clupea harengus	Whole, body, head gonads	Antioxidant

closed system. A piece of filter paper is placed on the surface, then picked up so that the top layers of drugs are not disrupted. The solvent is poured on the sample slowly at some specific time interval, allowing the bottom area to percolate easily and which is collected in the bottom of containers. This process followed for one complete day and fluid percolated through the plant sample is collected and followed by the evaporation of the sample to get the powder form to quantify the sample then is concentrated and sent for analysis which is further sent for production industries for the production of compounds at an industrial scale.

3. *Soxhlet apparatus*: This apparatus is used in the small-scale extraction of bioactive compounds in laboratory conditions. These steps are involved in bioactive extraction. The plant sample of *holanerrhea pubescus*, *terminalia elliptica*, or any other plant species such as mulberry which have high medicinal

properties is first dried in the microwave and then the powdered, dry powder of the plant sample is then placed in the pocket made up of a blotting paper like a pouch. This pouch is loaded in the main chamber of Soxhlet apparatus, the chamber that is attached to the condenser on the top, for the cooling effect, and in the bottom heating mantle for heating the sample. The solvent used here is ethanol or methanol. The phytochemicals in the sample are drawn into the solution and collected at the bottom, and after boiling the sample is cooled by the condenser. The process is continued until the drug or the phytochemicals are extracted and the extracted compounds in the flask are processed by evaporating and followed by the concentration method and sent for analysis [3].

2.2 Solvents Used in Extraction

In the process of extraction of bioactive compounds, different solvents are used. There are two types of solvents, namely, nonpolar solvents and polar solvents. The nonpolar solvents used in the extraction are cyclohexane, toluene, hexane, benzene, ether, chloroform, and ethyl acetate; hence, the products extracted from the nonpolar solvents are alkaloids, terpenoids, coumarins, fatty acids, flavonoids, and terpenoids [12]. The polar solvents used in the extraction are acetone, acetonitrile, butanol, propanol, ethanol, and methane. The products that are extracted by using polar solvents are flavonols, lectins, alkaloids, quassinoids, flavones, polyphenols, tannins, and saponins [12].

3 Process Development

The production of bioactive compounds has a commercial demand in the market; hence, there demand toward the plant tissue culture is at higher rates. The bioactive compounds in plants are obtained in the lower quantity with high-value pharmaceutical properties and food-beneficial properties. Hence, to get the bioactive compounds in the greater quantity for commercial use, biotechnologists go for the method called extraction of bioactive compounds facilitated by enzymes [13]. In an enzymatic method, there will be efficient extraction and a greater yield of bioactive compounds. In this method, specific lytic enzymes are used to damage the outer membrane of the plant cells to improve the productivity of the extraction [6].

The use of enzymes for the extraction of various bioactive compounds, for example, extracting vanillin from vanilla grape seed, extracting carotenoids either from tomato peel or marigold flower, extracting polysaccharides from *Sterculia foetida*, extracting oil from grape seeds, etc. has shown significantly good results. This method of extraction has greater potential and is commercially attractive [13]. Prior to the adoption of traditional extraction techniques, enzymes were utilized specifically to treat plant material

[7]. It is frequently necessary to use a variety of enzymes, such as pectinases, cellulases, also hemicelluloses, to break the structure [14]. Improving the extraction of bioactives from plants while maintaining the rigidity of the plant cell wall is challenging. These enzymes enhance cell wall permeability by hydrolyzing cell wall constituents, leading to increased bioactive extraction yields [13]. Extracts of fungi, bacteria, vegetables, or animal organs, also fruits can all be used to make enzymes. Understanding an enzyme's catalytic features and mode of action, ideal performing circumstances, and the enzyme or combination of the enzyme to utilize in an extraction application will help you use enzymes more effectively [11].

3.1 The Disintegration of the Cell Wall by the Action of Enzymes

It is the crucial step in the process of getting the bioactive molecules out of cells. Cells are the basic unit of life; cells store valuable bioactive compounds such as flavonoids, anthocyanin, carotene, and many more which has significant value in both food and nutraceutical industries; there is a variety of other classes of bioactive compounds from medicinal plants, which have pharmacological importance. Hence, it is important to extract them without denaturation and also to increase the extraction yield and productivity. Therefore, as the science is developing, there are various techniques that have been introduced for the easy extraction of phytoconstituents from various sources. The enzyme-assisted extraction is one such method that is commonly used nowadays to better intracellular compound extraction methods.

During the process of extraction of intracellular compounds from the cell, it is important to break open the cell so that it allows the intracellular compounds to come out of the cell. The capacity of the enzymes to degrade the cell wall component and also to disintegrate the systematic integrity of the plant cell wall forms the basis for enzyme-assisted extraction. When enzymes and substrates bind to one another, the shape of the enzyme molecule adapts as better as possible with the substrate to improve the interaction. The change in form could put the substrate under strain and stress, which could lead to the bonds breaking and speed up the reaction. When the substrate concentration is high, the enzyme can accelerate the process until the substrate concentration becomes limiting. The operational characteristics of each investigation of enzyme-assisted extraction are taken into consideration, including system pH, enzyme extraction time, substrate particle size, concentration, and reaction temperature. All study findings demonstrate that enzyme-assisted extraction leads to a decrease in extraction time and solvent volume in addition to an increase in yield and product quality (Fig. 1).

Bioactive compounds in plants are secondary metabolites of plants that have therapeutic or harmful effects on living things. Secondary metabolites are created in plants in addition to the basic biosynthetic and metabolic pathways for the substances

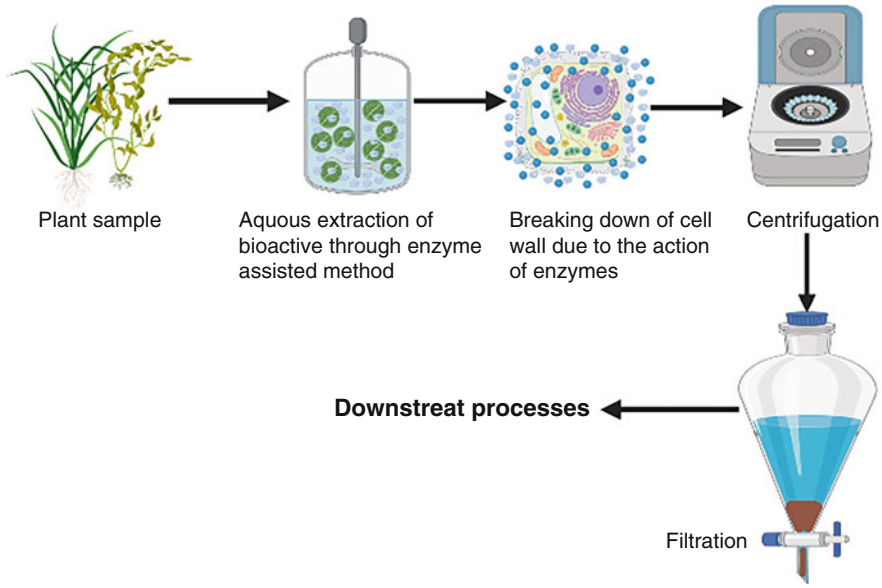
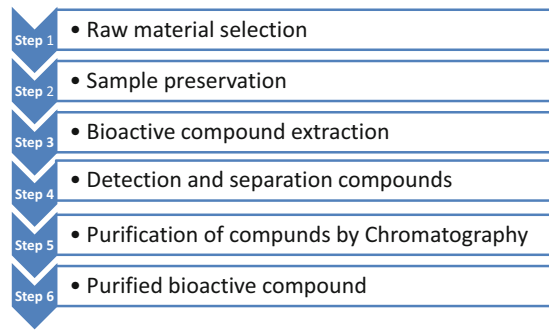


Fig. 1 Picture depicting enzyme-assisted extraction

related to plant growth and development. These by-products of the plant cell are not necessary for the regular operation of the plant, but some of them have been identified to play crucial roles in the survival of living plants, such as signaling and protection. It appears that most plant species can synthesize these compounds. But some chemical groups that make up a plant's bioactive compounds show toxicological and pharmacological effects on both animals and humans.

Bioactive substances can be used for a variety of purposes, including enhancing the nutritional, sensory, and technological qualities of conventional food, creating functional foods that have proven physiological benefits, creating nutraceuticals from food or agro-industrial waste, key nutritional components, and creating films for use as active, smart, and/or bioactive food packaging.

3.2 Steps Involved in the Extraction of Bioactive Compounds



The purified bioactive compounds can be sent for structural elucidation by NMR, FTIR, and LC-MS/GC-MS and biochemical characterization such as in vitro toxicity, in vitro evaluation, and clinical research.

3.3 Other Different Techniques Combined with EAE to Enhance the Extraction Process

Nowadays, the importance and commercial demands for the enzyme-assisted extracted bioactive compounds from plants are at a high rate. To fulfill the demands of the market, researchers are finding novel techniques, which are going to solve the problem with high commercial value and usage. Some of the novel techniques are microwave-assisted extraction, supercritical fluid extraction, and ultrasound-assisted enzymatic extraction. These techniques are combined with enzyme-assisted extraction to increase the efficiency of extraction to get more yield. These techniques are eco-friendly and efficient with a high yield for the commercial purpose to fulfill the demands of the market.

1. *UAEE* (*Ultrasound-assisted enzymatic extraction*):

   Ultrasonic irradiation was utilized to expedite the treatment of enzymes due to the increased effectiveness of enzyme-used reactions and the convenience of practical work. As innovative approaches for extracting bioactive compounds, enzyme-assisted extraction methods linked with ultrasonography have been devised [15]. Acoustic cavitation occurs when ultrasound waves flow through a solvent solution, resulting in increased extraction yield [16]. When ultrasonic waves are exposed to a solvent media, tiny vapor-filled bubbles form. It is named cavitation. Whenever the bubbles reach a particular structure, they explode forming in localized high temperatures and pressures. Whenever these bubbles break at the plant cells' surface, the high temperature and pressure released create liquid jets with shear forces that also are directed toward the plant cells' surface. The liquid jets and shear pressures that occur during this procedure lead to physical harm to the cell's wall or cell membrane's integrity. Ultrasound enhances cell wall penetration, allowing more solvent to penetrate the cell wall and leach compounds into the liquid phase. Furthermore, under ultrasonic treatment optimal conditions (proper frequencies and intensities) can result in increased enzyme work. This is owing to favorable arrangement shifts and structural rigidity, to facilitate bimolecular production [17].

   Ultrasound-assisted enzymatic extraction is found to be one of the easiest and quick methods of isolation. An indirect sonication (ultrasonic wash) and also direct sonication, or ultrasound horn can be used. The extraction yield in UAEE of bioactives is related to factors such as ultrasound power enzyme concentration. This technique will minimize the time taken for extraction and can be inculcated in commercial use.

The researchers discuss the source of bimolecular extraction, as well as the different aspects that influence ultrasonic extraction, including ultrasound strength, frequency, irradiation period, and extraction yield [15]. Researchers used an enzymolysis-ultrasound-aided extraction (EUAE) technique to extract polysaccharides from corn silk. The ultrasonic treatment will change the chemical makeup and morphological aspects of maize-derived silk polysaccharide. Moreover, it modifies the molecular weight distribution. This is proved based on the previous studies. The polysaccharide output improved from 4.56% to 7.10% under optimal extraction conditions. In addition, polysaccharides isolated using EUAE showed modifications in morphological aspects in the cell wall as well as an increased anticancer and antioxidant activity when observed side by side with polysaccharides isolated through boiling water extraction. Carotenoids were isolated using carrot sap utilizing ultrasound in conjunction with therapy using enzymes by intermittent radiation. This technique increases productivity without causing heat denaturation of carotenoids.

2. *Enzyme-assisted high-pressure extraction*:

   The high-pressure extract (HPE) technique comprises processing the plant matter with solvent, then treating the combination using isostatic ultra-high hydrostatic pressure and purifying the mixture to remove the solids [18]. To acquire the biomolecule of interest, the extracted extract is concentrated, dried, or filtered further. Plants undergo structural alterations as a result of high-pressure treatment due to physical damage to cell membranes, which increases cell wall permeability and phytochemical diffusion through solvents [19]. This results in a higher extraction rate as well as efficiency. In contrast to traditional extraction, HPE can easily separate heat-sensitive chemicals [20]. With this approach, volatile chemicals can be removed quickly and easily without degradation. Most biological biomolecules become more soluble under high pressure [21]. HPE operates at pressures ranging from 100 to 1000 MPa. When compared to other removal technologies, HPE operates at the maximum pressure, allowing it to extract greater phytoconstituent [22]. To investigate the extraction of chemicals from plants using natural sources, researchers combined the benefits of biochemical extraction at high pressures [23].

3. *Enzyme-assisted ionic liquid extraction*:

   Ionic liquids (ILs) are liquid at room temperature and are composed of bulky organic ligands and inorganic anions [24]. Because of their outstanding qualities of low vapor pressure, the flexibility of recycling, miscibility using common organic solvents, thermal and chemical stabilities, and high

solubility and extraction rate in organic compounds, they have recently gained prominence in a variety of sectors. Because of the pressing need for environmental preservation, ionic liquids are seen as safer alternatives to standard solvents in the extraction of biomolecules from plants. ILs have previously been used to extract bioactive substances including alkaloids, lignans, and polyphenols [25]. The viscosity of ILs is also important in their application for separating bioactive chemicals from plants**.** At high temperatures, the fluidity of ILs decreases. ILs can interact with both polar and nonpolar compounds and are helpful when paired with a variety of extraction methods [25]. Chemical composition, concentrations, moisture level, loading rate, pH, column temperature, and enzymes to target ratio are all crucial parameters to monitor during IL extraction. IL-assisted enzymatic extracting has been utilized successfully to extract a wide range of chemicals, including curcumins and phenolic compounds [26]. It has been demonstrated that ILs have a higher viscosity than standard organic solvents. The fluidity of ILs affects the stability and activity of enzymes. The researchers and the scientific community recently confirmed how enzyme conformation changes are delayed in very viscous IL solvents. This maintains enzyme stabilities for long periods. In general, high solvent viscosities lead to effective enzyme stability. The remarkable thermal stability of enzymes in viscoelastic ILs has opened the door to the extraction of pharmaceuticals from plants [26]. The durability of a broad range of enzymes, including thermolyzing, lysozyme, and chymotrypsin, was shown to be much higher in this solvent than in typical organic solvents. From a critical standpoint, enzymes in ILs exhibit good functional and temperature stability in many circumstances [27].

3.4 Types of Bioactives That Can Be Extracted Using Enzyme-Assisted Extraction Method

1. *Polyphenols*:

Plants with a phenolic composition one and phenolic ring (e.g., polyphenolic compounds or phenolic alcohols) include a diverse range of phenolics. The extraction of phenolic chemicals was done either traditionally using mixed solvents (acetone/water, dioxane/ethanol, biofuel, and methanol/water) or alkaline/acidic techniques. Antioxidants are a large class of phenolics that are among the most coveted bioactive chemicals [28]. They are quite interesting and also serve a variety of biological purposes. They disrupt oxidative cycles to prevent or delay oxidative damage to macromolecules. Many studies have shown that fruit peel, seeds, pomace, and leaves are high in polyphenolic chemicals [29]. Because of the possible toxicity of some chemical preservatives, researchers have made greater attempts to uncover and use naturally produced antioxidant properties from agricultural residues. The early extraction processes are critical during the separation of bioactive compounds

from vegetables and fruits. Among these procedures, dehydration and grinding have the greatest impact on removal capacity and mass transfer to obtain polyphenols. Drying, in general, entails the elimination of all bound water from the peel by enhancing the permeability of the cellular matrix, enabling diffusion rate and promoting interaction with enzymes, thereby improving phenol extraction. The extraction efficiency of polyphenols is affected by phenolic component properties, operational drying circumstances, and drying [30].

The citrus industry generates huge amounts of peel and seed wastes (up to 50% of total fruit weight), which could be the main sources of phenolic chemicals in certain peels. Taking this into account, total phenolic extract using food-safe enzyme cellulase, the bioactives from five distinct fruit peels (mandarin, Yen Ben lemons, grapefruit, orange, and Meyer lemon) were extracted. Similarly, Gomez-Garcia et al. performed cellulase-assisted extraction of antioxidative polyphenolic using grape (*Vitis vinifera* L.) leftovers in an attempt to valorize fruit waste. Not only did protease extraction produce more phenolics (O-coumaric acid as assessed by high-performance liquid chromatography-electrospray mass spectrometry), but it also demonstrated free radical-scavenging ability. These experiments show that enzymatic extraction of phenolics performs far superior to traditional approaches. Various methods are being employed to use agricultural industry trash as a renewable source for high value-added (primarily polyphenolic chemicals) products. The use of enzymes offers an alternate method for producing these beneficial chemicals from agro-industrial waste. Enzyme-assisted extraction has been used to extract antioxidant components from a variety of sources such as lemon balm (*Melissa officinalis*), red algal leftover (*Palmaria palmata*), *Agaricus blazei* Murill (rice bran), and pumpkin peel waste (*Cucurbita moschata*). Plant cell walls are largely made up of interconnected polysaccharides such as starch, cellulose, hemicellulose (xyloglucans), and pectin, which act as a barrier to the escape of intracellular chemicals [31].

Flavonoids are entrapped in polysaccharide complexes via hydrogen bonding and hydrophobic interactions. A combination of carbohydrate-hydrolyzing enzymes, including cellulose, hemicellulose, and protease, may be a more effective opportunity to sufficiently damage the cell walls, break down complicated interior storage materials, and therefore facilitate the release of free polyphenols. Some polyphenols are always covalently coupled with glucose in the form of glucosides with glycosidic connections. Glucosidase is capable of breaking the -1,4 glucoside bonds in glucosides. Cellulose, hemicellulose, and pectin can be hydrolyzed using the enzymes, cellulase, -glucosidase, and pectinase. To improve the extraction yield

of intracellular contents, enzyme-aided treatment was combined with solvent extraction. The authors pretreated Prunus species with various enzymes (cellulase, pectinase, protease, and alpha-amylase) before extracting anthocyanins. Flavonoids are entrapped within polysaccharide complexes via hydrogen bonding and hydrophobic interaction [32]. A combination of carbohydrate-hydrolyzing enzymes including cellulose, hemicellulose, and pectinase may be more effective. By solvent extraction, there is a possibility to adequately damage the wall of the cell and break down complicated inner storage. Cellulase produced the highest anthocyanin content when compared to other enzymes. Furthermore, the quantity of the targeted enzyme has a considerable impact on the extraction. Lower enzyme concentrations result in less enzyme interaction with the peels/pulp, resulting in less extraction of the desirable polyphenols. A larger concentration of enzyme, on the other hand, can successfully extract the necessary polyphenols. However, in some circumstances, using more enzymes for extract may result in final product inhibition. To evaluate the effects of varying cellulase concentrations (ranging from 1% to 10%) on the recovery of lycopene from pumpkin tissues, one researcher observed that as the enzyme concentration increased, lycopene yield increased up to 7% within the enzymatic reaction. In contrast, no notable decrease in anthocyanin extraction yield from saffron tepals was observed as the Pectinex content increased from 1% to 10%. However, the proper enzyme and its quantity are determined by the nature of fruit yield cell walls [33].

The combined impacts of numerous enzymes can result in greater cell wall breakdown, leading to higher removal yields. Several enzyme mixes are commercially available. The synergistic effect of the enzyme combinations Pectinex Ultra Clear and Lallzyme Beta for grapes extraction of juice of *Vitis labrusca* L. variety Concord was investigated. They found that when two enzymatic preparations were combined, it increased juice yield and the release of bioactive components compared to using individual enzyme preparations. To increase extraction yield, available commercial enzymes are typically mixed with two or more enzymes. Phenolic components and antioxidants were recovered from rice bran in one study by employing available commercial carbohydrates: AMG, Cell clast, Pentopan, Viscozyme, Termamyl, and Ultralow. The ferric-reducing capacity of phenolics released by all carbohydrates increased significantly (1.5–3.3 times). Among the enzyme mixtures listed above, Pentopan (a mixture of Endo-1-4-xylanase as well as feruloyl esterase, caffeoyl esterase, as well as pectinase) emerged to be the most efficient in increasing antioxidant activity, so although Cell clast, Ultralow, as well as Viscozyme

appeared to be more effective in increasing phenolic content as well as radical-scavenging activity. Commercial carboxylases successfully boosted phenolic acid extraction yield through enzymatic hydrolysis of cell-wall components as well as the release of free phenolic acids. Bioactive chemicals, particularly anthocyanins as well as other polyphenols, are affected by processing and subsequent storage. As a result, it must be considered while evaluating the possible health advantages of foods and beverages. Using different commercial enzymes, the researchers extracted phytonutrients from bilberry skin and examined the stability in the form of half-life values. Due to enzyme-assisted extraction, the half-life value increased (from 12% to 64%). Because of the electrostatic attraction between anthocyanin and hydrolyzed pectin molecules, this suggested good pigment stability. Other polyphenols (especially flavanols and polyphenols) were also produced after the significant enzymatic degradation of polysaccharide cell walls in bilberry skin. These polyphenols served as co-pigments to enhance anthocyanin retention, complexes (carbohydrates) can transform water-insoluble cell wall and cell membrane substances into water-soluble components (short polysaccharide fragments), resulting in a variety of bioactive characteristics observed. The chemical structure, structural morphology, and physiochemical properties of insoluble and soluble residues are critical in identifying optimal conditions to enhance extraction yield [33].

2. *Polysaccharides*:

Aside from polyphenols, polysaccharides are indeed the group of metabolites that have been investigated the most. Water-soluble, high-molecular-weight polysaccharides can modify the rheological properties of food and are frequently employed in a variety of food applications as stabilizers, emulsifiers, thickeners, and texture modifiers. Additionally, dietary fibers contain a wide range of bio-functional properties, including immunomodulatory, hematopoiesis-promoting, antioxidant, antibacterial, and anticancer activities, according to several recent studies [34]. The most typical technique for removing polysaccharides is traditional heated reflux extraction. In general, the recovery time and temperature have a significant impact on the yield of this approach. However, prolonged use at high temperatures can weaken polysaccharides, which in turn reduces their biological activity. The enzymatic extraction for bioactive biodegradable polymers has been extensively studied [21]. There are two ways to perform enzyme-assisted carbohydrate extraction. The first is to use enzymes capable of destroying biological membranes such as cell walls and membranes to aid in the isolation of desired polysaccharides. To facilitate extraction, enzymes that partially

break down desired polysaccharides to minute fragments are used. Alkaline was used to extract carrageenan from *Mastocarpus stellatus* (a type of protease). The gelling characteristics of the isolated polysaccharides were excellent [35]. The extraction procedure resulted in a combined extraction of important phytochemicals such as polyphenols adding value to the extract. As a result, enzymes can be used to selectively extract bioactive polysaccharides, potentially allowing for the targeted creation of certain gelation characteristics and desired physical features [22].

Another work used a response surface approach to do cellulase-assisted extraction of water-soluble Malva Sylvester's carbohydrates (MSP). The maximum MSP yield (10.40%) was obtained at 5.64% cellulase, 55.65 °C temps, 3.4 h, and 5.22 ph. These homogenous polysaccharide fractions were then purified using chromatography. In a dose-dependent pattern, the fractions dramatically improved antioxidant, anticancer (tested on HepG2 and A549), and antibacterial activity. Using enzymes thereby improves not only the removal efficiency but also the medicinal characteristics of polysaccharides. Because of their unique physical and biological qualities, seaweed (algal) disaccharides and polysaccharides are gaining popularity in the functional food and pharmaceutical industries. The researchers conducted enzyme-assisted carbohydrate extraction from the brown cyanobacteria *Ecklonia radiata* using a combination of six commercial enzyme mixtures: Viscozyme L, Cellclast 1.5 L, Ultraflo L, Alacalase 2.4 L, Neutrase 0.8 L, and Flavourzyme 1000 L. They discovered that total sugar output was unaffected by enzyme type or ph. The above-mentioned parameters, however, govern the molecular mass of the isolated polysaccharides. High buffer salt concentrations were discovered to impede polysaccharide extraction. As a result, buffers should not be used in enzyme-aided algal carbohydrate extraction for increased extraction efficiency [22].

It is commonly accepted that the chemical components, structural, molecular mass, and conformation of polysaccharides can influence their bioactivity and other therapeutic qualities. To separate polysaccharides from *Epimedium acuminatum*, hot water extraction was optimized. Nonenzymatic extracting and heating extraction, on the other hand, resulted in a lower polysaccharide yield (30.2%) as compared to enzyme-aided water extraction (82.4%). Furthermore, unlike previous methods, enzymatic water extraction enhanced time efficiency, reduced solvent usage, and functioned at lower extraction temperatures. SEM images demonstrated that after enzyme-assisted extraction, the cell walls became thin and disordered, facilitating mass transfer among polysaccharides and solvent and therefore increasing yield. The impact of various

extraction procedures on the makeup of both acidic and neutral pectic polysaccharides was investigated during bulk pectic extraction from agroindustry leftovers. The most common method for freeing pectin from plant sources is acidic extraction. Previous research has shown that the extraction method can cause significant pectin breakdown, resulting in a low yield and loss of gelling characteristics. Furthermore, they are used in crude form instead of purified pectin to cut production costs. The influence of different extraction procedures (acids, enzymes, and chelators) on the recovery and content of bulk pectic polysaccharides was studied using four distinct wastes as substrates: berry pomace, onions hulk, compressed pumpkin, and sugar beet pulp. Enzymatic hydrolysis employing Cell class, which contains cellulases (endoglucanase), was found to be successful in removing pectin through plant cell walls. Sugar beet pulp or onion hulk was discovered to be appropriate substrates for purifying pectin and manufacturing pectin-based products based on the mechanism of recovering pectic sugars following enzymatic extraction. In addition, the incubation conditions of the different substrates with enzymes were shown to be significantly milder and more time efficient than extraction with acidic and chelators. The enzyme extraction method has demonstrated an advantage over conventional procedures in the sustained extraction of polysaccharides with increased pharmacological of the extract. Complex enzymes have also been shown to increase polysaccharide output and extract quantity. Another benefit of enzyme-aided extraction is that less alcohol is required again for the precipitation of target polysaccharides from the reaction mixture, making the procedure more environmentally friendly.

3. *Oils*:

Oil extraction via seeds employs traditional procedures including mechanical press by hydrolytic method, solvent extraction, and extruder pressing. In certain circumstances, the oil can be obtained or produced directly from the fruits using a normal mechanical pressing and is taken without further procedures and experimental analysis, such as virgin olive oil. Hexane is commonly used for oil extraction due to its ease of recovery, low boiling point (63–69 °C), and high solubilizing ability. Unfortunately, due to health, safety, and environmental considerations, hexane is not a preferred solvent in the extraction process. Researchers are seeking alternatives to hexane that will not reduce oil yield or quality. As a result, the use of green solvents and fluids may be a viable alternative to oil extraction. Aqueous enzymatic extract (AEE) is an effective method for extracting oils from oil seeds. This approach is straightforward to use, consumes less energy, and is

economically viable. In general, oil droplets are surrounded by protein, which is a major component of the cell wall. Proteins and pectin were the main components of cell walls in soybeans and rapeseeds. As a result, degrading these components with proteolytic, cellulase, pectinase, as well as other enzyme mixes increased oil output. The chemical structure, anatomy of the cell wall, and placement of the oil droplet within the seed must all be considered while selecting enzymes [36]. Furthermore, in oil extraction optimization trials, the oil-to-water ratio must be considered in addition to general characteristics such as pH, temperature, and particle size. Enzymes as well as their concentration require moisture content to function, and the presence of low humidity in oilseeds causes the formation of a thick suspension, which inhibits enzyme activity [37].

In the literature survey scientifically, there are various examples and illustrations available in which an enzyme mixture has been proven to function synergistically to enhance and improve the extraction efficiency of required oils including phenolics from bay leaf (*Laurus nobilis* L.). By disintegrating plant cell walls, the enzyme mixture comprising cellulose, hemicellulose, and xylanase improved the removal efficiency of biomolecules of plant matrix. The extraction rate of essential oils was enhanced by 243%, 227%, 240.54%, and 248% when the substrate was treated using hemicellulose, cellulase, and xylanase individually and their ternary mixture. Further investigation found that essential oils increased the antioxidant activity of enzyme-pretreated substrates. The addition of enzymes altered the proportions of separate components and increased the number of oxygenated monoterpenes. As a result, the essential oils produced from enzyme-treated samples had higher antioxidant activity. Similarly, oil was extracted using bush mango kernel flour by treating it with commercially available enzyme mixtures such as Alkalize, Pectinex, and Viscozyme, yielding oil yields of 35%, 42.2%, and 68.0%, respectively. In addition to boosting oil extraction output, the enzymatic method increased oil sample quality by increasing the number of bioactive components (such as carotenoids and other phenolics) along with their antibacterial activity. The use of tannase increased the total phenolics in the hydrophilic and lipophilic fractions, resulting in the oil having a better antioxidant capacity. Essential oils with enhanced antioxidant capabilities and antibacterial activity have a high potential for usage in the food and pharmaceutical industries. Enzymatic processing was shown to drastically reduce the production of oil left in Chilean hazelnut food in a few situations [38].

Clove essential oil is one of the most important essential oils. Recently, research has concentrated on the pretreatment of clove buds' powder with enzymes including cellulase,

lignocellulose, pectinase, and amylase before extraction. For the recovery of clove oil, the typical procedure of steam distillation yields 10.1%. In recent investigations, enzymes specific for cell wall activity were utilized in preprocessing before extraction to enhance the purity and quantity of the phytochemicals recovered. The extraction of oil from discarded pomegranate seeds using protease (from *Aspergillus oryzae*) treatment yielded a more than 50% good return than the control samples in terms of quantity of extracted essential oil. SEM images of discarded pomegranate seeds were obtained before and after protease treatment to better understand the extraction mechanism. The micrographs of SEM revealed that even after protease therapy, the cell wall is damaged as a result of the enzymatic treatment, which renders the surface of the seeds very porous, facilitating the recovery of physiologically entrapped oil. Furthermore, the protease-derived oil had 1.4 times the phenolic content and 4% more antioxidant activity than hexane-extracted oil. The preceding example implies that pretreatment with enzymes before resource extraction can boost oil extraction yield. Enzymatic pretreatment before solvent extraction and mechanical pressing softens the cell wall and increases oil extraction [37]. This method reduces the potential of oil production in water emulsion following extraction.

The parameters for enzyme selection are determined by the location of oil inside the cellular structure as well as the chemical composition of the substances surrounding it. The simultaneous assessment of each of these criteria is critical in determining the appropriate enzyme combination for a particular oil-containing substrate. Despite its numerous benefits, the application of AEE is still limited due to the lengthy processing time and difficult drying process following enzymatic treatment. A substantial amount of the enzyme is required (usually more than 1% of the weight of an oilseed ingested), which contributes to the expensive expense. Furthermore, the lack of commercially available enzymes has hampered the development of these methods. An additional issue with AEE is the difficulty in avoiding emulsification of the oil extracted, which necessitates post-extraction demulsification step toward recovering and increasing oil yield. Tabtabaei and Diosady employed aqueous enzymatic emulsion de-emulsion approach to destabilize the oil-in-water emulsions to recover access to the oil before the industrial application [39]. The researchers used enzymatic demulsification therapy by several proteases and phospholipases in their work to test its ability to release free oil. The targeted emulsifiers are hydrolyzed. Protex 6 Land phospholipase therapies proved successful in collecting over 91% of the oil inside the emulsion.

3.4.1 Flavors and Colors

Colors and flavors improve the quality of a food product by influencing its visual appearance and taste. In the food sector, there is an ever-increasing need for natural flavors and colors. Synthetic dyes were suspected of emitting hazardous compounds that are highly polluting, allergenic, carcinogenic, and toxic to humans. Given the health and environmental concerns associated with industrial chemical dyes, researchers redirected their focus away from artificial (synthetic) colorants and toward the excitation of natural colorants derived from plant sources [40]. Colorants taken from roots, bark, foliage, nuts, berries, and flowers include anthocyanins, betalains, chalcones, chlorophyll, carotenoid, and flavones. Traditional natural dye extraction procedures include water and alkali extraction, fermentation, and solvent extraction. If the right enzymes are chosen and the operating conditions are tuned, enzyme-assisted extraction method has a lot of promise for pigment isolation. In the past, commercially accessible enzymes including cellulase, amylase, and pectinase were investigated. This method may hasten the extraction of pigments through tough and compact plant matter such as barks and roots. Conventional means of natural dye extraction, in addition to being time-consuming and ineffective, result in the co-extraction of unwanted compounds such as chlorophyll and waxes [41].

3.5 Enzyme-Enhanced Processes for Plant Materials

1. *Extraction of bioactive from by-products.*

   By enhancing the permeability of plant cell walls, enzyme-assisted extraction (EAE) makes it possible to extract pectin from waste and by-products [42]. Many phenol chemicals, such as flavonoids and anthocyanidins, can be extracted using enzymes [43]. For enzymatic treatment to be as effective as possible, factors such as enzyme activity, treatment time, substrate ratio, and particle size are crucial [44]. Scientists have addressed that ideal condition for extracting pistachio green hull. For the extraction of cellulose, tannases, pectinases, and their mixtures were utilized. Results have revealed that the three enzymes used simultaneously to extract phenolics gave the best result [45].

2. *Production of fermented drinks and plant-based drinks from grains.*

   The production of high-protein food items using grains and cereals, involves a process that requires the degradation of cell walls with the assistance of enzymes to obtain the desired food product. Yearly, dairy-related product consumption is growing by 10%. In the year 2019, America reached to the extent of 1.8 billion dollar spending on dairy products [46]. This is due to the high release of sugars forming the acceptant-sensory organoleptic features [47]. The enzymes

which are majorly used to start the earlier steps pectinase-denatured rapeseed fibers and cellulose for the growth of the cultures such as L. johnsonii L63, reuteri L45, Plantarum L47 are some examples for the starter culture and growth of bacteria for high production of nutrients in fermenter tanks [48]. Furthermore, plant-based fermented goods have antibacterial qualities, and their pH is lower than that of typical plant-based drinks, impacting product stability [49].

3.6 A Review of Enzymes and Factors Influencing Bioactive Extraction

Usually, bioactive compounds in natural products exist as either soluble or insoluble conjugates (glycosides). In food industries, for example, the majority of phenolics (24% of overall phenolic content) are found as bounded phenolics [50]. The majority of phenolic compounds are imprisoned inside cell wall polysaccharides such as cellulose, hemicellulose, and pectin, which are connected by chemical bonding and hydrogen bonds. Other phenolic acids create ether links with lignin via their aromatic ring hydroxyl groups and ester links with structural polysaccharides and proteins via their carboxylic groups [51]. Flavonoids are covalently bonded to sugar moieties via glycosidic bonds or carbon-carbon bonds. Tannins have a proclivity to create powerful complexes with proteins. Here, enzymes such as cellulase, hemicellulose, pectinase, and protease are used to solubilize the plant cell wall, consequently speeding the discharge of intracellular biomolecules [45]. Hydrolases, such as lipases, work in the water that is present inside the reaction system. They also act in the presence of additional substrates such as alcohols, amines, and oximes. During the separation of flavonoids in *Ginkgo biloba*, *Penicillium decumbens* cellulase outperformed *T. reesei* cellulase in the condition of maltose as the glycosyl donor. Cellulases and hemicelluloses can be used to separate oils and proteins. These enzymes attack the interior locations of the polysaccharide chains at random. This results in the formation of tiny oligosaccharides of varying lengths, which allow for the easy liberation of entrapped molecules [52].

The extraction efficiency is determined by the solvent system, temperature, enzyme mode of action, substrate availability, extraction length, enzyme loading, and pH condition. Each enzyme has a different optimal pH for enzymatic hydrolysis [53]. Many enzymes have an optimal pH that is close to the neutral pH of proteins. Because proteins are particularly insoluble in this pH range, biomolecule release may be hampered. As a result, pH must be selected in such a manner that not only does it inhibit enzyme action but also does not fall within the range of protein isoelectric point. Temperature, in addition to pH, is an essential aspect to consider during extraction [16].

3.7 Advantages and Disadvantages of Enzyme-Assisted Extraction Method

3.7.1 Advantages of Enzyme-Assisted Extraction Method

The enzyme-assisted extraction method is a highly efficient technique compared to the other extraction techniques as it takes very less time with the high productivity of extracted compounds. It requires the knowledge of the basic and simple methodology for the extraction of compounds. The extracted compounds will retain their structural and physiochemical and configure stability during and after the process of extraction. Consequently, the concept of "green chemistry" is being pursued. People are searching for an effective, ecologically friendly way to increase bioactive recovery rates. Due to its improved extraction capabilities and environmental friendliness, enzymatic extraction has demonstrated many benefits. Through this process, we can exactly figure out our required intracellular compound and can be extracted with high accuracy so that purity of the extracted compound is enhanced. The process required for the extraction of intracellular bioactive chemicals using this unique technology is regarded as lenient, meaning there are no precise requirements to be maintained. The cellular barrier that is the cell wall, which processes cellulose, hemicellulose, and pectin, can efficiently be degraded with enzymes, namely, cellulase, hemicellulose, and pectinase, respectively, without affecting the bioactives [54, 55].

3.7.2 Disadvantages of Enzyme-Assisted Extraction Method

- Costly equipment is needed; hence, this method is expensive compared to other extraction methods.
- We establish this technique at a small-scale level and cannot be used at the industrial level.
- This technique is not suitable for the extraction of by-products such as fibers, phenolic compounds, carotenoids, and anthocyanin.
- Some plants having different cell wall compositions rather than usual cell wall compositions will bring a difficulty to break open the cell wall so that it allows the intracellular compounds to come out of the cell. There are no such novel enzymes that are available to break open those cell walls with different biochemical compositions.
- The process of extraction and purification of enzymes for the treatment process is a difficult and crucial step, and it is difficult to store and maintain those enzymes in large quantity, which is the drawback in this method [56].

The industrial importance of bioactives: Bioactive compounds are having more importance in the food industries and pharmaceutical industries.

3.8 Some of the Pharmaceutical Activities of Bioactive Compounds

(a) *Antidiabetic activity*: Diabetes is concerned with a group of conditions characterized by a high level of blood glucose, commonly known as blood sugar [57]. Too much sugar in the blood causes serious health problems, sometimes even it may lead to death. In diabetics, there are two types: type 1 and type 2. Type 1 diabetes destroys by the immune system by mistake [58]. The reason is insulin binds to its receptor on target cells; hence, less glucose is taken into the cells so that more glucose stays in the blood. Therefore, this type of diabetes is called insulin-dependent. Another type, known as type 2 diabetes, is characterized by insulin resistance. This condition is often associated with factors such as obesity, a sedentary lifestyle, and an unhealthy disposition. Type 2 is related to endocrine metabolism. In such circumstances, plants or natural products containing antidiabetic properties, such as insulinogenic or secretagogue properties, hold significant promise and potential for the development of novel pharmaceuticals. There are so many plants with the antidiabetic properties. Some of them are *Acacia arabica, Aegle marmelos, agrimonia eupatoria, allium cepa, Allium sativum, Aloe vera, Azadirachta indica,* and *Benincasa hispida* [59]. The various parts of medicinal plants can treat diabetes in various ways, including insulin secretagogue activity, the insulin release from the pancreas, insulin-like activity, an increase in plasma insulin concentration, an increase in insulin binding to insulin receptors, a decrease in plasma triglyceride levels, insulin-sensitizing activity, and an antihyperglycemic mechanism to stimulate islet insulin release [60]. Additionally, a sizable population worldwide has switched to this complementary method of treating illness because of its varied flora, affordability, and simplicity of use with little negative effects. Studies show that medicinal plants are multitargeting and least likely to fail during treatment, which is supported by the evidence [61].

(b) *Anticancer activity*: Cancer is the result of uncontrolled, rapid cell division. Numerous types of cancer can be found [62]. Cancer is one the most deadly disease caused due to metabolism. This disease is not completely curable. We can just increase the life span of the patient with chemotherapy and some antibiotics [63]. Presently, ten million people lose their lives every year with this deadly disease, and this may exceed in the future according to the WHO (World Health Organization) [64]. Topoisomerase inhibitors such as irinotecan and doxorubicin and alkylating drugs such as oxaliplatin, carboplatin, and cisplatin are used in chemotherapy. Irinotecan's adverse effects include neutropenia and sensory neuropathy (side effects include nephron, gastrointestinal, cardiovascular,

pulmonary, and hematologic toxicity). Apart from their complexity, expense, and non-eco-friendliness, the aforementioned medications' main drawbacks are their side effects and toxicity because they also target normal cells [65]. In order to treat cancer naturally and herbally, a group of photochemical known as "vinca alkaloids," which were extracted from *Catharanthus roseus*, are used. Vinorelbine, vindesine, vincristine, and vinblastine are the four primary alkaloids found in vinca. Vinblastine and vincristine are particularly effective at stopping the cell cycle in metaphase and interfering with microtubule function. Currently, vinorelbine, vindesine, and vinfosiltine are semisynthetic derivatives of the vinca alkaloids. Consequently, we can predict a bright future for photochemical research because, over the next 10 years, it is anticipated that these compounds will completely change how cancer is treated [66].

(c) *Diuretic properties*: Today, heart disease, kidney disease, and excessive blood pressure are all fairly frequent. Acute renal failure, edema or an increase in blood calcium or potassium, acute left ventricular failure or heart failure, and acute pulmonary edema are common medical conditions that patients frequently experience. Different medications are used to treat these issues because they assist the body to excrete more electrolytes and urine, which helps to lessen fluid retention. In actuality, many medications have negative long-term effects and are unable to treat the threat of high blood pressure. In contrast, green plants with high flavonoid and polyphenol content have been discovered to have high salt and potassium excretion properties, including *Cynodon dactylon*, *Emblica officinalis*, *Kalanchoe pinnata*, and *Bambusa nutans*. Hence, many of the plants are shrubs are to be identified and used in the preparation of drugs for the curing of diseases [67].

4 Conclusion

Considering all the extraction methods of the bioactive compounds, enzyme-assisted method seems to be the most efficient method for extracting the bioactive compounds. The enzymes make the cell wall most permeable and which leads to the high yield of metabolites that are having more applications in the food and pharmaceutical industries [28]. This method consists of efforts of food technologists, food chemists, nutritionists, and toxicologists. For improving the level of release of bioactive compounds, synthesis of new enzymes and their purification is important.

Compared to earlier extraction methods, enzymes-assisted extraction is more efficient and reliable. Extracting the terpenoids, polyphenols, and lectins need more research work to uncover the hidden potential, without the release of toxic substances. The extraction methods should use metabolites, which are eco-friendly nature. The approach of genetic engineering also plays an important role in this process to produce on a larger scale. Still, there is a need for finding the available enzymatic processes for further enhancement of the yield of the bioactive compounds.

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Chapter 9

Pulsed Electric Fields as a Green Technology for the Extraction of Bioactive Compounds

Radhika Theagarajan, Susindra Devi Balendran, and Priyanka Sethupathy

Abstract

In recent years, several innovative extraction procedures have been developed to carry out more effective and sustainable extraction of bioactive compounds. Sustainability is a vital concept for social, technological, and economic advancement, which strives to establish a circular economy. Thus, the significance and demand of eco-friendly methods used for producing plant extracts is currently booming. However, the food industries are strictly expected to implement manufacturing techniques that are highly effective and energy-efficient because of the rising cost of energy and other utilities. Pulsed electric field (PEF) processing is very efficient, more eco-friendly and sustainable compared to conventional extraction techniques such as solvent extraction and steam distillation. Further, PEF is also a desirable and effective nonthermal method with improved functioning, extractability, and retrieval of phytochemicals with nutritional benefits. Thus, swapping the conventional techniques with PEF can minimize or eradicate the utilization of harmful solvents and utilize less energy and water, and in return preserve the environment for the future generations. Therefore, PEF extraction technology has been considered as a sustainable strategy and green technology to extract the bioactive compounds. Additionally, cutting-edge nonthermal extraction techniques such as PEF make it intelligible and more effective to identify, characterize, and analyze bioactive components. PEF also has various advantages when compared to conventional extraction techniques such as cost efficiency, reduced extraction times, and greater yields with less solvent use. This chapter focuses on the employability of pulsed electric fields as a suitable green technology for bioactive extraction.

Key words Pulsed electric field, Green technology, Nonthermal processing, Novel extraction technique, Bioactive compounds

1 Introduction

Pulsed electric field (PEF) technology, a nonthermal technique, employs electric pulses to mostly conserve foods with higher electrical conductivity such as liquid or semi-liquid foods. PEF treatment also increases the membrane permeability of food material's cell wall by inducing permanent irreversible perforation in the cell membrane using a pulsated electric field. PEF is purely utilized in

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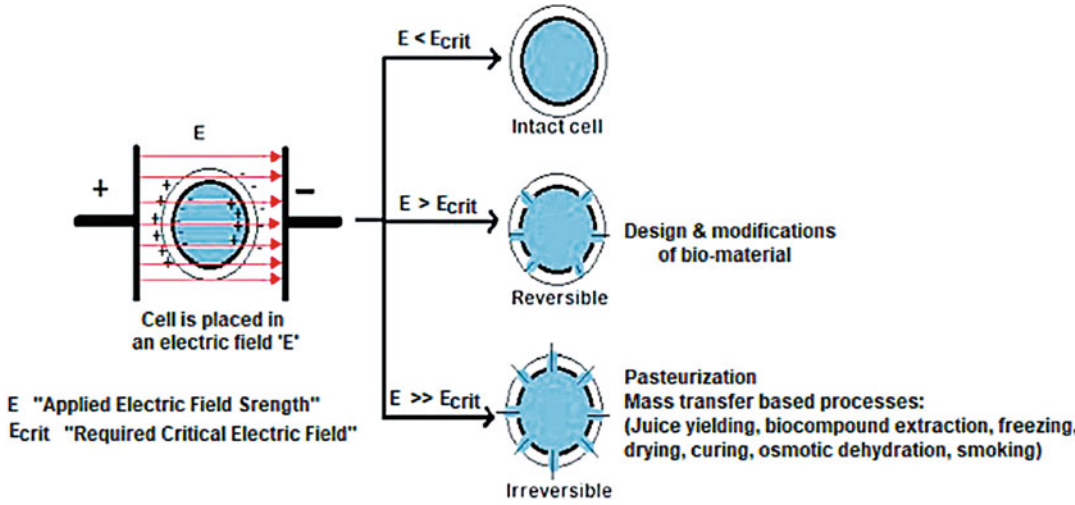

Fig. 1 Impact of PEF on the process of cell membrane permeabilization [2]

the food and nutraceutical industries to expedite extraction rates and to reduce the extraction time [1].

Extraction of bioactive components using a pulsed electric field (PEF) is viewed as a substitute for thermal methods, due to the absence of thermal destruction of bioactive compounds. Subsequently, the enhancement of nutrient benefit is also one of the main advantage of PEF extraction. Further, it also aids in the reduction and/or prevention of quality deterioration of bioactive food ingredients. PEF technology is depicted in Fig. 1. PEF results in the permeation of cell membranes when they are treated with short treatment times and with minimal energy consumption. The fundamental idea behind PEF technology revolves around passing the pulsed electric force through the cell membrane which generates a charge on the molecules, enabling them to separate based on charge mass resulting in the electroporation of the cell membrane and in return increasing the extraction yield significantly [3].

Moreover, PEF's continuous extraction process will aid in the proficient and rapid extraction of the components from biological tissue. Further, this novel nonthermal food processing approach has a great potential to be expanded to pilot plant and industrial stages. Also, the PEF extraction process is very quick and uses less organic solvent when put together as a continuous process. Also, pollution is prevented when using PEF continuous extraction. Additionally, the entire procedure can be completed at or slightly higher than the ambient temperature. Thus, thermal deterioration of bioactive compounds are minimized [4].

The nonthermal processing capability of PEF technology minimizes the adverse chemical reactions that emerge from the deterioration of bioactive components present in processed foods. Further, it has been used in several food industries and food-related sectors

for various applications such as food pasteurization, fruit juices clarification, and preservation. For instance, PEF is a great replacement for thermal pasteurization of liquid foods, due to the negative impacts associated with several other conventional thermal processing procedures.

This chapter discusses the major key technological advancements in PEF in the field of bioactive compounds extraction, as well as the significant advantages of the technology. Additionally, the fundamentals of PEF are discussed, and special emphasis was placed on technology evolution over a period with regard to applications in bioactive extraction [5].

2 Extraction Approaches in Nutraceuticals and Bioactive Extraction: Conventional and Novel Techniques

In recent times, bioactive components can be retrieved utilizing several novel extraction techniques such as solvent extraction, supercritical fluid extraction, subcritical water extraction, and PEF, enzymes assisted-, ultrasound-, and microwave- extraction methods. Moreover, the easier accessibility to these methods offers greater chance to employ any of them most effectively for the recovery of distinctive bioactive compounds [6].

In particular, the conventional extraction methods employ cell-damaging techniques which consume a large quantity of mechanical or thermal energy. Also, these processes lead to the formation of dangerous redox compounds over an extended period due to thermal degradation and oxidation. Additionally, these traditional methods are inadequate for selective extraction of particular bioactive compounds from the plant-based food materials. So, the quality of recovered products such as terpenes and terpenoids, alkaloids, carotenoids, phenolic compounds, polyphenols, proteins, and polysaccharides may be damaged or reduced by these standard methods. In recent years, numerous studies explore various sustainable nonthermal methods of extracting food components. These eco-friendly extraction techniques result in higher yields and supreme-quality of extracted products, since they utilize minimal to no organic solvents depending on the selected novel nonthermal extraction technique and also consume less time and energy [7].

The conventional extraction process of bioactive compounds has various unit operations such as soaking, maceration, boiling, grinding, magnetic stirring, water percolation, heat reflux, and Soxhlet extraction. These approaches have a variety of disadvantages and constraints, including excessive processing times, poor extraction recoveries, increased solvent consumption, and low extraction efficiency. Some of these also entail the possibility of thermally degrading thermolabile bioactive compounds. To

address the above-mentioned drawbacks of conventional extraction methods, many novel methods have been investigated. The efficiency and performance of newly developed technique in comparison to existing extraction techniques such as the Soxhlet method was demonstrated in a review work [8]. Therefore, it is essential to employ novel nonthermal techniques especially PEF as a preferable strategy to achieve improved extractability in order to overcome the significant limitations in the extraction process of bioactive; further, this study elucidates the efficacy of the PEF application for the nutraceuticals and bioactive extraction [8].

3 PEF-Based Bioactive Extraction Technique: A Sustainable Greener Technology

Sustainability is a key idea for social, technological, and economic advancement which aims to create a circular economy and maintain the ability to meet society's demands. Consumers in developed nations are becoming more and more demanding for a diet that is safe, wholesome, and appealing to the senses. PEF technology has been considered as a sustainable choice for the extraction of bioactives from foods due to the critical implications (higher energy and water usage) of conventional thermal extraction methods. PEF tends to support a strategic advantage for the food industry by advancing sustainable food processing without sacrificing product quality or safety. Technically, PEF technology's sustainability in each food industry is assessed through life cycle assessments (LCAs) [9].

Numerous studies have identified PEF technology as a smart substitute for products extraction and preservation (through pasteurization and sterilization) and also it can decrease or even prevent the generation of harmful chemical compounds. Indeed, PEF-assisted extraction methods adhere to all sustainability and green chemistry concepts. PEF is a green extraction method that makes it possible to extract natural colorants using nontoxic solvents rather than contaminating food and beverages with hazardous chemicals. PEF-assisted extraction technologies are much more energy-efficient than traditional methods in terms of sustainability concepts. One of the key features of this cutting-edge technology that encourages energy savings is the low treatment time observed in PEF-based operations. There are, however, assessment studies examining the environmental effects of PEF technology on various manufacturing sectors, such as bioactive component extraction procedures [10]. Therefore, it is crucial to concentrate on the sustainable attributes of the food processing industry, such as utilizing the PEF technique for the extraction process, rather than employing a conventional approach and delivering harmful residues to the ecosystem.

4 Role of PEF Processing System in Bioactive Extraction

PEF as a technology can cause permeabilization of cell membranes when applied for shorter times and it consumes low energy. The fundamental idea behind PEF technology is the electroporation of the cell membrane resulting in high extraction yield. The electroporation theory is the primary mechanism used in the exploration of PEF exaction applications. Generally, plant, animal, or microbial cells are briefly exposed to high-voltage electric field pulses that may cause the lipid bilayer and proteins in cell membranes to become unstable. Additionally, the plasma membranes of the cells become permeable to tiny molecules after being exposed to strong electric fields; this causes the cell to inflate and finally ensues the cell membrane to rupture. The charging mechanism at the membrane interfaces causes the potential of the transmembrane to rise when a sphere-shaped biological cell is exposed to an external electric field [11].

Electroporation produced by the dielectric breakdown of cell membranes is the central principle of PEF-assisted extraction. Due to the presence of free charges with opposing polarities throughout the membrane, it is believed that cell membranes operate like a capacitor with a lower dielectric constant and a natural transmembrane voltage. The accumulation of charges across the membrane increases the transmembrane potential when the external electric field is supplied. The potential is further raised by repeated exposures to electric fields, which causes electrostatic attraction between opposite charges to move across the membrane and narrow it. If the external field intensity increases past the critical breakdown voltage, which results in the creation of transmembrane pores, the membrane will break down [12].

The continuous and short voltage pulses induces a permeability in cell membranes that makes it easier for bioactive components to be released from the interior of the cells and this occurs when plant tissue is exposed to an electric field of moderate intensity (0.5–10 kV/cm) and remarkably reduced energy (1–10 kJ/kg), applied repeatedly in the form of very brief voltage pulses (generally from few second to 1 ms). PEF treatment may impose a selective permeability of the membranes (tonoplast and plasma membrane) while the cell wall persists due to its nonthermal action on foods, which enhances the purity and yield of the extracts. Therefore, PEF treatment has been demonstrated to improve the quantity and quality of the juice collected from fruits and vegetables when combined with mechanical extraction [13].

The effects of high-intensity PEF processing on physicochemical and antioxidant properties were evaluated. High-intensity PEFs (HIPEF) help in protecting the quality of juice throughout storage, which is preferable compared to heat treatment [3]. Figure 2 illustrates the processing system of PEF.

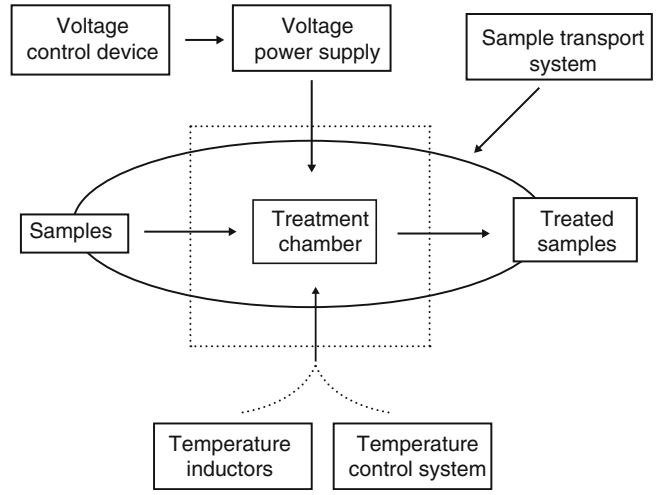

Fig. 2 Schematic illustration of the PEF treatment system [11]

4.1 Mechanism of PEF-Based Bioactive Extraction Unit

The application of rapid pulses (μs to ms) of moderate electric voltage (usually 0.5–20 kV/cm) to a suitable substrate situated between two electrodes is known as the pulsed electric field (PEF)-assisted extraction. The method has been used for preservation, enzyme, and microbial inactivation using high electric voltage (5–50 kV/cm). Particularly, in cell cultures and plant systems, low to medium PEF treatment intensities are frequently regarded as an efficient pretreatment technique for improving secondary metabolite extraction yields.

In the batch system of the PEF technique, the electric field intensity ranges from 100 to 300 V/cm, and in the continuous mode of extraction, it ranges from 20 to 80 kV/cm. Several hypotheses share two points of view regarding the putative PEF mechanism. One involves accelerating chemical reactions comprising numerous substances in the biological cell membrane to increase the solvent's solubility, and the other is the electroporation process (Fig. 3). An external electrical force is used in electroporation or electro-permeabilization to increase the permeability of cell membranes. A high-voltage electric field is positioned between the electrodes and food or any other targeted materials. By generating hydrophilic holes, which activate protein channels, the cell membrane is pierced. When high-voltage electrical pulses are applied to the sample, a force per unit charge known as the electric field occurs. When high-voltage electrical pulses are applied across the electrodes, the sample feels a force per unit charge known as the electric field. When the membrane no longer serves as a structural component, the plant material is removed [14].

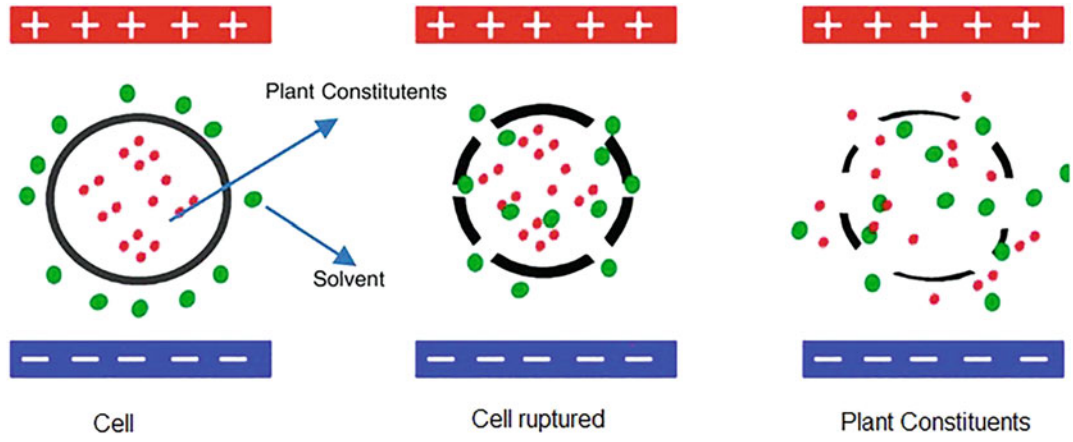

Fig. 3 Electroporation mechanism of extraction

4.2 Design and Fabrication of PEF-Based Bioactive Extraction Unit

The main PEF variables were intensively evaluated to determine the optimal PEF for the specific system to achieve the maximum polyphenol content of the extracts. For specific extraction duration, it was shown that the key PEF parameters that affect permeability are field intensity, pulse duration, and pulse period. For electroporation, the uniform electric field chambers expose each cell in the sample to the same electric field. High-yield intracellular chemical extraction is feasible when the field strength is sufficient and close to the ideal value. Due to the possibility that the best extraction yields could differ noticeably above or below the ideal value, the electric field strength set point must be determined by systematic experimental design [15]. Figure 4 shows the schematic representation of an ordinary PEF-based processing system for the treatments of food systems.

For instance, in a study conducted by Zhu et al. [16] PEF pretreatment for protein extraction was performed with a PEF generator system that was self-built, *Cardamine violifolia* was pretreated with PEF. A peristaltic pump was used to pump 4 g of sample, dissolved in 120 mL of water, at a rate of 1.35 mL/s into the treatment chamber. The operating parameters were as follows: The frequency was 1.01 kHz, the half-peak width was 48 s, and the pulsed electric field strength was 6.67 kV/cm. After six cycles, a NaOH solution was used to bring the pH value to 9.0 ± 0.2, and the treated solution was then extracted using conventional extraction (CE), ultrasound (US)-assisted extraction. After extraction, the extracts were centrifuged at 4000 rpm for 20 min at room temperature to get the supernatant.

Similarly, in a study performed by Athanasiadis et al. [17] 4.0 g of freshly cleaned plant material (not dried) was crushed into smaller pieces and combined with 80 mL of the solvent (at a ratio

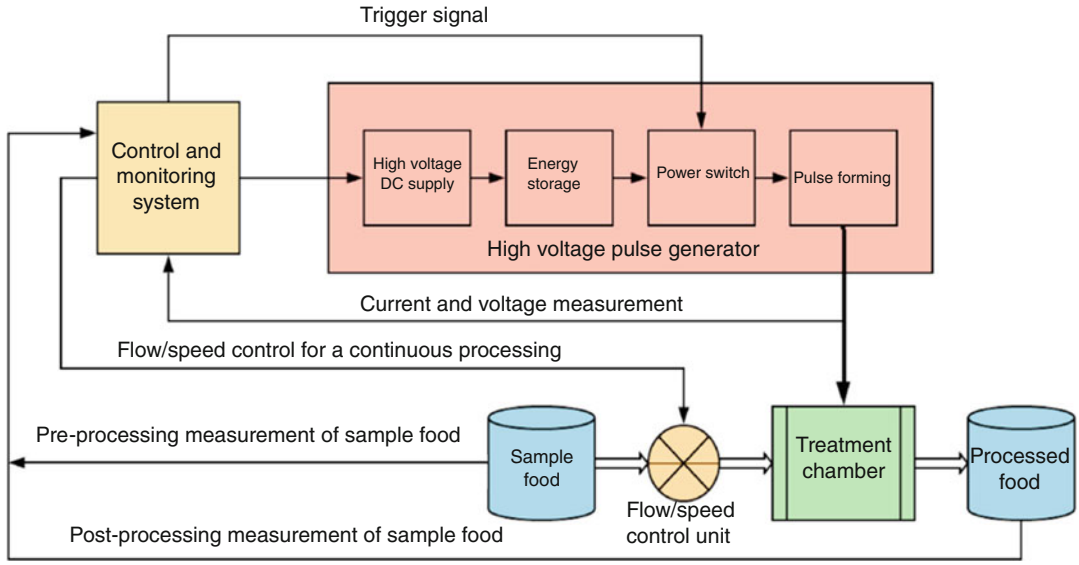

Fig. 4 Schematic representation of a typical PEF-based processing system for the treatments of food application [7]

of 20:1 mL/g) for the extraction of total polyphenols. 100% water, 25%, 50%, and 75% ethanol in water, and 100% ethanol were the different extraction solvents. The mixture was thoroughly mixed before being inserted into the PEF chamber for a 20 min extraction. 10 and 100 μs were used as the pulse durations for the extraction of total polyphenols. One (m) of the period (1000 Hz frequency) and 100 pulse cycles were accomplished. At 1.0 kV/cm, the electric field density was established. When the temperature was measured before and after PEF, there was no discernible difference (less than 1 °C). The mixture was put in a Falcon tube when PEF was finished, and it was centrifuged at 4500 *g* for 10 min. The supernatant was then immediately subjected to further studies.

4.3 Configuration and Requirements of PEF-Based Extraction Unit

The high-voltage pulses are delivered to the treatment chamber containing the food sample using a pulse modulator. To transmit the stored energy in an economically sound way, power switches are required. Most significantly, it has an impact on the electrical systems' overall design. Initially, various food sample components were extracted using batch devices with PEF pretreatment (for solid-liquid extraction). However, Yin et al. [18] successfully applied extraction in a continuous-flow treatment chamber. Since then, there has been a considerable advancement in technology that resulted in the invention of a continuous PEF extraction system, making it simpler to extract continuous products. According to reports, the PEF continuous extraction system has efficiently extracted fishbones, eggshells, tomato juice, and other materials [19]. However, it has not been widely implemented in food

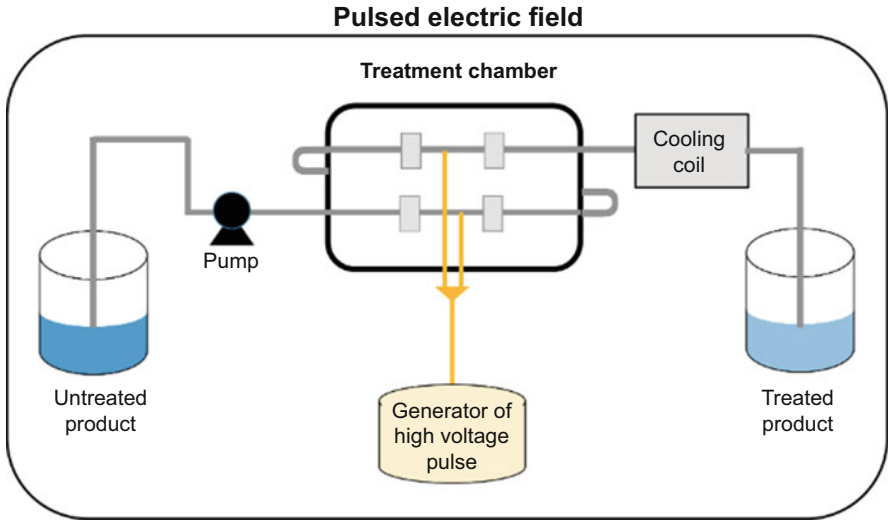

Fig. 5 Fundamental components of PEF processing treatment [10]

processing, though. When the electric field applied voltage and associated strength are higher than the necessary critical transmembrane potential, PEF-based extraction is possible. Figure 5 exhibits the basic components required for the PEF-based bioactive treatment.

The final application and the food that needs to be processed determine this crucial transmembrane potential. The development of pores takes place in the membrane of biological cells, including those of plants, animals, microorganisms, and algae, when the necessary critical transmembrane potential has been applied. Once the pores have formed and have a radius of around 0.5 nm, they may enlarge in response to the applied electric field, leading to irreversible electroporation, which is the disruption of the cell. After the applied voltage is removed in irreversible electroporation, the cell cannot move back to its initial location.

4.4 PEF—Batch and Continuous Treatment Chamber

The treatment chambers can be separated into batch treatment chambers (Fig. 6) and continuous treatment chambers (Fig. 7) based on the type of the treated product (solid, semisolid, liquid, and semiliquid). The latter form is far more practical for manufacturing applications since it enables the pumping through the chamber of liquid and semiliquid products. A central computer manages the process. It sets the parameters, manages the operation of the pump, and collects data from the sensors inserted within the chamber. The main issue with liquid products handled with PEF is the nonuniformity of the electric field distribution inside the treatment chamber, which is brought by the design of the chamber, the occurrence of bubbles and other contaminants, and the thermophysical characteristics of the product itself. As a result, some areas

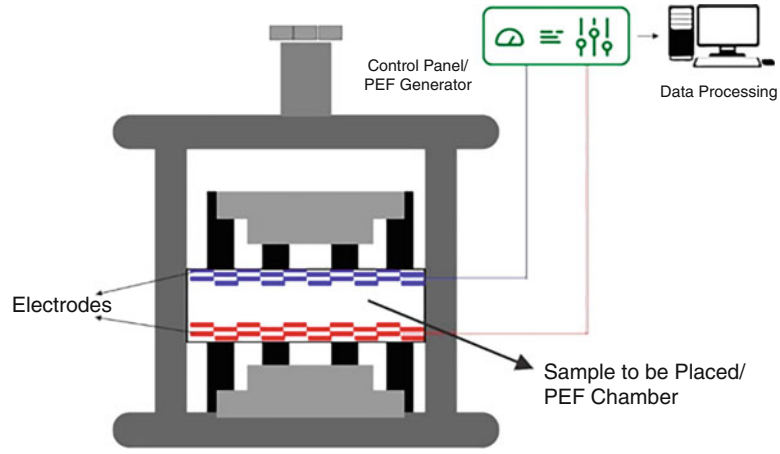

Fig. 6 Schematic representation of batch extraction system of PEF

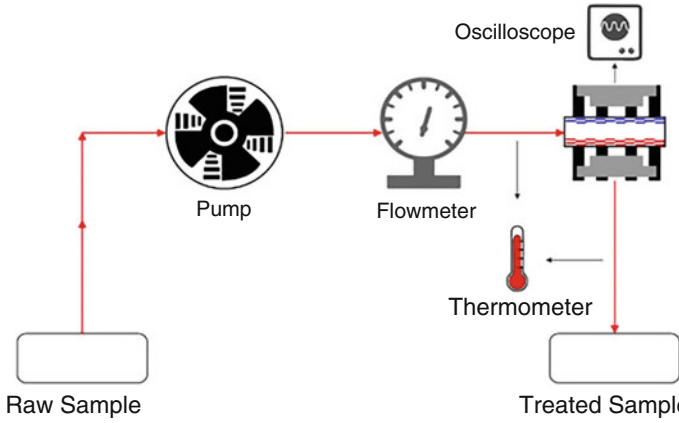

Fig. 7 Schematic representation of continuous extraction system of PEF

of the liquid volume may either receive inadequate or excessive treatment, typically in the center or in dead spots, often in boundary regions [20].

Generally, batch chambers are limited in the amount of liquid and solid food they can process. However, the productivity observed with the traditional continuous processing of liquid samples needed in industrial applications can be achieved in a dynamic chamber. Common electrode arrangements utilized in PEF include parallel, coaxial, and colinear electrode configurations. The adjacent plate pairs each electrodes and biaxial electric field lines [21]. However, compared to a setup with only two electrodes, this later configuration does not appear to offer any noticeable advantages. Multiple electrode rings on alternating potentials are segregated by insulating rings in colinear electrode designs. Two insulators and three conductors make up the colinear chamber. The

physical structure of the insulator positioned between the electrodes regulates the heterogeneous distribution of temperature and electric field intensity. This arrangement of electrodes offers a high resistance that is beneficial for continuous bioactive extraction and a significant treatment capacity with a smaller effective cross-sectional area of the electrodes. This design is advantageous for PEF systems since it requires limited current from the pulse modulator [7].

5 Factors Influencing the PEF-Based Bioactive Extraction

It is essential to recognize that the consequences during bioactive extraction will depend on the strength of a given pulse as well as the total damage sustained by a progressive succession of pulses that results in cell malfunction when thinking about the shape and spatial distribution of PEF therapeutic effects. Similar to charging a capacitor, the electric field-induced change in the cell transmembrane potential depends on the strength of the electric field and the length of the charge mobility. As a result, the strength of the applied electric field and the length of time that a certain pulse is exposed to the electric field can directly influence cellular effects at a specific segment (Fig. 8). For corresponding waveform characteristics (fundamental frequency, packet active period, and the total number of packets) and a specific electrode arrangement, an effective electric field limit may be outlined to account for changes in

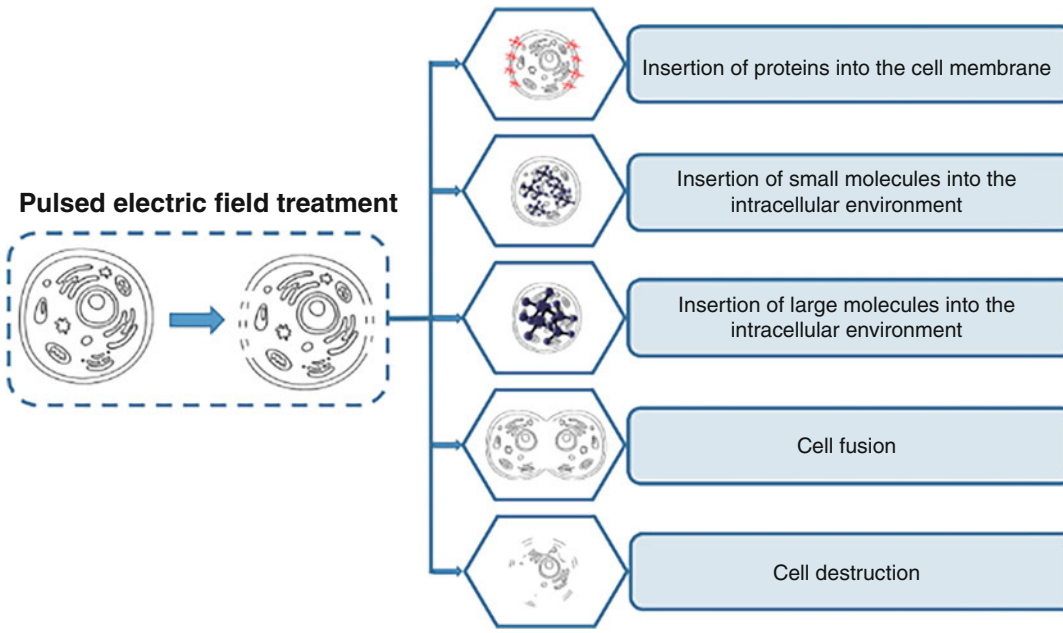

Fig. 8 Effect on the cell structure subjected to PEF [10]

treatment size, with changes to a delivered voltage having an impact on the electric field intensity. The length of the electric field pulse, for a given voltage, can also modify how long the cell is exposed to a different environment [10].

6 Overall Application of PEF Techniques in Bioactive Extraction

The sustainability of the world's food supply depends on the utilization of agro-industrial byproducts, which are abundant in naturally occurring bioactive chemicals. Numerous unique strategies have been developed and optimized to help with the effective and sustainable extraction of the bioactive alongside more traditional ways. Table 1 briefly elucidates the application of PEF in the extraction of various bioactive from different food systems.

7 Integrated Extraction Technologies in Combination to PEF

Integration of PEF and Ultrasonication (US) technology is reported to be the most extensively utilized strategy for enhancing bioactive extraction, since this method elucidates many notable instances, particularly in the field of bioactive extraction from food ingredients, as both strategies primarily focus on the cellular levels discharging of the food material and hence accelerate the extraction process.

A study conducted by Manzoor et al. [41] looks at the combined effects of PEF and US to assess the physicochemical properties, bioactive components, and chemical composition of almond extract. First, PEF was used to treat the almond extract, and then an US. In comparison to all other treatments, combined treatment (PEF-US) achieved the highest value of total phenolics, total flavonoids, concentrated tannins, anthocyanin contents, and antioxidant activity in DPPH. All of those treatments had a marginal differences in hue. Furthermore, according to FT-IR spectra, PEF-impact US on almond extract did not result in the production of any new carbonyl compounds, but rather in their concentration increases. This study showed that the PEF-US could help with volatile component stability improvement as well as bioactive chemical extraction.

Another widely administered technique along with PEF is the solvent treatment for improved bioactive extraction. A study by Quagliariello et al., [42] intends to show that PEF-assisted brown rice extraction results in higher antioxidant component yields, including oryzanol, polyphenols, and phenolic acids, as well as saturated and unsaturated fatty acid yields and cytotoxic effects on cancer cells. The first PEF-assisted extraction conditions were established by measuring the DPPH antioxidant activity and the cell permeabilization index using impedance. The cytotoxicity and

Table 1
Application of PEF in the bioactive extraction from various food systems

Category	Bioactive compound	Commodity	Treatment intensity					References
			EF: Electric field intensity *E*: Energy	*F*: Frequency	*P*: Pulse width *N*: Number of pulse	*S*: Solvent	*t*: Treatment time *T*: Temperature	
Fruits and vegetables	Phenolics, total anthocyanins, and antioxidant activity	Blueberry fruits (*Vaccinium myrtillus L.*)	EF: 1–5 kV/cm *E*: 10 kJ/kg	10 Hz	*P*: 1–23 μs	50% ethanol; 0.5% HCl, v/v	*T*: 20 ± 1 °C	[13]
	Carotenoids, anthocyanins, flavonoids, and phenolics	Date palm fruit extract	EF: 1, 2, and 3 kV/cm	10 Hz	*N*: 30		*t*: 100 s	[3]
	Polyphenols	Grape vine (*Vitis vinifera*); Greek mountain tea (*Sideritis scardica*) and Saffron crocus (*Crocus sativus*)	EF: 1.2–2.0 kV/cm		*P*: 10 μs		*t*: 1 ms	[22]
	Phenolic compounds	Cocoa bean shell and coffee silver skin	EF: 1.5–3 kV/cm for CBS and 1.30–4.40 kV/cm for CS		*N*: 500–1000	*S*: water for CBS; ethanol/water solution for CS	*t*: 5–20 μs	[23]
	Phenolic compounds	Brewers' spent grain	EF: 2.5 kV/cm	50 Hz	*P*: 10 μs, *N*: 500–2250	Ethanol/water	*t*: 14.5 s	[24]
	Phenolic compounds and flavonoid compounds	Onion	2.5 kV/cm	1 Hz	90 pulses 100 μs	Water	45 °C	[25]
	TPC, AA, and total monomeric anthocyanins	Blackcurrant	1318 V/cm		315 pulses	Cold pressing	10 and 22 °C	[26]

(continued)

Table 1
(continued)

Category	Bioactive compound	Commodity	Treatment intensity					References
			EF: Electric field intensity *E*: Energy	*F*: Frequency	*P*: Pulse width *N*: Number of pulse	*S*: Solvent	*t*: Treatment time *T*: Temperature	
Pigments	Betanin and vulgaxanthin	Red beetroot tissue	*P*: 4.38 kV/cm *E*: 4.10 kJ/kg		10 μs 10, 20, 30	Acidic buffer (pH: 6.5)	9.2 °C.	[27]
	Turmeric crude extracts	Rhizomes of turmeric	0.5–7.5 kV/cm		100 ms		20 ms	[28]
	Pigments	*Spirulina*	30 kV	300 Hz	4–32 μs			[29]
Green leaves	Polyphenols	Olive leaves	1 kV/cm	1000 Hz	1000 μs	Aqueous ethanol	30 min	[30]
	Phenolics	Fresh rosemary and thyme by-products	1.1 ± 0.2 kV/cm	10 Hz	67 bipolar pulses of 30 μs	Aqueous NaCl	40 °C	[31]
	Bioactive compounds	Custard apple (*Annona squamous*) leaf extract	Electric field strengths (2–6 kV/cm) pulse energies (45–142 kJ/kg)	100–300 pulses		Ethanol (70%, v/v)	2.5–5 min	[32]
	Antioxidant activity	Drumstick (*Moringa oleifera*) *plant*	7 kV/cm	37 kHz		Aqueous extraction	40 min	[33]
By-products	Nonpurified bioactive extracts	Peach pomace waste	0.8–10 kV/cm	0.1 Hz	4–30 monopolar pulses of 4 μs	Ethanol: water 70:30	20–22 °C	[34]
	Total phenolic contents	Cocoa bean shell (CBS) and coffee silver skin	1.5–3 kV/cm	50 Hz	500–1000	0.1% formic acid and 100% methanol	5–20 μs	[23]

	Polyphenol and volatile compounds	Grape stems	1 kV/cm	1 Hz			30 min	[35]
	Phenols, flavonoids, and antioxidant compounds	Fresh thinned peaches	EF: 0–5 kV/cm *E*: 0.61–9.98 kJ/kg	1 Hz	*P*: 30–150 μs *N*: 10–50	Water	*t*: 3 μs *T*: 15–35 °C	[36]
Algae	Polyphenols and carbohydrates	Brown macroalga (*Alaria esculenta*)			0, 720 pulses	Ethanol concentration (0%, 15%)		[37]
	Starch	Green macroalga (*Ulva ohnoi*)	1 kV/cm	3 Hz	200 pulses			[38]
	Phycocyanin and protein	*Spirulina platensis*	15–25 kV/cm	2–6 Hz			1 μs	[39]
	Pigments and total phenolic compounds	*Tetraselmis chuii* and *Phaedoactylum tricornutum*	1–4 kV/cm 100 kJ/kg	2 Hz	45–400 pulses	Aqueous or dimethyl sulfoxide (DMSO)	100 ms	[40]

anti-inflammatory capabilities of PEF have then been assessed in the human colon cancer cell line HT29. The findings demonstrate that PEF-assisted extraction increases the quantity of bioactive chemicals in comparison to untreated extracts. Additionally, brown rice PEF extracts significantly reduce interleukin production and gene expression in colon cancer cells, suggesting that they could be used as a natural antioxidant. Therefore, it appears that incorporating PEF pretreatment into the solvent extraction process of brown rice bioactive is a promising strategy to greatly increase their biological activity.

Further, this review focuses on the idea that combining nonthermal technologies is a beneficial alternative strategy in the bioactive extraction sectors; however, it is economically less cost-effective than using them individually. Apart from these techniques, various researchers have discovered that this cutting-edge technology works well in the extraction process when combined with other methods such as osmotic shock and mechanical press [43]. Moreover, Table 2 depicts the applications of other nonthermal processing combined with PEF for the extraction of bioactive components.

8 Benefits of PEF-Based Bioactive Extraction

The application of PEF-based extraction has become established during the last ten years in both bioactive extraction and novel food processing techniques. Compared to many of the present methods used in the bioactive extraction, PEF-based solutions are more efficient and sustainable for extraction. The numerous application of pulsed electric fields is due to its advantages in terms of cost and the environment; PEF processing is more acceptable because it uses less specific energy per processed product. For instance, PEF-treated juices are purer than untreated juices, which is probably due to the careful extraction of penetrated cells. Similarly, PEF-based extraction frequently has higher selectivity for certain bio-compounds, is quicker, more sustainable, causes less temperature rise, and uses less energy. Therefore, PEF technologies are known to provide increased earnings and perhaps be able to effectively produce bioactive components with added value from food waste [43].

Moreover, the use of PEF as a nonthermal approach for food processing is one of the key areas of research in the context of biofuel methods. Although cooling is required to retain a low temperature of the treated product during PEF treatment, it should be noted that the energy of the electric pulses generates heat owing to Joule heating. However, this phenomenon can be used in a delicate preservation procedure. The inactivation efficiency is increased by combining high temperature with PEF membrane

Table 2
Integration of PEF and other nonthermal techniques in extraction of bioactive compounds

Commodity	Bioactive compounds	Integrated treatment	Treatment conditions	Significant findings	Reference
Canola seed cake	Total phenolic content, total flavonoids	Microwave and PEF	Microwave: Liquid to soil ratio of 6.0 and 633.3 W for 5 min PEF-assisted extraction: 30 V, 30 Hz, 10% ethanol concentration and 10 s	Due to their reduced solvent usage, shorter extraction times for moderate microwave power, lower electroporation voltage and frequency, and greater efficiency in extracting polyphenols	[44]
Almond extract	Condensed tannins, anthocyanin, total phenolics, total flavonoids	PEF and US	PEF: Flow rate of 40 mL/min, 18 kV/cm electric field strength for 500 L/s, 1 kHz pulse frequency; US: 40 kHz ultrasonic frequency, 200 W radiation, and 35 °C temperature for 20 min	Permeabilization, increases the yield, extraction efficiency, and the extraction of intracellular metabolites. Due to the release of bound phenolics mediated by cavitation-induced cell membrane rupture, ultrasound improves the bioactive component	[41]
Grape stem	Polyphenols	PEF and US	PEF: low electric field strength of 1 kV/cm (30 min). US: 35 kHz frequency and a 320 W high frequency peak	Compared to solely ultrasound-assisted extraction-derived extracts, this integrated technique helps to improve the yield of volatile compounds in the extracts	[35]
Spirulina	Phenolics, chlorophyll, and carotenoids	PEF and pressurized liquid extraction (PLE)	PEF: 44 pulses, 3 kV/cm, 99 kJ/kg PLE: preheating time of 1 min, heating time of 5 min, flush volume of 60%, nitrogen purge time of 60 s, extraction pressure of 103.4 bars, extraction temperature of 40 °C, extraction time of 15 min	PEF has the power to damage the structural and functional integrity and obliterate the microalgae's adherent filaments, which helps to facilitate more effective PLE extraction. The polyphenol content is increased by this integrated technique (PEF + PLE)	[45]
Ziziphus lotus fruits, leaves, and roots	Total phenolics, chlorophyll, and carotenoids	Supercritical fluid extraction and PEF	–	Extraction of pharmaceutical drugs and nutritional supplements from natural sources, such as aromatic and medicinal plants, spices, and herbs	[46]

electroporation. Enzymes and bacteria are mostly inactivated in research on the usage of PEF [47].

High-voltage impulses induce the cell membrane to rupture, allowing tiny molecules to get through and leading to the swelling and breaking of the cells. For items that are liquid or semisolid, such as soups, liquid eggs, or fruit juices, PEF can be employed. In 2005, fruit juices produced using this method was made available on the US market. For solid items, the industry that processes potatoes has seen the most success with PEF technology. PEF alters the structural stability of tissues, causing a better-controlled release of intracellular substances such as reducing sugars or amino acids involved in Maillard reactions, which lowers the risk of acrylamide content in fried or cooked potato products [48].

It is claimed that those certain electrical factors, such as pulse frequency and the make up of the processed product (such as the presence of halides), have an impact on the quantity of metal discharged from the electrodes. Unfavorable electrode reactions can be prevented or at least avoided by determining the ideal conditions for PEF treatment on an industrial scale, as well as the electrode material and geometry. The high-voltage pulse generator, treatment chamber, fluid-handling system, control, and monitoring devices make up the majority of a typical PEF machine. The initial component provides the necessary shape, duration, and intensity for the high-voltage pulses. A pair of electrodes in the treatment chamber is used to apply the generated pulses, and the product being treated is then positioned between them [20].

9 Future Perspective of PEF-Based Bioactive Extraction

Consumers today demand fresh, healthy products that can maintain their nutritional profile during storage, which highlights the significance of creating novel and green processing methods [49].

The PEF-based extraction has been created as an alternative to traditional extraction techniques, offering benefits such as faster processing, greater extraction yield, fewer extract contaminants, and reduced solvent and energy usage. However, there are still certain defects in the PEF-based extraction method. As a result, the PEF-based extraction still experiences a lot of obstacles that will need to be solved in the future. Research regarding validation of the PEF's extraction mechanisms and creation and assessment of its extraction kinetics models are highly required. Similarly, PEF mechanism and kinetics are still up for debate and require more research. For future industrial use, a deeper comprehension of the mechanism and the development of a scientific model of PEF-assisted extraction unit are necessary. Further, to scale up its extraction technique for industrial applications and optimize the treatment chamber shape of PEF, more research is required. The

extraction system's industrial applicability will be facilitated by further development of the technology. In the future, we anticipate that PEF-based extraction will be a competitive method for bioactive material extraction. More research is required on bioactive and nutraceuticals extraction inferred from food processing industries. These surpluses might constitute excellent alternatives for the creation of innovative functional foods. Consequently, it is essential to research components extracted, with the PEF extraction technique providing substantial support [4].

According to earlier studies, it has been demonstrated that PEF treatment can increase the effectiveness of extraction in plant items; however, the outcomes vary depending on the type of extraction medium used. Therefore, in future research, the following aspects should be considered when applying the PEF in the extraction of bioactive compounds: When employing the PEF technology, materials properties of the diverse plants and extracted chemicals should be taken into account. Even though the PEF technology has some advantages in the extraction processes, it is still necessary to develop optimal processing to integrate with certain other methods, and a compatible extraction strategy should also be put into consideration. This is due to the differences in the food materials administered and processing parameters of the PEF equipment [11].

10 Conclusions

The demand for natural products extracted from plants and other materials is booming, especially for those nutrients derived from by-products, as a result of customers' concerns about conceivable hazards in the extracts and health risks associated with employing organic solvents as an extraction medium. Therefore, researchers are increasingly focusing their attention on investigating more complex and efficient green-extraction processes as a result of the rising demand for sustainably extracted bioactive compounds. Likewise, PEF technology is a recently developed nonthermal method that has been mainly used in the food industry for various applications. The PEF procedure used to extract bioactive and nutraceuticals has evolved recently. Moreover, the dielectric breakdown theory is the chief principle for bioactive extraction on a theoretical basis using PEF and is one of the widely recognized mechanisms. There has not been much research conducted on the mechanism underlying PEF dependent bioactive extraction, however, these techniques have been used as assisting technique for the improved extraction.

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Chapter 10

Pulsed Electric Field Extraction

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Abstract

An efficient nonthermal food processing method is called pulsed electric field processing. It involves applying a short-duration, high-voltage pulse to a food product that is sandwiched between the two electrodes. This method is used for microbial inactivation in food, making them last longer without the need for preservatives or thermal treatments. This method results in many benefits for foods, such as protection of their original nutritional value, taste, color, freshness, and flavor. Apart from these common benefits, there are many other benefits of pulsed electric field (PEF) for specific foods. The PEF treatment can increase the number of bioactive substances that can be extracted from plant tissues and their by-products, including vitamins, minerals, polyphenols, anthocyanins, and plant oil, as well as the soluble intracellular matter from microorganisms. In this chapter, we examine how the PEF method is used in the extraction of bioactive compounds as well as their applications in the food and nutraceutical industries.

Key words Pulsed electric field, Nonthermal, High-voltage pulses, Extraction yield, Microbial inactivation, Bioactive compounds, Intracellular matter

1 Introduction

One of the extending and enticing uses of high-voltage engineering techniques is the pulsed electric field (PEF) technique. It is a cutting-edge processing method that does not require any thermal energy [1]. A pulsed electric field's effect on a biological cell's electric potential gradient between its inside and exterior improves the conductivity and permeability of the cell membrane [2] and controls or prevents the devaluation of the quality of the food compounds [1]. By heating food to a temperature between 60 and above 100 C for varying lengths of time, thermal processing serves as a common method for biological stabilization. Although the extra energy can be used to eliminate or prevent harmful germs, numerous unexpected secondary reactions reduce the nutritional and sensory quality of food [3]. Numerous studies have shown that pulsed electric field (PEF) technology can be used to produce safe,

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high-quality foods that include considerable amounts of chemicals that are good for health [2]. PEF technology can be used to process both solid and liquid foods, as well as semisolid foods. Although minimal information is offered about the impact of PEF on food composition, quality, and acceptability, most researchers have mainly concentrated on the element of food preservation with particular reference to microbial control. Recent research has been done to assess the potential of PEF for increasing the efficiency of food processing, including enhancing juice extraction and intensifying food dehydration or drying [4]. In addition, increased emphasis has been given recently to the use of pulsed electric fields to extract beneficial components from food waste and by-products through osmosis, squeezing, and drying. It reduces the negative effects of standard heating methods. Important chemicals have been successfully separated, intensified, stabilized, and dehydrated without losing their nutritional characteristics using PEF technology, a possible alternative to traditional techniques. Pulsed electric field (PEF) has just been introduced as a method to stress the plant cells, which will boost the extraction process by enhancing the production of active ingredients [5]. Traditional extraction techniques typically take a long time, use a lot of solvents, and involve heating. There has recently been an increase in the demand for novel extraction techniques that are also energy- and environmentally efficient to improve mass transfer processes, and the quality of the extract, and to minimize extraction time and solvent consumption while avoiding the use of organic solvents. High-voltage electrical discharges (HVED), pulsed electric fields (PEF), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), high-pressure extraction (HPE), and microwave-assisted extraction (MAE) are some of these new techniques that have shown promise in improving the overall yield and selectivity of biomolecules from various vegetal matrices [6]. In reaction to the administration of pulsed electric fields of high intensity and duration between microseconds and milliseconds, cell membrane permeabilization may happen briefly or permanently. Since the usage of PEF has drawn significant interest in several scientific fields, including cell biology, biotechnology, medicine, and food technology, the effects of PEF on biomembranes have been intensively investigated [7].

2 General Overview of PEF and Its Working Principle

The PEF has enormous advantages and benefits in different perspectives at different sectors. It can be exclusively used for the preservation of different types of food. Through this process, it follows its methodology and also it is utilized for the extraction of various kinds of bioactive compounds that are produced by plants and microorganisms. Therefore, this technique is being abundantly

utilized in food, nutraceuticals, and laboratory extraction of bioactive compounds [6]. We all know that every living thing in the world is made up of various types of cells and that a cell is the fundamental structural unit of life. As a result, cells serve as the storage units for all the valuable materials needed for an organism's growth and development. If we want to obtain something valuable from an organism, we must target its basic cell. From the cell, we can get our desired product, for example, any bioactive compound from the plant cell. So if we want to take out the desired product, one has to disrupt the cell in order to allow the required compounds to come out of the cell. To accomplish this, we require a revolutionary technology that does not harm the organism, saves time, costs less money, and is simple to use. Pulsed electric field extraction is one such advanced technique; hence, the name itself states that the PEF technology works based on the utilization of short pulses of high electric fields for a very short interval of time (milliseconds), the electric property associated with each point in space when the charge is present in any form is known as electric field. Here, the sample from which the bioactive compound has to be extracted or any food product that has to be processed should be kept between the two electrodes, which generate high electric field pulses of about 100–300 V/cm in batch mode and 20–80 kV/cm in continuous mode of extraction [5]. This process depends on the number of pulses that we pass through the sample placed between the electrodes [4]. The space between the two electrodes to place the sample is known as the treatment gap of the chamber. Typically, there are two ways to help the extraction of valuable bioactive compound from the cell of an organism [7]: one is to achieve the solubility of the solvent in a biological membrane one has to speed up the chemical-based reaction from various compounds and the another one is electroporation of cell membrane [8]. Electroporation is the method of permeabilization of the cell to extract bioactive compound, by the external application of short-term high-voltage electrical impulses, which will help to increase the permeability of the cell membrane [5]. This happens through the formation of hydrophilic pores. When a cell is suspended in an electric field, an electric potential flows across the cell membrane [9], and subsequently the high voltages will cause the pores in the cell membrane of that cell and thus cellular structural functioning will be lost and the desired product can be easily extracted [10]. We can apply electric field in various ways like oscillatory square waves, unipolar triangular, or bipolar pulses. The electroporation that occurs is either temporary or permanent, but depending on the application, this effect can be controlled [11]. Irreversible electroporation increases the extraction process; sometimes, this cell permeability depends on the size and geometry of the cell [12]. According to certain research, electrical fields with strengths between 0.1 and 10 kV/cm are sufficient for fragile plant tissues such as the pericarp or mesocarp of a few fruits, while seeds and

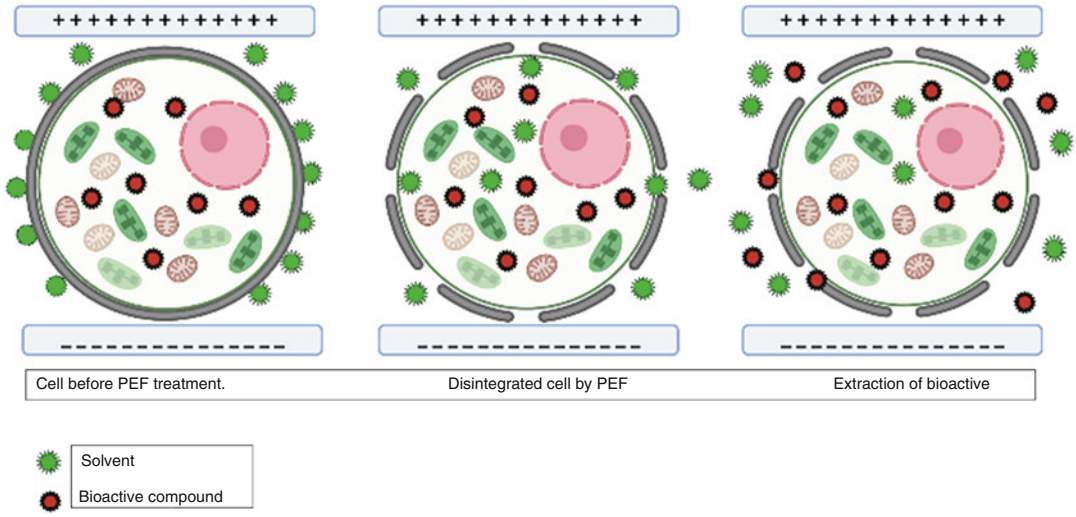

Fig. 1 Cellular disintegration and the extraction of bioactive compound from the ruptured cells upon the application of pulsed electric field

other hard materials require higher intensities, between 10 and 20 kV/cm, to be extracted effectively [13]. It is still unclear how electroporation caused by PEF works at the molecular level. The most widely recognized theory, however, is based on Sale and Hamilton's "transmembrane potential ($\Delta\Phi$) breakdown model" [14]. A high amount of transmembrane potential develops because of the accumulation of electric charges on the membrane of the cells as and when it is sandwiched between the electric field–producing electrodes. But the cells have a critical endurance limit, that is, without disruption plasma membrane of a cell can withstand some level of electric field strength. When it crosses or exceeds, the cell membrane gets disrupts [15]. Increased membrane permeability causes cells to disintegrate, while an increase in mass transfer rate facilitated the release of intracellular components [5]. High electric field strength affects the electroporation rate, type of waveform, duration of time, and the type of sample taken [16]. One can also perform this method at various temperature ranges such as ambient, sub-ambient, and above ambient type of temperatures (Fig. 1) [4].

3 Equipment Design of PEF-Assisted Extraction

To achieve this complex process, that is, the high voltage at a very short interval of time, engineers have designed equipment that has all the necessary components to meet our required purpose. The entire unit of PEF consists of a generator that produces very high-voltage pulses, a monitoring and control system, and a fluid managing assembly with a treatment chamber. To convert alternate

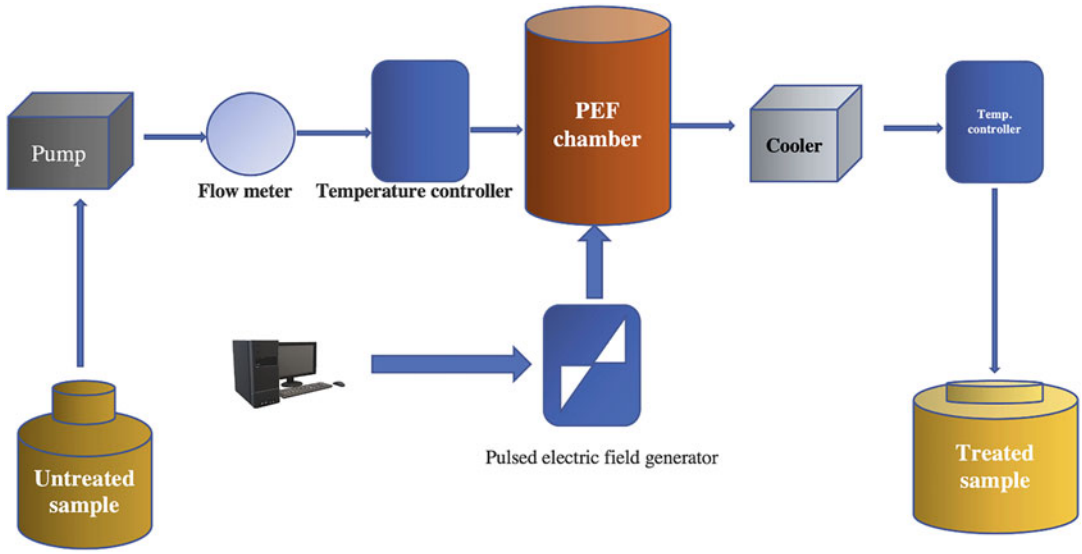

Fig. 2 Design of pulsed-field extraction system

current into direct current, it has a converter/charger [5] and a device which helps to store the energy in the generator. To produce electrical pulses, scientists have designed the switch that helps in the on and off mechanism of a high-voltage circuit. It consists of a capacitor that is to be continuously monitored if there is any interruption in the voltage flow [3]. The PEF unit consists of two main electrodes in the treatment chamber where our desired sample to be treated is placed, among them, one electrode is connected to a generator that produces very high voltage and another one is attached to the ground. An electric field is generated because of the difference in the electric potential on either side of the membrane. In addition, this depends on the type of electrode and the distance between those electrodes and the sample placed in the treatment chamber (Fig. 2).

The effect of the electric field depends on other factors such as the design or the layout of the treatment chamber, the nature of electric pulses, and the conductivity of a product. The PEF extraction methods can be categorized into the following type of extraction methods based on the way of performed [7].

1. Batch method of PEF extraction
2. Continuous method of PEF extraction

3.1 Batch Method of PEF Extraction

In the batch method of extraction, the unit contains a pretreatment chamber for solid-liquid extraction. The cylindrical vessel of polypropylene is fixed in the pretreatment unit of PEF. It consists of two electrodes made of stainless steel, which are parallelly arranged, and the distance between them is 10 mm. Electrical field strength, shape

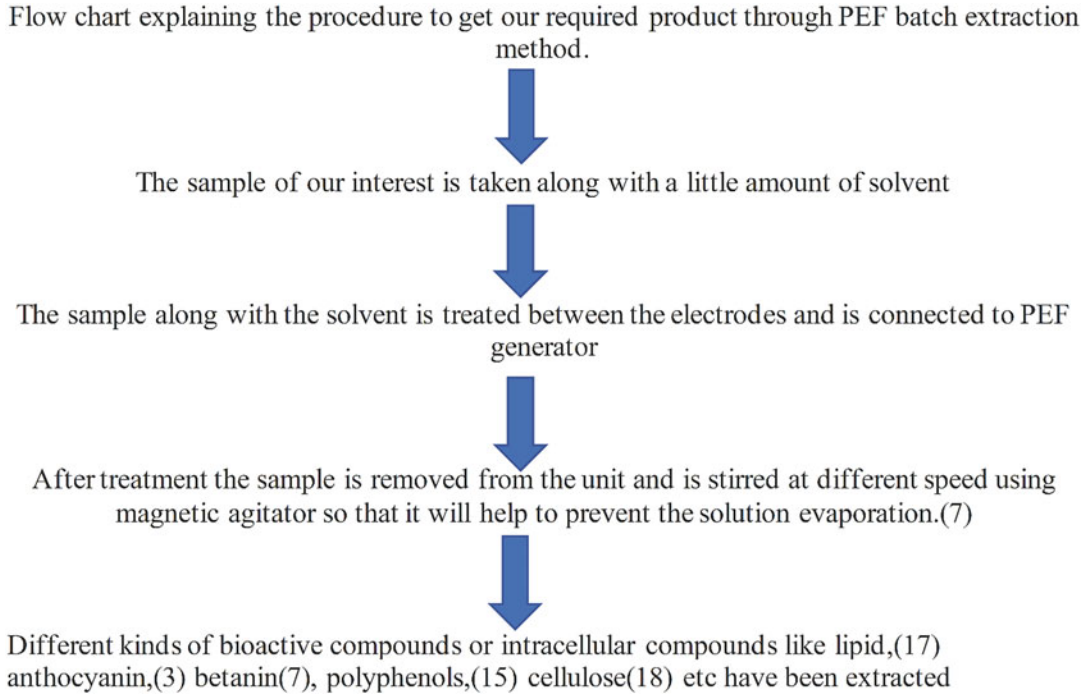

Fig. 3 Flowchart explaining the PEF batch extraction method

number, the width of the pulses, and frequency are the most important parameters one should consider during processing [5] (Fig. 3).

3.2 Continuous Method of PEF Extraction

In order to carry out the extraction process in a well-organized manner in an industrial level, the continuous method of PEF extraction has been developed. The batch method of PEF extraction gave a better result only in terms of better extraction, but it took considerably high operating time due to the low capacity of the batch method. To overcome this problem, a new arrangement and design is developed. The first extraction from this system was done by Yong Guang et al. [3] to extract the polysaccharide from the *Rana temoraria chensinensis* David in 2006 [17]. They got a good result that PEF extraction gave 55.59% compared to usual extraction methods. Nowadays, this method is extensively used in laboratories to get various kinds of animal and plant by-products such as fishbone, eggshell, and tomato juice [5].

Normally, the continuous PEF extraction system contains a generator to produce high electric pulses; a treatment chamber to place the desired sample to be treated; a product handling system; a controlling system; output voltage reader (oscilloscope), which can read about 40 kV pulse; and the frequency can be adjustable from 40 to 3000 Hz. In this method, at constant fluid speed, the mixture of solvent is sucked into a treatment chamber through a pump

(peristaltic pump). During the extraction procedure, the thermostat controls the cooling coil's temperature (25 °C) in the water bath. Nowadays coaxial and cofield continuous PEF units are abundantly used as they are user-friendly [5, 7, 10].

4 Factors Affecting the PEF Extraction

Factors such as nature of the solvent, composition of the sample, size, shape, conductivity, pH, extracted compound size, and their location in the cell affect the PEF extracting system. The properties of the cells and tissues also have a significant influence over the effectiveness of pulsed electric field extraction [11, 18]. In case of low ionic strength, the extraction procedure is improved. The cytoplasmic system is impacted by ionic strength because of cell shrinkage and pore formation. Even so, the conductivity of the matrix has a substantial impact on how the electric field behaves as it moves through the matrix [19]. As it influences the physical characteristics of the target molecule, such as, surface tension, viscosity, and solubility, EFS is a critical parameter in determining the degree of extraction [20]. Ensuring that electric fields are distributed evenly throughout the treatment chamber is crucial. Electric field energy is transmitted as square, bipolar, exponentially decaying, and oscillatory pulses. Due to their high energy and lethal performance, exponential square wave pulses are among those that are frequently used in the PEF extraction procedure. Square waves are also the most typical waveform used in the extraction process. Although the PEF intensity varies depending on the food's characteristics, typically 12–45 kV/cm is sufficient to remove the essential ingredients from food. With an increase in the EFS, the extraction of target compounds also gets better because of the considerable energy transfer in the food sample. Additionally, Lin et al. [21] found a significant increase (5.042 0.04 to 6.996 0.03 mg/mL) in the calcium malate extraction from an eggshell while using PEF at 0 to 10 kV/cm EFS. The PEF treatments, according to the scientists, accelerated the interactions between ionic groups and electrons with increased kinetic energy, speeding up the extraction of calcium malate and malic acid. Another critical element influencing the PEF extraction process is treatment temperature [22]. As a nonthermal process, the PEF extraction technique works at or close to room temperature. Higher temperatures typically cause liquid solvents' viscosity to diminish, which is harmful to the extraction process. Another criterion to gauge PEF effectiveness is treatment time (pulse numbers and width) [19]. The product's temperature could rise with a longer treatment period, though. In addition, choosing the right solvent is essential for better PEF extraction. Numerous aspects, including solubility, conductivity, and solvent polarity will affect the extraction process.

The extraction rate was ultimately boosted by the higher solvent conductivity because it facilitated cell membrane electroporation. The strong polarity of the solvent and the extract's high solubility in the solvent both accelerated mass transfer and extraction rates [5, 23].

5 Factors Impacting the Effectiveness of Pulsed Electric Field Treatment

The physicochemical characteristics of the biomaterial (cell or tissue), the treatment medium, and the pulse parameters all have an impact on the potency of PEF treatment.

5.1 Tissue Parameters

The effects of electroporation depend on the physical and chemical characteristics of the cell and the tissues, such as the composition, thickness, and size of the cell membrane. The value of the transmembrane potential depends on the type of tissue and the rigidity of the membrane; it is inversely proportional to the size and rigidity of the cell. Soft plant tissues typically require a moderate strength of the electric field, whereas hard tissue parts require a comparatively high amount of electric field strength to rupture the cell membrane by exceeding transmembrane potential. The larger field strength necessary is 20 kV/cm, and the moderate quantity of electric strength required is (E 5 0.12 kV/cm) [15].

5.2 Media Parameter

According to certain research, the medium's conductivity and ionic potential may have an impact on the membrane's ability to separate. According to existing lines of evidence, the conductivity of the medium is inversely proportional to cell mortality in the case of microbe inactivation and gene transfection. However, several investigations have shown the exact opposite phenomenon. In extraction studies, the medium's conductivity is generally unaffected. The plant tissues or dietary by-products do, however, contain large amounts of extracellular ionic compounds (salts) when suspended in the aqueous environment during PEF treatment, which may change the cell size (owing to osmosis) and electrical conductivity. The heat generated during the PEF treatment may have a favorable or negative effect on extraction efficiency. Larger field amplitude pulses can be employed in low-conducting mediums to achieve the appropriate level of electroporation [14].

5.3 Pulse Parameter

The degree of electroporation may be influenced by the pulse's electric field strength, polarity duration, and frequency. For parallelly arranged electrodes, the electric field strength can be defined as the ratio of voltage and the distance between the two parallelly arranged electrodes.

Electric field strength (E)
= Applied voltage (V)/Distance between two electrodes (d) [4].

By creating irreversible electroporation, the high electric field strength for a long time results in better tissue damage. Typically, the medium electric field strength beyond the critical field and longer pulse durations are best for electroporation of plant cells.

As far as the pulse shape is concerned, it also has a significant effect on the membrane disintegration and extraction of intracellular compounds from the cells. Due to its energy efficiency and ability to produce homogenous electric fields, square wave pulse has been used in the majority of laboratory-scale experiments. Oscillatory pulses are not advised since cells are only marginally injured under these circumstances because they are continuously exposed for a long period [14, 24].

As far as pulse polarity is concerned, typically bipolar pulses are found to be more efficient than those monopolar pulses. The bipolar pulses are more efficient because in that mode the direction of the provided electric field will be continuously reversed. In addition, it processes half-negative and half-positive pulses. Overall studies indicate that bipolar pulses with a square form may result in a higher extraction yield [15, 25].

6 Extraction of Intracellular Bioactive Compound from Plant Sources Through PEF-Assisted Method

Researchers' interest in organism extraction has increased as a result of the PEF treatment's reputation as a viable method. In addition to soluble intracellular matter from microorganisms, the PEF treatment can boost the extraction yield of bioactive compounds from plant tissues and their by-products, such as polyphenols, anthocyanins, and plant oil [1].

6.1 What Are Bioactive Compounds?

Secondary metabolites of plants that have medicinal or toxic effects on living organisms are known as bioactive substances in plants. In addition to the primary biosynthetic and metabolic pathways for compounds related to plant growth and development, secondary metabolites are produced in plants. These by-products of plant cells are not required for daily plant function, but some of them are found to have important functions in living plants, including signaling and protection. The majority of plant species appear to be able to produce these substances. But some of the chemicals and bioactive compounds from the plants have pharmacological and toxicological effects on humans and animals [21].

Plants are a large reservoir of various kinds of bioactive compounds; these are the extra constituents that are rich in nutritional value. Therefore, it is important to utilize these compounds in

various fields of medicine as well as food and nutraceutical industries. Scientists have studied that these bioactive compounds show antidiabetic, anticancerous, and antioxidant properties so that they can be utilized to cure cancer [26], to increase bone strength, and to build the strong immune system [21, 27]. As these bioactive compounds have commercial importance, it is important to extract them from the cell of the plant, which is a very big task. Hence, scientists have done various types of research to find out the cost-effective, and less harmful, methods to enhance the productivity of these compounds from the plant sources. The novel technology called pulsed electric field extraction is found to be more efficient compared to the conventional method of extraction of bioactive compounds [2].

When the cell is exposed to an electric field, the dipole nature of the cell membrane will separate the molecules based on their charge under the electric field. Initially, when the pulse of high voltage is passed through the cell for a very short interval of time, it weakens the lipid bilayer of the plasma membrane of the cell and also the proteins present in the plasma membrane [28]. Eventually, the high electrical pulses will create the temporary pore in the plasma membrane, which leads to the transfer of large molecules along the cell membrane. As the outer membrane of the cell encloses or shields the intracellular membranes, the optimal level of pulses will not affect the intracellular membranes [2, 25]. In order to achieve increased extraction of bioactive compound from the desired sample, it should be treated with the optimal level of pulsed fields, which leads to the reversible electroporation on the cell membranes of the cell. The temporary pore formed by the pulses enables the mass transfer of intracellular compounds into the surrounding solution, primarily due to the high membrane permeability [29] for the movement of an electric field it should need a media, that is, ions as the foods contain several ions which help to provide conductivity to the product to a certain extent. However, the PEF method is most abundantly utilized nowadays for liquid foods as the electric current can easily pass through the liquid media from one point to the other point due to the presence of charged molecules [4].

Free radicals are one of the most dangerous oxygen-containing molecules with an uneven number of electrons. This property allows free radicles to react easily with other molecules in the body. Free radicles can cause a chain of chemical reactions in our body, which may damage the cell, and it may be harmful to our body. Moreover, this may later lead to tissue degradation and followed by various kinds of diseases such as heart ailments, cancer, and atherosclerosis-related diseases [30]. To overcome the harmful effects of free radicles in the body, antioxidants are utilized, which will play a major role in protecting the cells of our body from free radicle damage by neutralizing the free radicles by donating the

electrons. The bioactive compounds present in the intracellular matrix of food, animal, and plant cells possess these antioxidant properties. By using the pulsed electric field extraction system, one can easily extract valuable bioactive compounds from the cells [2, 19, 25].

Table 1 shows the application of the optimal value of PEF for the extraction of various bioactive compounds from plant sources (Table 1).

Table 1
Utilization of the PEF method in the extraction of bioactive compounds from various sources

S. No	Bioactive compound	Product	The optimum value of the PEF application	References
1.	Polyphenol	Red grape pomace Orange juice Orange peel Tea leaves Green grapes	E-400 V/cm, n-2 tp −2000+_1 μs, delta t-20 ms E-35 kV/cm, f-800 Hz, t_p-4 ms, t_{PEF}-750 ms E-5 kV/cm, n-20, t_p-3 μs, t_{PEF}-60 μs E-0.9 kV/cm, t_{PEF}-0.5 s μs E-10 kV/cm, t_{PEF}-100 μs, n-15	[31] [32] [33] [34] [35]
2.	Anthocyanin	Merlot grapes Raspberry Potato Red cabbage Blueberry	U-7 kV, f-178 Hz, t_{PEF}-150 s E-3 kV/cm, t_{PEF}-15 μs, n-420 E-3.4 kV/cm, t-3 μs, n-35 t_{PEF}-105 μs, 8.9 kJ/kg E-2.5 kV/cm, t_{PEF}-15 μs, n-50, W-15.63 J/g E-3 kV/cm, W-10 kJ/kg, f-10 Hz, t_p-20 μs	[36] [2] [37] [38] [39]
3.	Carotenoids	Carrot Tomato Orange juice	E-0.6 kV/cm, t_{PEF}-3 ms, t_p-20 μs, f-5 Hz E-25 kV/cm, t_{PEF}-110 μs E-35 kV/cm, f-800 Hz, t_p-4 ms, t_{PEF}-750 ms	[40] [41] [42]
4.	Lycopene	Tomato Watermelon	E-1.5 kV/cm, n- 40, t_p- 500 μs E-35 kV/cm, f- 200 Hz, t_p- 50 μs	[43] [44]
5.	Lutein	Microalgae	E-25 kV/cm, t_{PEF}-150 μs	[45]
6.	Alkaloids	Korean monkshood Potato peel	E-20 kV/cm, n-8, t_{PEF}-60 s E-0.75 kV/cm, t_{PEF}-600 μs	[46] [47]
7.	Betulin	Mushroom	E- 40 kV/cm, t_p- 2 μs	[2]
8.	Protein	Microalgae	E-38 kV/cm, f- 158 Hz, t_p-232 μs	[2]
9.	Polysaccharides	Corn silk	E-30 kV/cm, t_p- 6 μs	[48]
10.	Lipid	Microalgae	U-45 kV, E- 45 kV/cm, t_p-10 s, t_{PEF}-30 s, n-3	[49]

6.2 PEF-Assisted Extraction of Bioactive Compounds from Fruits and Vegetable Sources

Fruits and vegetables are the reservoirs of valuable antioxidants, minerals, vitamins, and fiber [50]. These contain beneficial antioxidants such as phenolics, carotenoids, and anthocyanin [51]. Hence, these compounds have commercial and medicinal importance, and it is necessary to extract them. To achieve this, PEF method plays an important role in the extraction process. There are various other types of traditional methods of extraction of bioactive compounds such as the Soxhlet extraction method, maceration techniques, and hydrodistillation [52]. However, all these methods are time- and energy-consuming and are also less productive. Various studies have been undertaken to compare the extraction yield between normal extraction methods and PEF-assisted extraction methods. In all the cases, the PEF-assisted extraction gave the best result compared to normal traditional methods. One of the study findings showed that PEF-assisted extraction has improved the release of vitamin C, anthocyanin, and also increased antioxidant property of the grape juice when compared to the normal untreated sample [2, 5, 53]. Hence, the extracted sample had a good composition of phytochemicals and helped to prevent the oxidation stress of the cell [53].

Another study accounts for good quality extraction of phenols and flavonoids from onion through PEF extraction. As the cells have a plasma membrane, which affects the mobility of intracellular substances among other cells, it is a difficult and time-consuming process to extract the required compounds by traditional methods of extraction. However, in contrast, in the PEF method, the high-voltage pulses will cause a plasma membrane permeability by forming a pore so that the substance inside the cells can be easily removed out, leading to high yield in a short interval of time [54].

Similar studies show that the PEF extraction method provides a greater yield of polysaccharides, proteins, and polyphenolic compounds from white button mushrooms when compared to usual thermal treatments [5].

6.2.1 From Plant Leaves

One of the research studies has been done by Khursheed Ahmad Shiek and his team about the examination of extraction yield of bioactive compound from the leaf of custard apple. They prepared the extraction by adding 70% of ethanol along with the help of a pulsed electric field. Results showed the increased cell integration index in the extraction of custard apple when they provided a pulse electric field of 6 kV/cm of about 300 pulses with the energy of 142 kJ/kg for about 5 min. They also found that the productive yield of extraction through the PEF method has shown an additional higher percentage of 5.2% than those of untreated extracts by the PEF method, which is 13.28%. The concentration of chlorophyll A and B in the pulsed electric field–treated custard apple leaf extraction was found to be very less or negligible [55].

Drying is an essential pretreatment before the lab and a time-intensive process of extracting tea polyphenols from tea leaves. In this study, pulsed electric field technology was employed to dehydrate phenolic compounds instead of the conventional thermal technique. Evaluating the impact of various PEF settings on the total polyphenol production from fresh tea leaves and a solid-liquid extraction, Zhibin Liu and their team made this observation. By applying PEF treatment with an electric field intensity of 1.00 kV/cm, 100 pulses of 100 s each, and 5 s of pulse repetition—which supplied 22 kJ/kg and raised the temperature by 1.5 °C—it was possible to further explore the kinetics of green tea catechin extraction. Results showed that compared to oven drying, PEF pretreatment nearly quadrupled the extraction rate [56].

Another case study was done by Segovia et al. for the extraction of polyphenols in *Borago officinalis* L. leaves. They observed that PEF treatment of about 300 Hz frequency with 30 kV voltage has enhanced the extraction yield of polyphenols from 1.3% to 6.6%. They have also found that it also enhanced the oxygen radical absorption capacity from 2.0% to 13.7%. In addition, they have observed that PEF treatment took less time for the extraction and also it increased the antioxidant property of the extract [5].

6.2.2 Extraction of Bioactive Compounds from Plant Seeds

There is a variety of seeds from different plants that have numerous beneficial applications in the day-to-day life as well, as they have commercial and medicinal importance. The seeds include groundnuts, cotton, safflower, and sunflower. These are the most important commercially valuable seeds as they are the reservoir of oil and fat [57]. These fats and oils contain triacylglycerol, which plays a vital role in enhancing the immune system of the body [58]; moreover, these oils are the main source of calories and vitamins. Usually, the oil from these seeds is extracted by squeezing the seeds. Once the oil is extracted, the press cake is then subjected to solvent extraction. This is usually done by normal Soxhlet extraction methods by using a large quantity of hexane [59]. However, nowadays, the PEF extraction method is incorporated at an industrial level to increase the extraction yield without any negative impact on the nutritional value of the product [5, 60].

Studies show that from the PEF extraction method oil yield of 55.9% from sunflower seeds [5], an yield of 0.105% with a 50% increase in essential oil from damask rose flower seeds [5], 48.24% with hexane (40 mL) solvent from sunflower seeds, [55] 22.66 kg/100 kg from Arroniz variety of olive fruit seeds, [61] and 85.5% oil yield from Nocellara del Belice variety of olive fruit seeds [62].

6.2.3 Extraction of Bioactive Compounds from Herbs and Spices

Soxhlet extraction is the most popular approach for obtaining bioactive compounds from plant sources. Other classical extraction methods include maceration, etching, and cohobating. However, the commercial production of essential oils has historically relied

heavily on steam distillation [63]. The conventional methods are intricate multistage procedures that use more organic solvent, take longer time, and use more energy while losing analytes. These elements contribute to the limited selectivity of traditional extraction techniques. By increasing osmotic dehydration with less energy input and improving nutrient recovery, the current PEF approach, on the other hand, improved the extraction capacity of bioactive compounds from metabolically active tissues. Furthermore, solvent diffusion and freeze-drying made PEF extraction easier [64].

6.2.4 Extraction of Bioactive Compounds from Microorganisms

Microorganisms, including bacteria, yeast, and algae, are excellent sources of extremely valuable substances such as enzymes, pigments, and nutrients. Since the majority of these chemicals are found inside the cell, it is crucial to isolate and refine them before using. Microorganisms are capable of a wide range of reactions and may adapt to a broad spectrum of environmental factors. They can be introduced into the laboratory from nature to build advantageous molecules using inexpensive materials such as carbon and nitrogen. Bioactive compounds produced by bacteria are extremely advantageous for human nutrition and health due to their biological activity. The screening of naturally occurring microbial products for the creation of novel medicinal medicines has advanced in research [65].

Several studies have shown that PEF-assisted extraction of bioactive compounds from various microorganisms, such as *Arthrospira platensis*, fresh abalone (*Haliotis discus hannai Ino*) viscera, fresh microalgae *Auxenochlorella protothecoides* studied, which has resulted in increased extraction of 151.94 ± 14.22 mg/g, 42.35%, 175.20 mg/100 mL, and 97%, respectively [66–68].

6.2.5 Extraction of Bioactive Compounds from Food Wastes

In the recent times, due to the increase in the population and the discovery of various food products, there is also increasing in the rate of food waste. Food waste may be agricultural, bakery, dairy, and industrial; these food wastes are nowadays leading to different types of pollution around the world. Hence, the scientists propound ways to reduce food waste and utilize them for beneficial aspects. Most of the food wastes from agricultural and dairy foods are a large reservoir of valuable bioactive compounds such as carbohydrates, phenolic acids, flavonoids, anthocyanin, terpenoids, limonoids, lipids, catechins, tannins, vitamins, alkaloids enzymes such as amylase, cellulase, pectinase, and invertase. Hence, it is important to extract those valuable bioactive compounds without simply discarding them. Conservation of energy and production of energy are the need of the hour. As science and technology have developed, scientists have invented many extraction methods,

Table 2
Bioactive compounds from fruit wastes

Name of the fruit	Waste type	Bioactive compound extracted	References
Citrus fruits	Peel, seeds	Carbohydrates, limonoids	[69, 70]
Apple	Pomace	Carbohydrates, phenolic acids, flavonoids, anthocyanins, dihydrochalcones, triterpenoid	[70–72]
Mango	Kernel seed Peel	Flavonoids, phenolic acids, tannins, xanthanoids, catechins, hydrolysable Carotenoids	[70]
Banana	Peel	Flavonols, catechins, catecholamines, phenolic acids	[70]
Elderberry	Branch waste	Phenolic acids, flavonols, anthocyanins	[70]

Table 3
Bioactive compounds from vegetable wastes [70]

Name of the vegetable	Waste type	Bioactive compound extracted
Carrot	Discarded carrots	α-carotene, β-carotene, lycopene lutein, lutein γ-tocopherol
Potato	Pulp and peel	Carbohydrate Phenolic acids Glycoalkaloid Carotenoids
Beetroot	Pomace Aerial parts including stem and leaves	Phenolic acids Flavonoids Betalains, phenolic compounds
Cauliflower	Leaves and stem	Phenolic acids Flavonoids Isothiocyanate Proteins
Broccoli	Industrial residues: stalks and floret Waste from agriculture activity: leaves	Phenolic acids Flavonoids Glucosinolates

which have been already discussed. Pulsed electric field extraction is one such effective method that is commonly used at the industrial level for the extraction of bioactive compounds from food wastes; it is mentioned in Tables 2 and 4.

Bioactives extracted from different types of food waste are listed in Table 2.

Bioactive compounds extracted from vegetable wastes are listed in Table 3

Table 4
Bioactive elements found in dairy, marine, and animal waste products [70]

Industrial product	Waste type	Bioactive compounds
Meat products	Blood: plasma hemoglobin cuttings and trimmings Horns Bones Skin	Bioactive peptides from protein hydrolysate Bioactive peptides from protein hydrolysate Collagen hydrolysate
Marine products	Salmon nasal cartilage Heads, tails, and shrimp shells Salmon skin and trimmings	Astaxanthin Polyunsaturated fatty acids Ω3 Proteoglycans Bioactive peptides from protein hydrolysate
Dairy products	Whey Colostrum	Bioactive milk Galactooligosaccharides Lactoferrin Oligosaccharides

Bioactive elements found in dairy, marine, and animal waste products are listed in Table 4 [70].

7 Utilization of the PEF Extraction Method in Food and Nutraceutical Industries

The demand for safe and high-quality food items has led to the development of numerous unique food processing processes in recent years. Consumers today have high standards for the items' olfactory quality, usability, and nutritional worth. They also place a high value on the utilization of environmentally sustainable food-producing methods. The demand for products that resemble freshness and for food produced using environmentally friendly practices, as well as the growing customer interest in food with high nutritional value, all stimulated the incorporation of pulsed electric field (PEF) technology into food production [6, 73].

Pulsed electric fields (PEFs) have been utilized successfully and safely on a range of different products in the food and bioprocessing industries. In Germany, Ukraine, and Moldova, the first studies on the use of pulsed electric fields (PEFs) were published in the 1950s, but it took decades for the technology to be used in industrial settings. Since the 1990s, more than 20 research organizations have been investigating the basic mechanisms of action, influencing variables, and potential applications [74].

Applications of the pulsed electric field (PEF) can be used to disintegrate biological tissues or microorganisms. There are many uses for this technology, including improving mass transfer during extraction or drying processes and mild food preservation. The method has acquired its initial commercial uses. The reliability

and cost-effectiveness of the equipment have improved thanks to the development of equipment based on semiconductor technology. The technology is moving toward more widespread industrial use.

8 Benefits in Food Industries

Due to its exceptional effectiveness in microbial inactivation and quality preservation, the food processing and preservation industries have paid close attention to PEF technology. Gene transfection, drying, pasteurization, and juice extraction from fruits and vegetables have all been accomplished with this technology. Predominantly, this method is employed in industries during freezing, drying, food preservation by microbial inactivation, spore, and enzyme inactivation, starch modification, etc., over the past few years [14].

8.1 Inactivation of Microorganisms

The inactivation of microorganisms is crucial for extending the product shelf life in the field of food processing. To do this, a cutting-edge technique called pulsed electric field is used. High-voltage electric impulses are utilized in this method to make the membranes of the bacteria permeable. Here, the microorganisms' membrane serves as a capacitor that is filled with a dielectric liquid. There is a transmembrane potential difference when a high voltage is applied. When the crucial threshold value, or 1 V, is reached, electroporation occurs, which results in the membrane of the microorganisms rupturing and killing it. These days, this technology is often used to destroy bacteria in liquid food products, although this method is only suitable for materials with poor conductivity and no air bubbles, such as milk, soya milk, fruit juice, and wine [75].

9 French Fry Manufacturing Saves Water and Energy by Utilizing the PEF Method

PEF has created new benchmarks for the manufacturing of French fries and chips. The method replaces previously used pre-heating and reduces water and energy consumption by up to 90%. PEF is applied cold, with treatment periods of less than 1 s, as opposed to gradually warming the substance up to 60 °C, loss of turgor pressure and tissue softening during hydro jet or slicing cutting resulting in a clean cut are the main advantages of treatment with an intensity of less than 1 kJ/kg. Less product breakage and feathering are caused by the smooth cut, which improves product quality and yield and enables the use of various product configurations or building materials. Additionally, a smoother product surface

means that up to 10% less oil will penetrate the product. More than 90 PEF systems are currently in operation by the global fries industry [76].

9.1 Perfect Vegetable Chips

Vegetable chips are a fad, but it can be difficult to make them consistently well. However, the use of PEF results in significant quality improvements along the entire production chain: less starch is discharged into the processing water, slicer blade wear is reduced, and so on. Users of PEF also get from 1% to 2% yield boost. Quality benefits range from better crunch to improved color to more of the original vegetable's natural flavor. PEF makes it possible to use raw materials and shapes that were not before. Accelerated moisture release following PEF permits lower frying temperatures in less time, preserving the color of natural food while reducing the development of acrylamide for products such as sweet potato, carrot, parsnip, or beetroot chips [77].

10 PEF Enhanced Extraction in Different Food Items

Extracellular materials are extracted during numerous food processing processes. PEF-induced cell membrane permeabilization can be used to improve extraction yield in the extraction of fruit juices, sugar, colors, pigments, and oils. PEF can be used to replace enzymatic maceration or mechanical disintegration in the generation of juice. A lower extraction temperature can be used in the sugar sector, and in the processing of olive oil, yield increases of up to 10% and shorter malaxation durations have been noted.

A higher extraction yield of anthocyanins and polyphenols from processed grapes allows for a significant reduction in maceration times and an improvement in production capacity. Two distinct equipment configurations can be used depending on the type of raw material. Pipe systems with diameters ranging from 50 to 200 mm can process materials such as vegetable mashes, semisolid products, or precut products. Beets, tubers, and entire fruits are examples of solid food items that are usually processed on PEF belt systems [78].

10.1 Premium Quality Drying

One of the earliest methods for preventing microbial development and preserving a food product's quality is drying. The process is typically constrained by moisture diffusion. PEF removes this restriction, enabling a decrease in temperature and a reduction in drying protocol time as it opens the cell membrane. For the majority of plant-based products, a drying time reduction of about 20% is achieved because water may migrate from the product, preventing the formation of a core crust and product shrinkage. Less adverse effects on product color, shape, flavor, and energy savings result from lowering the drying temperature. Additionally, PEF

treatment can be used with any drying process, including traditional drying by air, drying by vacuum, and drying by osmotic or freezing method [77].

10.2 Peel Removal: The Easy Way

PEF also has an impact on the structural qualities of plant matter. Fruits, kernels, and seeds can vary in their elastic characteristics and peel attachment after treatment. The tomato skin may be readily removed without any connected tomato flesh following treatment at 2 kJ/kg. Increased flexibility improves kernel integrity and reduces breakage during shelling for nuts and seeds such as cashews [79].

10.3 Superior Quality Juices

PEF not only affects microbial cells but also plant-based cells. Microbial inactivation results from the loss of membrane barrier function in yeasts, molds, and bacteria. To ensure the juice's microbiological safety and to provide a particular shelf life, the PEF treatment reduces the microbial load of the juice. Reduced treatment temperature and duration are the main advantages over traditional thermal processing. Thus, heat-sensitive flavors, colors, and nutrients are unaffected. PEF can be run continuously and is simple to integrate into existing processing lines, in contrast to other nonthermal processing choices. The majority of industrial PEF lines in the juice business use this technology since it works well with mild temperatures. With inlet temperatures ranging from 30 to 40 °C and maximum product temperatures well below 60 °C, the majority of industrial PEF lines in the juice industry employ preheating and cooling systems that are already in place or that have been specially designed for them. Compared to a high-temperature, brief treatment, the temperature reduction—typically more than one z-value—leads to a premium appearance and flavor [80].

10.4 Use of PEF in Meat Processing

Treating animal cells such as meat has positive impacts as well. PEF modifies the structure of meat to make it more softer and delicious, whereas tumbling mechanically can speed up the process. The production process is more efficient and meat quality improves as a result of the about 50% faster brine uptake following PEF treatment of the meat [74].

11 Application of PEF in Nutraceutical Industries

Foods or food additives with medicinal qualities are known as nutraceuticals. Nutraceuticals are categorized into three types, namely, nutrients, herbals, and dietary supplements. The dietary components with well-established roles, such as vitamins, minerals, amino acids, and fatty acids, are collectively known as nutrients, extracts, and concentrations made from herbs or other botanical products have come under herbals and dietary nutraceuticals are

the oral products that contain dietary ingredients that are meant to enhance the flavor of the foods we eat. Mostly all these nutraceuticals come from organic materials, mostly plants and microbes [81]. "Nutraceuticals" is a term that is occasionally used interchangeably or in conjunction with the terms "functional foods," "bioactive chemicals," "natural food additives," and "dietary supplements." To give a variety of health benefits and to ward against diseases, certain proteins, fatty acids, fiber, plant extracts, and secondary metabolites have been employed as nutraceuticals. The global nutraceutical industry is constantly growing as a result of the rising demand for functional foods and nutraceuticals, particularly in industrialized nations. To develop nutraceuticals, businesses are concentrating on mild and effective (in terms of productivity and purity) technologies. Some of the important applications of plant bioactive compounds in nutraceutical compounds are mentioned in Table 5 [14].

11.1 Extraction of Polyphenols

The most significant class of nutraceutical chemicals is thought to be dietary polyphenols. They are secondary metabolites generated by plants, and they are important for oxidant, pathogen, and UV radiation defense mechanisms. In humans, polyphenols display a wide range of biological effects, including anti-inflammatory, anticancer, and antiaging effects. Foods such as vegetables, fruits, seeds, oils, tea, wine, and chocolate often include dietary polyphenols. More than 8000 phenolic compounds in vascular plants have been discovered so far.

Applying an electric field of 450 V/cm for 10 ms with a specific energy of under 3 kJ/kg improved the yields of polyphenols from apple mash [86]. PEF with 5 pulses per second at 30 to 60 kV (at 35 C) doubled the number of polyphenols recovered from grape skin [86]. It was discovered that certain polyphenols needed to be isolated from grape pomace and peeled using PEF because they could not be extracted using alternative methods [87]. To maximize the recovery of polyphenols from fresh tea leaves, several parameters, including pulse duration (0.05 s), several pulses [88], pause between pulses (PBP, 0.5–3 s), and pulse intensity (0.4–0.9 kV/cm), were evaluated [86].

11.2 Extraction of Nutraceuticals from Microalgae

According to some studies, PEF is used to extract various important chemicals from microalgae (*Chlorella vulgaris, Chlamydomonas reinhardtii, and Dunaliella salina*). In dietary supplements, nutraceuticals, and food additives, lipids derived from microalgae can be a valuable source of essential fatty acids. Some research studies and patents have revealed the possible use of PEF for the microalgae's pretreatment to boost lipid production. After PEF treatment, lipid droplets may remain inside cells. However, algal lipids can be extracted with suitable solvents following the separation of other extractives [89].

Table 5
Plant bioactive compounds and their function and applications in nutraceutical industries [82–87]

Food source	Extracted bioactive compound	Function and benefits in nutraceuticals
Red wine, grapes	Isoflavonoids Polyphenols	Immunomodulator, antiosteoporotic, anticancerous, antioxidant property
Wheat	Derived immunopeptides, wheat gluten	Enhance the activity of natural killer cells
Oats	Dietary fiber	Helps in the achievement of lipid lowering
Flaxseed, soybean and soy-based products, tea legumes, cabbage	Phytoestrogens	Antiosteoporotic, anticancerous, antiproliferative, antiestrogen
Cauliflower, broccoli, sprouts, Brussels, onions, garlic	Diallyl sulfides, isothiocyanates, glucosinolates	Anticancer, immunomodulator, antimicrobial, detoxification
Corn, carrots, papaya	Carotenoids	It acts as an antioxidant immunomodulator
Seeds, nuts, vegetable oil	Phytosterols, tocotrienols, and tocopherols	Lipid lowering, immunomodulator, antioxidant
Tender coconut	Phytosterols, triglycerides	Anti-inflammatory, antihelminthic, antioxidant, antinociceptive, antitumor, antimicrobial, antifungal, analgesic, antiarthritic, antibacterial, antipyretic, antidiarrheal, hypoglycemic, antiseizure, hepatoprotective, cytotoxicity, nephroprotective, and antiosteoporosis effects, vasodilation
Chlorella vulgaris	Uncharacterized peptides	Hemopoiesis, activation of humoral immune functions, activation of monocyte and macrophage system
Garlic	Ajoene, allicin	Anticancer, antimicrobial, helps to lower cholesterol; antistatic and antibiotic properties
Rice	Phenolic compounds, alkaloids, essential oils, aromatic carbons	It acts as anti-inflammatory, hypocholesterolemic, helps to prevent cancer, nematicide, antihistaminic, antiarthritic, anticoronary, antieczemic, and antiandrogenic Activities
Turmeric	Curcuminoids	Antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, antibacterial, antifertility, antidiabetic, anticoagulant, antifungal, antiviral, antivenom, antifibrotic, antiulcer, antiprotozoal, hypocholesteremic, hypotensive

(continued)

Table 5
(continued)

Food source	Extracted bioactive compound	Function and benefits in nutraceuticals
Fenugreek	Apigenin, isovitexin vitexin	Antioxidant, lipid-lowering activities, hypoglycemic
Cinnamon	Polyphenols, cinnamaldehyde Catechins	Antibacterial, anti-inflammatory, antifungal
Black Pepper	Piperidines, piperine	It helps to improve digestibility Utilized to treat vertigo, indigestion, congestion, asthma, diarrhea, fever. It has antimicrobial activity
Ginger	Shogaols, gingerols	Anti-inflammatory, anticancer, antioxidant, neuroprotective, antimicrobial, respiratory protective, cardiovascular protective, antidiabetic, antiobesity, antinausea, and antiemetic activities
Citrate fruits	Vitamin C Flavonoids	Antifungal, antibacterial, anticancer, anti-inflammatory, cardioprotective, hepatoregenerating
Natural honey	Coumaric acid, chrysin, luteolin, abscisic acid, apigenin, caffeic acid	Regenerative, anti-inflammatory, antifungal, antibacterial
Allium sativum	Allicin	Antioxidant, antibacterial, and arteriosclerosis
Green leafy vegetables and germinated grains	Betaine (trimethyl glycine)	Homocysteine
Ananas	Bromelain	Prevent heart disease, arthritis, and inflammation
Capsicum annuum	Capsaicin	Antioxidant
Strawberries and raspberries	Ellagic acid	Anticancer
Curcuma longa	Curcumin	Alzheimer's disease, anticancer

11.3 Extraction of Various Bioactive Compounds from Plants Having Nutraceutical Value

There are several bioactive compounds from plants that can be used as nutraceuticals and can be extracted through the various available extraction techniques including pulsed electric field extraction. The chemical compounds such as allicin from *Allium sativum*, betaine (trimethyl glycine) from green leafy vegetables and germinated grains, bromelain from *Ananas* sp., capsaicin or trans-8-methyl-N-vanillyl-5 noneamide from *Capsicum annum*, ellagic acid from strawberries and raspberries, curcumin from *Curcuma longa*, omega 3 fatty acids from *Linum* spp., and resveratrol especially high in grape skin were extracted and are giving a huge contribution to the nutraceutical industries [82].

12 PEF Aided Extraction: Pros and Cons

As PEF-assisted extraction method is a novel method of bioactive extraction that uses high-voltage pulses to disintegrate the cell wall and cell membrane to allow the intracellular compounds to come out of the cell. During the process of PEF extraction, the electrical pulses will generate the heat because of joule heating, therefore, the cooling down the processes product during PEF treatment to balance a low temperature. Hence, this process can be implicated in the process of gentle preservation. High temperature and PEF membrane electroporation together enhance the inactivation rate. Products, namely, fruit juices, soups, or liquid eggs can all be processed using PEF, as it involves the inactivation of enzymes and microorganisms by breaking the cell membrane and making it permeable to small molecules thus leading the microorganism's death by utilizing high-voltage pulses. Because no harmful chemical reactions have been found, the pulsed electric field technology is widely regarded as being safe for humans [6]. Other important advantages of the PEF extraction method include benefits such as selective extraction, speedy extraction, and clean extraction. The PEF's operational parameters are easy to manage. It is possible to change operating parameters (such as electric field intensity, pulse duration and number, and the size of membrane pores) so that only the desired components can be released while keeping other components inside the cell. The recovery factor is greatly increased under ideal circumstances with the shortest extraction time. In addition, it does not involve a drying or dehydration process, which lowers the operational cost. It does not affect the quality of the food, which has been processed. Highly scalability, and with this novel technique, one can reduce the amount of solvent [90].

Some limited drawbacks have been considered with the industrial level of PEF extraction, which is depicted. Air bubbles in the treatment chamber can affect the uniformity of the PEF treatment

and hinder the dielectric breakdown. Depending on the strength of the applied electric field, the cell membranes during the electroporation mechanism can be irreversible or reversible. The gap between the electrodes and applied electric strength has a significant impact on the PEF's effectiveness [90]. Additional constraints encompass cost, limited availability in commercial units, and reduced effectiveness in treating solid foods, as it relies on the electrical conductivity of the food. Hence, it is better suited for liquid food treatment. Another significant drawback involves the release of heavy metals and toxic particles from outdated electrodes, which subsequently impacts the extraction and processing techniques [6].

13 Application of Bioactive Compounds in Food and Nutraceutical Industries

Bioactive substances have become important food constituents for maintaining health and preventing disease. Noncommunicable diseases are on the rise as the population ages and become less physically active. In some circumstances, bioactive chemicals are thought to be an intriguing alternative to traditional illness-preventive treatment methods [83]. This is being supported more and more by customers' growing demand for natural products as they look for long-term solutions to improve the quality of life through individualized nutrition. The creation of innovative solutions for this sector depends on an understanding of the chemistry of natural products and their mechanistic approach. According to research, poor lifestyle choices and stress raise the risk of several ailments, including cerebrovascular diseases, cardiovascular diseases, infections, and diseases such as cancer. The expanding understanding of how nutrition impacts health has resulted in a significant increase in the demand for functional foods and nutraceuticals. For their antibacterial qualities as well as humoral- and cell-mediated immunological functions, specific bioactive components have been added as supplements to functional foods, nutraceuticals, and pharmaceuticals, where their biological activities can aid in disease control and prevention. As a result, the therapeutic potential of many food ingredients has grown in significance. Secondary plant metabolites with biological properties such as antimicrobial activity, enzyme detoxification regulation, antioxidant activity reduced platelet aggregation, immune system modulation, anticancer property, and hormone metabolism activity are successfully used (Fig. 4) [83].

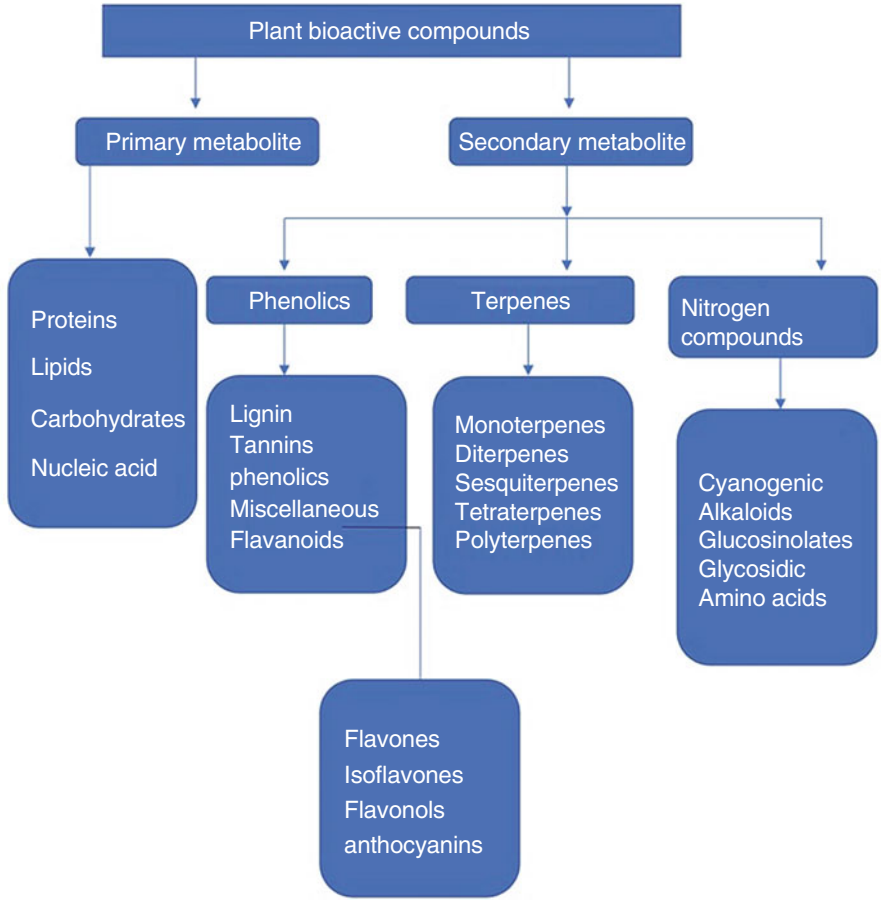

Fig. 4 Classification of plant bioactive compounds

14 Bioactive Compounds Extracted from Animal Sources and Their Application in Nutraceutical Industries

Animals are a rich source of bioactive substances with different types of biological functions to maintain healthy human life. The production of these bioactive chemicals might either be necessary for an animal's survival or increase its value to other living things. To stop, lessen, or treat many diseases and their associated symptoms, numerous natural chemicals have been identified, and classified, from animal sources and are used as dietary and medical supplements.

Some of the bioactive compounds from animal sources and their application are listed in Table 6.

Table 6
Bioactive compounds from animal sources and their application [83, 88, 91, 92]

Food source	Extracted bioactive compounds	Function and benefits in nutraceuticals
Meat	Fatty acids, minerals, vitamins, peptides	Antioxidant activities and antihypertensive
Fish	Proteins, fatty acids, polyether, peptides, enzymes and lectins, polysaccharides	Antithrombotic, immunomodulatory, antimicrobial, anticancer, and antioxidant activities
Egg	Avidin, ovalbumin lysozyme, ovomucin, ovotransferrin, phospholipids	Antimicrobial, immunomodulatory, antihypertensive, and anticancer activities
Milk	Whey protein	Helps to modulate both innate and adaptive immune responses

15 Conclusion

This chapter summarizes the revolutionary technologies that have been developed in recent decades for the recovery of our interesting chemical compounds from various sources, which process significant value in different sectors. The pulsed electric field extraction method for various bioactive and functional compounds represents an innovative, eco-friendly, user-friendly, energy-efficient, time-saving approach with high yield potential. Its role is crucial in producing significant quantities of essential compounds with nutritional, pharmaceutical, and medicinal value, benefitting the global population for sustainable, healthy living. Since plants, animals, and microorganisms process valuable bioactive compounds, it is necessary to extract and purify them with high accuracy without wasting them. Hence, new technologies should be invented, which should be more efficient in terms of yield, time, and energy. One such technology in the present era is pulsed electric field extraction. In summary, this method finds efficient industrial application for improved extraction of intracellular functional compounds from living organisms. These compounds are subsequently purified and used in the food processing and nutraceutical industries to enhance both nutritional and pharmaceutical aspects, thereby benefiting food quality and the development of nutrient and pharmaceutical products.

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Chapter 11

Case Studies and Application of Different Novel Extraction Methods

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Abstract

This chapter focuses on the case studies of the bioactive compounds that is defining the promising pathway of compounds that can be used as functional units. This chapters allows to gain an insight of the steps taken to make the optimization of the parameters and synthesis pathway. This chapter also focuses on the selection of the material, sorting, and process of extraction. The highest percentage of bioactive compounds required to achieve this extraction process and the point of consideration in implementation are also taken into considereation A case study on polyphenols, alkaloids, and terpenoids will be discussed. This chapter focuses on the novel extraction method by which an efficient amount of bioactive compounds are obtained. The main focus is to explore the extraction of bioactive compounds from inexpensive resources or residues that give cost-efficient products. Various novel methods of bioactive compound extraction and their application in different domains will be discussed. This chapter provides a wholesome view of the steps in the bioactive compounds by case studies and will carry forward to the novel extraction methods.

Key words Bioactive compounds, Polyphenols, Novel extraction method, Application, Case study, Alkaloids, Terpenes

1 Introduction

Bioactive compounds are defined as the nutrients and non-nutrient components that are present in the food matrix whether it is of vegetable or animal that is leading to physiological effects [1]. The compounds are secondary metabolites, mostly hydrophobic and poorly soluble compounds. The properties of nutrients are seen in terms beyond their classical nature [2]. These compounds are emerging as the key components in the food-related and medicine-related components that are leading to outstanding contributions to health status and in the prevention of diseases and also promote green technology. In recent times, it is seen that the population is getting aged and involvement in physical activity is little to moderate, which is increasing the potential chances of noncommunicable diseases. The trend is leading to the increased

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demand for natural products that are boosting consumer needs. Reduction in cost, efficient time, and amount of solvent used is an effective method over traditional method [3, 4]. Along with these variable options of bioactive compounds are present, presenting the sustainable option for improving the better quality of life. The main focus of the development of bioactive compounds is to increase personalized nutrition. With the application of the knowledge of chemistry in natural products, a fruitful outcome is derived that is termed as the bioactive compounds. These compounds are considered as the extra nutritional constituents that are there in a very small portion. Some examples of bioactive compounds are carotenoids, flavonoids, carnitine, choline, and so on. The occurrence of bioactive compounds is found in a variety of foods and plant materials that can be extracted by microwave-assisted extraction [5]. Most of the bioactive compounds are having antioxidant, anticarcinogenic, anti-inflammatory, and antimicrobial properties. Following are some of the crucial compounds listed that will improve the quality of life and allows the prevention of noncommunicable diseases.

- Phenolic compound: It is comprised of a sub-category of flavonoids that are present in almost all plants. Cereals, legumes, nuts, and olives are a few materials that are having an abundance of flavonoids. The category of compounds is having antioxidant activities that impose a favorable impact on cardiovascular risk factors [6].
- Phytoestrogens: It is found in flaxseed, whole grains, fruits, and vegetables. The compounds are displaying the dual nature that are the antioxidant properties and estrogen at the molecular levels. Plant-based compounds that are having estrogen-like properties and isoflavones, stilbene, coumestan, and lignan [5, 7].
- Carotenoids: It is one of the efficient free radical scavengers present that is showing potent antioxidant compounds. The majority is found in fruits and vegetables such as apricot, carrot, mangoes, and pumpkins.
- Glucosinolates are the natural form present in many pungent plants such as mustard, cabbage, and horseradish. The role is seen in the induction of phase 1 and 2 enzymes and inhibition of enzyme activation, and currently ongoing research is going to investigate the role in the mitigation of cancer.
- Vitamins are the influential nutrient present that any organism requires in a limited amount. The compounds are not synthesized by the human body but must be obtained by diet. It offers a diverse role in various metabolic activities and health functions [8]. Activities such as regulation and catalysis are offered by vitamins, and they also act as an antioxidant (*Malva sylvestris* leaves).

Area of application: Bioactive compounds are used for many purposes as there are numerous functional and structural properties that are displayed. One of the potential areas is Neutraceutical as vegetable-based proteins [9]. It is a combination of nutrition and pharmaceutics that comprise the properties of medicinal and other (in terms of physiological benefits). The use of Neutraceuticals leads to the improvement in health factors, delays the process of aging, prevents chronic disease, increases the expectancy of life, and supports the structure and function of the body [7]. Great advantages are seen in complex curative disorder that relates to oxidative stress. It includes the allergy, Alzheimer's cardiovascular conditions, diabetes, cancer, and obesity. The food industry is the second largest existing domain of bioactive compounds where food bioactive compounds are referred to as the extranational constituents that are occurring in a very small percentage of food [10]. It encopasses different functional categories that act as flavonoids need to be mention. Morphine, quinine, and nicotine are a few examples of bioactive compounds.

Functional bioactive compounds are classified into the following categories that are produced by plants and grouped among polyphenols(one of abundant class), triterpenes, phytosterols, and polysaccharides, lupine, oleanane, and ursane, which seem to possess anticancer agents. Terpenoids, also called isoprenoids, are considered the second largest group of secondary metabolites. They are particularly abundant in living cells and organisms [11]. It is one of the responsible components present for the fragrance of plants and fruits. Beta-carotene or carotenoids are grouped as the constituents that are depicting an important part of the human diet [12]. They will be serving as the direct precursor of vitamins A and E. The function played by carotenoids is in terms of providing resistance against diseases and enhancing the immune system [13]. Sulforaphane, a compound that is an important agent found in broccoli, leads to the anticancerous effects [14]. Apart from this, a polysaccharide that is a polymeric chain of the monosaccharide lies and is linked with the glycosidic bonds. Taking an adequate amount of functional bioactive food is very favorable and important for the growth of cells [15]. At the same time, consideration needs to be taken that the excess is not there. Otherwise, it will lead to mutation and toxicity. Relation of a bioactive compound with the human system and health: An imbalance in the production of the reactive oxygen species in the system leads to the creation of oxidative stress. To reduce the impact, the reactive oxygen species aim to make the promotion of the antioxidant and co-factors interaction that allows the maintenance of health and will prevent aging and age-related disorders. Antioxidants are obtained by the bioactive compounds resulting in the deactivation of the free radicals and serve as the redox biomarker that controls the redox state of the functional proteins. With the evidence, it is found that bioactive compounds

hold a great place in human welfare. This chapter focuses on "Case studies and application of different novel extraction methods." The gain of insight into the case studies will help in enhancing the overview that what leads to the discovery of new bioactive compounds [16]. The section is followed by a description of novel extraction processes that define the effectiveness and efficiency of various processes and what research going on that makes increases the output of the techniques [17]. Understanding the extraction process allows the transformation on an industrial scale.

Case studies will be discussing the process of extraction of the various classes of bioactive compounds, their parameters, obtained products, and optimization of the process that is resulting in the high yield. The case studies section will provide information about polyphenols, terpenoids, carotenoids, and so on. One of the classical case studies is of polyphenols; it is one of the most abundant classes of bioactive compounds that exist. It is a plant-based food category lies that is possessing the ability of antioxidant, anti-inflammatory, antimicrobial, and cardioprotective [18]. Polyphenols are found in several components that are there in the normal diet and will contribute to the metabolization, transport, and distribution to the target organs [11]. The case study will be giving evidence that what are the sample preparation techniques is there that increase pre-prepare the sample. Extraction techniques are comprised of the recovery of analytes with the intake of the classical and advanced emerging techniques that is supporting the high yield of the polyphenol bioactive compounds. After this comes the cleanup which will be making the elimination of the atoms and entities that are interfering with the functioning of the bioactive compounds. The last step is evaporation which is reducing the extract volume. Similarly, the case study of catenoid, terpenoids, and polysaccharides is going to be discussed that highlights the similarity and difference in the process of recovery, gives facts about the unique properties of the bioactive compounds, and what will components used in the extraction process that is evolving in the yield percentage of 80–90%. Furthermore, a novel extraction method will be discussed and its application in various areas. The case study will be forming a basis that how the extraction process happens while the next section allows the understanding that what are the factor lies that make contributes to the optimization of the extraction process that makes it suitable for the pilot scale or industrial purpose. Novel extraction techniques are designed in such a manner that extraction of bioactive compounds will take place with less solvent consumption and extraction time. It improves the extraction quality and extraction yield. Extraction is the foremost step that lies in the differentiation of the desired natural products by the selected raw material. Out of all extraction processes, solvent extraction is the most widely used method [19]. It comprises the stages that are as follows penetration of

solvent into a solid matrix, dissolution of the solute into the solvent, diffusion out of the process from the solid matrix, and extraction of the solute [20]. In this process, the factor that comes into the role is the diffusivity and solubility of the material that is facilitating the extraction of the bioactive compounds. The law of similarity and inter miscibility is the basic principle of extraction protocol. The laws will be used to scale up the production of bioactive in food industries and pharmaceuticals [21]. Extraction is an advantageous process that is adding value to industrial growth. The ongoing trend of bioactive compounds due to emerging consumer needs will lead to the motivation to perform the new experiment and optimize the process of extraction via nanocarrier [17].

2 Case Studies on the Nutraceuticals and Bioactive Compounds

2.1 Case Study 1: Fruit Seed-Based Bioactive Compounds for the Formulation of Nutraceuticals

It has been evidenced that bioactive compounds comprise many applications in the culinary industries as well as pharmaceutical industries. The long interest is in the functional feature of the diverse fruit seeds such as the tomato, guava, dates, as well as apple. Some of the bioactive compounds in this include bioactive peptides, lycopene polysaccharides, phytochemicals, and vitamins [22]. These are abundant in fruit and its by-products and have many health benefits. The role of bioactive compounds that are obtained from the tomato and the seeds is that they are processed into cans, products, and sauces. The seeds and the peels comprise the bioactive compound named beta-carotene as well as lycopene. They have a diverse phytochemical composition and the seeds of tomatoes are known to be a natural source of antioxidants and phenolic compounds and carotenoids. Another one is peel and pulp which has lycopene, flavonoids, and phenolic acids. It can be done by the methods of crushing, heating, and stirring. The content of moisture was 8.5%, fat was 20%, ash was 3.1%, and dietary fiber was 35.1% in the tomato. When in the case study the phytochemical analysis was done, the presence of the 14 flavonoids was there such as quercetin, kaempferol, and isorhamnetin. They have the power to alter many numerous cellular signal transduction pathways as well as stimulation of endogenous defensive activities are there [23, 24]. They have anticancer, antibacterial, antimutagenic, and stimulating endogenous defensive activities. They are also known to have cardioprotective and antiplatelet properties. In this case, there were many approaches used, such as the valorization of unused parts in tomato, and there were methods for extraction of the carotenoid compounds. The seed extracts of tomatoes have antibacterial properties, and pharmaceutical and food sectors can use them for the preservation of food and microbial deterioration is there. The waste from tomatoes can be significantly used by industries to develop various by-products or functional foods, as well as

the processing manufacture of tomatoes can benefit from it [25]. It was discovered that the peels are high in lycopene, fiber, and phenols, and the seeds are high in crude protein and fat. Investigation were carried out for their roles and applications as food sources and bioactive phytochemical constituents are the nutritional, pharmacological, therapeutic uses, functional properties, and bioactive contents of the seeds of various fruits. Allaqaband et al. [1] show that alkaloids, carotenoids, flavonoids, glycosides, saponins, terpenoids, tannins, steroids, and polyphenolic compounds, which have anti-inflammatory, antioxidant, anticancer, antidiabetic, antihyperlipidemic, anti-obesity, neurological disorders, cardiovascular, skin diseases, and chronic diseases properties, are essential bioactive components found in seeds.

2.2 Case Study 2: Pomegranate Seed Oil

Pretreatment of seed will lead to the production of the phytosterol composition. In addition, it holds the ability of antioxidant. For the extraction of the bioactive compounds, seed oil is extracted with the mechanical procedure. It will not lead to the effective results, apart from this other extraction procedure will help. The extraction procedure is known as the important step and it puts a great impact on analysis as well as characterization. There are majorly four steps that are used in food analysis such as sample preparation, data acquisition, data analysis, and statistical integration. The sample preparation and/or treatment, the extraction process, the extract cleanup, and in most cases, extract concentration by evaporation are all done in the first phase [26]. Depending on the purpose of the study and the targeted analytes, the second stage involves the collecting of data utilizing high-resolution analytical techniques, such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography/mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR). The equipment software processes the data acquisition in the third stage. Depending on the goal of the study, the final phase may include multivariate statistical analysis, which might include, among other things, analysis of variance (ANOVA), principle components analysis (PCA), partial linear square analysis mixed with discriminant analysis, and cross-validation. As per the case study, there are four strategies for the treatment and extraction of the bioactive compounds from the food matrices. It includes the treatment, in which the different methods are milling, filtration, drying, hydrolysis, centrifugation, as well as adjustment of pH. In the extraction part, analytes will be recovered from the sample, and the classical advanced emerging techniques are used. In the cleanup process, the elimination of the compounds is done with the analysis. The different methods that can be used include liquid-liquid extraction, dispersive solid-phase extraction, as well as solid phase extraction. There are then advanced extraction techniques that comprise microextraction techniques and these comprise a wide range of applications. The different features are

simplicity, versatility, as well as high extraction efficiency and environmental friendliness [27]. It is used for the determination of polyphenols. Some of the relevant improvements have been done and it was done by the QuEChERS method, which comprises the food matrices. Ultrasound-assisted extraction involves the use of US radiation and it has devices such as sonoreactors, probes, and water baths. Microwave-assisted extraction is such that, it is based on the solvent heating that occurs due to the interaction of polar molecules in the media. It includes dipole rotation and ionic conduction. Electrotechnologies are nonthermal methods of pasteurization and sterilization in the food industry. They use the current that flows across samples to electroporate membranes and harm living tissues. The electrical field induces electropermeabilization and the release of the target analytes by causing charge accumulation and transmembrane potential. Water is used as a solvent in subcritical water extraction (SbFE), a pressurized liquid extraction method, which speeds up the mass transfer and extraction rate from the solid matrix [28]. High- and ultra-performance liquid chromatography is one of the most widely used techniques for the separation, identification, and quantification of analytical processes. It is difficult to analyze and characterize the bioactive compounds found in nature due to the wide variety in their number and chemical makeup. Câmara et al. [11] advanced and developed extraction procedures that are faster, more sensitive, and reproducible, employed to address several extractions and analysis/characterization-related shortcomings. The direct analysis functionality of some ambient approaches has proven to be very helpful for their direct analysis, identification, and characterization. The next-green extraction methods present a great extractive performance regarding polyphenols.

2.3 Case Study 3: A Case Study of Raspberry Fruit Pomace—A Bioactive Compound

The bioactive compounds are known to be found in fruit biowaste as well as they provide low-cost, integrated, and environment-friendly alternatives. The fruit named raspberry is known to be rich in antioxidant compounds which inculcate fiber, anthocyanin, and ellagitannins. The content of water is 80% and carbohydrates are predominantly found. The chemical properties are dependent on the edaphoclimatic conditions. The majority of antioxidants in raspberry fruit pomace are squandered during juice manufacturing (RFP). According to reports, RFP has 77.5% of the total nutritional fiber found in fresh fruit [29]. The RFP also still contains a significant amount of phenols. Tocopherols and -linoleic acid are both abundant in the seeds. A sizable amount of biological potential is also lost when RFP is wasted, including its antioxidant, antiproliferative, and antihyperglycemic properties. Due to its capacity to "spare" heat-sensitive chemicals, cheap cost, wide availability, and efficiency, traditional maceration is the most widely used extraction

method for the exploration of berry fruits and their biopotential. Investigating the total phenolic, flavonoid, and anthocyanin content is the basis for the case study. The case study is based on research into the antioxidant activity and total phenolic, flavonoid, and anthocyanin content of *Rubus idaeus L. pomace* obtained in the Mediterranean region, specifically Montenegro. The effectiveness of conventional maceration and ultrasound-assisted extraction as extraction procedures were compared [30]. Moreover, a high-performance liquid chromatography analysis was used to assess the polyphenolic profile of the obtained extracts. The plant material and the sample preparation include the raspberry which was harvested. The usage of fruits was in the extraction of juice by the destoning machine. The two different extraction methods that were used here were ultrasound-assisted extraction as well as conventional maceration. Initially, the homogenization of pomace was comprised of formic acid and methanol.

The methods that were used in the evaluation of the pomace extracts were the colorimetric Folin-Ciocalteu method. The units in which the results were expressed include Gallic acid per volume. The flavonoid content was determined by the aluminum chloride colorimetric method. The pH differential method is used for determining the anthocyanin content. The original procedure for the ferric reducing antioxidant power test (FRAP) was followed. Using an Agilent Technologies 1100 liquid chromatograph fitted with a diode-array detector, the chemical characterization of the analyzed extract and the quantification of the chosen components were carried out both before and after hydrolyses. Even specimens of the same plant species having different phenolic contents and environmental factors such as climate, soil type, proximity to the shore can affect the outcomes. Some plant species tend to create more phenols than the same species growing in conditions that are different from those seen in colder climes, higher elevations, and more dry environments [31]. Lysis was carried out of several significant polyphenolic compounds, including gallic, p-coumaric, caffeic, chlorogenic, ellagic acid, and quercetin. According to the results, the raspberry pomace's output of total phenolics, flavonoids, and anthocyanins—particularly caffeic, chlorogenic, ellagic, and gallic acids—was increased by ultrasound-assisted extraction (UAE). The production of quercetin and p-coumaric acid is maximized during traditional maceration. Raspberry fruit pomace has a great industrial potential for biowaste valorization and may be helpful in the creation of dietary supplements that are high in antioxidants [32].

2.4 Case Study 4: Bioactive Potential of Phenolic Compounds: A Case Study

The in vivo bioavailability of phenolic compounds is increased when they are delivered via a carrier vehicle, and the quantity of biotransformations that restrict the expression of bioactivity is decreased. The bioavailability of these substances is increased

when probiotic yeast biomass, such as *Saccharomyces boulardii*, is used for assimilation [20]. In these situations, the biological action may be accomplished by directly affecting the microbial pattern in the colon. The biotransformation into an intermediary molecule occurs during this process. Following the evaluation of the bioactivity and chemical characterization of the natural substrate represented by medicinal herbs, new sources of biologically active chemicals have been discovered [6]. The vegetal material, the product conditioning mode, the meteorological circumstances, and the makeup of the soil all influence the number of useful chemicals (such as polyphenolcarboxylic acids) that are present. The utilization of six dried samples of *A. linearis* leaves, *P. cupana* seeds, *A. chilensis* berries, *I. paraguariensis* leaves, *S. aromatic* cloves, and wild berries as alternatives for treating diseases brought on by oxidative stress was examined and chosen. The studies were carried out using a high-pressure liquid chromatograph called ELITE-LaChrom and analytical scales for DAD (Diode-Array Detection) detectors. By measuring the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity and the chelating activity, the antioxidant capacity of each hydroalcoholic extract was evaluated. The distribution of bioactive components was associated with some nutraceuticals' capacity to mitigate the physiological effects of oxidative stress (like phenolic acids and flavonoids) [33]. The discovery of the crucial point of contact prompted the development of novel methods for the exploitation of bioactive substances, which may ultimately increase the target compound's bioaccessibility and bioavailability. Additional research should be done to verify this aspect. This in vivo indicator demonstrated the product's potential. Understanding the stability of the functional components and the significance of the pattern and amount of these chemicals in vivo came about as a result of the ability to digest yeast cells. Thus, one viewpoint made clear by this study was the distribution of the full range of nutraceuticals in the evaluated product—rather than increasing the concentration of a compound. Bioassimilation and bioavailability of phenolic compounds characterize the in vivo complex effect that phenolic compounds exert on contact with eukaryotic cells in addition to the increase in oxidative stress stability [34, 35]. Because there was a species-specific link, a correlation between bioactive substances and various activities in vitro/in vivo was not found in the case of high levels. Dabulici et al. [17] provide information about the capacity of probiotic biomasses to release useful components will be determined by a precise understanding of bioassimilation and bioavailability processes, which will also improve the formulation of nutraceuticals.

3 Different Novel Extraction Methods

There is a versatile range of structures and functionalities of natural bioactive compounds present. It will serve as the excellent pool of the atoms and molecules that will be supporting the production of nutraceuticals, functional food, and additive. In nature, some of the compounds are found in abundance like polyphenols while the rest are found in very low levels. To make the extraction of compounds, the use of the chemical synthesis processes is leading to unprofitable outcomes. As in setting up the chemical reaction, the process comprises one or more than one reaction that is aiming to make the conversion of the reactant from the starting material to multiple products [36]. One of the setbacks that are found in chemical synthesis is the use of material and maintaining conditions such as pressure, temperature, and ions. Therefore, the outcomes are presenting an insufficient option at the pilot scale. Taking the focus on the massive harvesting of bioactive compounds in the desired amount will occur by developing technologies. It overcomes the challenges of the screening and production of the compounds. Advanced technologies have been developed that will be commonly used for the extraction of bioactive compounds [37]. A variety of innovative and novel technologies is there that integrate the use of natural resources and methods that is economically advantageous to chemical synthesis. Conventional liquid-liquid or solid-liquid extraction and the advanced include pressurized-liquid extraction, subcritical and supercritical extractions, and microwave- and ultrasound-assisted extractions are commonly used technologies present [36]. In addition, enzyme-and instant controlled pressure drop-assisted extractions are currently been used. The advantages that the technology offers are in terms of the release of the compounds from the matrix. In the future, the innovative approach will be leading to an increase in the production of the specific condition that is further used as a nutraceutical and as a bioactive compound (like supercritical carbon dioxide extraction) [32]. This section will be discussing the novel extraction method that is followed by a comparative analysis of the Soxhlet extraction.

3.1 Solvent Extraction Technique

In this extraction technique (HPLC), different organic solvents are introduced to effectively size the raw material that will take up the desired components such as anthocyanins. These components are preferring the properties of anticancerous agents and anti-inflammatory [38]. For this, samples will undergo centrifugation and then be filtered for the removal of the solid residues [39]. The extract that is obtained is used as an additive, supplemental food, and as an ingredient in functional food. Low processing cost and ease of operation will be serving as the advantage over other

techniques. Agro-waste (fruit peel, food, and grains) is found to be the potential source for the extraction of nutraceutical and bioactive compounds.

The procedure of using solvent extraction is as follows: selection of the plant material. Taking fresh fruit without any physical or microbial damage is figured out. Extraction protocol in which the peel of the fruit will be boiled will be in a ratio of 1:20 (water). Further, it is homogenized and filtered out under a vacuum with the use of the Whatman paper [38, 40]. The sample will be lyophilized and kept at a freezing temperature. The next step is determining the total phenolic component, gallic acid is used as the standard one that is mixed with the Folin-Ciocalteu reagent (1:1), 7.5% (w/v) sodium carbonate before the UV-vis spectrometry (760 nm) that will be repeated for five times. After this, FRAP (Ferric Reducing Antioxidant Power Assay) is done that is leading to the formation of the calibration curve. Sample extracts and standards were analyzed with the DPPH Free Radical Scavenging Capacity [41]. Further, analysis of the compounds will take place with the HPLC technique. It will determine the detection in different wavelengths that is 280, 320, and 370 nm. Once, the HPLC is performed; statistical analysis will be done that is allowing the presentation of the results in the form of standard deviation and variance [42]. The correlational data will be calculated with Pearson's coefficient.

3.2 Supercritical Fluid Extraction

It is one of the environmental-friendly technologies present that is used for the extraction of bioactive compounds. The reason that lies behind the high efficiency is the use of natural matter which are plants and algae. One of the qualities offered by the technique is final product extraction, which is very easy due to solubilizing lipophilic substances [42, 43]. In the process of extraction, the material is placed in a container that is comprised of the pressure and temperature control that is needed for maintaining certain conditions. The protocol is comprised of the dissolution of the fluid that is transported in the separator. The product further gets collected in the tap that is there in the separator. The main focus of the technique is a selection of the supercritical fluid that is allowing a wide range of compounds to get extracted [44]. For the extraction of the compounds, the plant material has been used in the ground state and will be placed in the vessels, CO_2 gas is used that undergoes high temperature and pressure [45]. After that, a pump forces the supercritical phase CO_2 to get into the extraction vessel where it meets the plant and breaks the trichomes and allows the plant material to get break down. Over the period, carbon dioxide based techniques have gained a significant place in extraction that whether it is the use of nontoxic compounds or giving a cost-effective process the techniques also gained a significant value that contributes in the growth of various industries [13]. Supercritical

carbon dioxide is serving as the novel extraction method as it will be strengthening the antioxidant extraction process by making the rise in superior abilities of the cells.

3.3 Subcritical Water Extraction

This technique called the subcritical water extraction technique is an alternative technology that is used for the extraction of the phenolic compound from different food. Subcritical water also refers to the temperature of the water which is between 100 and 374 °C, the pressure is also high and it is maintained in a liquid state. The major advantage of the SCW over any other conventional extraction technique is that it requires a short extraction time, the solvent cost is low, the quality of extraction is high, and the major advantage is that it is environment friendly. Subcritical water extraction and microwave-assisted technology is the most effective engineering approach and also it offers some environment-friendly techniques so several other plant components can be extracted [46]. An author Tunchaiyaphum has extracted some phenolic compound from the mango peels with the help of SCW technology. The amount of phenolic compound, which is present in the mango peel, was much higher than using the Soxhlet extraction technique. Hence, SCW extraction is also known as an alternative green technology for phenolic compound extraction from agricultural waste. It also substitutes the conventional method using the organic solvent. There are mainly eight extracted phenolic compounds and these are gallic acid, caffeic acid, chlorogenic acid, syringic acid, protocatechuic acid, benzoic acid, p-hydroxyl benzoic acid, flavonoid, and coumaric acid [47].

3.4 Enzyme-Assisted Extraction

There are a number of methods of enzymes that can be used for the extraction of bioactive compounds from food waste. However, the main source of extraction of the antioxidants is the plant tissue [48]. The plant cells contain polysaccharides and these are hemicellulose, cellulose, and pectins, all these act as barriers so the intracellular substance can be released. There are several enzymes such as cellulase, xylanase, beta-glucosidase, and beta-glucose held to degrade the cell wall structure and then depolymerize the plant cell wall polysaccharide [49]. As water is used as a solvent, in place of chemicals, it is an eco-friendly approach that is used for the extraction of some bioactive compounds along with oil. Several bioactive compounds are released from the plant cell following the cell disruption and extraction method. It also optimizes enzyme preparation and it is done either alone or in the form of a mixture. The enzyme-assisted extraction is a highly promising technique when compared to conventional solvent-based extraction. It is mainly the ability of the enzyme to catalyze the reaction, and mild processing conditions should be used in an aqueous solution. It leads to the production of high efficiency molecules that is cost-efficient and organic rich [50].

3.5 Extraction with the Help of Ultrasound

The extraction done with ultrasound is a relatively simple and effective technique when it is compared to the traditional extraction method. All the bioactive compounds are obtained from natural products. The ultrasound method induces a much greater method in which diffusion of the solvent in the cellular material is done. Adopting this technique improves the mass transfer and along with it disrupts the cell wall and facilitates the release of bioactive compound. The amount of extraction is highly influenced by the ultrasound frequency, which mainly depends on the plant material which needs to be extracted. Wang et al. [51] have demonstrated the use of ultrasound-assisted extraction. In this method, the extraction of three dibenzyl butyrolactone lignans, which are trichloride, hemislienoside, and action, is done from *Hemistepta lyrate* [52]. To determine the corresponding extract, high-performance liquid chromatography is been done. In another study by Fahmi et al. [53], the extraction efficiency of the four isoflavone derivatives is studied; these are glycerin, daidzin, genistin, and malonyl genistin from the soybean. To carry out this extraction method mix-stirring method is used. As this technique also used ultrasound, it has been known to improve the extraction yield and it depends on the solvent. Azka et al. [54] have extracted anthocyanins and other phenolic compounds from the grape peel. This is also done using ultrasound technology as it takes care of even the smallest details. Pineiro et al. [55] have optimized and validated ultrasound-assisted extraction while extracting stilbenes from grape canes.

Using this method, the stilbenes in the grape cane will be extracted in 10 min. The temperature required will be 75 °C with an ethanol concentration of 60% in the extraction solvent. From the study, it was concluded that the grape cane by-product is a potential source of the bioactive compound and it plays a vital role in the food and pharmaceutical industry [56]. Aguilo Aguayo et al. [57] also studied the effect of ultrasound technology while extracting water from a soluble polysaccharide. These are obtained from the milled and dried product which is generated from *Agaricus bisporus*. It has been observed that beta-Glucan has been obtained in a specific amounts such as 1.01 and 0.98 g/100 g dry mass in the particle size of about 355–250 and 150–125 μm, respectively; these are the by-products of mushroom. The highest extraction was done at 4.7% and this was achieved when there is an extraction time of 15 min, with an amplitude of 100 um and 1 h of precipitation in about 80% ethanol [58].

3.6 The Microwave-Assisted Extraction (MAE)

This is a new extraction method that mainly combines microwave and traditional solvent extraction. It is a highly advantageous technique due to the short extraction time, high extraction rate, and low cost of the traditional method. However, the major advantage of ultrasonic-assisted extraction and solvent extraction is that the

plant metabolite requires a shorter interval of time. Padmapriya et al. [59] stated that the extracted mangiferin presented in the *Curcuma amada* uses ethanol as a solvent. The mangiferin which is extracted has increased to 500 W; however, it has decreased when the microwave power was increased. An adequate mangiferin yield of about 41 μg/ml is obtained with an extraction time of 15 s with a microwave power of 500 W [60]. Kerem et al. [61] extracted saponins from the chickpeas by using MAE and found that this method is far better and superior to the Soxhlet extraction. However, to complete this process adequate time and energy must be required and utilized. The chickpea saponin has exhibited that prominent inhibitory activity should be used against *Penicillium digitatum* along with additional filamentous fungi. The *Mangifera indica* leaves have also been extracted from the mangiferin by Kulkarni and Rathod [62]. They used microwave-assisted extraction conditions and used water as a solvent. The maximum-assisted extraction condition which is used has adopted water as a solvent. However, the maximum extraction yield which is obtained is 55 mg/g and it is obtained with an extraction time of about 5 min [63]. The solid-to-solvent ratio which is used is 1:20 and the microwave power required is 272. When sequential batch extraction and Soxhlet extraction are compared, it has been noticed that MAE has increased the yield of extraction in a much shorter period. It has also reduced the solvent requirement time to a much shorter level when compared to the conventional method. Smiderle et al. [64] have also studied MAE along with pressurized liquid extraction (PLE) as an advanced technique to obtain polysaccharides mainly biologically active beta-glucan. This component is obtained from the *Pleurotus ostreatus* and *Ganoderma lucidum*; these are the fruiting bodies and in this beta and alpha glucan have been detected in all the extracts. In another study by Kolář et al. [65], MAE is optimized in response to the surface methodology to enhance the extraction of polyphenols from the basil. It has been then found that the extraction can be done with 50% ethanol, at a microwave power of 442 W, and with an extraction time of only 15 min. Under this condition, basil liquid extraction is obtained and it contains 4.299 g GAE/100 g polyphenol and 0.849 g catechin which is equivalent to about 100 g of DDW of total flavonoid [66].

Hence, from the above discussion, it can be concluded that the microwave-assisted method offers several advantages when compared to another extraction method. This is a highly efficient method because it takes less extraction time, is less labored, and is highly sensitive; hence, it makes it a favorable method of extraction in the bioactive compound.

3.7 Pulsed-Electric Field-Assisted Extraction (PEFAE)

This method is a highly novel extraction technology that is used for the extraction of bioactive compounds. This is a preferred method because less energy is required in it and also it is an environment-friendly solvent. This method is a nonthermal extraction process, as all the natural compounds can only be recovered at a minimum temperature so quality and nutrition value can be obtained. Electroporation is the primary mechanism that has been followed behind the pulse electric field extraction method. In this process of PEFAE, the electric energy is utilized to create nano- or micro-poration of the cell membrane so all the bioactive compounds which are present in the cell plasma can be extracted easily [16]. An electric pulse is necessary for the transfer of the molecules or the ions from inside the cell to the cell membrane as it acts like an insulator [67].

In another study, it was observed that the apple peels were treated by the pulsed electric field using several electric intensities and time to extract the phenolic compound. The extraction is analyzed using several electric conductivity and confocal laser extraction. Results were obtained depending on the cell integration index along with electric field intensity. This study has also confirmed that higher the intensity, the cell integration will be constant, and the soluble matter recovery will be higher. Parniakov et al. [68] has stated that the yield of the antioxidants, proteins, and carbohydrates obtained from the mango peel was maximum when PEFAE is at the intensity of 13.3 kV/cm. This process is carried out with extraction at a 50 °C temperature for about 5 h at about 6 pH [69].

3.8 CE (Combination of Extraction) Process

All the extraction technologies which are discussed above are mentioned and used in combination. When all the methods are used, this reduces the extraction time, increases extraction yield, and overcomes any limitation if present. The pre-treatment of any fruit by-product by using the ultrasound or enzyme before any CE process. Likewise, two or more emerging extraction processes are used in combination such as UAE and SFE.

Techniques	Solvents	Advantage	Disadvantage	Application
Soxhlet extraction	Ethanol (highest) Hexane (lowest)	Low processing cost and easy operations	The disadvantage is seen in terms of using toxic solvents and extended time to carry out the process. In addition, it also requires the evaporation and concentration step for recovery	Anticancerous agents and anti-inflammatory and functional foods

(continued)

Techniques	Solvents	Advantage	Disadvantage	Application
Subcritical water extraction	Acetonitrile, methanol, ethanol, liquid ammonia, and dichloromethane	Short extraction time, the solvent cost is low, and the quality of extraction is high	When high water flow is applied, it increases extract volume and lowers the concentration of the final extract	Extraction of bioactive compounds from *Orostachys japonicus* known as rock pine
Enzyme-assisted extraction	Methanol or ethanol and polyphenol	Eco-friendly approach	Enzymes relatively expensive, cannot break plant cells completely, not feasible	Use specific enzymes to destroy the cell wall of the source material and hence improve extraction yield
Extraction with the help of ultrasound	Acetone and dichloromethane	Improves mass transfer	A low quantity of oil is produced	Effective in cell wall disruption and mass transfer
Microwave-assisted extraction (MAE	Ethanol	Short extraction time, high extraction rate, and low cost of the traditional method	High maintenance cost in commercial-scale settings	The active component of medicinal plants uses microwave energy
Pulsed-electric field-assisted extraction (PEFAE)	Ethanol-water mixture and propylene glycol	High yield utilizes fewer solvents and energy, and it saves a lot of time compared to traditional extraction methods	High maintenance	Increase extraction yield

3.9 Interpretation

Bioactive compounds are the type of chemical composition occurring in plants and animals in small amounts. A required percentage is needed for the efficient functioning of an individual. Bioactive compounds are abundant in nature and considered superfoods. The demand and growing interest of consumers lead to the inclination of the scientific community toward the discovery of versatile compounds. To extract a single molecule from a plant source, a series of steps is going to take place. This is a tedious process that requires the sources and costs that make the availability of the compounds to get restricted. At the same time, it is important to overcome the restriction as the survival of an individual is dependent on the consumption of bioactive compounds. Bioactive compounds are comprising ample amounts of molecules that are significantly contributing to the production of nutraceuticals. It will act as the functional moiety in the variable industries whether it is the food industry or pharmaceutical industry. Nutraceuticals

hold variable properties that are seen in terms of physiological activities and will be helping in the prevention of cancer therapies. The journey of bioactive compounds to the nutraceutical requires many steps that allow the conversion into a functional form.

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Chapter 12

Pressurized Liquid Extraction for the Isolation of Bioactive Compounds

Rakesh Barik, Sinoy Sugunan, and Mohd Affendi Bin Mohd Shafri

Abstract

Pressurized liquid extraction (PLE), an advanced extraction technique, employs solvent extraction at elevated temperatures and pressures, consistently under their individual critical points, so the solvent is sustained in the liquid state during the entire extraction process. As a result of utilizing these exact conditions of pressure and temperature, an alteration in the physicochemical properties of the solvent arises. It is an alternative and advanced preparation technique compared to conventional extraction methods in many areas, such as environmental, food, and pharmaceutical analysis. Medicinal plants are the sources of numerous compounds that can tackle numerous diseases when they are used in a reasonable combination. Every single plant contains one or more major bioactive compounds that are responsible for various biomedical functionalities. This chapter summarizes the application of the PLE technique in extraction and phytochemical analysis. The various advantages offered by this technique, such as low solvent usage, less preparation time, high extraction efficiency and better reproducibility, have made it a better alternative for the extraction and analysis of phytoconstituents.

Key words Extraction, Phytoconstituents, Efficiency, Selectivity, Solvent, Analysis

1 Introduction

Pressurized fluid extraction (PFE) or pressurized liquid extraction (PLE) is an innovative extraction technique consisting of liquid solvents at higher temperatures and pressures to formulate samples for analysis by either gas chromatography or liquid chromatography. This process is also called accelerated solvent extraction (ASE), pressurized hot solvent extraction (PHSE), subcritical solvent extraction (SSE), pressurized fluid extraction (PFE), high-pressure solvent extraction (HPSE), and high-pressure high-temperature solvent extraction (HPHTSE) [1].

The PLE was developed from Soxhlet extraction, established by the German inventor Franz Ritter von Soxhlet in 1879. This method was immensely successful in obtaining solutes even from solid samples, which were previously impervious. Hence, the

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Soxhlet method could become a reference standard in analytical extraction for more than a century. Novel extraction techniques, such as SFE (supercritical fluid extraction), PLE, and other processes, were developed and demonstrated cleaner and more efficient than Soxhlet extraction [2].

Pressurized liquid extraction is analogous to Soxhlet extraction, but the solvent condition inside the PLE cell extends to the supercritical region during extraction, ensuring efficient extractions. The raised temperature alters the sample more soluble and accomplishes a higher diffusion rate, while the high pressure keeps the solvent below its boiling point [3]. Solvents could penetrate solid models in an enhanced way at elevated pressures and temperatures, lowering solvent usage. A pressurized liquid extraction, while compared to a traditional Soxhlet extraction, exhibits a lessening of extraction time to approximately to 20 min from 18 h and a decline in total organic solvent consumption to 80 mL or less of organic solvent from 300 mL [4].

The effectiveness of the extraction procedure generally varies with the three correlated facets of the matrix, mass transfer, and solubility. Concerning the matrix, its nature and the constituent of interest to be extracted and its position within the matrix have an influence [5]. The elevated temperature enhances solubility properties and mass transfer between the plant matrix and the extraction solvent, leading to improved extraction kinetics. Lowering of solvent viscosity facilitates the plant matrix's hydration and increases the bioactive compounds' solubility. Higher temperature also leads to the breakdown of bonds or interactive forces in the matrix (dipoles, van der Waals, and hydrogen bridges), accelerates the release of compounds, and produces high extraction yields [6].

1.1 Basic Principles of Pressurized Liquid Extraction (PLE)

The PLE method is a quick extraction technology due to the direct interaction between the liquid solvent and the particles of the plant matrix under high pressure and subcritical temperature conditions to extract the constituents of interest effectively. The efficiency of the extraction technique mostly depends on the three correlated facets of the matrix, mass transfer, and solubility. Related to the matrix, its nature and the molecule of interest to be extracted and its position within the matrix have an effect [7]. Elevated temperature significantly enhances solubility properties and mass transfer between the plant matrix and the extraction solvent, resulting in better extraction kinetics. Solvent viscosity decreases, facilitating the plant matrix's hydration and increasing the bioactive compounds' solubility. High temperature also causes the breakdown of bonds or bonding forces in the matrix (dipoles, van der Waals, and hydrogen bridges), facilitates the release of compounds, and produces high extraction yields [8].

The PLE system is an extraction method designed to perform the extraction of constituents from multiple samples simultaneously. The PLE system provides high recoveries and exceptional precision for all analytes in a small amount of time. Inexpensive stainless-steel extraction cells with end cap filtration keep working costs at a minimum. Optional disposable end cap filtration enhances productivity and prevents valuable time. This advanced method (PLE) is preferred over other conventional methods of extraction due to the following reasons [9]:

1. Decreased Solvent Costs
   PLE uses as small as 15 mL of solvent compared to more than 500 mL of solvent required to perform Soxhlet extractions.
2. Lowered Running Costs
   Speedy extraction and cleanup and decreased solvent use and waste lower the running costs by around 70%.
3. Enhanced Productivity
   The entire extraction and cleanup could be carried out in less than 30 min. Conventional methods could consume 10–16 h.
4. Decreased Solvent Leftover
   The PLE system reduces solvent waste by utilizing solvents in an efficient way.
5. Purge Cross-Contamination
   Optional low-cost disposable extraction cells and Teflon filtration end caps guarantee trouble-free extraction and exclude the risk of cross-contamination.
6. One-Step Sample Extraction and Cleanup
   The optional in-line cleanup segment performs the comprehensive sample extraction and cleanup in one step with augmented speed and reduced cost.
7. Automated Operation and Documentation
   Real-time software lets plotting six pressure channels and six temperature data channels simultaneously. This powerful feature permits automatic documentation of all extraction data. Temperature and pressure data can be overlaid, printed in graphic or tabular format, and stored for future reference.
8. Modular Assembly Provides for Easy Maintenance
   The modular design of the PLE system mixed with its exposed plumbing facilitates effective system maintenance. The PLE system's channels are designed to operate independently. This adaptability ensures easy replacement with no down time.

9. Leakage and Clog-Free Operation
   Simple design combined with large bore plumbing enables the PLE system for *n* operation free from clogging and leakage.
10. Numerous Extractions
    The programming of varying pressure and temperature lets the extraction of a variety of compounds.

Pressurized fluid extraction is comparable to Soxhlet extraction, except the solvents are used near their supercritical region with increased extraction properties. In that physical region, the elevated temperature enables higher solubility and increased diffusion rate of lipid solutes in the solvent. By keeping the solvent below its boiling point, the high pressure allows an enhanced solvent penetration in the sample [10]. Thus, PFE permits a high extraction efficiency with a decreased solvent volume and a quick extraction time. That technique is called accelerated solvent extraction (ASE). Dionex first developed this method and validated it on a commercially available automated extraction system (Dionex ASE).

With the similar solvent blend employed in the Folch procedure, the elevated pressure solvent extraction of total lipids in poultry meat decreased the consumption of solvents and the time extraction. Concurrently, it has given comparable lipid recoveries and fatty acid compositions.

The PFE device contains an extraction cell (1 up to 100 mL) kept at a temperature between 80 and 200 °C into which a solvent is injected and held at 10–20 MPa for some minutes. Then, the extract is pushed into a collection vial by a second volume of solvent, and finally, the complete solvent is driven with an inert gas flow [11].

In the beginning, PFE was used for environmental contaminants in soils, sediments, and animal tissues, but it is currently used for food (meat, seeds, feeds), pharmaceutical products, and several other biological samples. This technique successfully replaced the Folch extraction for oxysterols in food. The polar and nonpolar lipids' efficiency of extractions with pressurized solvents (hexane, methylene chloride, isopropanol, ethanol) was assessed in corn and oats kernels. The solvent polarity and temperature properties were tested on the recovery of total lipids, triglycerides, glycolipids, and phytosterols [12].

1.2 Mechanism and Components of Pressurized Liquid Extraction (PLE)

The PLE involves circulating the solvent through the extraction cell or column with a high-performance liquid chromatography (HPLC) pump where the plant matrix is placed to remove the bioactive molecules of interest [13]. The pretreated and conditioned sample within the extraction column is exposed to the designated temperature using an electrothermal liner while being compressed to the specified pressure. In order to stabilize the system and facilitate solvent diffusion through the plant matrix,

the pressure and temperature are kept constant, marking the beginning of static extraction [14]. Afterward, the required pressure and solvent flow rate are maintained, commencing dynamic extraction. The extraction procedure is conducted through multiple cycles. At the conclusion of the process, the extraction column containing the sample is substituted with a new column consisting solely of an inert element. The system is then cleaned by pumping out the solvent and passing nitrogen or carbon dioxide through it [15].

As a result of the constraints posed by commercial equipment, the only feasible method to handle liquid samples is by converting them into solids, typically achieved through the addition of an absorbent or adsorbent substance. The extraction process for analytes from semisolid and solid samples can be outlined using the following five steps [16]:

1. Dampening of the sample (analytes to be extracted and matrix) with menstruum
2. Dislodging of compounds from the matrix (including or not the breakdown of chemical bonds)
3. Dissolution of the compounds in the menstruum
4. Dissemination of the compounds from the matrix
5. Propagation across the immediate solvent layer enveloping the matrix, culminating in the bulk solvent

The extraction efficiency is contingent upon both kinetic and thermodynamic factors. Consequently, the efficacy of extraction is influenced by three interconnected facets [17]:

A. Matrix effect
B. Mass transfer
C. Solubility

The characteristics of PLE are subject to several factors that impose limitations, including the careful selection of temperature, pressure, flow rate, and extraction duration required for achieving comprehensive extraction.

2 Instrumentation

The measurement process is simple to perform the liquid extraction process. Anti-corrosion products should be used, as high pressure (35–200 bar) and temperature (room temperature to 200 °C) are frequently used [18].

The most common representation of the instrument is shown in Fig. 1. It comprises of a pump, solvent container a furnace with the extraction cell, valves and chokes, and a collection vessel.

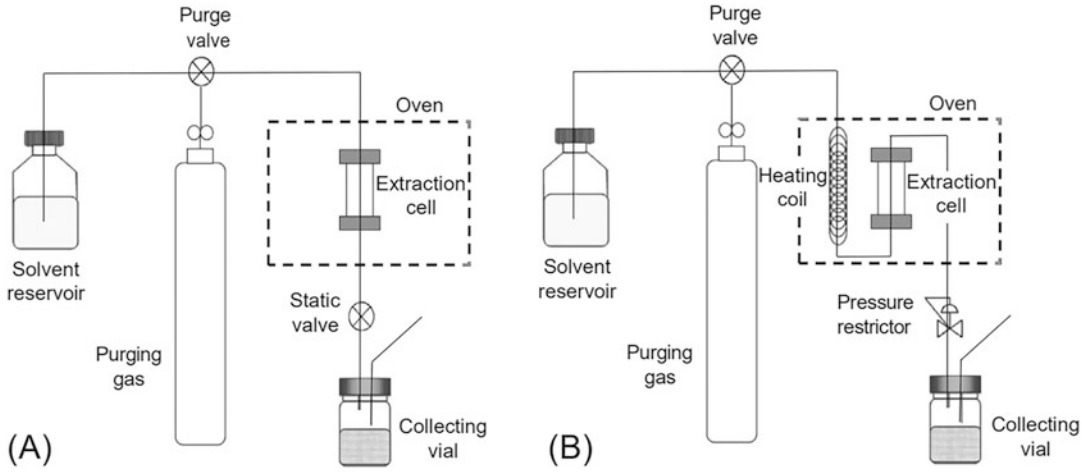

Fig. 1 Representative diagram of a pressurized liquid extraction (PLE) system showing configurations for the development of static system (**a**) and a dynamic system (**b**)

The solvent tank is first connected to the high-pressure pump. The pump introduces the solvent into the system and helps remove the extract when the process is complete. The extraction process takes place inside the extraction cell. If needed, a filter paper is inserted into the stainless-steel extraction cell, followed by the sample, which is sometimes mixed with a dispersant [19].

The cell is manually or automatically placed in the oven supported by valves and restrictors to control the entire pressure throughout the extraction process. Finally, there is a collection vial placed at the end of the extraction system. Instrumentation can be more or less sophisticated depending on the process requirements. For example, a solvent controller is needed if there are multiple solvent reservoirs (to obtain solvent mixtures), or an inert gas loop (usually nitrogen) can help purge the menstruum from the lines after extraction. In addition, a cooling bath can be used for the collection vessel, which lowers the temperature of the extractant to minimize thermal degradation. A dynamic pressurized liquid extractor requires a somewhat more complicated high-pressure pump to control solvent flow, solvent preheat coils, and a pressure limiter (backpressure regulator) or micrometer valve instead of a static open or close valve as in static pressurized liquid extractor [20].

3 Factors Affecting Pressurized Liquid Extraction (PLE)

3.1 Effect of Temperature

Temperature is the most important factor because it changes the solvents' physical and chemical properties and affects the extraction efficiency. Temperature lowers the dielectric constant of the solvent and changes its polarizability. It also reduces the viscosity and density of the menstruum, enhances diffusion and penetration

into the matrix, and causes a higher mass transfer rate. Breakdown of the structure of the vegetable matrix is brought about by high temperatures, which reduces the surface tension between the menstruum, sample, and the compounds during the extraction process. This change is desirable to form cavities in the menstruum into which the desired compounds are transported [21].

On the other hand, very high temperatures bring about the degradation of thermolabile compounds. Too high temperatures (>150 °C) may lead to the formation of toxic compounds such as hydroxymethylfurfural (HMF) [22]. Hence, proper optimization of temperature is essential after knowing the nature of compound to be extracted, to avoid undesirable compounds, and get high extraction yields [23].

3.2 Effect of Menstruum

Chemical affinity of the compound to be extracted for the extraction solvent is the essential factor for proper selection of the menstruum. This leads to high diffusion and mass transfer leading further to high extraction yields. Additionally, the menstruum should be nontoxic or less toxic in nature, inexpensive, accessible, and easily disposable. The most environmentally acceptable solvent system is the water–ethanol mixture [24].

3.3 Effect of Pressure

High-pressure technology is a significant merit in the entire process of pressurized liquid extraction as it maintains the menstruum in the liquid state even after subjecting to temperatures above their respective boiling points [25]. The highest pressures result in higher extraction yields because they help hydrate the plant matrix by keeping the solvent in a liquid state. However, their effect is less than that of temperature, since air bubbles can form at higher pressure, which reduces the solubility of the compounds in question [26].

3.4 Nature of the Plant Matrix

Plant matrices are usually subjected to unique treatments such as air drying, freeze drying, grinding, and screening before extraction. Drying type and conditions directly affect the extraction yield. The small size of the model makes it easy to change sizes. The larger contact area of the material with the extraction solvent improves the adsorption and separation of the target compounds. However, in some studies, it is recommended not to reduce the particle size too much in order not to prevent the diffusion of the solvent from the compressed structure [27].It is also essential to know the nature (nature and moisture) of the target compound in the plant matrix. Many studies have shown that samples with high water content can improve performance compared to dry models. This may be due to cell disruption during drying, inhibiting the release of the target compound. However, other studies have shown that water competes with solvent extraction by reducing the recovery of bioactive compounds in plant matrices [28].

3.5 Effect of Extraction Time

The time the solvent is in contact with the matrix at a specific temperature, pressure, and flow rate is the extraction time. Matrix structure, type of target composition, temperature, pressure, weight, etc. are some of the factors affecting its efficiency. Many factors affect it. The static or dynamic extraction method is the most specific parameter matrix to understand the extraction time required to separate bioactive compounds from plants [29].

3.6 Impact of Energy and Environment

The extraction process uses high pressure and temperature, which means more energy. But it is lower than other extraction methods such as supercritical fluid extraction or traditional Soxhlet extraction. According to some reported studies, the chemical effects related to the type of solvent applied in the extraction of Rosemary plants and Soxhlet extraction shows that PLE is less energy-intensive than Soxhlet extraction. However, the temperature of PLE is higher (183 °C vs. 78 °C for Soxhlet) [30].

3.7 Chemical and Sensory Factors

Pressurized liquid extraction using specific menstruum such as ethanol and others can produce some undesirable compounds such as hydroxymethylfurfural (HMF), a nonenzymatic brown indicator [31]. Similarly, HMF was associated with the induction of colon cancer precursors in mice but was not toxic as found in some laboratory tests. Ethanol concentration had a significant effect on the overall performance of the PLE process. The lower the ethanol content of the PLE extract, the higher the recovery of phenolic acids and flavanols. Therefore, it is recommended to recover at ethanol concentrations of 15%, 32.5%, and 50% for phenolic acids, stilbenes, and flavanols, respectively. The extract showed highest total polyphenol content and antioxidant activity at 150 °C and 32.5% ethanol [32].

4 Advantages and Disadvantages of Pressurized Liquid Extraction Technique

The benefits of this approach are demonstrated by applying a solvent at the right pressure and temperature to interact with plant molecules and extract them via mass transfer and solubility [33]. Pressurized liquid extraction uses solvents that are GRAS-type and environmentally benign, such as water and alcohol. In comparison to current procedures, which employ harmful solvents, it also utilizes less extraction solvent [34]. This extraction technique is carried out using equipment that is basic, practical, and easy to operate. Many of them feature semiautomatic designs that can be connected to instruments for analytical measurement and separation, such as liquid phase chromatography (HPLC) and gas chromatography (GC) [35]. Additionally, to increase extraction efficiency, this novel technique can be used in conjunction with other techniques including supercritical fluid extraction (SFE) and ultrasonic technology.

5 Applications

5.1 Isolation of Tocopherols

Tocopherols were obtained from apple seeds and kiwi fruit using this technique. A more pure extract was obtained, and the recovery rate was similar to or higher than that of the existing method. Extraction parameters optimized to maximize the effects of grain tocopherols and tocotrienols or carotenoids from microalgae have been described [36–38].

5.2 Determination of Organic Pollutants

Pressurized liquid extraction technique has gained popularity as a green extraction method for analyzing a variety of organic pollutants, including personal care products and pharmaceuticals, nanoparticles, flame retardants, and endocrine-disrupting chemicals, which are frequently found in environmental samples since it was first introduced as an official US Environmental Protection Agency (EPA) method for determining persistent organic pollutants in solid environmental samples [39].

The majority of applications concentrate on removing organic pollutants from sediment and sewage sludge [40]. For the extraction of persistent organic pollutants from nonbiological materials such as soils, sediments, silt, and dust, analytical methods based on the pressurized liquid extraction method have been developed. Furthermore, comparable types of samples have revealed the presence of numerous novel contaminants, including nitrosamines [41], alkylphenols, bisphenol A, and UV filters [42]. Using pressurized liquid extraction to create sludge samples, researchers were able to find additional contaminants that have been classified as endocrine disruptors, such as homologs of bisphenol and bisphenol A [43], hormonal steroids, and flame retardants such as brominated and chlorinated flame retardants [44].

Similar to other extraction methods, the identification of the target pollutants can be hampered by the coextraction of nontarget analytes from the pressurized liquid extraction matrix. Due to this, a post-extraction cleanup step is often necessary before the determination stage [45]. This can be accomplished using various solid phase extraction cartridges, gel permeation chromatography, or packed chromatographic columns. Sulfur is a characteristic elemental interference in soil and sediment matrices, and in some applications, pressurized liquid extraction processes employ the right adsorbents in the extraction cell to retain it [46]. The pressured liquid extraction throughput is greatly increased by the option of including in-cell cleanup. In order to get clean extracts for the examination of nonpolar compounds such as polycyclic aromatic hydrocarbons and flame retardants such as polybrominated diphenyl ethers, silica gel was thus demonstrated to be a successful sorbent. In order to determine UV filters, activated carbon was effective in removing sulfur under reducing circumstances [47].

Emerging pollutants in cosmetics, personal care items, and environmental pressured liquid extractions include fragrance allergies, UV filters, and cosmetic preservatives. Pressurized liquid extraction was shown to be a successful method in this instance for removing these compounds from pressurized liquid extraction cosmetic matrices [48]. Pressurized liquid extraction-based technologies enable simultaneous in-cell derivatization and extraction of multiclass cosmetic preservatives (such as parabens, triclosan, bronidox, and bronopol) before GC-MS analysis [41]. Hence, in a study of dust analysis, parabens and triclosan were found after eliminating nonpolar interferences with hexane at low temperatures and pressure and extracting the target analytes with polar solvents [49].

5.3 Estimation of Pesticides

Although the advantages of this technology have been reviewed for the study of biological and food samples, pressurized liquid extraction applications were initially focused on the extraction of environmental pollutants [50]. Since this technique enables the simultaneous extraction of many residue types with a wide range of polarity, numerous applications for identifying pesticide residues have been documented [51]. Thus, samples of animal and vegetable tissues are analyzed using pressured liquid extraction. Pesticides are typically found in nonfat foods with a medium to high water content, such as fruits, vegetables, and cereal-based diets. As a result, it is frequently necessary to add a drying agent (such as sodium sulfate or diatomaceous earth) [52, 53].

Pressurized liquid extraction has been used to identify a wide range of pesticide residues in various agricultural and food matrices, such as honey [54], organophosphorus pesticide residues in corn [35], pyrethroid residues in feed samples [55], multiclass pesticide residues in food commodities and grain, or herbicide residues in soybeans [56].

By combining this method with gas chromatography/high resolution isotope dilution mass spectrometry, common organochlorine, organophosphorus, and pyrethroid pesticide residues in herbal liquid extractions such as tea may be accurately determined [57].

The analytical complexity of tea sample matrices can be reduced by using pressurized liquid extraction followed by gel permeation chromatography due to the high concentration of caffeine, pigments, polyphenols, etc., in tea samples. Applying a selective pressured liquid extraction technique, many pesticides as well as other lyophilic pollutants can be recovered and identified from lipid-rich matrices. The homogenized lipid sample is then placed in the extraction cell on top of basic alumina, silica gel, and florisil. The target chemicals are then extracted utilizing a single automated process employing a 1:1 (v/v) dichloromethane/hexane ratio [58].

Comparative assessment of pressurized liquid extraction performance and other pressurized sample preparation procedures such as supercritical fluid extraction or the traditional Soxhlet extraction revealed higher recoveries of pesticides by pressurized liquid extraction, compared with conventional analytic techniques, such as QuEChERS and buffered ethyl acetate extraction.

Pressurized liquid extraction methods also provided superior performance for extracting pesticide residues [59]. In these cases, the removal of lipids and other co-extractable materials was achieved by adding fat-retaining sorbents to the pressurized liquid extraction cell, such as florisil, alumina, or sulfuric acid–impregnated silica gel [60].

5.4 Determination of Toxins

Numerous fungi create a group of toxicologically significant poisons known as mycotoxins. Through the use of pressurized fluid extraction, mycotoxins, such as zearalenone, ochratoxin A, and aflatoxin, have been discovered in a variety of foods [61]. Before analyzing mycotoxins using chromatography, a cleaning procedure is typically advised. Compared to traditional sorbents, molecular imprinted polymer solid phase extraction, which is based on customized polymers with particular binding sites corresponding to the target chemicals, is more selective. This idea led to the successful detection of two *Alternaria* mycotoxins, such as alternarisol and alternariol monomethyl ether, in tomato samples using a modest amount of methanol (8 mL per sample) and a short period (13 min each sample) [62].

5.5 Determination of Metals

Metal and organometallic compounds have been determined using liquid extraction under pressure using Envi-Carb-based dispersing agents and speciation of polar arsenic species in Seafood. The recovery of four arsenic species was evaluated using silica, C-18, sea sand, diatomaceous earth, and alumina as a cleanup agent. On the other hand, Envi-Carb was used as a cleanup sorbent and dispersing agent to extract Mg, Al, Ti, Cu, Ag, Sn, and Pb in lubricating oils without any additional cleanup step [47].

5.6 Estimation of Antibiotics

The release of pharmaceutical products into the environment has raised issues concerning their occurrence, fate, and effects on the biota. Antibiotics are an essential group of pharmaceutical products widely used in human and animal health care, which are reportedly ubiquitous compounds in the aquatic environment. Pressurized liquid extraction is a reliable technique for extracting antibiotics and other drug residues associated with suspended solid matter [63]. Multiresidue analysis of sulfonamide antibiotics and their acetylated metabolites in soils and sewage sludge can be performed using fully automated pressurized liquid extraction methods [64]. In these methods, a subsequent step for preconcentration and purification is required. The extraction of quinolone and

sulfonamide residues, such as lomefloxacin, enoxacin, sarafloxacin, enrofloxacin, sulfadiazine, sulfamethoxydiazine, and sulfa dimethylpyrimidine, in fish and shrimp was carried out by pressurized liquid extraction using diatomaceous earth as a dispersing agent and acetonitrile as the extraction solvent [65].

5.7 Standardization of Polyphenols

Pressurized fluid extraction is one of the most widely used techniques for extracting polyphenolic compounds from various sources such as food, vegetables, seafood, and agro-industrial by-products. Whether acidified or not, the hydrogen ethanol mixture (EtOH $> 50\%$) is the preferred solvent for the extraction of polyphenols by liquid extraction. In addition, the temperatures of heat- and cold-resistant phenolics are generally 40–60 and 75–220 °C, respectively.

One study [62] used an optimized pressurized liquid extraction to extract antioxidant phenoliccompounds from defatted peanut shells using ethanol aqueous solution (60.5% v/v) as the solvent at a temperature of 220 °C for a time period of 12.2 min. Under these conditions, extracts with high phenolic yield like phenolic acids and glycoside flavonoids were obtained.

Another study used pressurized liquid extraction method to extract polyphenolic compounds with antioxidant activity from *Rubus fruticosus* L. residues [66].

Anthocyanins were the main components recovered by using water: ethanol (50 : 50) as extraction solvent in a dynamic extraction mode at 100 °C for a time period of 30 min.

Another example is the extraction of monomeric anthocyanins and other phenolic compounds from grape (*Vitis vinifera*) pulp by continuous liquid extraction [67]. The extraction was divided into two consecutive parts to recover the different groups.

The first step was performed at 40 °C using water/ethanol (50% w/w) pH 2.0 as solvent, and the second step was performed at 100 °C using water/ethanol (50% w/w). The process yielded two different sources: one rich in anthocyanins (first step) and one rich in other phenolic compounds (second step). The low temperature of the first step prevents thermal degradation of the anthocyanins before the second step, while the low pH aids the extraction yield.

In the second step, efficient extraction of phenolic compounds was found as high temperature increased the extraction of heat-stable phenolic compounds.

Another interesting example is the recovery of biflavonoids and anthocyanins from the dried fruit of Brazilian pepper (*Schinus terebinthifolius* Raddi) after a defatting step in a continuous pressure liquid extraction process [68]. The first step was to use petroleum ether at 60 °C for 6 min. In the second step, phenolic compounds were extracted from the stone fruit and dried fruit exocarp using acidified ethanol (5% v/v acetic acid) utilizing a static

extraction cycle (10 min each) at 75 °C and 100 °C. This continuous supply of fluid facilitates the selective extraction of phenolic compounds such as naringenin, biapigenin, and methylated anthocyanins.

A combined application of pressurized liquid extraction and ultrasonic-assisted extraction was used for the extraction of anthocyanins from *Rubus fruticosus*, *Vaccinium myrtillus*, and *Eugenia brasiliensis* [69]. In this process, the sample is subjected to preliminary sonication before being extracted by pressurized liquid extraction. These samples were mixed by using hydroethanol solution (50% or 70% ethanol) as menstruum and processed in an ultrasonic bath at 80 °C for 8 min.

Pressurized liquid extraction is also used in biorefinery processes to extract polyphenolic compounds. One study [70] carried out the biorefining of hemp (*Cannabis sativa* L.) residues by sequential supercritical carbon dioxide and pressurized liquid extraction along with enzyme-assisted extraction. Lipophilic fraction rich in cannabidiol and cannabidiolic acid was obtained in the supercritical process, the pressurized liquid extraction method gave a flavonoid-rich fraction, and the enzyme-assisted process gave a sugar-rich fraction. The liquid extraction biotreatment process was divided into two successive stages. In the first step, acetone was used as the solvent and hydroethanol solution (4:1 v/v) was used in the second step. Each step was performed at 100 °C for 45 min (3 cycles × 15 min).

In another study [71], a continuous method was developed for the isolation of bioactive compounds (human aromatase inhibitors) from *Cicer arietinum* seeds using liquid extraction, countercurrent chromatography, and preparative liquid chromatography.

The pressurized liquid extraction was performed using aqueous ethanol (60% w/v) at 80 °C for 5 min. Thereafter, the extract was transferred into the two chromatography sample loops. Both the chromatography separations were optimized based on the polarity of the active compounds already characterized in the pressurized liquid extract.

The complementarity between countercurrent chromatography and preparative liquid chromatography allowed the isolation of 11 bioactive flavonoid-type compounds. This novel continuous extraction method is effective and can be applied to other bioactive compounds in various food or plants [72].

Recovery of phenolic compounds from various parts of medicinal plants has been made possible by pressurized liquid extraction. The high temperatures and pressure and the right menstruum bring about rapid and effective extraction of compounds of different polarities [73].

Various fruits, vegetables, oils, such as *Hibiscus sabdariffa* calyces, *Sclerocarya birrea* stem, pomegranate peel, sweet cherry stem, and olive oil, have been subjected to pressurized fluid extraction to

isolate their phytochemicals and their by-products generated in the process of production [74].

In these studies, surface response technique was used to optimize phytochemicals combined with advanced extraction techniques to improve the process of bioactive extracts.

Cassia grandis (Fabaceae), also called as Carao or Red flower, is a legume native to Central and South America. The pods are edible and the seeds are used to make chocolates [75]. Research reports point to the antioxidant properties of the seeds, which explains their use in traditional medicine [76]. Some of these functions may be related to their use in bioactive substances such as phenols, flavonoids, and tannins [77].

Many authors have reported the biological activities of phytochemicals belonging to this chemical group [78]. Since scientific information shows the extending and medicinal properties of the content of phenolic compounds, they can be used to create food antioxidants or as ingredients in nutraceuticals.

A research aimed at identifying and optimizing the extraction of phenolic compounds from *C. grandis* seeds was performed by combining advanced extraction techniques and analysis platforms. Response surface technique of liquid chromatography was combined with electrospray to extract oil from oil time-of-flight Mass spectrometry [79].

For the extraction, the solvent was degassed for 15 min to remove oxygen to prevent oxidation. For each extraction, the sample was mixed with sand and loaded into a stainless-steel extraction cell.

The selection options are sandwich type (5 g sand + mixed sample – sand +5 g sand). Cellulose filters are installed at both ends of the pool to prevent clogging with metal frits. The above extraction procedure was used and the resulting product was collected in glass bottles. These extracts were rapidly cooled to room temperature, filtered, and evaporated in vacuo.

Using Statgraphics Centurion XV software version 15.1.02, the response surface method was used to evaluate the effect of wastewater on the recovery and yield of phenolic compounds.

The design pattern used is a basic mixed design model with two pivot points and two levels (maximum and minimum) for each independent variable. Temperature, percentage of ethanol, and extraction time were chosen as independent variables, and the experimental design consisted of a total of 14 experiments [80].

5.8 Isolation of Terpenoids

Pressurized liquid extraction technology has been used recently for the extraction of terpenoid compounds from various sources including plants and microorganisms [81]. Owing to the chemical diversity and polarity, different solvents and temperature ranges are required.

However, different solvents and temperature ranges are required due to their chemical diversity and polarity. The commonly used solvents include ethanol, water, hydroethanolic mixtures, and ethyl acetate. Certain renewable solvents such as 2-methyltetrahydrofuran at temperature ranging from 40 to 160 °C are also used.

In a study [82], various pressurized hot water extraction parameters such as time, temperature, and the frequency of cycles were optimized for the recovery of terpenoids such as steviol glycosides, carotenoids, and other bioactive compounds from *Stevia rebaudiana* Bertoni leaves.

Optimal conditions for the extraction of terpenoids were 160 °C and 30 min (10 min per cycle), demonstrating that the technique was found to be efficiently used to recover thermally labile and nonpolar to polar components in Stevia leaves.

Pressurized liquid extraction has been used to extract other terpenoids such as carotenoids from microbes. One such instance is the extraction of additional carotenoids, including hydroxylated and nonhydroxylated salinixanthin forms, from the marine bacteria *Rhodothermus marinus*, under pressurized liquid extraction conditions using ethanol as the solvent for 6 min (3 cycles of 2 min each).

A different investigation extracted carotenoids and chlorophylls from the microalgae *Chlamydomonas* sp. using pressured liquid extraction [83]. In this instance, the pressured liquid extraction extract's primary carotenoid was identified as lutein under the most stringent circumstances (100% ethanol, 40 °C for 20 min). However, the chlorophyll/pheophytin content of this extract was likewise high. The synthesis of terpenoids was studied chemically using pressurized liquid extraction.

Neochloris oleoabundans microalgae were used in a research study [84] that used pressurized liquid extraction as a reference extraction method to examine the effects of various culture conditions such as effects of nitrogen, light intensity, and carbon supply, on the total carotenoid and carotenoid composition. Also assessed was the pressure liquid extraction extracts' capacity to inhibit the proliferation of human colon cancer cells. At 100 °C and a static extraction time of 20 min, ethanol was used for the extractions. Pressurized liquid extraction helped to create the ideal circumstances for the cultivation of large quantities of carotenoids with antiproliferative activity, such as lutein, carotenoids monoesters, and violaxanthin.

Since different solvents can be used depending on the terpenoids' chemical characteristics, pressurized liquid extraction is a flexible method for terpenoids. In this regard, 2-methyltetrahydrofuran was first assessed for the pressured liquid extraction of a number of carotenoids from *Chlorella vulgaris* [85]. For the extraction of xanthophylls (violaxanthin, astaxanthin, lutein, and canthaxanthin) and carotenoids (Carotene and

lycopene), a mixture of 2-methyltetrahydrofuran and ethanol (50: 50 V/V) was heated to 110 °C for 30 min. To identify and characterize high-value chemicals from natural sources, a multianalytic platform with pressurized liquid extraction was also included.

To generate extracts from *Physalis peruviana* L. calyces that are rich in withanolide, in vitro antioxidant assays and Hansen solubility criteria were suggested [86]. In this investigation, pressured liquid extraction solvents were chosen based on the Hansen solubility parameters technique and target molecules 4-hydroxywithanolide E and withanolide E. The extraction temperature, ethanol, ethyl acetate, and their combinations were assessed in relation to the amount of withanolide present in the pressured liquid extraction extracts. The best results were obtained using a 75:25 v/v mixture of ethanol and ethyl acetate heated to 125 °C. The development of integrated solutions to increase process selectivity toward the recovery of target compounds has been accelerated by the quest for terpenoids with biological activity.

To obtain carnosic acid and carnosol-enriched rosemary (*Rosmarinus officinalis* L.) extracts with antiproliferative activity on colon cancer cell lines, another study developed an integrated pressurized liquid extraction followed by supercritical antisolvent fractionation at pilot plant scale and compared the process with other sub- and supercritical methods. The pressurized liquid extraction and supercritical process began with the production of a hydroethanolic extract under pressurized liquid extraction conditions (80:20 v/v, 150 °C, 20 min). Based on the antisolvent properties of SC-CO_2 in aqueous systems, the pressurized liquid extraction extract was then diluted with water and fractionated. High levels of phenolic terpenes were detected in the fractions produced by pressurized liquid extraction and supercritical antisolvent fractionation, and they also demonstrated antiproliferative solid activity [87].

5.9 Extraction of Lipids

The extraction of lipids is one of the principal uses of pressured liquid extraction. This technique has been used to extract lipids from a variety of sources and chemical structures utilizing low- or medium-polarity solvents such as hexane, (+)-limonene, ethyl acetate, methyl acetate, ethanol, and hydroethanolic combinations. The temperature used for lipid extraction typically ranged from 90 to 220 °C. For the purpose of resolving issues with traditional extraction techniques utilizing hazardous organic solvents, pressurized liquid extraction was assessed as an environmentally friendly method for isolating edible oils. For the effective extraction of 3-rich oil from *Echium plantagineum* seeds utilizing hexane-free processing methods, pressured liquid extraction, microwave-assisted extraction, and ultrasound-assisted extraction have recently been examined [88].

A range of solvents, including water, ethanol, ethyl acetate, hexane, and mixtures of ethanol and water, were utilized at temperatures between 60 and 200 °C. In a recent application, the effect of (+)-limonene on lipid recovery in various microalgae (*Arthrospira platensis*, *Phormidium* sp., *Anabaena planctonica*, and *Stigeoclonium* sp.) was studied using this method. A mixture of limonene/ethanol (1:1 by volume) under pressure liquid extraction conditions (200 °C for 15 min) was the selective solvent for obtaining lipid extracts rich in valuable fatty acids from the sources evaluated [89]. A sequential pressurized liquid extraction approach was also used for lipid fractionation [90].

Another recent study developed a four-step sequential method using pressurized liquid extraction to extract and fractionate lipid compounds from *Nannochloropsis gaditana* [91]. This method was based on increasing the temperature progressively and decreasing solvent polarity through sequential steps. In the first and second steps, the polar compounds (i.e., carbohydrates and peptides) were eliminated using water and hydroethanolic mixture (5% v/v) at 90 °C. In the third and fourth steps, lipid compounds were fractionated using hexane/ethanol mixture (3:1 v/v) at 120 and 150 °C, respectively. This method allowed to obtain fractions enriched in neutral and polar lipids such as triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, and glycolipids.

In another study, pressurized liquid extraction with methyl acetate was used for oil extraction from *Crambe abyssinica* H. seeds. The technique at 140 °C in a dynamic process (solvent flow 3.0 mL min^{-1} × 30 min) provided a high extraction efficiency with a fatty acid composition similar to commercial *C. abyssinica* oil obtained by mechanical pressing. Thus, the oil obtained by pressurized liquid extraction had good quality and was found suitable for biodiesel production [92].

5.10 Isolation of Volatile Oils

Pressurized liquid extraction has also been used to extract essential oils from plants. The most common method used for this application is pressurized hot water extraction due to its high efficiency and "green and clean" status for essential oil extraction. Due to the large chemical composition of essential oils (terpenes, alcohols, ethers, oxides, aldehydes, ketones, esters, amines, phenols, heterocycles, etc.) in this technology, the temperature and cooling are typically within 50–200 °C. The ability of this method to extract essential oils has been analyzed and compared to hydrodistillation and Soxhlet extraction methods [93].

In a study, pressurized hot water extraction method was used with optimized conditions of temperature and flow rate for the isolation of volatile oils from *Matricaria chamomilla* leaves. The optimal conditions of temperature and flow rate (150 °C and 4 min mL^{-1} for 120 min) gave the best quality yield (14%) comprising of α-bisabolene oxides, β-trans-farnesene, and α-bisabolol oxides A–B [94].

The same approach was used to obtain volatile oils from *Coriandrum sativum* L. seeds [95]. The optimized conditions were 125 °C, 0.5-mm particle size, and 2.0 mL min^{-1} of water flow. The extraction process showed an important volatile oils yield (14.1%); however, hydrodistillation (21.7%) and Soxhlet (19.4%, using hexane as solvent) methods presented the best performance. Nevertheless, it is worth mentioning that this technique obtains higher quality volatile oils, since small amounts of hydrocarbons are extracted. Another study used different extraction techniques (hydrodistillation, Soxhlet, supercritical fluid extraction, and pressurized hot water extraction) to extract volatile oils from *C. sativum* seeds [95]. In this case, supercritical fluid extraction (sc-CO_2 at 40 °C and 300 bar for 4 h) presented the best quality and yield; however, under pressurized hot water extraction conditions (200 °C for 20 min), it was possible to obtain an extract of volatile oils rich in polyphenolic compounds with a higher added value. Solvents other than water have also been explored for essential oil extraction under pressurized liquid extraction conditions.

In a recent study, ethanol, ethyl acetate, and hexane were evaluated for efficient extraction of α-bisabolol using pressurized liquid extraction and ultrasonic extraction methods from the wood of *Eremanthus erythropappus*.

α-Bisabolol is an important essential oil in many plants and is used in skin preparations, cosmetics, fine perfumes, and shampoo coatings. The highest purity content of α-bisabolol (64.23%) was obtained under pressurized liquid extraction conditions (55 °C, 20 min extraction) [96].

6 Recent Advancements in Pressurized Fluid Extraction

6.1 Sequential Biorefining

Recently, a combination of alternating supercritical fluid extraction and pressurized fluid extraction methods has been successfully used to isolate and purify bioactive components from waste. Combining the two methods allows for the use and complete separation of all bioactive compounds from waste.

In another study of cherry stems, the resulting extract was found to contain 42 compounds out of which 20 compounds were unknown and found suitable for incorporation into foods and nutraceuticals [97].

6.2 Microencapsulation and Nanoencapsulation by Combining Pressurized Liquid Extraction and Supercritical Fluid Extraction

The use of supercritical fluid extraction as a microencapsulation tool for rapid expansion of supercritical solutions is a useful program in which active ingredients and coatings are dissolved in supercritical fluid as solvents. The supercritical fluid containing the solvent is held at high temperature before expanding through a capillary device or orifice nozzle. At this point, supersaturation occurs and causes the layer of material placed on the active ingredient to dissolve and form microcapsules. In addition to obtaining

bioactive substances, lipid extracts, which are useful when used in any food, are stored in microcapsules. There are other techniques, such as supercritical melt micronization and microencapsulation, which use supercritical antisolvents or combine the coating material (coating, fluid bed coating) with supercritical CO_2 [98].

7 Conclusion

Hence, pressurized fluid extraction is a technique performed to extract solid or semisolid samples using organic solvents. Elevated temperatures are used to increase the kinetics of the extraction process while applying high pressures to maintain the organic solvents in the liquid state. Compared with traditional extraction techniques, it is unique because extractions are performed rapidly with reduced solvent use. It can reduce the extraction time down to 20 min per sample versus hours using Soxhlet and reduce solvent consumption to 30 mL per sample.

It is a recent technique of extraction of analytes from solid samples. The extraction efficiency is widely influenced by factors such as temperature, pressure, type and volume of menstruum, and addition of other reagents. Further derivatization reactions can be coupled for improving the analytical applications of this method. The rate of reaction and throughput are highly enhanced. Faster time of reactions reduces the exposure of labile samples to air and light.

Recent technological developments in the design of equipment at the industrial level contribute largely to the broadening of utilization of this technique in various fields.

Hence, an exhaustive understanding of the mechanisms of recently developed extraction techniques becomes necessary for promoting their use as cost-effective and environment-friendly measures to isolate bioactive-rich compounds.

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Chapter 13

Fruit Waste: Potential Bio-Resource for Extraction of Nutraceuticals and Bioactive Compounds

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Abstract

Globally, fruits and vegetables generate almost half of total food waste, which has become a major environmental concern. Though the ample amounts of fruit by-products are considered as industrial waste and usually disposed of or used as animal feed and biofuel, they can be a great source of nutraceuticals and bioactive compounds. Studies have suggested that bioactive compounds from fruit by-streams can be extracted using conventional methods such as solvent extraction, maceration, and enzyme-assisted extraction and emerging technologies such as supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, ultrasound-assisted extraction, and electric pulse field. This chapter discusses the potential of extraction of nutraceuticals and bioactive compounds from fruit waste and their possibilities for further application in the food, feed, cosmetic, and pharmaceutical industries, with their future perspectives.

Key words Fruit waste, Valorization, Bioactive compounds, Nutraceuticals, Extraction methods

1 Introduction

Each year, more than 1.3 billion tons of edible food are wasted globally, which is equivalent to nearly one-third of the entire amount of food produced and more than enough to feed one billion people. Less developed nations account for 44% of global food waste during the post-harvest and processing stages of the food supply chain, while developed nations in Europe, North America, Oceania, Japan, South Korea, and China account for the remaining 56% of these losses, of which 40% occur at the pre- and postconsumer stages [1]. According to a United Nations report in 2017, the world population is estimated to reach 9.8 billion by 2050, and studies show that the world will need 70–100% more food by that time. The only way to attain food security is by

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minimizing food waste and food loss. Apart from food security, food waste leads to monetary losses. Around the globe, the loss from food waste accounts for $750 billion, which would directly affect farmers' and consumers' incomes. The final disposal of food waste in landfills (uncontrolled methane release) as well as the production, processing, manufacture, transportation, storage, and distribution of food all contribute to the emission of greenhouse gases. Additional unfavorable externalities brought on by food loss and waste include nutrient depletion, soil erosion, salinization, and air and water pollution [2].

Fruit wastes, including citrus fruit skins, pineapple leftovers, sugarcane bagasse, and other fruit residues (mostly peels and seeds), are produced in enormous amounts in metropolitan areas because of high consumption and industrial processing. One of the main causes of municipal solid waste (MSW), which has become a more challenging environmental issue, is fruit waste. Such wastes are disposed of by landfilling or incineration. However, both techniques impose various risks to the environment as well as human health by releasing methane or secondary pollutants such as furans, dioxins, and acid gases [3, 4]. In order to minimize those hazards, recovering the bioactive components from fruit wastes, notably the phenolic compounds, and fully utilizing them in food, pharmaceuticals, and cosmetics seems to be crucial. Furthermore, value addition to agri-food waste is quite cost-effective and has minimal impact on the environment [5]. Epidemiologically, consumption of enough fruit and vegetables (10 servings or more per day) is confirmed to prevent the inflammation and oxidative stress that are linked to heart disease and diabetes, both of which have significant mortality rates worldwide. The presence of secondary metabolites such as fibers, carotenoids, anthocyanins, and phenols in fruits and vegetables exhibit various antioxidant, anti-inflammatory, and anticarcinogenic as well as biological activities [6–9]. These secondary metabolites are intended to help the plants grow and increase their capacity for survival (resistance to environmental stress, illnesses, and UV radiation). More than 15% of phenolic concentrations are found in the skin of oranges, grapes, and lemons, as well as the seeds of mangoes, avocados, and jackfruit than in fruit pulp. However, a total of 55 million metric tons of biowaste are anticipated to be produced during each processing stage, including 5.5 million metric tons from the processing of fruits and vegetables, 6 million metric tons from canning and freezing, 5–9 million metric tons from the processing of wine, and other sources. These losses occur from different compositions such as under-ripe, over-ripe fruits and vegetables or inedible parts including peels, rind, seeds, core, rag, stones, pods, vine, shell, skin, pomace [10]. Along with the fruit pomace, secondary metabolites such as fibers, carotenoids, anthocyanins, and phenols are lost during manufacturing [11].

Food waste can be valorized into value-added goods using a sustainable process that lowers the amount of food waste dumped in landfills and reduces greenhouse gas emissions. Food waste valorization is a growing sector as people become more conscious of the negative environmental effects of food waste disposal. Numerous decision-makers have embraced the concept of valorization, and the results of their actions have been sustainable projects in the social, business, and industrial spheres of society. Since valorization is still in its early stages, these judgments must be carefully considered; in addition, it is necessary to do extensive, difficult assessments of the product's life cycle, life-cycle costs, and social life cycle [12]. Successful bioactive chemical extraction from fruit or vegetable wastes is feasible, and their enormous potential in pertinent industries can be realized through the manufacture of various dietary supplements and functional foods as well as food additives and food preservatives [13, 14]. To make use of this bioactive-rich waste, extraction techniques should be novel, simpler, sustainable, and cost-effective, and there have been continuous efforts to identify such techniques. Some safer and more environmentally friendly extraction methods are supercritical fluid extraction, pulsed electric field, hydrodynamic cavitation, etc. Such extracted bioactive have been currently utilized in food as coloring and flavoring ingredients and have been identified as safe (GRAS) [15]. By adding bioactive from fruit and vegetable waste, several examples of functional food products, including extruded snacks, bakery goods, beverages, breakfast cereals, and dairy products, have been effectively produced [16]. Incorporating these bioactive compounds has also prevented meat from spoiling by improving the appearance and softness of the meat and providing an antibacterial impact [17]. Dried fruit pomace can be added to bread, cakes, muffins, cookies, biscuits, and other baked goods to increase their dietary fiber content. Although the byproducts of the processing of fruits are all included in the same category in this study, each by-stream has difficulties for valorization. Utilizing fruit processing byproducts raises the issue of microbial deterioration caused by excessive moisture content [18, 19]. Drying of byproducts for processing can lead to a concentration of pesticides. Additionally, even at modest levels of water activity, molds can still produce mycotoxins. The hazards that need to be considered before being valued can also vary greatly across different product sections including stems, leaves, and skins. The use of these by-streams as animal feed is the focus of most current research on the subject. Data on safety must be compiled if they are to be utilized in food applications, and existing food safety legislation must be expanded to cover these by-products.

2 Fruit Byproducts

Portions of fruits, vegetables, and other food products are wasted or lost because of the morphological qualities of the product, improper handling techniques, or discarding for a variety of reasons. Depending on the commodities and morphological components, such as leaves, roots, tubers, skin, pulp, seeds, stones, pomace, etc., there are different amounts and types of wastes and losses. When it comes to waste, apples produce 89.09% of the finished goods during slicing and 10.91% of seed and pulp as byproducts [11]. The main processing waste left over from making apple juice is called apple pomace. Apple pomace mostly consists of peels/flesh (95%) and stem (1%), with 2–4% of seeds also present. Pectins, cellulose, hemicelluloses, and lignins make up 35–60% of the dietary fiber in apple pomace. Banana peels, which make up about 35% of the weight of the fruit, have drawn attention in recent years because of their high concentrations of phenolics (hydroxycinnamic acids), flavonoids, phytosterols, carotenoids, anthocyanins, biogenic amines, vitamins (B3, B6, B12, C, and E) and dietary fibers. Currently, industries use avocado to make guacamole, spreadable puree, fresh and frozen chunks, and oil. Additionally, processing produces a lot of by-products (peel and seed), which consist of 33% of the fruit. Given their high concentration of phenolic acids (hydroxybenzoic and hydroxycinnamic acids), condensed tannins (procyanidins), and flavonoids (flavonols) with recognized antioxidant and anti-inflammatory actions, avocado peels and seeds have a high bioactive potential. Papaya biomass, which includes the seeds and skin, is wasted to a greater than 20% extent. Around 30% of the oil in papaya seeds is made up of mostly palmitic, stearic, oleic, and linoleic acids, as well as tocopherols and carotenoids, with beneficial nutritional and functional qualities. Papaya seeds are an excellent source of beneficial compounds that can be used to create food additions or supplements [20]. Mandarins produce roughly 16% peels and 84% of the finished product after being peeled [11]. There are a lot of byproducts produced, primarily peel and seeds, which can make up as much as 50% of the original fruit weight after processing citrus fruit to produce juice. Peel (60–65%), internal tissues (30–35%), and seeds (0–10%) are among the by-products. Citrus peel contains dietary fiber, polyphenols, essential oils, and vitamins, all of which have the potential to be bioactive. The dry weight of peels can be up to 70% dietary fiber. Citrus by-products are a rich source of natural flavonoids, such as hesperidin, naringin, and narirutin, flavonols, such as rutin and quercetin, flavones, such as diosmin and tangeretin, as well as several other compounds, such as flavanones, flavanone glycosides, and polymethoxylated flavones, which are specific to citrus. The peel is rich in phenolic acids, particularly hydroxycinnamic (ferulic,

p-coumaric, sinapic, caffeic, and chlorogenic) and hydroxybenzoic (vanillic, p-hydroxybenzoic, and gallic). In the process of processing pineapples, 14% of the peels, 9% of the core, 15% of the pulp, 15% of the top, and 48% of the overall product are produced [11]. Pineapple peel (PP) wastes include a significant amount of commercial bioproducts, including pectin, dietary fiber, and enzymes, which might be used for value-added products [21]. Mango processing yields roughly 11% peels, 13.5% seeds, 18% inedible pulp, and 58% finished product. Even more than the fruit pulp, mango peels and seeds contain significant amounts of bioactive substances such as dietary fibers, phenolic compounds, carotenoids, and vitamins [11]. Around 5–9 MMT of solid waste is produced annually by the processing of grapes and wine, which accounts for 20–30% of all processed materials. The amount and soluble/insoluble ratio of dietary fiber in grape pomace varies by variety and includes hemicelluloses, cellulose, and pectin. The main polyphenols are phenolic acids, flavanols, flavonols, stilbenes, and anthocyanins. About38–52% (dry weight) of grape pomace is made up of grape seeds, which make up 2–5% of the weight of the fruit. Grape seeds also include polyphenols, primarily gallic and caftaric acid, catechin, epicatechin, epicatechin gallate, and procyanidins B1 and B2, in addition to lipids (such as linoleic acid), proteins, carbs, and vitamin E. By cold-pressing seeds, seed oil can be produced while keeping more nutrients that are healthy. Approximately 6 MMT of solid waste from the canning and freezing of fruits and vegetables is produced each year, with 20–30% of that waste being made up of leaves, stalks, and stems. The waste of jackfruit is mainly from rind and seeds, which accounts for 50–70%. Jackfruit peel is reported to be rich in cellulose, pectin, protein, and starch comprising about 27.75%, 7.52%, 6.27%, and 4%, respectively [22]. The weight of the dragon fruit is comprised of 22–44% skins. Betalains, phenolics, and dietary fibers like pectin and oligosaccharides have been identified as the key bioactive constituents in dragon fruit peels. The identification of and possible health advantages of betalains have drawn the most interest of these [23]. Guava is wasted mainly from core, seeds, and skin. Seeds of guava are an important byproduct while processing juice. According to reports, seeds have a higher high-fat content than core and pulp. Studies have shown that guava seeds' mono- and polyunsaturated fatty acids have several positive effects [24]. Nearly one-third of the watermelon is made up of the watermelon rind (WMR) [25]. Alkaloids, saponin, cardiac glycosides, flavonoids, phenols, lipids, proteins, citrulline, fiber, carbs, vitamins, and mineral salts are all present in the WMR. WMR has a wide range of advantages for metabolic health, including antioxidative, antihypertensive, antidiabetic, hypolipidemic, and vasodilating properties, according to epidemiological research. Therefore, there should be a possibility for this solid waste to be transformed into usable products that can be added to the human

diet [26]. To conclude, foods enriched with health-improving ingredients (phenols, carotenoids and other colors, vitamins, dietary fibers, among other things) may be created through the right use of waste materials obtained from horticultural products in order to mitigate environmental issues and promote human health [11].

3 Bioactive Compounds in Fruits with Potential Health Benefits

Fruit processing produces two different types of waste: solid waste made up of pulp remnants, rind, peel, pomace, skin, seeds, and stones; and liquid waste made up of juice and wash waters, which has a high proportion of bioactive compounds and a higher potential to be used as functional ingredients in other formulations. Due to ignorance and lack of understanding of the need of turning "waste into wealth," these wastes are typically dumped or are merely used as a feed source. The fruit's varied parts, such as the peel/skin or the kernels, are concentrated with distinct bioactive substances in varying amounts. Various studies have found that fruit kernels and skins are rich sources of phytochemicals and essential minerals. For instance, the phenolic content of the skin and seeds of many fruits and vegetables, such as grapes, lemons, oranges, and avocados, as well as the seeds of jackfruit, mangoes, and peaches, is significantly higher than that of the pulp [27–30]. Bioactive compounds like phenolic compounds (phenolic acid, carotenoids, flavonoids), bioactive proteins (peptide isolate, amino acids), biogenic amines, fatty acids, fibers, and others can all be found in abundance in fruit waste byproducts (Table 1). Phytochemicals, phytosterols, and essential oils, for instance, can all be found in large quantities in fruit seeds. Pectin, priceless fibers, and minerals are also present in the peels [31]. The bioactive substances reduce the risk of developing heart-related illnesses, cancer, cataracts, Alzheimer's, Parkinson's, and aging-related disorders. These substances work defensively against chronic diseases, limiting the development of carcinogenic molecules and balancing the immune system because of their high antioxidant and antibacterial activity. These substances are advantageous whether taken as dietary supplements or as an ingredient in functional foods. In addition to their benefits for nutraceuticals, natural antioxidants and colorants may be preferable to synthetic antioxidants in the processing and pharmaceutical industries [32–34]. The most abundant bioactive molecules present in fruit wastes are enzymes, oils, carotenoids, vitamins, polyphenols with minor portions of biologically active proteins, and biogenic amines. The presence of these bioactive molecules in different portions of fruit and their potential health benefits with examples is discussed in a later session.

Table 1
Summary of bioactive compounds in major fruit waste

Fruits	Parts	Major bioactive compounds and antioxidant activity	Concentration	References
Apple	Pomace	Malic acid	1.08 g/100 g	[63–66]
		Total polyphenol	4620 mg/ kg	
		Quercetin, phloridzin, phloretin	3.31 mg/g	
		Chlorogenic acid	6.89 mg/g	
		Flavonoids	2153–3734 mg/kg dw	
		Anthocyanins	50–130 mg/kg dw	
		Dihydrochalcones	688–2535 mg/kg dw	
	Seed	Linoleic acid	63.76 g/100 g	[64, 67]
		Phloridzin	2.96 μg/g	
		Oleic acid	34.84 g/100 g	
		Antioxidant activity (DPPH)	0.71 μg TROLOX/g	
		Total phenolic acid	1.61 μg GAE/g	
		Hydroxybenzoic acid	2.99 mg/g	
Banana	Peel	Total phenolic content	872.7 mg of GAE/100 g DM	[68–74]
		Gallocatechin	91.9 mg/100 g DM	
		Epigallocatechin	65.9 mg/100 g DM	
		Gallic acid	47 mg of (GAE)/g DM	
		Rutin	973.08 mg/100 g DE	
		Myricetin	11.52 mg/100 g DE	
		Kaempferol	9.30–28.80 μg/mL	
		Isoquercitrin	10.47–14.54 μg/mL	
		Naringenin	8.47 mg/100 g DE	
		Ferulic acid	1.63–60 mg/100 g DM	
		Cinnamic acid	1.93 ng/g	
		Alpha-hydroxycinnamic acid	40.66 ng/g	
		Sinapic acid	10.29 ng/g	
		p-Coumaric acid	8.05 ng/g	
		Dopamine	1.17–1.72 mg/g DM	
		L-dopa	0.31 mg/g DM	
	Stem	Phenols	5.5743 mg/kg	[75]
		Phytate	1.2967 mg/kg	
		Hemaglutinin	1.8814 mg/kg	
		Ascorbic acid	0.44 mg/mL	
		β-Carotene	0.066 mg/100 mL	
		Lycopene	0.006 mg/100 mL	
		Total flavonoid	0.032 mg EQ/g	
		Saponin	14.494%	
		Alkaloid	0.347%	
		Flavonoid	0.253%	
		Tannin	67.594%	
		Hydroxyl scavenging activity (IC 50%)	0.543%	
			1.318%	
		Chelating effect of ferrous iron (IC 50%)	1.296%	
		Hydrogen peroxide scavenging activity (IC50%)		

(continued)

Table 1
(continued)

Fruits	Parts	Major bioactive compounds and antioxidant activity	Concentration	References
Citrus (orange)	Peel	Phenolic acids	103 mg/100 g	[76]
		Gallic acid	3 mg/100 g	
		Hydroxybenzoic acid	2 mg/100 g	
		Chlorogenic acid	12 mg/100 g	
		Syringic acid	2 mg/100 g	
		Vanillic acid	2 mg/100 g	
		Rosmarinic acid	8 mg/100 g	
		Trans-2-hydroxycinnamic acid	4 mg/100 g	
		Trans-cinnamic acid	2 mg/100 g	
		p-Coumaric acid	34 mg/100 g	
		Ferulic acid	33 mg/100 g	
		Flavonoids	33 mg/100 g	
		Epicatechin	4 mg/100 g	
		Catechin	4 mg/100 g	
		Rutin	14 mg/100 g	
		Naringin	7 mg/100 g	
		Flavone	3 mg/100 g	
	Seed	Total phenolic compounds	5.60 mg/kg	[77]
		Total carotenoids	7.85 μg β-carotene/g	
		Phytosterol	158.21 mg/100 g	
		Tocopherols	153.67 mg/kg	
Dates	Seed	Lignin	24.34%	[67, 78]
		Cellulose	20.63%	
		Hemicellulose	13.49%	
		Protocatechuic	7.9 mg/100 g	
		Caffeoylshikimic	28.3 mg/100 g	
	Date press cake	Total phenolic content	11.79 mg of GAE/g	[79]
		Total flavonoid content	1.89 mg of quercetin/g	
		IC50		
		Cupric reducing antioxidant capacity	1.42 mg/mL	
		Ferric reducing antioxidant potential	1.57 mg Vit C/g	
		Chelating effect	9.32 mg Vit C/g	
			7.87%	
Guava	Peel	Total phenolic compounds	589.49 mg GAE/100 g	[67]
		Anthocyanin	121.85 Eq. mg CyGE/100 g	
		Total flavonoids	374.05 mg de catechin/100 g	
		Carotenoids	4.47 mg of β-carotene/mL	
	Seed	Vitamin C	87.44 mg/100 g	[67, 80]
		Carotenoids totals	1.25 mg/100 g	
		Soluble dietary fiber	0.39 g/100 g	
		Insoluble dietary fiber	63.55 g/100 g	
		Linoleic acid	75.25%	
		Oleic acid	11.40%	
		Palmitic acid	6.6%	

(continued)

Table 1
(continued)

Fruits	Parts	Major bioactive compounds and antioxidant activity	Concentration	References
		2-Chloroethyl linoleate	44.53%	
		2-Pentanone 4-hydroxy-4-methyl	23.56%	
		n-Hexadecanoic acid	9.85%	
		Tocopherol (α-tocopherol)	654 ppm	
		Carotenoids (β-carotene)	19.24 ppm	
		Phytosterol (stigmasterol, campesterol)	420 mg/100 g 9.6 mg/100 g	
		Vanillin	3.9 mg/100 g	
		Vanillic acid	2.4 mg/100 g	
		Cinnamic acid	9.4 mg/100 g	
		Cinnamaladehyde	1048.9 mg/100 g	
		β-sitisterol	82.6 mg/100 g	
		γ-tocopherol		
Jackfruit	Peel	Phenolic content	10.0 mg CE/g	[81, 82]
		Flavonoids	158 mg GAE/g	
		Gallic acid	12.08 μg/g	
		Ferulic acid	13.41 μg/g	
		Tannic acid	5.73 μg/g	
	Seed	Polyphenols	243 mg GAE/100 g	[81, 82]
		Flavonoids	2.03 mg EC/100 g	
		Tannins	0.06–0.229 mg/100 g	
		Gallic acid	1.105 mg/100 g	
		Ferulic acid	0.216 mg/100 g	
	Flesh	Gallic acid	19.31 μg/g	[81, 82]
		Ferulic acid	2.66 μg/g	
Papaya	Peel	Gallic acid	18.06 μg/g DW	[83, 84]
		Caffeic acid	29.28 μg/g DW	
		P-coumaric acid	38.16 μg/g DW	
		Ferulic acid	95.46 μg/g DW	
		Quercetin	3.17 μg/g DW	
	Seed	Total phenolic content	1080 mg GAE/100 g DW	[83, 84]
		Total flavonoid content	117.7 mg GAE/100 g DW	
Pineapple	Peel	Phenolic content	5803.221 mg GAE/g	[85–87]
		Gallic acid	31.76 mg/100 g DW	
		Catechin	58.51 mg/100 g DW	
		Epicatechin	50.00 mg/100 g DW	
		Ferulic acid	19.5 mg/100 g DW	
		Bromelain	13.158 μm/mL	
		Carotenoids	1.82 μg/g DM	
	Core	Phenolic content	1543.51 mg GAE/g	[86, 87]
		Bromelain	24.13 μm/mL	
		Ferulic acid	19.5 mg/100 g DW	
		Carotenoids	0.35 μg/g DM	
	Crown	Total phenolic content	41.34 mg GAE/g DM	[86, 87]
		Total flavonoid content	33.69 mg QE/g DM	
		Bromelain	113.79 μm/mL	

(continued)

Table 1
(continued)

Fruits	Parts	Major bioactive compounds and antioxidant activity	Concentration	References
Pomegranate	Peel	Total phenolic content	18–510 mg/g DW	[88, 89]
		Tannin	193–420 mg/g DW	
		Flavonoids	84–134 mg/g DW	
		Gallic acid	123.79 mg/100 g DW	
		Ellagic acid	35.89 mg/100 g DW	
		Caffeic acid	20.56 mg/100 g DW	
	Seed	Total polyphenol	11.84 mg GAE/g	[90]
		Total flavonoid content	6.79 mg RE/g	
		Total anthocyanin	40.84 mg CGE/g	
		Tannins	29.57 mg TAE/g	
Tomato	Peel	Chlorogenic der	33–141.10 mg/kg	[91–93]
		p-Coumaric	07.38–26.58 mg/kg	
		p-Coumaric der	16.70–101.99 mg/kg	
		Quercetin	5.04–13.68 mg/kg	
		Rutin	107.06–410.13 mg/kg	
		Rutin der		
		Naringenin	36–109.75 mg/kg	
		Lycopene	73.52–287.62 mg/kg	
		B-carotene	167.43 mg/kg DW	
		Lutein	55.20 μg/g DW	
		Tocopherols	0.65–1.54 mg/100 g	
		Caffeic acid-glucoside isomer	1.62 g/100 g dw	
		Caffeic acid	0.74 mg/100 g	
		Syringic acid	0.55 mg/100 g	
		Di-Caffeoylquinic acid	0.547–1.122 mg/100 g	
		Tri-Caffeoylquinic acid	0.812–1.113 mg/100 g	
			0.591–0.662 mg/100 g	
	Seed	Gallic acid	0.11–6.94 mg/100 g	[91, 94–99]
		Ferulic acid	1.67–9.08 mg/100 g	
		Kaempferol	<0.001–2.01 mg/100 g	
		Quercetin		
		Rutin	<0.001–0.90 mg/100 g	
		Coumaric acid		
		Phloridzin	0.065–3.53 mg/100 g	
		Phloretin	2.58 mg/100 g	
		Procyanidin B2	1.35 mg/100 g	
		Naringenin	26.72 mg/100 g	
		Myricetin	76.62 mg/100 g	
		Caffeic acid	0.16–0.35 mg/100 g	
		Vanillic acid	0.34–0.88 mg/100 g	
		Sinapic acid	0.95–2.19 mg/100 g	
		Chlorogenic acid	2.01 mg/100 g	
		Lycopene	1.82–3.56 mg/100 g	
		ß-Carotene	0.05–1.41 mg/100 g	
			1.6–16.70 mg/100 g	
			0.093–5.500 mg/100 g	

(continued)

Table 1
(continued)

Fruits	Parts	Major bioactive compounds and antioxidant activity	Concentration	References
Watermelon	Rind	Total phenolic content	385–507 mg CAE/kg	[100]
		Polyphenols	63.33 mg TAE/g	
		Citrulline	24.7 mg/g	
		Flavonoids	1.105 mg RE/g	
	Seed	Total phenolic content	0.087 mg GAE/g	[101–105]
		Sinapic acid	152.330 μg/mL	
		Gallic acid	2.559 μg/mL	
		Caffeic acid	1.33 μg/g	
		Lignans pinoresinol	1.02 μg/g	
		Alkaloids	28.33 mg/g	
		Flavonoids	0.67 mg/g	
		Tannins	50.6–64.5 mg/g	

3.1 Enzymes

Enzymes are used in a biochemical reaction as a biocatalyst, with several health benefits that aid in the digestion process by a partial breakdown of complex biomolecules, that is, fat, carbohydrate, and protein, into its smaller building blocks which ultimately makes our stomach easier to breakdown and absorb necessary nutrients. The most abundant enzymes in the fruits are amylase, protease, and lipase. Amylases are enzymes responsible for the breakdown of carbohydrates into their simpler sugar, whereas proteases are responsible for the breakdown of protein molecules into their small peptides as well as in the simpler form of amino acids, and lipases are responsible for the breakdown of fats into three fatty acids along with a glycerol molecule [35]. Some examples of fruits that contain enzymes are:

- Pineapple consists of protease enzymes, especially bromelain.
- Papaya also consists of protease enzymes called papain.
- Mango consists of amylase enzymes.
- Banana consists of amylase and glucosidases.
- Avocado consists of lipase enzymes and polyphenol oxidase in small quantities.
- Kiwi fruit consists of a protease called actinidain.

Lack of these enzymes in the stomach causes difficulty in the breakdown process and can lead to digestive issues, or problems like food intolerances in some cases. Thus, consuming fruit enzymes that are natural digestive enzymes can help to get rid of this problem. There are several additional health benefits of fruit enzymes as these are found to act as antioxidants having anti-carcinogenic effect and also have been proven to maintain cardiovascular health,

increase the buffering capacity of blood by maintaining the pH level of the bloodstream, and increase the body's immune system to prevent common illness and several forms of infections [36–39].

3.2 Oils

Fruit waste, especially seed/kernel parts, has a higher amount of bioactive oil concentrated in it as compared to other parts. In the research conducted by da Silva and Jorge [40] for different fruit seeds, the lipid content ranged from 7.0% to 40.4%. Kernels/seeds from different citrus fruits, apples, guava, tomato, grape, mango, pumpkin, passion fruit, orange, melon, kumquat, etc. contain a different proportion of fatty acids in their oil. These oils are potential sources of essential fatty acids. Major fatty acids in fruit oil are palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidic acid (C20:0). For example, in mango seed oil, steric acid (C18:0) and oleic acid (C18:1) are the major fatty acids. While in papaya, mango, and soursop seed oil, oleic acid (C18:1) and linoleic acid (C18:2) are the major fatty acids. These oils are utilized more frequently because of their greater health advantages in the chemical, pharmaceutical, cosmetic, and food industries, as well as for the direct development of functional foods. Fruit seeds and their oils have added nutritional and health benefits due to phytonutrients and phytochemicals, which are natural chemicals that take part in a variety of biological processes, improve health status, or prevent or treat sickness situations. Similar advantageous effects have also been connected to other substances, including phenolics, tocopherols, and carotenoids as well as lipids like FA, sterols, or polar lipids. Fruit seed oils have been shown to have antioxidant, antiproliferative, anti-inflammatory, and anti-diabetic properties. Tocopherols act as primary antioxidants, as they donate one hydrogen atom to the peroxide radical, thus, interrupting the chain oxidative process. There are 4 types of tocopherols mostly present in fruit oil, namely: α-Tocopherol, γ -Tocopherol, β- Tocopherol, and Δ-Tocopherol. α-Tocopherol is the most active homologous in humans, and it performs the biological role of vitamin E. Phytosterols and phytostanols present in fruit oil perform hypocholesterolemic activity after ingestion by reducing cholesterol absorption by the intestine.

3.3 Carotenoids

Carotenoids are present in abundant amounts in both edible and inedible portions of fruits and vegetables, but significantly higher in inedible portions like peels and pomace. Different types of conventional and novel green techniques are used to extract carotenoids from fruit waste. Carotenoids in higher concentration have been successfully extracted from many fruits wastes, that is, carrot biowaste (8.27 mg/100 g), mango pulp (2.18 mg/100 g), passion

fruit peels (1.18 mg/100 g), seabuckthorn pomace (28.3 mg/100 g), red jalapeno pepper (230.54 mg/100 g), tomato byproducts (631.1 mg/100 g), sweet peppers (41.37 mg/100 g), etc. [41]. The amount of total carotenoids ranged from 0, in mango seed oil, to 49.40 mg β-carotene/g, in papaya seed oil [40]. These are lipid soluble components, mainly exerts pro-vitamin A function, along with this they support the immune system, protect skin from harmful UV radiations, fasten wound healing process, and show antioxidant capacity, as they protect the cell against lipid oxidation, preventing the risk of degenerative diseases, such as cancer, heart diseases, and cell degeneration [41]. So, these properties made carotenoids to be used in the food, pharmaceutical, cosmetic, and feed industries.

3.4 Vitamins

Fruit waste is a rich source of several water-soluble vitamins, including thiamine, niacin, riboflavin, vitamin C, and niacin, as well as lipid-soluble vitamins, including vitamins A, D, E, K, and carotenoids [42–44]. All three antioxidant vitamins A, C, and E can be found in abundance in berries. Ascorbic acid can be found in a wide variety of fresh fruits [45]. Vitamin C plays a crucial role in the prevention of cancer because of its potent antioxidant action, which shields our cells from oxidative damage. It is an effective electron donor in biological systems in addition to possessing redox potential. Vitamin C lowers oxidative stress on the stomach's mucosa, DNA damage, and inflammation via scavenging reactive oxygen species (ROS). By converting nitrous acid in the stomach to nitric oxide and generating dehydroascorbic acid, it also prevents gastric nitrosation and the creation of N-nitroso compounds. And finally, it strengthens the host's immune system. It inhibits stomach cell growth and induces apoptosis, which directly affects the growth and virulence of *Helicobacter pylori* [46]. The B-complex vitamins (thiamine, riboflavin, and niacin) are crucial cofactors in biochemical processes and are necessary for healthy skin, normal body growth and development, appropriate heart and neuron function, and the production of red blood cells. Since individuals who suffer from heart failure have a vitamin B deficit, vitamin Bs are directly engaged in energy metabolism, and there is growing interest in their potential to prevent heart failure [47]. Antioxidant-rich vitamins, such as lipid-soluble vitamins A, D, E, and K, can reduce the risk of cardiovascular disease, cancer, and neurological disorders [48]. Red and purple fruits have significant quantities of anthocyanins, which have bacteriostatic and bactericidal activity against many pathogens (including *Staphylococcus* sp., *Klebsiella* sp., and *Helicobacter and Bacillus*). Anthocyanins possess a variety of biological effects, including anti-tumor, anti-inflammatory, antioxidant, anti-diabetic, and neuroprotective [49].

3.5 Alkaloids and Polyphenols

Alkaloids and polyphenols are secondary metabolites of plants which act directly by reducing activity, scavenging the free radical and indirectly by chelating the prooxidant metal ions. Alkaloids are water soluble compounds containing one or more nitrogen atom in their molecule and possess significant biological activity. They directly interact with neurotransmitters and results in various psychological and physiological responses in human body [50]. Caffeine, theobromine, pipernine, quinine, capsaicin, solasodine, solamargine, and solasonine are some examples of alkaloids commonly present in fruit waste [51]. Among all phenolics, dietary phenolics, that is, polyphenols, flavonoids, and phenolic acids, are considered to have major health benefits and preservative action in food formulations. These compounds are rich in peel, rind, and seeds of fruit waste. The major phenolic compounds present in fruit seed oils are p-Coumaric acid, salicylic acid, and quercetin, with a significant amount of gallic acid, catechin, caffeic acid, and epicatechin in some fruits. Peels of citrus fruits contain bioactive components and have been used traditionally in various places to treat cough, muscle pain, digestive issues, and skin inflammation. Peels of pomegranate contain punicagranine, an anti-inflammatory pyrrolizine alkaloid. Annona crassiflora fruit peels' polyphenol-rich fraction exhibits antioxidant properties that may find use in the treatment of diabetes in clinical settings [52].

3.6 Bioactive Polysaccharides and Dietary Fibers

These part of the fruit waste does not have a nutritional function as they are resistant to enzymatic digestion in our body but has a vital role in digestive function commonly known as cellulose and noncellulosic polysaccharides, that is, pectic substances, hemicellulose, gums, mucilages, and other noncarbohydrate portions, such as lignin, categorized as dietary fiber [53]. These compounds possess several health benefits, such as regulating the feces output; reducing the risk of obesity by providing a satiety effect, diabetics, and hypertension; and lowering the occurrence of colorectal cancers by trapping mutagenic substances and not allowing them to reach the bloodstream [54].

3.7 Bioactive Protein and Peptides

The nonedible portion of fruits is a good source of bioactive proteins. For example, peels of citrus fruits ranged from 2.5% to 9.0% protein content [55, 56], whereas peels of papaya, kiwi, and avocado fruit were found to be 1.55%, 1.79%, and 1.57%, respectively [57]. Some enzymes have been discovered, which exert bioactivity like actinidin from the seeds of kiwi fruit [58], vicilin-like protein watermelons seeds [59], and leptin from seeds of jackfruit [60]. Citrus natural peptides have been investigated as a potential source of novel atheroprotective medicines due to their comparative benefits over small molecules in the creation of anti-inflammatory and cardioprotective drugs.

3.8 Biogenic Amines

The biogenic amines represent the nitrogenous compounds resulting from several enzymatic reactions, such as reductive amination, decarboxylation, transamination, and degradation of the corresponding precursor amino acids. Vasoactive amines, that is, tryptamine, histamine, and tyramine have a positive role in blood pressure control [61]. Five amines have been found in the study conducted by [62] for pineapple, papaya, guava, mango, and passion fruit, that is, spermidine, putrescine, agmatine, serotonin, and spermine. The total amine levels varied from 0.77 to 7.53 mg/100 g in mango and passion fruits, respectively. Among these amines, spermidine was detected in every test fruit sample, whereas spermine and putrescine were detected in most of the samples. Agmatine and serotonin were found in abundant amounts in passion fruits, papaya, and pineapple. Passion fruit seemed to be a good source of polyamines spermine and spermidine, with 2.43 and 3.05 mg/100 g, which has an important role in maintaining health, growth, and antioxidant activity and regulating membrane permeability. Serotonin governs your mood and is responsible for happiness. It also controls when you sleep and wake up, helps you think, keeps your mood stable, and regulates your sexual drive. It is also linked to the stomach to mediate reflex action and to lower the risk of thrombosis.

4 Extraction Technologies for Bioactive Compounds from Fruit Waste

In order to utilize the bioactive compounds from fruit waste, extraction is the most important step. Generally, extraction is carried out using different solvents, acids, alkalis, steam diffusion, and hydro-distillation. Selection of method of extraction depends on the bioactive compound to be extracted, source (raw materials) and desired purity. Extraction of bioactive compounds could be affected by several factors, such as extraction conditions (temperature, pressure), part of the fruit (peel, pomace, seed), particle size, extraction time, and solvent used for extraction. The methods and condition of extraction play a significant role in terms of purity, yield, time of extraction, cost-effectiveness, and effect on the structure of bioactive compounds [106]. Therefore, the extraction method and parameters should be selected based on the target bioactive compound and source (raw material). Bioactive compounds from fruit waste can be extracted mainly by conventional extraction techniques and emerging/novel extraction techniques. Extraction is an effective way to obtain bioactive compounds. However, high temperature and long extraction duration cause instability and loss of functionality of extracted bioactive compounds. Figure 1 shows the schematic diagram of different extraction technologies for the extraction of bioactive compounds from fruit waste. Table 2

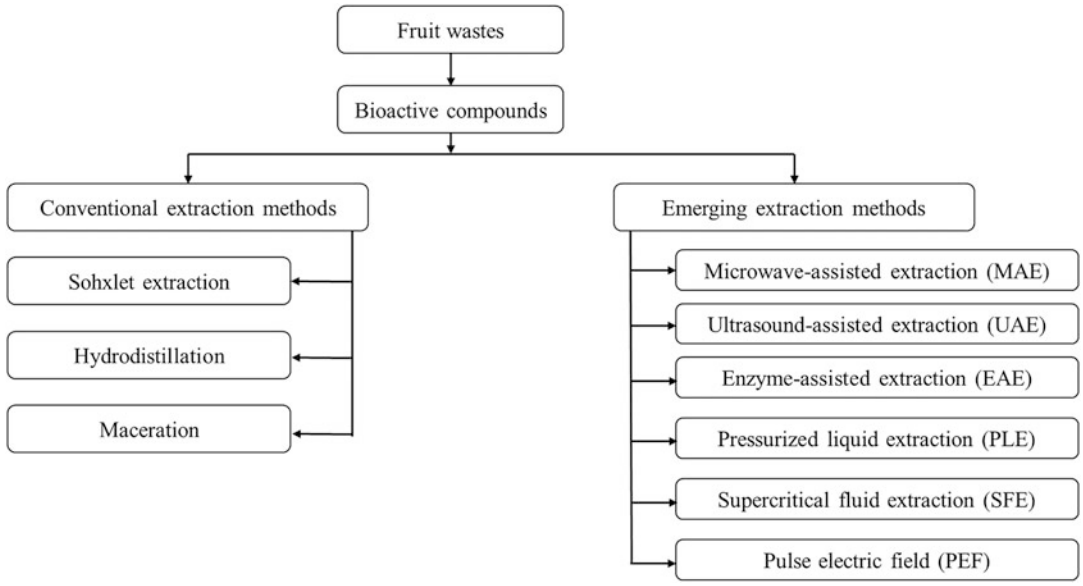

Fig. 1 Schematic diagram of different extraction technologies for the extraction of bioactive compounds from fruit waste

shows the extraction of bioactive compounds from different fruit waste using conventional as well as emerging technologies. Both technologies are discussed below.

4.1 Conventional Extraction Technologies

Conventional extraction techniques are the traditional extraction methods that are in use since ancient times and are the most frequently used techniques for extracting bioactive compounds from plants. The main principle of this extraction technique is thermal treatment and solvent extraction. Most of the conventional method depends upon the extraction potential of solvent and application of heat or agitation. Moreover, the process is strongly dependent on the polarity of the extracting solvent. However, this method has low efficiency, longer extraction time, and high temperature and requires a large volume of organic solvent [13, 128]. Most common conventional extraction techniques include (a) Soxhlet extraction, (b) hydro-distillation, and (c) maceration.

4.1.1 Soxhlet Extraction

Soxhlet extraction has been used as a classical method of extracting bioactive compounds from plant parts since ancient times. Though the Soxhlet extraction method was designed for lipid extraction, this method has been popularly used to extract bioactive compounds from plants part including fruits. The advantages of Soxhlet are it is simple and inexpensive. Nevertheless, this is time-consuming and requires a large amount of solvents; thus, it is not environmentally friendly. Alcohols, mostly ethanol and methanol,

Table 2
Extraction of bioactive compounds from different fruit waste using conventional and emerging technologies

Extraction technique	Fruit waste	Bioactive compounds	Extraction conditions	Results	References
Soxhlet extraction	Grape peel	Phenolic compounds		80 mg GAE/g	[107]
Maceration	Grape and orange residue	Phenolic compounds		67.1 and 146.6 mg GAE/g (dry weight)	[108]
	Tangerine peels	Total phenolic content	40 °C, 80% methanol		[109]
	Peels of apple, apricot, avocado, banana, custard apple, dragon fruit, peach, pear, pineapple, plum, pomegranate	Phenolic compounds	4 °C, 70% ethanol, 120 rpm agitation	0.45–25.2 mg GA/g	[110]
	Mango seed kernel	Total phenolic content	50% ethanol	107.7 mg GAE/g	[111]
Microwave-assisted extraction (MAE)	Pomegranate peel	Phenolic compounds	600 W	199.4 mg GAE/g dry weight	[112]
	Pomegranate peel, carob fruit peel, banana peel	Polyphenols			[113–115]
	Avocado peels	Phenolic compounds	500 W, 95.1 s	281.4 mg GAE/g dry weight	[116]
Ultrasound-assisted extraction (UAE)	Grape seeds	Total phenolic contents	53.14% ethanol, 46.03 °C and 24.03 min	Higher yield	[117]
	Seeds and peels of avocado	Trans-5-O-caffeoyl-D-quinicacid, procyanidin B1, catechin, and epicatechin	Ethanol-water solvent, 15 min		[118]
	Fermented grape pomace	Malvidin-3-O-glucoside, peonidin-3-glucoside, petunidin-3-gucoside, and delpinidin-3-glucoside	24 kHz		[119]
	Pomegranate peel	Phenolic content and total flavonoids	140 W, 30–40 kHz, 30 min	69.87 and 7 mg/g	[113]
	Tomato paste	Lycopene	98 W (microwave), 40 kHz, 50 W, 86.4 °C, 365 s	Extraction yield 97.4%	[120]
	Grape cane byproducts	Stilbenes	75 °C, ethanol (60%), 10 min		[121]

(continued)

Table 2
(continued)

Extraction technique	Fruit waste	Bioactive compounds	Extraction conditions	Results	References
Supercitical fluid extraction (SFE)	Mango peel	Flavonoids and carotenoids	30 MPa, 40 °C, carbon dioxide flow rate 1.1 L/min, 7.7 h		[122]
Pulse electric field (PEF)	Grape byproducts	Anthocyanins	3 kV/cm	2 folds higher than control	[123]
	Mango peel	Mangiferin, quercetin, ellagic acid	13.3 kW/cm, 60 °C, 1000 kJ/kg	Extracted with high clarity and colloidal stability	[124]
Enzyme-assisted extraction (EAE)	Guava leaves	Phenolics	Cellulase or beta-glucosidase-assisted extraction	Yield increased by 103.2%	[125]
	Pistachio green hull	Phenolic compounds	Cellulase, pectinase and tannase, pH 4.0, 37 °C	Yield increased by 112%	[126]
Pressurized liquid extraction (PLE)	Pomegranate peel	Phenolic compounds	3 MPa, 126.1 °C, solvent-solid ratio 54.8 mL/g, 18.5 min		[127]

are the most common solvents used for extraction of bioactive compounds. Other solvents used include chloroform, carbon tetrachloride, chlorobenzene, acetone, and acetonitrile [129]. The selection of solvent depends on several factors, such as target compounds, toxicity, cost, and polarity. This extraction technique is widely used to extract alkaloids, terpenes, terpenoids, and phenolic compounds. However, this process produces undesirable residue, and the extract obtained might undergo oxidative transformation during solvent removal. Moreover, this method requires longer extraction time and a large amount of solvent [130]. The process is mainly affected by solubility, mass transfer, and solid material characteristics. A small quantity of dried sample is kept in thimble, and the distillation beaker is filled with appropriate solvent. As the solution attains overflow point, it is aspirated from thimble holder and transferred to the distillation flask. The extract is held in this combination, which transfers it into the liquid in bulk. The extract solute stays in the distillation flask, whereas the solvent stays with the solid sample. The process is repeated continuously until the extraction is complete [11]. Caldas, Mazza [107] performed a comparative study of conventional (Soxhlet) and nonconventional extraction methods to obtain phenolic compounds from grape peel. The authors reported that the Soxhlet method (80 mg GAE/g) resulted in higher extraction yield than nonconventional methods (around 65 mg GAE/g). However, the phenolics recovered nonconventionally possessed the highest purity.

4.1.2 *Hydro-distillation*

Several bioactive compounds including oils are extracted using hydro-distillation technique. Water and steam distillation, water distillation, and direct steam distillation are three different types of hydro-distillation methods. In this extraction technique, the sample is completely immersed in boiling water. This process undergoes different physicochemical processes such as hydrodiffusion, hydrolysis, and thermal decomposition. However, this technique is not appropriate for heat-sensitive compounds as they might be lost or degraded during the extraction process. The advantages of this extraction technique are: no use of organic solvents and shorter extraction time.

4.1.3 *Maceration*

Maceration is a cost-effective extraction technique popularly used at the household level to obtain bioactive compounds from plant parts. In this method, ground samples with small particle size (for increased surface area) and appropriate amount of menstruum (solvent) are mixed in a closed container. Then the mixture is pressed and an extract-rich liquid is strained. Occasional stirring is performed to intensify the solvent diffusion and separate concentrated bioactives from sample surface. Maceration is one of the best methods to extract thermolabile (heat-sensitive) bioactive compounds [131]. Different types of maceration techniques are: simple

maceration, double maceration, and triple maceration. Soto, Quezada-Cervantes [108] extracted 67.1 and 146.6 mg GAE/g (dry basis) of total phenolic content from grape and orange residues, respectively. A higher amount of total phenolic content was extracted from tangerine peels with maceration at a temperature of 40 °C using 80% methanol as solvent [109]. Suleria, Barrow [110] extracted phenolic compounds using maceration in the range of 0.45–25.5 mg GAE/g from peels of apple, apricot, avocado, banana, custard apple, dragon fruit, grapefruit, kiwifruit, mango, lime, melon, nectarine, orange, papaya, passion fruit, peach, pear, pineapple, plum, and pomegranate with 70% ethanol, agitation at 120 rpm and 4 °C. Furthermore, Lim, Cabajar [111] compared maceration at different concentrations of ethanol to extract total phenolic contents from mango seed kernel and found that maximum extraction (107.7 mg GAE/g) with 50% ethanol. Overall, the factors affecting the yield and efficiency of conventional extraction techniques are the type of solvent used, polarity of bioactive compounds, and mass transfer rate.

4.2 Emerging Extraction Technologies

Conventional extraction technologies possess some pitfalls, such as the use of large quantities of costly solvents, longer extraction time, thermal degradation of heat-sensitive bioactive compounds, low extraction yield, and low purity and selectivity. Therefore, to overcome those limitations of conventional extraction methods, novel/emerging extraction technologies have been developed. Emerging extraction technologies reduce the use of harmful organic solvents, increase extraction rate, reduce energy use, increase heat and mass transfer, improve extraction yield, and reduce processing steps [132, 133]. Recently, emerging technologies such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and pulse electric field (PEF) are considered effective for the extraction of bioactive compounds from fruits.

4.2.1 Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) reduces the amount of solvent used and extraction time as compared with conventional extraction methods. Microwave-assisted extraction involves direct application of nonionizing electromagnetic waves in the range of 0.3–300 GHz to food samples. The common frequency used is 2450 MHz, which is equivalent to 600–700 W energy. MAE generally possesses several steps because of heat and mass gradients generated into the matrix, which includes (a) penetration of the solvent into the matrix, (b) solubilization and/or breakdown of the components, (c) transport of the solubilized compounds from the insoluble matrix to the bulk solution, and (d) separation of the liquid and residual solid phase [134, 135]. This method can reduce extraction time and volume of solvent required, and improve

recovery of bioactive compounds as compared with the conventional Soxhlet extraction method because the localized pressure and temperature can enhance the penetration of solvents into the plant matrix and disrupt the cell wall to release bioactive compounds [136–138]. MAE is considered an efficient and fast method to extract bioactive compounds from plant matrices. Extraction of compounds results because of dipole rotation and ionic conduction by heating the sample in the meantime. Factors to be considered for the extraction of bioactive compounds using microwave are the amount of solvent, solid-solvent ratio, microwave power, dielectric properties of samples, sample moisture content, stirring effect, the temperature of extraction, and time. The stability of the extracted compound depends on the microwave power used, extraction time, and temperature. Most importantly, water, instead of organic solvents, is sufficient to use as solvent in this extraction process. The most common solvent used in microwave extraction is ethanol, which is used either alone or with water.

The sample usually should be dried and powdered prior to MAE as milling improves the extraction of phenolics. The decrease in particle size increases the surface area of the sample in contact with the solvent and reduces diffusion distances, resulting in greater mass transfer and yield [139]. While if the particle size is too small (<250 μm), a cleaning step might be required because this will make it difficult to separate the extract from the residue [135]. MAE has been widely used and studied for the extraction of bioactive compounds such as carotenoids, polyphenol, etc. from fruit byproducts. Studies have reported that pectin is the most extracted compound from citrus fruit waste and byproducts (citrus residues, peel, and bagasse) using MAE technology [140]. Kaderides, Papaoikonomou [112] used microwave-assisted extraction to obtain phenolics (199.4 mg GAE/g dry basis) from pomegranate peel.

MAE has been proved to be an effective process to extract highly intact bioactive compounds with better recovery than conventional methods. Recently, MAE has been gaining attention and is considered an excellent green extraction technique. The MAE is more effectively applied for the extraction of short-chain polyphenols, such as phenolic acids and flavonoids, which remain stable at microwave heating of 100 °C [141]. However, polyphenols that are polymeric with hydroxyl conjugates, such as tannins, or are heat sensitive, such as anthocyanins, could undergo structural damage during microwave treatment [142].

Previous literature have suggested that polyphenolic compounds from wastes such as pomegranate peel [113], carbo fruit peel [114], and banana peel [115] were extracted efficiently using MAE. Furthermore, coupling MAE with other extraction technologies could increase extraction yield and reduce cost when the

combination is designed with statistical and modeling resources such as RSM [143]. Trujillo-Mayol, Céspedes-Acuña [116] combined MAE with UAE for the extraction of bioactives from avocado peels; the combination included 15 min of sonication at 60 C, followed by 95.1 s of microwave irradiation (500 W). The combination resulted in maximum yield of total phenolic content (281.4 ± 0.2 mg GAE/g dry extract) and had higher efficiency compared with sonication and microwave application.

4.2.2 Ultrasound-Assisted Extraction

Ultrasound-assisted extraction (UAE), also known as sonication, uses a sound intensity of 5–1000 W/cm^2 sound wave with frequency from 20 kHz to 100 MHz [144]. Ultrasound creates compression and expression in the medium. This induces acoustic cavitation by forming collapsing bubbles in the solvent, generating localized pressure, which can permeabilize the cell wall, release intercellular content into the solvent, and improve mass transfer. Ultrasound-assisted extraction reduces the extraction time and volume of solvent required to extract bioactive compounds. Many process parameters such as the composition of the solvent, solvent-solid ratio, particle size, pressure, moisture, ultrasonication time and temperature, influence the extraction process and yield. Studies have suggested that use of frequency higher than 20 kHz affects the physicochemical properties of the extracted compounds because of formation of free radicals [145, 146]. Organic solvents, namely, ethanol, methanol, acetone, and isopropanol, are frequently used. To protect bioactive compounds from thermal degradation, a low temperature of below 50 °C is recommended. Ghafoor and Choi [117] used UAE to extract bioactive compounds from grape seeds and found that UAE significantly increased the yield of total phenolic contents, antioxidant activities, and anthocyanin content. Bioactive compounds, namely, trans-5-O-caffeoyl-D-quinicacid, procyanidin B1, catechin, and epicatechin, have been extracted from the seeds and peel of avocado with ethanol-water as solvent and extraction period of 15 min [118]. Moreover, phenolic compounds and anthocyanins such as malvidin-3-O-glucoside, peonidin-3-glucoside, petunidin-3-gucoside, and delpinidin-3-glucoside were extracted by Barba, Brianceau [119] from fermented grape pomace using UAE. Pectins, carotenoids, and lipids have been successfully extracted using UAE [147–149]. Furthermore, previous studies have suggested that UAE requires less energy, reduces extraction time, and improves extraction yield compared to conventional extraction technologies. More and Arya [150] extracted bioactive from pomegranate peel and reported higher yield with noticeable antioxidant and bioactive content. Similarly, phenolic content and total flavonoid content of 69.87 and 7 mg/g, respectively, with 67% antioxidant activity were obtained from pomegranate peel using UAE at

140 W power, 30–40 kHz frequency for 30 min [113]. The ultrasound-assisted extraction technology could be combined with other extraction technologies to increase extraction yield. Previous studies have reported that ultrasound-assisted extraction when combined with supercritical fluid extraction for the extraction of phenolic compounds from blackberry bagasse doubles the extraction yield than only using supercritical fluid extraction [151]. The extraction yield of lycopene from tomato paste of 97.4% was obtained when microwaves (98 W) were combined with ultrasonication bath at 40 kHz and 50 W, 86.4 °C, and extraction time of 365 s. However, 89.4% lycopene yield was obtained with UAE [120]. Piñeiro, Marrufo-Curtido [121] optimized and validated the ultrasound-assisted extraction process for rapid extraction of stilbenes from grape canes. The optimal conditions were extraction time of 10 min, extraction temperature of 75 °C, and ethanol as extraction solvent (60%). The study suggested that grape cane byproducts were potential sources of bioactive compounds. Different from conventional methods, UAE possesses the advantage of low operational temperature, which results in less thermal degradation and reduced extraction time.

4.2.3 Supercritical Fluid Extraction

Supercritical fluid is a hybrid media that possesses combined properties of liquid and gases in a single phase. Supercritical fluid extraction (SFE) does not require toxic organic solvent; hence, it is considered a green technology. The advantage of using supercritical fluids for recovery of bioactive compounds from fruit waste is that supercritical fluid has low viscosity and high coefficient of diffusion as compared to conventionally used liquid solvents. These properties promote efficient extraction of bioactive compounds by allowing the fluid to penetrate into the matrix to a greater extent. Fluids such as carbon dioxide, water, methanol, ethylene, ethanol, n-butane, and n-pentane at pressure and temperature above their critical points are used in SFE. The most used fluid in supercritical fluid extraction is carbon dioxide as it is relatively inexpensive, nonpolar in nature, nontoxic, and nonflammable. The low polarity of carbon dioxide enhances the extraction of nonpolar or small polar biomolecules such as lipid and volatile compounds [152]. However, supercritical fluid extraction with carbon dioxide could efficiently extract nonpolar or mid-polar compounds (essential oils and carotenoids). The yield and purity of extracted bioactive compounds from fruit waste depend on several factors, such as temperature, pressure duration, solvent flow rate, amount of co-solvent used, co-solvent flow rate, the nature of fruit waste, and prior processing technique (drying, lyophilization, etc.). Available literature suggests that supercritical fluid extraction has advantage over conventional extraction in terms of selectivity, compound stability, easy recovery, and time and energy saving. Pham [122]

extracted nonpolar flavonoids and carotenoids from mango peel using SFE at pressure 30 MPa, extraction temperature 40 °C, carbon dioxide flow rate 1.1 L/min, and extraction time of 7.7 h. Extraction with supercritical fluid extraction technique is harmless to both food components and human consumption. Moreover, toxic solvents used in other extraction are completely avoided, and the high energy required is reduced; hence, SEF has no environmental impact. In recent years, supercritical carbon dioxide has been considered the standard, powerful, and green extraction technology to valorize fruit waste because of safety and purity of extracts [11].

4.2.4 Pulse Electric Field Extraction

Pulse electric field extraction technology is an energy-efficient and environmentally friendly process that uses high voltage pulses of electricity, which is used in food processing to prolong shelf-life. However, it has been equally used for the extraction of bioactive compounds. In PEF treatment, the food sample is subjected to electrical resistance of 20–80 kV/cm for very short time (<1 s) to produce high energy discharges. When the electric pulse is applied to plant tissue, it increases tissue softness through electroporation of cell membranes. Electroporation depends on energy, time, and number of pulses. The pore size and wall/membrane disintegration increase with the intensity of electric pulses; the main factor in play to govern optimization of PEF extraction is electric field/mass ratio [144, 153–155]. Studies have suggested that use of PEF for bioactive compound extraction improves the extraction yield. Corrales, Toepfl [123] reported that the use of PEF to extract anthocyanins from grape byproducts enhanced the yield of anthocyanins, and PEF could also be used to selectively extract targeted bioactive compounds. Moreover, the extraction depends on pH, extraction time, electric field strength, pulse shape, pulse width, pulse frequency, food matrix density, and the chemical properties of the compound to be extracted. Parniakov, Barba [124] reported that application of pulse electric field–assisted extraction significantly increased the extraction yield of polyphenol (mangiferin, quercetin, ellagic acid) from mango peel. Moreover, Delsart, Ghidossi [156] extracted anthocyanins and polyphenols from grape skin using PEF and observed that the extraction yield of these compounds was higher than other extraction techniques. Plum and grape peels [157], orange [158] and lemon [159] peel residues, or olive pomace [160] are additional examples of byproduct valorization by PEF-assisted polyphenol extraction. PEF has several advantages over conventional extraction technology, which include: (a) no water removal or dehydration of samples, (b) additional chemicals not needed, (c) no heating needed, (d) less time-consuming, (e) scalability. However, more research is still needed on the effects of pulsed electric fields on pulsed matrices.

4.2.5 Enzyme-Assisted Extraction

Enzymes are also used to extract bioactive compounds from food waste including fruit waste. Enzymes such as cellulase, pectinase, hemicellulase, xylanase, β-gluconsae, β-glucosidase, and alcalase can decompose the cell wall materials (pectin, hemicellulose, and cellulose) of fruit waste and can assist in the extraction of bioactive compounds [161]. Since EAE utilizes water as solvent rather than organic solvents, it is considered as the most ecologically friendly extraction technique. Factors that should be considered during enzyme-assisted extraction are type and concentration of enzyme, extraction temperature, particle size, water-to-solid ratio, time, and pH. More specifically, pH and temperature are critical to activate catalytic potential. Further, the efficiency of enzyme-assisted extraction could be maximized when assisted with other processes such as ultrasound, microwave, and so on. Wang, Wu [125] treated guava leaves with cellulase or beta-glucosidase-assisted extraction and found that the extraction of phenolics increased by 103.2%. Enzyme-assisted extraction is gaining popularity in the valorization of polyphenol-rich matrices from food-agroindustry since it is an environmentally friendly approach [162]. Similarly, Ghandahari Yazdi, Barzegar [126] used the combination of different enzymes, cellulase, pectinase, and tannase, at optimal conditions (pH 4.0, 37 °C) to extract phenolic compounds from pistachio green hull and compared to the conventional control extract. They observed that the yield of phenolic compounds increased by 112%, and the antioxidant capacity was found to be 71% higher than extract without enzyme treatment [126, 163]. Treatment with cellulase and tannase to recover polyphenols from Syrah grape pomace increased the recovery of phenolics (up to 66%) as well as the antioxidant activity (up to 80%), compared to classic hydroalcoholic (50:50) extraction at 50 °C for 6 h [163, 164].

4.2.6 Pressurized Liquid Extraction

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), was introduced as an extraction technology by Dionex Corporation for the extraction of anthocyanin from seaweed [139]. PLE is also a green extraction technology that uses water instead of organic solvent and reduces the amount of solvent used to extract bioactive compounds from fruit waste. The pressurized liquid extraction technique employs pressure (between 5 and 15 MPa) to keep the solvent at a temperature higher than its boiling point. PLE, solid-liquid extraction technique, uses pressurized solvents at high temperature (>100 °C). The high temperature and pressure applied increases the solubility and mass transfer rate of bioactive compounds. The high pressure used aids the penetration of the solvent into the pores of the solid material and the high temperature facilitates diffusion of the solvent into the solid material. PLE has been frequently applied for the extraction of phenolic compounds, carotenoids, and tocopherols from fruit waste. Xi, He [127] have extracted phenolic compounds from pomegranate peel

with PLE at 3 MPa, 126.1 °C, solvent-solid ratio 54.8 mL/g, and extraction time of 18.5 min. The extraction of bioactive compounds using pressurized liquid extraction technique is the function of factors such as solvent polarity, toxicity of solvent, particle size, mass transfer rate, sample moisture content, temperature, pressure, and extraction time.

5 Current Application and Challenges

Fruit waste contains a variety of dietary, nutraceutical, and medicinal bioactive compounds. In the current scenario, these bioactive compounds are used as novel functional ingredients for enrichment and fortification in different food products for formulation of functional and nutritional products, in the chemical industry, bioremediation, energy, cosmetics industries, and pharmaceutical and nutraceutical applications. These bioactive compounds have substantial value to the food sector as being used as food and nutrient supplements, as colorants, and as an additive in meat and meat products for their preservative and enhancing action [165–167]. Polyphenols are used as antioxidants and colorants in different industrial applications. Fruit oils consist abundant quantity of antioxidants and fatty acids, due to which they are used in salad dressings, frying oil, food formulations, and skin care products as they possess anti-ageing, anti-inflammatory, and skin reconstructive properties. The bioactive chemicals derived from these oils may also be useful to the pharmaceutical and cosmetic sectors in addition to the food business. Several natural essences are being produced utilizing bioactive components of fruit waste, which are used as flavoring agents in pharmaceutical, food formulations, culinary, cosmetics, and detergent industries, that is, vanillin (4-hydroxy-3-methoxy benzaldehyde), furaneol (2,5-dimethyl-4-hydroxy-3 (2H)-furanone), ethyl acetate, decanal, citral, limonene, glucose and fructose, different esters, aldehydes, alcohols, acid, and lactones, etc. [11, 168, 169].

The present research trend is most inclined toward the isolation and identification of bioactive compounds but, there are limited studies conducted for the study of their toxicological effects, digestibility, bioaccessibility, bioavailability, and metabolism. So, these facts limit the potential use of bioactive compounds in industrial applications. For exploiting the value of isolated bioactive, that is, pigments, vitamins, polyphenols, oils, enzymes, etc., in vitro and in invo studies are essential. Studies are being conducted on the extraction, isolation, identification, purification, and characterization of bioactive from a single source only; still, there is a lot to go to understand the effect of the mixture of raw materials on the bioactivity of extract, whether the extract will show the antagonistic

or synergistic effect, as fruit processing industries creates waste combined of multiple sources. So, a sustainable approach will be utilizing the natural bioactive compounds in industrial applications from fruit wastes after a proper investigation of their bioactivity.

6 Conclusion and Future Perspectives

Based on the available literature and sophisticated state-of-the-art technologies bioactive compounds in fruit waste can be identified and quantified precisely. Fruit waste with higher content of specific bioactive molecule could be utilized for extraction. The advancement of food processing technologies opens the opportunity to utilize the increasing amount of fruit waste as a source of bioactive compounds, which eventually will contribute to economy and waste management. Moreover, the processing waste of fruit from industries and other different stages of food supply chain is very high; therefore, the disposal of such waste is a serious issue. If not disposed of properly, they might be hazardous to the environment. On the other hand, such waste could be a potential bioresource to extract bioactive compounds. This chapter discussed several conventional as well as emerging extraction technologies employed to obtain bioactive compounds that have high potential to be used as nutraceuticals and dietary supplements. In conclusion, the bioactive compounds present in the side stream of fruit processing have great potential for application in food and nutraceutical industries as bio-preservatives, stabilizers, and nutraceuticals agents. The utilization of fruit byproducts will help fruit producers gain extra revenue from fruit residue and help to mitigate the issue of proper disposal of fruit waste.

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Chapter 14

Plant Seeds: A Potential Bioresource for Isolation of Nutraceutical and Bioactive Compounds

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Abstract

The greatest difficulty facing the cosmos now is the ability to survive in excellent health. The idea that nature preserves plant seeds when they are dormant and have been sitting in the soil for a long time is the foundation for the current notion. Bacteria, fungus, and virus attack are deterred from attacking seeds with the assistance of antimicrobial phytochemicals, plant defensins, other associated antimicrobial biopeptide components, plant prebiotics, probiotics, postbiotics, etc., prepared by the seeds during its journey towards the matured plant. Nature uses this technique to safeguard the offspring. We merely need to understand what nature is trying to tell us. The aim of the chapter is to take us through this message of nature followed for the well-being and growth of plant life cycle. Readers shall be acquainted with the current state of knowledge on plant seeds as a potential bioresource for the extraction of nutraceutical and bioactive chemicals. Moreover, the chapter will take the reader through Mother Nature's ways of green extraction techniques like sprouting, germination, and fermentation for the health benefits of human beings.

Key words Plant seeds, Antimicrobial biopeptide, Prebiotics, Probiotics, Postbiotics, Green extraction, Sprouting, Germination, Fermentation

1 Introduction

Healthy survival is the current biggest challenge for the universe [1]. When it comes to people, animals, and plants, good survival is relevant in this context. In the end, it applies to the entire cosmos. Ecological equilibrium is the name we can give it [2]. The largest difficulty the universe is currently facing is keeping this balance. Humans are involved in the hunt for answers to address this imbalance that is causing the turmoil in regard to everyone's health since they are morally responsible beings with more active minds than animals and plants [3]. Researchers are deeply involved in figuring out the answers for leading a healthy lifestyle, whether it is by

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medication, preventative measures, physical activity, yoga, eating habits, etc. It is indeed time to reflect, comprehend, and pinpoint the natural processes that have been in place to preserve ecological balance. Let us imagine the seeds of a plant in relation to this. The survival and lifespan of plant seeds will be key topics of the present chapter. A plant's whole defense system [4], which consists of a number of defense mechanisms, keeps the seeds and, ultimately, the progeny free from illness. In this context, we'll take a closer look at a number of seed defense systems that are involved in homeostatic regulation and defense mechanisms throughout the plant's life cycle [5, 6]. In this sense, both edible [7] and non-edible plant seeds [8] could be considered.

In order to comprehend seed defense mechanisms and the significance of plant seeds as a bioresource for the extraction of nutraceutical and bioactive substances [9], it is important to prospect a wide range of bioactive components, including antimicrobial biopeptides like defensins, thionins, lipid transfer proteins, cyclotides, snakins, and hevein-like proteins [10]. When thinking about health and the innate immune system, it's also critical to look into microflora-based defense mechanisms like prebiotics, probiotics, and postbiotics connected to seeds [11]. The reader should pay particular attention to how nature uses green extraction and isolation techniques. The current chapter focuses on the in situ green extraction methods that seeds use. The crucial ones are germination and sprouting [12]. These are the bioprocesses for enhancing the digestibility and bioactivity of the components [13]. Most of the inactive or dormant components get activated during germination/sprouting process. The most notable one is conversion of difficult to digest crude proteins into easily digestible amino acids by various enzymes which get activated during the process. The most crucial activity, fermentation, which forms the basis of innate immune system of universe [14], also requires special attention because it is Mother Nature's green extraction strategy for preserving the ecological balance. The concept of fermentation should help readers comprehend how prebiotics and probiotics fit into the rules governing plant health and how it could be correlated in terms of making maximum beneficial use of plant seeds for health benefits of human beings. The present chapter will definitely activate thought process for the complete cost effective and appropriate utilization of the components from seeds with zero waste aspects [15] and health benefits. The present chapter discusses seed to plant life cycle helping us realize the nature's unique arrangements to explore the homeostatic regulations and to think if we can make beneficial use of it for the well-being of the universe alongside with appropriate management of ecological homeostasis. Figure 1 represent the schematic for understanding in situ green extraction systems of nature and activation of nutraceutical &

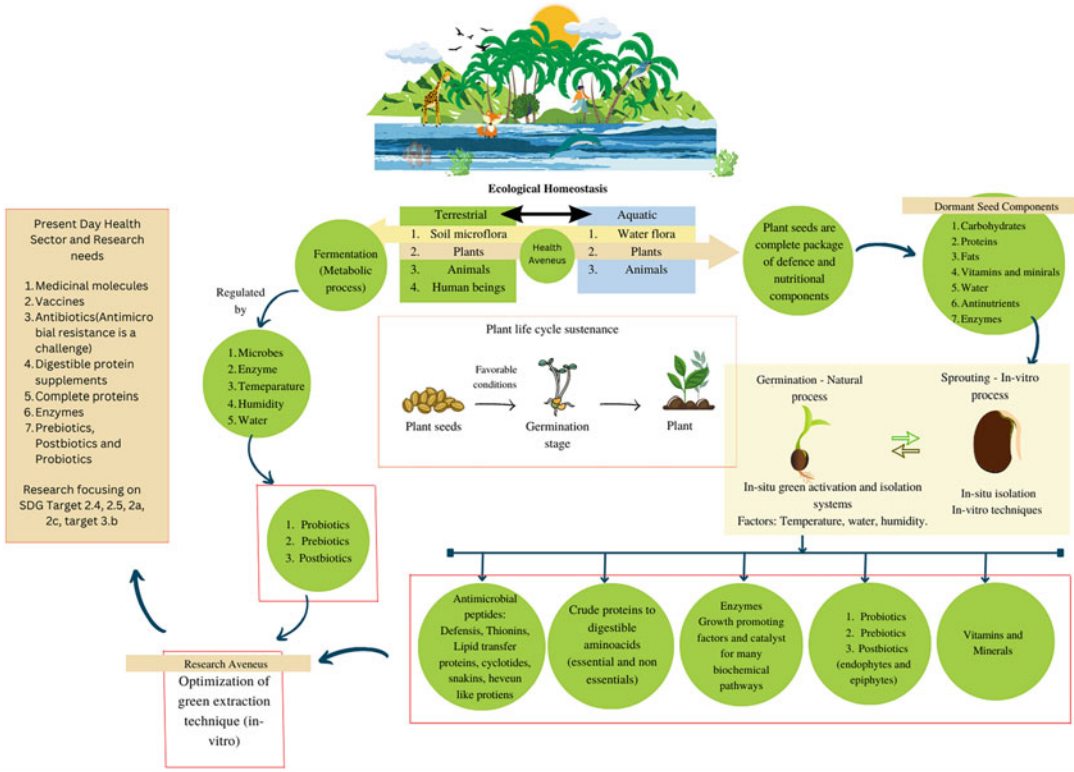


Fig. 1 Roadmap for understanding *in situ* green extraction systems of nature

bioactive resources from the plants seeds. Further, how these bioactive compounds involved in defense and healthy growth of plant, which could be beneficially utilized for commercial exploitation of the products for human health benefits.

1.1 The Established Mechanisms Used by Nature to Preserve Ecological Homeostasis

In ecology, homeostasis refers to an ecosystem's capacity to preserve its overall stability despite any disturbances. It is the result of interactions between species on an ecological level as well as biodiversity. The attributes of a system consider the entire system. It is independent of the species that make up the system. Complex systems frequently exhibit homeostasis. Ecosystems are kept in a state of balance, but since they are such a very diversified system, they cannot be totally stable. In an ecological system, negative feedback controls the maintenance of equilibrium. The adverse feedback negates the environmental change. One of the main causes of negative feedback is resource limitations. For instance, when a resource is scarce or nearly exhausted, the death rate of those who use it rises or the birth rate falls [16, 17]. Our main concern in this case is ecological balance, which includes health issues.

Homeostasis is described in regard to this as a self-balancing mechanism through which biological systems retain stability while adjusting altering external environment. Any organism can keep internal conditions that are more or less constant in order to adapt to and survive in challenging exterior environments. The principle of homeostasis has evolved into the main organizing tenet of physiology as a result of the mounting challenges of existence and ongoing medical research conducted worldwide. Understanding this self-regulating process is necessary in order to properly appreciate how ecological homeostasis controls both health and sickness in this intricate environment. Disruption of homeostatic processes is the first sign of sickness. Therefore, human health-related research must be focused on restoring these homeostatic circumstances by cooperating rather than competing with nature [18]. Resource constraint in terms of health is lack of understanding of how nature contributes to the human wellbeing indirectly through its well-planned happenings and resources. Ecosystems are the planet's life-support systems—for the human species and all other forms of life. Ecosystem services are indispensable to the well-being and health of people everywhere. Fresh water, food, fiber, fuel, biological products, nutrient and waste management, processing, and detoxification, control of infectious diseases, cultural, spiritual, and recreational services, and climate regulation are a few of the ecosystem services that should be mentioned.

The most important ecosystem function nature offers to preserve the health equilibrium of the universe is the management of infectious diseases [19]. The existence of plant seeds is the most significant illustration of the ecosystem service that nature has given to humans. The various chemical components that are present in seeds and variety of roles of seed components during the plant's life cycle make up the entire package of defense mechanisms in plants. The treasure contained in the form of diverse biologically useful components enables the seeds to last longer and contributes to the continuation of the generations. We must comprehend the idea underlying plant seed survival and longevity in order to comprehend the significance of seeds.

1.2 Concept Behind Plant Seed Survival and Longevity

Seeds are a crucial component of the ecosystem process because they enable plants to adapt to changing environments, maintain existing populations, and establish new populations in suitable areas through a scatter network. Thus, seeds play a variety of significant roles in the physiology, ecology, and geography of plants [20].

Two key factors, seed lifetime and seed dormancy, are used to control seed quality. The most important quality of the seed is its resistance to deterioration. Seed dormancy, which limits seed germination under unfavorable conditions and prevents the plant from concluding its life cycle without losing its ability to grow in the appropriate conditions, serves as the plant's protective mechanism.

According to Dalling et al. (2011), seeds make use of four strategies for resisting decay: (i) physical barriers that make seeds impervious to pathogens; (ii) endogenous chemical defenses of seeds; (iii) chemical defenses of beneficial seed-microbial interactions; and (iv) rapid seed germination [21, 22]. A crucial mechanism for seed survival and longevity in the soil is the array of inducible enzyme-based biochemical defenses that are present on the exterior and inside of seeds. Since they may be applicable to other defense enzymes as well as to a variety of plant species and habitats, enzyme-based biochemical defenses may have wider ramifications [22]. In the framework of this chapter, the themes that demand consideration are chemical and microbiological defense systems.

2 Chemical Defense System of Seeds

Structural and chemical traits influence predator rate prior to incorporation in the soil. Seeds contain diverse secondary chemicals, mostly at concentrations much higher than elsewhere in the plant. Chemical constituents found to be effective against microbial infection in the soil include phenolic compounds, alkaloids, antimicrobial peptides, etc. Consistent with an antimicrobial role, chemical defenses often are allocated primarily to seed coats rather than embryo or endosperm tissue protecting seeds from pathogen infection at the pre-dispersal stage [23]. Excellent insights into the idea of seed defense are provided by research done in 2018 by team Zalamea et al. In the seeds of pioneer species (pioneer tree species from lowland tropical forests in Panama), they studied dormancy-defense syndromes and trade-offs between physical and chemical defenses. The physical and chemical properties of seeds determine their ability to survive in the soil seed bank in the presence of pathogens and predators. Seeds can survive in soil and even decades after being dispersed, seeds can still germinate and recruit [24]. Research data from Zalamea et al. (2018) mentions two methods that can be used to create both short- and long-term persistence of seeds. Impermeable seeds exhibit physically dormant defensive syndrome while permeable seeds exhibit chemical defense implying physiological dormancy. The quiescent defensive syndrome is mirrored by the decreased level of chemical and physical defenses in transient seeds. Overall, they discovered a connection between seed defense and seed dormancy, indicating that environmental forces on seed persistence and delayed germination may be able to select for trait combinations defining various dormancy-defense syndromes [24].

When it comes to soil borne infections, the phenolic chemicals that plants release from their roots and seeds frequently exhibit strong antifungal, antibacterial, and antiviral properties. The phenolic group of metabolites consists of terpenoids,

phenylpropanoids, cinnamic acids, lignin precursors, hydroxybenzoic acids, catechols, coumarins, flavonoids, isoflavonoids, and tannins, among others [25]. *Trigonella foenum-graecum* is a Leguminosae plant that is widely farmed in India and Egypt. Trigonelline alkaloid was initially discovered in the seeds of this species. *Salmonella enteric* and *Escherichia coli* are both susceptible to the antibacterial effects of the coffee alkaloid trigonelline. Alkaloids are crucial for plant protection in both sweet and wild types of Lupinus. Lupin seeds contains about 5% quinolizidine alkaloids, which are poisonous to insects [25]. Quinolizidine alkaloids (QAs) are poisonous secondary metabolites that are present in the genus Lupinus, some of which are significant grain legume crops. QAs provide the plants with protection from insect pests. Based on measurements of QA translocation and total QA levels in reproductive tissues, it has been hypothesized that of the QAs that accumulate in seeds, half are generated within the seed and half are translocated [26]. In relation to this chemical defense association of seeds let us have a look at bioactive compounds of seeds which are actively involved in defense systems associated with seeds and plants throughout the life cycle of plant.

2.1 Antimicrobial Peptides in Seeds

The barrier defense mechanism of plants includes antimicrobial peptides (AMPs). They have been isolated from the roots, seeds, flowers, stems, and leaves of numerous different species, and they have actions toward both phytopathogens and organisms that are harmful to humans, such as viruses, bacteria, fungi, protozoa, parasites, etc. The antibacterial action of AMPs has drawn a lot of attention due to the rise of drug-resistant infections. Plant AMPs are thus seen as promising antimicrobial substances with significant biotechnological uses. Plant AMPs are thought to have a considerable impact on both plant growth and development as well as pathogen defense mechanisms. Plant AMPs are divided into various groups and share characteristics including a positive charge, disulfide links (which stabilize the structure), and a method of action that targets multiple sites on the plasma membranes, intracellular components of pathogen, and outer membrane structures [27]. In the case of a mechanism involving membrane breakdown, it advances by forming membrane pores, which causes ion and metabolite leakage, depolarization, stoppage of the respiratory processes, and cell death. Plant AMPs can be classified as anionic (AAMPs) or cationic peptides (CAMPs) depending on their electrical charge. The cationic residues electrostatically draw negatively charged molecules, such as anionic phospholipids, lipopolysaccharides, or teichoic acids, causing the peptide to assemble on the surface of the membrane. When concentration reaches a certain point, the collapse starts [28, 29]. These peptides are categorized into distinct families mainly on the basis of their amino acid sequence, identity, number of cysteine residues, and their spacing.

They exhibit a wide range of functions ranging from direct antimicrobial properties to immunomodulatory effects [28, 29]. The main families of AMPs comprise defensins, thionins, lipid transfer proteins, cyclotides, snakins, and hevein-like proteins, according to amino acid sequence homolog [28].

Note Representative examples of plant seed antimicrobial peptides (AMPs) isolated using germination process with their classification and bioactivities are given in Table 1.

2.1.1 Defensins

Plant defensins are the vast antimicrobial peptide superfamily found across the plant world [27]. Plant defensins are small, highly stable, rich in arginine, lysine, and cysteine residues, that constitute a part of the innate immune system. These are large family of cationic host defense peptides (HDP) [30]. The first plant defensins were isolated from wheat *Triticum aestivum L* and barley *Hordeum vulgare* and initially classified as gamma thionins. Plant defensins are small basic, cysteine-rich peptides ranging from 45 to 54 amino acids with low molecular weight about 5 kDa. Biological activities reported for plant defensins include antifungal, antibacterial, proteinase, and insect amylase inhibitor activities [28]. Additionally, it has been reported that these compounds mediate abiotic stress and zinc tolerance, inhibit protein synthesis, impair ion channel function, and hinder microbial, root hair, and parasitic plant growth, change the redox state of ascorbic acid, stimulate the perception of sweetness, act as epigenetic factors, influence self-incompatibility, and support male reproductive development [31]. Plant defensin genes produce precursor proteins that target a signal at the amino terminal and a pro-peptide at the c-terminal (CTPP) and have a mature defensin domain.

This CTPP is optional; some defensins may contain it while others do not. Class II plant defensins are defined as peptides with a c-terminal pro-peptide signal (CTPP) of 27–33 amino acid residues. Glutamic and aspartic acids, which have a negative charge and balance the positive charge of the defensin domain, are prevalent in these amino acid residues. Class I is assigned to the other class that is deficient in these signals. Class I plant defensins are only detected in the seeds, but class II plant defensins are reported to be abundantly produced in both the reproductive and vegetative sections of the plant [32–34].

Activation Process of Defensins in Seeds

The study projects carried out by the co-researcher Terras F et al. in 1995 provided illuminating insight into the activation mechanism of defensins for the execution of their functional tasks. *Raphanus sativus*-antifungal protein-1 (RsAFPl) and Protein-2 (RsAFP2), two homologous, 5-kD cysteine-rich proteins found in radish seeds, both demonstrate strong antifungal action in vitro. In their

Table 1
Representative examples of plant seed antimicrobial peptides (AMPs) isolated using germination process and their classification and bioactivities

Sr. no.	Plant seed	AMP	Peptide	Function	Reference
1.	Radish seeds [*Raphanus sativus*]	Defensins	5-kD cysteine-rich proteins, *Raphanus sativus*-antifungal protein-1 (RsAFPl), and Protein-2 (RsAFP2)	Antifungal activity against foliar pathogen *Alternaria longipes*	[35]
2.	Lentil seeds [*Lens culinaris*]	Defensins	Lentil seed defensin termed as Lc-def has 8 cysteines forming four disulfide bonds. A 74-residue predefensin contains a putative signal peptide (27 amino acid) and a mature protein	Activity against *Aspergillus niger* (Aspergillus niger causes sooty mold on onions and ornamental plants)	[96]
3.	Pearl millets, [*Pennisetum glaucum* (L.) R. Br.] cultivars IP18292	Thionins	Thionins (PR protein-13) are a class of Cys-rich polypeptides of about 5 kDa	*Sporangia. graminicola* zoospores lysis	[39]
4.	Black cumin seeds [*Nigella sativa* (L.)]	Thionins	Nigellothionins-dominant peptide, NsW2 with 8-Cys motifs	Growth inhibitory activity against filamentous fungi and yeasts	[97]
5.	Rice seeds [*Oryza sativa*]	Lipid transfer proteins	OsLTPL36, a homolog of putative lipid transport protein	Seed quality seed development and seed germination	[45]
6.	Castor bean [(*Ricinus communis* (L.)]	Lipid transfer proteins	Nonspecific lipid transfer protein, (nsLTP)	Regulation of fatty acid β oxidation through enhancement of acetyl CoA oxidase activity in glycosomes (nsLTP as acetyl CoA carrier-energy source and helps in cellular respiration	[98]

(continued)

Table 1
(continued)

Sr. no.	Plant seed	AMP	Peptide	Function	Reference
7.	Lentil seeds [*Lens culinaris*]	Lipid transfer proteins	Lc-LTP 1–8, (92–93) amino acid residues, with four disulfide bonds	Antibacterial: Inhibit growth of *Agrobacterium tumefaciens. (causative agent of crown gall disease)*	[99]
8.	Sweet violet seeds [*Viola odorata*]	Cyclotides	Peptides with cyclic, cystine knot structural motif	Act as defense and storage proteins	[47]
9.	Alfalfa seed [(*Medicago sativa* (L.)]	Snakins	MsSN1 has a putative signal peptide of 25 amino acids and possesses a snakin/GASA domain (Pfam02704) containing 12 cysteine residues in conserved positions within a conserved C-terminal region	Antibacterial and antifungal activity, helps in plant innate immunity	[51]
10.	Quinoa seeds [*Chenopodium quinoa*]	Hevein-like proteins	Type III lectin precursor, CB-HLPs (chitin-binding hevein-like peptides – cysteine-rich peptides) chenotides, tandem repeats of the mature peptide domains, with a cleavable Gly/Ala-rich linker consisting of 18 amino acids	Inhibition of phytopathogenic fungi	[55]

research, it was shown that these proteins are found in the cell wall and are more common in the lining of the outer cell layers of several seed organs. Additionally, following breakdown of the seed coat, RsAFPs are selectively released during seed germination. The number of proteins produced is sufficient to provide a microenvironment that inhibits fungal development surrounding the seed [35]. There is also documented additional material that supports the idea that external stimuli activate defense mechanisms. It was discovered that when a vegetative plant tissue is injured or detects

pathogens, a number of dynamic defense mechanisms can be activated. In response to attempts by fungus hyphae to penetrate the cell wall, newly produced carbohydrate material may be deposited at the location [35, 36]. Furthermore, injury and elicitor therapy can cause preexisting cell wall proteins to become oxidatively cross-linked [37]. At early germination, when the seed coat, an efficient physical barrier against microorganisms, is destroyed and the tiny seedling is exposed to the earth, is a particularly vulnerable time. The importance of this event in relation to seedling defense against fungal infections was evaluated using radish seed as a model system for studying the release of Rs-AFPs during germination. A bioassay was carried out in which seeds were permitted to sprout on a medium promoting the formation of a fungal colony. A halo of growth inhibition surrounded the seed as the borders of colony grew closer to it. After their seed coat is damaged, either by germination or by mechanical incision, radish seeds produce a proteinaceous antifungal chemical [35].

2.1.2 Thionins

Thionins are a family of antimicrobial peptides that are found in the seeds, stems, roots, and leaves of cruciferous plants, mistletoe, and cereals. They have a low molecular weight (approximately 5 kDa) and are highly concentrated in arginine, lysine, and cysteine residues. Thionins are poisonous to yeast, fungus, and bacteria. It was proposed that their activity resulted from the lysis of the membranes of adhering cells. Antifungal activity shown by thionins, is a result of electrostatic interactions between positively charged thionins and negatively charged phospholipids in fungal membranes, which lead to the creation of pores or a particular contact with a particular lipid domain [28]. The typical 5 kDa, basic thionin peptide with three or four disulfide bridges is obtained by first producing them as preproproteins and then processing them. Transcriptomes of more than 1000 plant species have been sequenced as part of the "one thousand plant transcriptomes initiative" (1KP project). New thionin sequences were sought using the data. The four types of thionins that were previously identified, only two classes and their variants are presently recognized using the data from the 1KP study. Due to the fact that every variant was linked to either class 1 (eight cysteines) or class 2 (six cysteines). Eighteen versions in total were found by the 1KP project with all the distinct variants [38].

Activation Process of Thionins in Seeds

Mobilization of thionins is very clearly elucidated by Chandrashekhara S. et al. in 2010. Purified thionin from pearl millet was tested on the fungus *Sclerospora graminicola* that causes downy mildew disease and was more successful in rupturing the membrane, pointing towards a potential toxicity mechanism. It was shown that resistant cultivars expressed thionin transcripts at a higher level than susceptible cultivars. Immunofluorescence studies have

revealed strong fluorescence in vascular locations, confirming its systemic translocation during contact, as well as in epidermal regions, confirming its involvement in halting pathogen entrance. Additionally, localization studies using the coleoptile epidermal peeling of resistant and susceptible cultivars of pearl millet revealed that thionin protein was more abundant in seedlings of the resistant cultivar that had been inoculated with *S. graminicola* than in uninoculated seedlings at the papillar region of the cell wall. In susceptible inoculation samples, no discernible localization was found [39]. Thionins are created from bigger precursors, including a C-terminal peptide and a signal peptide that have undergone post-translational processing. These characteristics demonstrate that thionins are released following pathogen infection, which results in antimicrobial activity at the site of pathogen entry and stops the pathogen from spreading the infection region [39, 40]. In conclusion, this study showed that thionin is crucial for pearl millet systems' ability to fight the downy mildew infection [39]. Similar studies were done on wheat and barley thionins against bacterial strains including *Clavibacter michiganensis subsp. Sepedonicus* and fungal pathogens such *Rosellinia nectatrix, Colletotrichum lagenarium, Phytophthora infestans, Fusarium solani, Thievaliopsis paradoxa, and Drechslera teres.* Thionins have been shown to have an impact on several cell types, and there is evidence that these proteins can alter the permeability of yeast membranes [41, 42].

2.1.3 Lipid Transfer Proteins

Nonspecific lipid transfer proteins(LTPs) are small, cysteine-rich proteins that have a variety of functional roles in the growth and development of plants, including the production of cutin wax, adhesion of pollen tubes, cell expansion, seed development, germination, and adaptation to changing environmental conditions. LTPs have a hydrophobic cavity with eight conserved cysteine residues that allows for a wide range of lipid-binding specificities. Many LTPs serve as positive regulators of plant disease resistance because they are members of the pathogenesis-related protein 14 family (PR14) [43]. Researchers are interested in LTPs for three basic reasons. Plant LTPs have two distinct properties. First, they can bind and transfer lipids, which is how they received their names and were grouped into one class. The second characteristic is that LTPs are protective proteins that are part of innate plant immunity. The third characteristic is that one of the most therapeutically significant classes of plant allergens is represented by LTPs [44].

Activation Process of Lipid Transfer Proteins in Seeds

In the studies reported in 2015 by Wang et al., a homolog of a putative lipid transport protein was revealed to exhibit unique expression in the developing rice seeds. Homolog was predominantly expressed in developing seed coat and endosperm aleurone

cells, according to transcriptional profiling and in situ hybridization studies [45]. By forming complexes with diverse lipid molecules, LTPs are thought to be involved in the activation and control of many signaling pathways in plants. One of the classes of signal mediators in plants are oxylipins. Oxylipins also control the procedures for neutralizing the harmful byproducts created during stress. The concurrent activity of lipoxygenase and allene oxide synthase results in the formation of covalent complexes between the barley LTP1 and oxylipin of 9(S),10-epoxy-10,12(Z)-octadecadienoic acid during seed germination, according to studies on the grain. This connection may point to a cooperative role for LTPs and oxylipins in the control of the signaling pathways that activate the mechanism that protects plant cells from damage under stress [44, 45].

2.1.4 Cyclotides

A cystine knot made up of three disulfide bonds stabilizes the globular microproteins known as cyclotides, which have a distinctive head-to-tail cyclized backbone. Compared to other peptides of comparable size, they exhibit excellent stability to chemical, thermal, and biological degradation because of their distinctive circular backbone topology and knotted arrangement of three disulfide links. Multiple cyclotides (between 10 and 160) are typically present in every tissue of a single plant, including the flowers, leaves, stems, roots, and perhaps even the seeds [46].

Activation Process of Cyclotides in Seeds

Slazak, B., et al. (2020) described experiments involving variations in the quantity of cyclotides in developing seeds of *Viola odorata*. To soften the seed coating, sterilized seeds were placed in a beaker with wet paper and kept at 4 °C for 7 days. The seed coating was then taken off, and the seeds were placed on half-strength MS media that had been thickened with 7 g/l agar. Three distinct stages of seedling development—the seed, a germinated seedling with endosperm, and seedlings that utilized the entire endosperm—were collected and freeze-dried after seeds were grown in a culture chamber. The growing seedling appeared to ingest cyclotides found in the seed endosperm. It was found that the cyclotide pattern found in various tissues and surroundings is shaped by degrading processes. The findings show that various cyclotides have distinct functions, some of which are related to defense and others which are related to storage proteins [47].

2.1.5 Snakins

Snakins are typically tiny, positively charged, cysteine-rich proteins with a molecular weight of about 7 kDa that play a number of roles in plant defense responses, including antimicrobial action against a wide variety of phytopathogens and animal diseases. Snakin-1 (StSN1), the first recognized Snakin peptide, was isolated from potato tubers and given the name Snakin because it had sequence features with snake venoms. The 12 cysteine residues that are

always present in the conserved GASA (Gibberellic Acid Stimulated in Arabidopsis) domain in the C-terminal portion of the Snakin family peptides give them their distinctive properties [48, 49]. Genes from the Snakin/Gibberellic Acid stimulated in Arabidopsis (GASA) family are important for plant growth and development as well as protection against pathogens. In one study by Deng M. et al. in 2021, the effect of Snakin-2 (SN2) on tuber dormancy and sprouting was examined. This work used a transgenic technique to regulate the level of SN2 expression in tubers, and it showed that StSN2 strongly impacted tuber sprouting, silencing StSN2 caused dormancy to be released, and overexpressing tubers had a longer dormant period than the control [50].

Activation Process of Snakins in Seeds

Understanding the activation and use of Snakin peptides for antifungal action in alfalfa seeds was made possible by Garcia et al. (2014). Sterilized seeds of both transgenic and wild types were put in petri dishes with 1% agar water under 16 h of light (100 moles/m^2s) and 25 °C.

Plants were moved to 1:1 vermiculite: perlite and kept in magenta vessels to preserve humidity after being incubated at 25 °C with a 16 h photoperiod for a month. *Phoma medicaginis* was sprayed onto all aerial tissues of two-month-old plants to inoculate them. The percentage of sick leaflets was examined 30 days after vaccination, and 60 days later, the number of regrowing plants and the percentage of heavily defoliated plants were counted. Snakin-1 produced by alfalfa (MsSN1)-overexpressing alfalfa transgenic plants exhibits increased antimicrobial activity against virulent fungal strains without changing the nitrogen-fixing symbiosis, paving the way for the development of efficient alfalfa transgenic cultivars for biotic stress resistance [51].

2.1.6 Hevein-Like Proteins

A family of antifungal plant AMPs known as hevein-like antimicrobial peptides (AMPs) contain a chitin-binding site that interacts with the chitin of fungal cell walls. Hevein-like peptides are members of the AMP family that are structurally related to the antimicrobial peptide hevein, which is found in the latex of the *Hevea brasiliensis* (Willd. ex A. Juss.). Müll. Arg. Chitin and similar oligosaccharides can bind to hevein-like AMPs' chitin-binding site. It is widely accepted that antifungal action is mediated by binding to chitin and similar oligomers found in fungal cell walls [52, 53]. Antimicrobial peptide (AMP) derived from cycad (Cycas revoluta) seeds, Cy-AMP1, has been isolated and characterized. Antimicrobial peptide Cy-AMP1 was shown to have chitin-binding ability in one of the researches conducted by Seiya Yokoyama et al. in 2008, and the chitin-binding domain was found to be conserved in knottin-type and hevein-type antimicrobial peptides. To investigate the function of the chitin-binding domain, *Escherichia coli* was used

to generate and purify the recombinant Cy-AMP1. Cy-AMP1 mutations drastically reduced their antifungal activity compared to native Cy-AMP1 and completely lost their capacity to bind chitin [54].

Activation Process of Hevein-Like Proteins in Seeds

Another work by Shining Loo 2021, examined the hyperstable antifungal Chitin-binding hevein-like peptides (CB-HLPs) chenotides found in quinoa seeds (*Chenopodium quinoa*). The biosynthesis of chenotides was shown to be novel and to belong to a new family of cleavable hololectins, which were designated as type III lectin precursors. These precursors were tandem repeats of the mature peptide domains with an 18-amino-acid cleavable linker between them. Chenotides can also stop the growth of phytopathogenic fungi because they link to chitin. It has been noted that the presence of chenotides, which are naturally occurring anti-microbial agents, in quinoa may be the underlying cause of the grain's prolonged shelf life and unintentional selection as a staple food throughout human history [55].

3 Microbial Defense System of Seeds

The life cycle stage of seed germination and seedling development is where all prior encounters with microbiota may ultimately have an impact. The interactions of seed-associated bacteria with the soil microbiota during seed germination may have the greatest effects on overall plant fitness. The soil microbiota, together with the endophytic and epiphytic seed microbiome, are all activated during seed development. During the crucial seed-to-seedling stage of the life cycle, imbibition and germination can produce a spermosphere environment (microbiome zone in the soil surrounding a germinating seed) that is either advantageous or deleterious for further plant life cycle [56–58].

3.1 Endophytes of Seeds

The genetic spread of endophytes across generations of hosts can occur via seeds. Seed endophytes are founders of the juvenile plant microbiome and can aid host defense during seed germination and later phases. Endophytes are symbionts that live inside plant tissues, including seeds. The findings were reported by Khalal and Raizada 2018, by using dual culture assay methodology wherein they examined the in-vitro antagonistic effects of endophytes from seeds of various cultivated cucurbits against significant soil-borne pathogens like *Pythium aphani dermatum*, *Phytophthora capsici*, *Fusarium graminarium*, and *Rhizoctonia solani*. For the purpose of inducing plant defense, the endophytes were also examined in-vitro for their ability to secrete volatile organic compounds. It was discovered that fungal infections were suppressed when extracellular ribonuclease activity was also examined. These findings demonstrate that the

microorganisms that are packaged inside the seeds of grown cucurbits have a great ability to inhibit illness [56]. The term endophyte" refers to a significant collection of many and diversified plant symbionts that exist asymptomatically and occasionally repeatedly within plant tissues without doing any damage or transmitting illnesses to their host plants [57]. The complexity and dynamism of seed microbiomes are the result of intricate interactions with microbes throughout the plant life cycle. Exciting new potential for research into plant-microbe interactions are being revealed by the diversity and dynamics of seed microbiomes. The interactions that seeds have with microbes, notably fungus, bacteria, and viruses, have an effect on their tenacity, resistance to diseases, emergence from dormancy, and subsequent seedling vigor [58, 59].

The endophytic microbiota (i.e., those microbial species that live in internal seed tissues and are vertically transmitted to progeny seedlings). The primary symbionts known as seed endophytes have been shown to have the potential to impact the development of secondary symbioses in maturing hosts, according to one of the important findings by Ridoutel et al. in 2019. On *Phialocephala fortinii* early root colonization of winter wheat seedlings, the most prevalent primary symbionts had priority effects. The primary symbiont controls not only the effectiveness of early *P. fortinii* colonization but also, indirectly, the fitness of the seedling because *P. fortinii* is very beneficial for wheat seedling growth [60]. The germination and survival of seeds are affected by soil-borne fungus that have a host affinity and host-specific effects. Plant species have a greater impact on the organization of seed associated fungus communities than do soil types, forest features, or time in the soil.

These fungi have effects on seed viability and germination that are specific to their hosts [61]. A distinct pattern has evolved from culture-based studies demonstrating the diversity of bacteria and fungus recovered from seeds, leaves, and roots, while the microbiomes of individual seeds are still rarely investigated. The transmission of various fungal endophytes in the seed and needles of *Pinus monticola*, the western white pine, was examined by Ganley & Newcombe (2006) using a sequence-based methodology. Seven hundred and fifty surface-sterilized needles yielded the enormous quantity of 2003 fungal endophytes. Eight hundred surface-sterilized seeds yielded 16 endophytic isolates [62].

3.2 Epiphytes of Seeds

Epiphytic microbiota should be distinguished when talking about seed microbiomes (i.e., those microbial species that colonize seed surfaces and may or may not become internalized within seed tissues and transmitted either vertically or horizontally). Seed epiphytes are fairly diverse [63]. The endophytic microbiota may frequently arise from different seed tissues or environmental sources than those of the epiphytic microbiota, which makes this distinction rather artificial given that endophytes can become

epiphytes and vice versa. In contrast to those associated with the seed coat, which are expected to be far more diverse and transported horizontally, microorganisms associated with the embryo and endosperm are more likely to be transmitted vertically [58, 59]. It is evident from research on the endophytic and epiphytic seed microbiome that maternally transmitted microbes have a significant impact on how the core microbiome of plants develops [63]. Additional evidence comes from the next-generation sequencing of seeds, which shows that most fresh seeds contain extremely few bacterial and fungal operational taxonomic units [60]. Microbiome of plant further regulates the overall plant defense theory.

Note Representative examples of plant seed germination/sprouting for isolation of endophytes and epiphytes are given in Table 2.

4 Green Extraction and Isolation of Bioactive Components

The idea underlying plant seed survival and longevity is explained by the whole defense mechanism of plants. The main areas to be researched in order to comprehend the significance of plant seeds as a bioresource for the extraction of nutraceutical and bioactive compounds are antimicrobial biopeptides, enzymes, and microflora-based defense systems involving prebiotics, probiotics, and postbiotics associated with seeds. It is the nature's special manner of using these parts to carry out the defense mechanism. Nature uses eco-friendly extraction and isolation processes. The procedure greatly enhances ecological homeostasis while preserving the overall health of ecosystem. This is what is referred to as an in situ green extraction system, and it is used to carry out the defensive mechanism either horizontally or vertically to ensure the survival of seedlings as well as the expression of this defense-related genetic makeup throughout the life cycle of plant. Mother nature can teach us a lot that will benefit the future of humanity. Growing new generations using seeds has been the method used throughout the plant era to start new generations.

In addition, during germination and fermentation, beneficial components are activated in situ and the life cycle of a healthy plant continues, seeds play the role of protection from pathogens by activating a variety of defensive components. Along with defense components, nutritional components are also increased or activated. Concepts based on a similar intentional activation process in- vitro could be a practical strategy to employ the components from seeds with potential for functional and dietary benefits.

Table 2
Representative examples of plant seed germination/sprouting for isolation of endophytes, epiphytes, amino acids, enzymes, probiotics, prebiotics, with their classification and bioactivities

Sr. no.	Plant seed	Bioactive component	Function	Reference
1.	Fruits and seeds-*Cucurbitaceae* family (examples: melon (*Cucumis melo*), cucumber (*Cucumis sativus*)	Endophytes: *Lactococcus*, *Pantoea*, *Pediococcus*	Anti-oomycete activity	[56, 100]
	Water melon (*Citrullus lanatus*)		Activity against t *Podosphaera fuliginea* responsible for powdery mildew on cucurbits	
	Squash and pumpkin (*Cucurbita* sp.)		Diverse nutrient acquisition and growth promotion activities to the hosts	
	Seeds belonging to Phyla (*Firmicutes*, *Proteobacteria*) and one class within each phyla (Bacilli, γ-proteobacteria, respectively)		These microbes may lead to novel seed inoculants to assist sustainable food production	
2.	The asymptomatic wheat [*Triticum aestivum* (L.) cv.] Heixiaomai NO.76) seeds (sterilized sprouted seeds)	Endophytes: Dominant bacterial genus identified are *Erwinia* and *Rhizobiales*) and dominant fungal genus identified is *Emericella*	Growth and disease resistance of wheat plants (antibacterial and antifungal)	[101]
3.	Seeds of Brassica (*B. juncea L. Czern., B. rapa L., B. napus L.*) and Triticum (*T. aestivum L., T. turgidum L. subsp. durum (Desf.) Husn.*)	Epiphytes: Total epiphytic microbial load of 10^6–10^8 bacterial genomes g^{-1} seeds was observed. Examples: *Pantoea agglomerans*	Antimicrobial biocontrol agent	[102]
4.	Soybean (*Glycine max*), Lentil (*Lens esculenta*), Black gram (*Vigna mungo*), Green gram, (*Vigna radiata*) Bengal gram, (*Cicer arietinum*) Groundnut (*Arachis hypogaea*), Pea bean (*Phaseolus vulgaris*)	Enzymes proteases	A protease is an enzyme that catalyzes proteolysis, breaking down proteins into smaller polypeptides or single amino acids, and spurring the formation of new protein products	[74]

(continued)

Table 2
(continued)

Sr. no.	Plant seed	Bioactive component	Function	Reference
5.	Sword bean seeds (*Canavalia gladiata* (Jacq.) DC.)	Enzyme α-amylase	*α-Amylase* is an enzyme that hydrolyses α bonds of large, α-linked polysaccharides, such as starch and glycogen, yielding shorter chains thereof	[103]
6.	Germinating oil seeds (*Brassica napus L.*)	Enzyme lipase	Lipases are versatile enzyme that catalyzes the hydrolysis of ester linkages, primarily in neutral lipids such as triglycerides	[104]
7.	Pigeon pea seeds (*Cajanus cajan*)	Amino acids	Nutritional quality and bioactivity of seeds increases. Germination helps in increase in essential and non-essential amino acids, digestibility of crude proteins increases by conversion to amino acids	[105]
8.	Lentils (*Lens culinaris*), Mung beans, (*Vigna radiata*), Peanut (*Arachis hypogeal*)	Probiotics: *Lactobacillus* and *Bifidobacterium*	Beneficial effects on human physiology	[61]
9.	Faba bean (*Vicia faba L.*), Lentil (*Lens culinaris*), Common bean (*Phaseolus vulgaris*), and Cowpea (*Vigna sinensis*)	Prebiotics Raffinose family oligosaccharides account for 67.3, 63.2, 53.0, and 51.0% of the total soluble sugars in cowpea, faba bean, lentil, and common bean, respectively Other oligosacchrides like Verbascose in faba bean and stachyose in the other three legumes	Serves as food for gut flora, undergo anerobic fermentation in large intestine	[106]

4.1 Germination vs. Sprouting

Germination is the process by which a seed transforms into a new plant after achieving the conditions necessary to end its dormant state. The only seeds that germinate are those that have an embryo in them. The process of germination causes a seed to grow into a seedling, which subsequently forms the plumule and radicle. The post-germinative growth of the seedling is thought to occur after seed germination, which is regarded as the beginning of the first developmental phase in the life cycle of higher plants. When the environment is right, a seed will begin to germinate in response to factors like light, temperature, soil elements and well-understood chemical pathways. The mature seed resumes growth during the intricate process of germination, switching from a maturation- to germination-driven programme of development and subsequent seedling growth. By definition, a seed's germination process begins with the absorption of water and is finished when the radicle emerges from the surrounding structures [64].

When a monocotyledonous plant seed germinates, the coleorhiza is the first component to emerge from the seed coat, however when a dicotyledonous plant seed does the same, the radicle emerges first. The pace at which seeds are absorbing water determines how quickly they are germinating in both groups. Phase I of the process begins with a dry seed rapidly absorbing water until all of the seed tissues are saturated. Phase II is then followed by a modest intake of water, but phase III is followed by a significant uptake of water that is associated to the completion of germination. Phase II is the most significant and is connected to a number of cellular and physiological processes, including DNA repair and the translation of both newly generated and stored mRNAs. Both enhanced metabolic and cellular activity define Phase II. In order to generate seedlings, embryo cells must decide whether to re-enter the cell cycle or remain arrested during the germination stage. When a seed germinates, the quiescent seed's stalled cell cycle is released [64].

Understanding the notion of germination of the particular seed will help you extract functional and neutraceuticals components from the seeds while taking the same procedure emulating natural seed germination process into consideration. By optimizing the many factors related to the process completion, components could be activated, extracted, and isolated. Sprouting may be viewed as a commercial green extraction method that could be optimized for component isolation.

"Sprouts" (Regulation (EC) No 208/2013) are "the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed" [65, 66].

The American Association of Cereal Chemists (AACC) and United States Department of Agriculture (USDA) have agreed on the following definition of "sprouted grains":

> "Malted or sprouted grains that retain all of the original bran, germ, and endosperm shall be regarded whole grains provided sprout growth does not exceed kernel length and nutrient values have not been depleted. It is important to designate these grains as whole grains that have been sprouted or malted" [66, 67].

The aforementioned descriptions of germination and sprouting help us to realize that, during the sprouting process, seeds are soaked and transformed into digestible forms so they may be used as food sources, while during germination, a plant grows out of a seed structure. The fundamental difference between sprouting and germination is that the latter refers to the process by which seeds are purposefully compelled to sprout or germinate in order to suit commercial needs.

4.1.1 Understanding the Germination Process to Optimize Extraction by Sprouting Process

Understanding the natural germination process of seed is required for the optimization of sprouting for commercial use. Most agricultural seeds need water, a suitable temperature, and a good gaseous atmosphere for germination. Dormancy is a significant element in the emergence of weeds but has minimal effect on the seedling emergence of the majority of commercial crops. Additional germination-promoting elements for weed seeds to consider are sunshine and nitrate [68]. Let us have a look at various factors impacting the germination/sprouting process.

Effect of Water Uptake/ Imbibition

Three phases of water intake by the seed are typically observed: a rapid initial phase, a lag phase with little additional uptake, and finally a second phase of rapid water uptake linked to radicle emergence. Although metabolism begins before seeds achieve their maximum moisture content, imbibition is recognized as a physical process and is associated with the first stage of water uptake. Initial water intake is propelled by matric pressures brought on by the hydration of protein and starch bodies, cell walls, and other cellular components. There is an increasing dependence on osmotic potential, which is defined by the concentration of dissolved solutes, as the physiological range of water levels is approached. The viability of seeds and the success of seedling emergence can both be significantly harmed by the rate of early water intake. Rapid ingestion can harm both directly and indirectly through a favorable interaction with chilling injury. The quality of the seed coat and other factors of seed vigor directly influence how much harm has been done. By modulating permeability, the seed coat and other tissues can also play a significant regulatory role in water intake. Germination is

often documented when radicle growth is first noticed since germination is strictly terminated when it begins at the conclusion of the lag period of imbibition. In most species, desiccation tolerance is gradually lost during the growth of the radicle after germination, making the beginning of growth an important phase in the process from seeding to seedling emergence. Each seed in the population will go through this crucial stage at a different time [68].

Effect of Temperature and Water Potential Threshold

Temperature and water potential together have a significant impact on the percentage of seeds that will germinate, germination time, and spread of timings within the seed population.

(a) *Temperature*: Although seeds can sprout at a variety of temperatures, the maximum percentage of germination is often reduced at the extremes of the temperature range. Because of this, various seeds within a population may have varied thresholds for high and low temperatures. Germination rate, which is the reciprocal of germination time for each individual seed in the population, rises from a base temperature to an ideal temperature, after which it declines to a ceiling temperature that represents the upper limit of its tolerance [68].

(b) *Water*: It has been demonstrated that the pace of development toward 50% germination is linearly proportional to water potential, much like with temperature. A scale similar to thermal time called hydro time can be used to describe how seeds react to various water potentials [68].

Effect of Oxygen on Germination

One of the limiting elements in seed germination is oxygen since germination necessitates the metabolism of storage chemicals, which depends on respiration. Oleg A. Kuznetsov and K.H. Hasenstein studied the germination of flax seeds in relation to oxygen requirements in their 2003 investigations. In trials with controlled atmospheres, as the oxygen concentration in the atmosphere was dropped, the length of roots and percentage of germination fell. Seeds absorbed water but did not germinate after 2 days in environments with less than 5% oxygen. At 10% oxygen, germination was nearly as high as that of the controls (21% O_2), however the root length was decreased to less than 50%. The seeds grew when the temperature was 27 °C, which is ideal for the growth of flax seedlings.

At constant temperature, the root length grows linearly. A steady oxygen supply and an even number of seeds per chamber affected the germination rate, demonstrating the importance of oxygen for the best possible seed germination [69, 70].

Effect of Light on Germination

Effect of light on seed germination and seedling form of succulent species from Mexico were both shown in studies by Joel Flores et al. in 2015. According to previous research, the adult plant height and

seed mass of cactus plants are related to the amount of light required for seed germination. Twelve species and two varieties of one species from the Southern Chihuahuan Desert were subjected to germination experiments with and without light in order to better understand the seed photosensitivity of desert species from the *Asparagaceae* (subfamily *Agavoideae*) and *Cactaceae*. The photoblastic neutrality of all species was assumed. Eleven species showed comparable seed germination in both light and darkness, and three taxa (*Mammillaria compressa* and the two types of *Ferocactus latispinus*) demonstrated more germination in the presence of light than in the absence of it. Higher seed mass reduced dependence on light, making it a crucial element. These results provide credence to the idea that tiny seed mass and light requirements have co-evolved as a means of ensuring germination [71]. Effect of light on seed germination of eight Wetland Carex Species, published by Kettering et al. in 2006, found that seeds of *Carex brevior* and *Carex stipata* grew more than 25% faster in continuous darkness. The eight species showed a wide range of germination responses after being exposed to various durations of white light. For about 50% of germination, *Carex brevior* needed about 15 min of white light, whereas *C. hystericina*, *C. comosa*, *C. granularis,* and *C. vulpinoidea* needed about 8 h. Red light has taken over the function of white light in all species. All species, with the exception of *C. stipata*, had their induction of germination upon exposure to white or red light reversed by far-red light [72].

4.1.2 Extraction by Sprouting

Sprouting for Isolation of Antimicrobial Peptides

The study highlights of Kamala Golla and coresearchers 2016 are considered here for representing isolation of antimicrobial peptides using sprouting technique. The presence of short peptides with antimicrobial peptides was extensively examined in 50 distinct types of germinating seeds. Proteins were extracted using both liquid nitrogen and phosphate buffer (PBS) treatments after selected seeds were germinated on brown sheets over a period of time. Small peptides of less than 10 kDa were formed by 5 kDa flow through, and the same was validated by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis). *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa* were the four clinical isolates against which the short peptides that had been extracted were tested for antibacterial efficacy. *Staphylococcus aureus* (MTCC 9542), *Escherichia coli* (MTCC 1698), *Klebsiella pneumoniae* (MTCC 10309), and *Pseudomonas aeruginosa* (MTCC6458) were the reference/standard organisms employed in this investigation. All cultures were subcultured on nutrient agar at regular intervals and preserved at −20 °C and 4 °C, respectively, by suspending them in 10% glycerol. Using 1% mercuric chloride, the surfaces of jowar, paddy, millets, foxtail millets, red gram, green gram, black gram, ground nut, pea, field bean, and wheat seeds were sterilized. Depending on the seed variety, the

seeds were soaked in distilled water for 6–12 h before being placed on sterile brown paper and allowed to germinate for 2, 4, 6, 8, 10, 12, 14, or 16 days, respectively, at 26 °C. A 10% moisture reduction was achieved by drying ungerminated and germinated seeds at 600 °C. Before usage, these samples were stored in tightly packed polyethylene bags at 40 °C. Using a pre-chilled mortar and pestle and a phosphate buffer pH 7.5, dried seeds were pulverized. The supernatant underwent additional salt precipitation before being cutoff, separated, and utilized to estimate total protein and antibacterial activity. The sample was stored at −20 in a freezer after being centrifuged for 15 min at 10,000 rpm. After partial purification using the ammonium sulphate precipitation technique, samples were collected for antimicrobial tests. According to the findings, germinating seeds of soya, barley, maize, jowar, and wheat had more antimicrobial peptides that act on gram-positive and gram-negative bacteria [73].

Note Representative examples of plant seed antimicrobial peptides (AMP's) isolated using germination process with their classification and bioactivities is given in Table 1.

Sprouting for Isolation of Enzymes

Akhtaruzzaman et al. [74] reported the isolation and characterization of protease enzyme from leguminous seeds using sprouting/germination process. According to the mentioned study, leguminous seeds can be a source of proteases for use in industry. Seven different types of leguminous seeds—soybean, lentil, black gram, green gram, Bengal gram, groundnut, and pea bean—were used in the study to identify and characterize the proteases. Temperature and pH were found to affect protease activity. Maximum specific activity was seen in the pH profile of proteases between 7.5 and 9.0. The seeds were cleaned separately and immersed in distilled water for overnight germination at room temperature. Due to their low fat content, all seeds were then ground using an electric homogenizer without the use of acetone, with the exception of soybean and groundnut. To remove the fat, cold acetone was used to homogenize soybean and groundnut. The homogenates were then finely pulverized and blended for 3 h with chilled 10 mM Tris–HCl buffer at pH 8.0 containing 2 M NaCl in a pre-chilled mortar. The extracted mixtures were put through gauge filters, and the filtrates were centrifuged for 10 minutes at 10,000 rpm below 4 °C. The estimated extracellular protein content and further purifications were done using the collected supernatant. For overnight precipitation, the collected supernatants were saturated with 50% solid ammonium sulphate. After precipitation, they underwent a 30-min centrifugation at 10,000 rpm at 4 °C. The precipitate that had been collected was dissolved in 10 nm Tris–HCl buffer (pH 8), dialyzed against the same buffer, and then centrifuged at 5000 rpm for

10 min. For the characterization and testing of a particular activity of enzyme, the supernatant was employed as crude enzyme. Assay of protease activity procedure for the leguminous seeds using hemoglobin and casein as substrate were performed. The researchers concluded that leguminous seeds could be a source of proteases for industrial purposes [74].

Sprouting for Isolation of Amino Acids

The purpose of this study reported by Sulieman, M.A., 2008, was to investigate how sprouting affected the cultivars' chemical, compositional, energy, and amino acid contents. Three varieties of Sudanese lentils—Rubatab, Nadi, and Selaim—were sprouted over 3 and 6 days. The dried and ground seeds were sprouted. A determination was made regarding how sprouting altered the proximate makeup and amino acid content. While food energy and Nitrogen Free Extract (NFE) dropped during sprouting. The amino acid content of the seeds after sprouting was observed to change, and it was found that there was a little variance between cultivars. For the Selaim cultivar, sprouting for 3 days increased the proportion of all essential amino acids, with the exception of methionine, and decreasing amino acid content was shown when the sprouting duration was extended to 6 days, as was the case for histidine, lysine, and arginine. This outcome was likewise seen for the Rubatab cultivar, except for methionine and lysine, where the amount of essential amino acids rose as a result of sprouting. The essential and non-essential amino acids were enhanced in the Nadi cultivar after 3 days of sprouting. All cultivars of lentils generally had low levels of sulphur amino acids like methionine and cystine.

Each cultivar's bulk was divided into three equal pieces, the first of which served as a control (unsprouted seeds), the second of which was given 3 days to sprout, and the third of which was given 6 days to do so.

The seeds were steeped in distilled water for 2 h at room temperature prior to sprouting. In sterile petri dishes lined with damp filter paper, sprouting was done for 3 and 6 days at 4 °C. Samples were dried at room temperature at the conclusion of each sprouting phase and crushed to pass through a 0.4 screen for a future chemical analysis. For further study, the unsprouted seed control groups were crushed and stored at 4 °C. The amount of total nitrogen, crude fiber, and ash in a sample of unsprouted and sprouted seeds were examined. The study found that the proximate composition and food energy values of lentil underwent a notable alteration as a result of germination. Before germination, lentil had a low concentration of amino acids containing sulphur. Essential and non-essential amino acid levels increased as a result of germination [75].

Sprouting for Isolation of Probiotics Strains

According to a research by Manju Pathak (2013), *Bifidobacterium* sp. and *Lactobacillus* sp. were found in the seeds of lentil (*Lens culinaris*), mung bean (*Vigna radiata*), and peanut (*Arachis hypogeal*), and their populations grow as the seeds germinate. For peanuts, the highest bacterial colony count was found in seeds that had been soaked for 8 h, whereas for mung beans and lentils, seeds that had been soaked for 8 h and then germinated for 24 h had the greatest results. It was concluded that these seeds can serve as a source of *Lactobacillus* and *Bifidobacterium*, and the process of germination can be used to improve them. The seeds were cleaned three times before being immersed in RO (Reverse Osmosis) water for 8 h. After 8 h of soaking, seeds were retained in a damp muslin cloth for a further 24 and 48 h of germination. The raw seed samples (R) were taken without soaking, the 8-h sample was taken after soaking in water for 8 h, the 24-h germinated seed sample was collected after germination for 24 h after soaking for 8 h, and the 48-h germinated seed sample was collected after germination for 48 h after soaking for 8 h. Appropriate moisture and 28 °C temperature were maintained during germination. After screening, the optimal dilution of 1.25 g of sample seeds was taken for investigations. Each sample was spread on MRS agar (De Man, Rogosa and Sharpe agar) medium which was used as the growth medium. Every sample was applied to the MRS agar medium, which served as the growth medium and had a pH of 6.2 ± 0.2 at 25 °C. Colonies were counted each day while the plates were incubated at 37 °C on days 1, 2, and 3. The method used to determine the number of viable cells on each plate was colony counts. Genus-specific PCR (*Polymerase chain reaction)*, a catalase test for *lactobacillus*, a lysozyme resistance test for *bifidobacterium*, and other tests were carried out [76]. The outcomes show signs of endophyte activation during the germination phase.

Note Representative examples of plant seed germination/sprouting for isolation of amino acids, enzymes, probiotics, prebiotics, with their classification and bioactivities are given in Table 2.

4.2 Fermentation as Green Extraction Process

An environmentally friendly extraction method is fermentation. The metabolic process of fermentation involves the action of enzymes to cause chemical alterations in organic substrates. The metabolic system that controls homeostasis in both the human body and the natural world is based on fermentation, and ecological equilibrium is reliant on this concept. Without using high heat, ultrasonic wave, or other radiation sources for extraction, which typically damages a number of bioactive ingredients, fermentation followed by maceration improves the leaching of plant secondary metabolites from the matrix. Additionally, different extraction techniques require the use of chemical solvents that can vary in polarity and include ethanol, ethyl acetate, chloroform, petroleum

ether, n-hexane, etc. However, aqueous (water) solvent is primarily needed for fermentation [77]. Since no external chemical solvents need to be used, a gradient of successively created alcohol or acids during fermentation aids in a better extraction of active chemicals. Therefore, compared to other extraction techniques, fermentation has been seen as an environmentally friendly procedure. Additionally, the fermentation process eliminates unwanted carbohydrates from the herbal ingredient, improving the formulation's bioavailability. The bacteria function as probiotics in fermentation and improve the bioavailability of existing secondary metabolites. Different enzymes produced by various micro-species (bacteria, fungus, and yeast) for the breakdown of the cell matrix play a significant role as fermentation initiators creating particular byproducts. One of the oldest medical systems in India, Ayurveda makes extensive use of both single and multi-herbal medications and formulations, which are described in numerous Ayurvedic texts and the Ayurvedic Formulary of India (AFI). By using various extraction techniques, these formulations transport the active components of herbs into menstruum fluids. "Sandhana kalpana" (Asava and Aristha) is a special Ayurvedic dosage form that involves fermentation [77–80].

Fermentation has been used to produce food for a very long time. Edible seeds are essential to the human diet and have numerous health advantages, such as some beans and cereal grains. Edible seeds and their products are frequently fermented using a variety of microorganisms, such as lactic acid bacteria, molds, and yeasts, which are known as generally recognized as safe (GRAS) microbes. New bioactivities and altered bioactive components can both result from fermentation. Overall, fermented edible seeds and their products have more bioactive components, especially natural phenolics and gamma-aminobutyric acid, and they have a variety of bioactivities, including antioxidant and anticancer effects. As a result, they can be suggested as a significant component of the human diet or developed into functional foods to aid in the prevention of specific chronic diseases [81].

The by-products or metabolites produced as a result of integration of prebiotics with probiotics in the fermentation process today are found to be taking stand as postbiotics. The fermentation end product contains prebiotics, probiotics, vitamins, minerals, short chain fatty acids, cell lysis products, etc., that could act as treatment strategies in case of dysbiosis (human flora disturbance) conditions. Moreover, these combination products of fermentation could also make the alternatives for the development of prophylactic approaches in the form of nutritional postbiotic health drinks, powders, etc., considering the homeostasis balance of the body.

4.2.1 Preliminary Guide to Fermentation Procedures

There are two types of fermentation, naturally occurring and inoculated, depending on the source of the bacteria used in the process. According to the amount of water in the system, fermentation can also be classified as either solid-state (SSF) or liquid-state fermentation (LSF) [82–84]. Before fermentation, edible seeds frequently require pretreatment, such as soaking, cracking, grinding, sifting, and boiling. [84] These processing techniques are frequently put together in combination. Prior to natural fermentation, seeds cannot be cooked or autoclaved since doing so will partially or totally eliminate any bacteria already present on the seeds. The most frequent bacteria utilized in edible seed fermentation as starter culture and inoculum are lactic acid bacteria (LAB), including *Lactobacillus (Lb.) acidophilus, Lb. brevis, Lb. bulgaricus, Lb. casei, Lb. fermentum, Lb. johnsonii, Lb. paracasei, Lb. plantarum, Lb. reuteri, Lb. rhamnosus, Lb. rossiae, Lb. Streptococcus (Sc.) thermophilus, Bifidobacterium (Bb.) animalis, Bb. infantis, Lactococcus (Lc.) lactis, and Weissella (W.) paramesenteroides* [81–84]. In addition, *Bacillus (B.) subtilis* has also been commonly used to ferment edible seeds [83]. Additionally, cultures of fungi (molds) used for fermentation are *Aspergillus (A.) oryzae* [85], *A. awamori, A. sojae, A. niger, Agrocybe (Ac.) cylindracea, Cordyceps (C.) militaris, Coprinus (Cr.) cinereus, Grifola (G.) frondosa, Ganoderma (Gd.) austral, Gd. neo-japonicum,* etc. Cultures of yeasts, such as *Issatchenkia (I.) orientalis, Saccharomyces (S.) cerevisiae, and S. boulardii*, etc., have also been employed to ferment edible seeds [81, 86]. The amount of inoculum in the starter culture is essential for the fermentation process. Inoculation of 1% to 10% (bacteria (mL)/sample (mL or g)) of the starting culture (10^8 cfu/mL) has been used regularly in SSF and LSF of edible seeds, and their products, with 10^6 to 10^7 cfu/mL LAB in original samples, have been used for LAB fermentation. In SSF of edible seeds for *B. subtilis* and fungal fermentation, inoculation of 5% (bacteria (mL)/sample (mL)) of the starting culture (10^5/g sample) has been employed most frequently [81, 83]. To carry out the fermentation effectively, the inoculum needs to be optimized. The fermentation efficiency is influenced by a number of variables, including fermentation temperature, duration, humidity, and other circumstances [81]. Other factors controlling the fermentation process which are reported to be optimized are temperature, humidity, stirring/shaking speed, aerobic or anaerobic conditions, pH, fermentation time, etc. [81].

Living creatures are profoundly impacted by temperature variations. The sensitivity of enzyme-catalyzed reactions to minute temperature variations is very high. As a result, the environment temperature frequently affects the metabolism of poikilotherms organisms whose internal body temperature is influenced by it [87]. Every degree of temperature count. Temperature is key to fermentation success. The optimum temperature range for yeast fermentation is between 32 °C and 35 °C. Every degree above this

range depresses fermentation. Yeast can usually tolerate short-term fluctuations in temperature [88]. However, operating above ideal temperatures for longer stretches of time can significantly have impact on fermentation and secondary metabolite production. Humidity control is one of the important parameters to be considered during solid state fermentation process. The study reported by Taimí Carrasco et. Al, 2003, shows the requirement of humidity as indicator of fermentation success. Humidity is one of the determinant indicators in the dynamic of the solid-state fermentation. Impact of humidity on actions of enzymes has been predicted in the study which ultimately found to have impact on the production the secondary metabolites required during the process [89]. Agitation plays an important mixing and shearing role in fermentation processes. It not only improves mass and oxygen transfer between the different phases, but also maintains homogeneous chemical and physical conditions in the medium by continuous mixing. On the other side, agitation can cause shear forces, which influence microorganisms in several ways, such as changes in morphology, variation in growth and metabolite formation and even causing damage to cell structures. Aeration determines the oxygenation of the fermentation process, and also contributes to mixing of the fermentation broth, especially where mechanical agitation speeds are low. Aeration not only supplies the necessary oxygen for cell growth, but also eliminates exhaust gas generated during the fermentation process. However, higher aeration rate results in a reduction in the volume of fermentation broth. Oxygen supply is necessary for growth of microorganisms in aerobic fermentation, but some microorganisms may be affected by oxygen toxicity at excessive oxygen concentration. So agitation optimization based on type of fermentation is the ultimate requirement [90]. So as the supportive information, the following data could be considered for fermentation optimization process.

Natural fermentation has been reported to control the temperature at 30, 37, or 42 °C which is probably associated with the main microbes carried by different seeds. In addition, the temperature commonly controlled at 37 °C for LAB fermentation while fermentation using *B. subtilis* and fungi has mostly been employed at 30 °C probably due to the optimum growth at this temperature. For fermentation time, several hours to several days have been reported, while 48 and/or 96 h are most commonly used for edible seed fermentation. In addition, it is better to control the fermentation humidity at 90% to 95% if possible which can provide a relatively moist air condition for the growth of microbes. SSF of edible seeds and bean milk fermentation are generally performed quiescently, while LSF of edible seeds is commonly carried out by continuous shaking/stirring, with a speed of 200 to 450 rpm which can accelerate the growth of microbes, increase the interaction between

microbes and substrates, and enhance the efficiency of fermentation. Similarly, adding sugars (1–2%) to the fermentation system, such as glucose or sucrose can provide an extra energy source to accelerate the growth of microbes. Moreover, fermentation can be performed under aerobic or anaerobic conditions, dependent on the species of microbes involved. For example, it is better to perform LAB fermentation in an anaerobic or microaerophilic environment. Overall, the fermentation condition is critical for the efficiency of fermentation and needs to be optimized for fermenting different products [81, 91].

4.2.2 Fermentation for Isolation of Postbiotics

Fermentation is the most prevalent postbiotic source in the food industry. The presence of postbiotics can be found naturally in several milk-based and other products like kefir, kombucha, yogurt, and pickled vegetables, etc. [91]. Postbiotics are functional bioactive substances that are produced during fermentation in a matrix and can be used to improve health. Postbiotics can be thought of as a catch-all name for all synonyms and related terms of various components of microbial fermentation. As a result, postbiotics can consist of a wide range of components, such as metabolites, short-chain fatty acids (SCFAs), microbial cell fractions, functional proteins, extracellular polysaccharides (EPS), cell lysates, teichoic acid, muropeptides derived from peptidoglycans, and pili-type structures, among others. The use of postbiotics may enable active bacteria to become more potent or transform them into useful components. In addition, postbiotics get around the technical problems of colonization effectiveness and maintaining the microbes in the product at a high dose. As a result, it is easier to deliver the active substances where they are needed in the gut, the shelf life is increased, and perhaps packing and transportation are also made simpler [92]. The structure and operation of the commensal human gut microbiome can be influenced by postbiotics produced during fermentation. They also aid in inhibiting possible pathogens while giving the local microbial population the substrates it needs to produce SCFAs. The following are some potential advantages of fermented foods and beverages: Kefir's organic acids, bacteriocins, carbon dioxide, hydrogen peroxide, ethanol, and diacetyl all have antimicrobial properties. Kombucha's low pH and high acetic acid concentration also inhibit the growth of pathogens. Meanwhile, conjugated linoleic acid in sauerkraut may have potential health benefits. Similar types of chemicals are produced during grain fermentation along with proteolytic activity by lactic acid bacteria, which transforms wheat proteins into bioactive peptides (postbiotics). Vitamin B12 is produced during the fermentation of soybeans. Fruit fermentation with *Lactobacillus plantarum* produces phenolic compounds and a number of organic acids [93].

Studies conducted in 2017 by Sasithorn Sirilun et al. showed the advantages of fermented soybeans mediated by lactic acid bacteria. By acting as an antioxidant and increasing the quantity of isoflavones in their aglycone forms, lactic acid bacteria-mediated fermentation improved the fermented soy broth quality. Additionally, it stopped the development of coliforms in fermented soybean. East Asian nations consume large quantities of fermented soybean products, which are important sources of bioactive chemicals. Examples include cheonggukjang (Japanese natto), doenjang (soy paste), ganjang (soy sauce), and douchi. A range of new compounds, the majority of which have health benefits, are produced when cooked soybeans are fermented with bacteria (*Bacillus* spp.) and fungi (*Aspergillus* spp. and *Rhizopus* spp.) [94].

Note Representative examples of seed fermentation for isolation of prebiotics, probiotics, and postbiotics are given in Table 3.

5 Experimental Case Report from Our Own Laboratories

*5.1 Experiments with Sprouted Flaxseeds (*Linum usitatissimum, *in the Family Linaceae)*

Sprouted flaxseeds were explored for prebiotic and postbiotic properties: It was an attempt to develop, In- vitro biorelevant media and time simulation probiotic proliferation methodology to determine prebiotic potentials of flaxseed powder. The research ultimately came up with excellent findings for prebiotic and probiotic potentials of flaxseed. Prebiotic potential of flaxseed powder was tested using *Bacillus* coagulans *SNZ 1969* marketed probiotic wherein it was found that flaxseeds act as excellent prebiotic supplement for the growth of probiotics, similarly at the outset it was also observed that endophytes from the seeds of flaxseeds colonize in the presence of MRS agar media, provided strict sterile conditions were maintained to avoid environment contamination. It was concluded that fermented flaxseed powder could be effective postbiotic supplement which could be explored further in postbiotic supplement development [95].

*5.2 Experiments with Sprouted Ragi (Finger Millet) Seeds (*Eleusine coracana *in the Family Poaceae)*

In our another unpublished research on comparative evaluation of prebiotic and probiotic benefits of sprouted and non-sprouted ragi seeds, it was observed that sprouted ragi seeds grains comparatively have excellent prebiotic properties. Sprouted ragi seeds and sprouted fermented ragi seeds also showed excellent prebiotic and probiotic potentials. It was also observed that endophytes from the seeds of Ragi seeds colonize in the presence of MRS agar media, provided strict sterile conditions were maintained to avoid environment contamination. Which could further be explored for identification and isolation of probiotics and postbiotic components from the sprouted and sprouted fermented seeds.

Table 3
Representative examples of seed fermentation for isolation of prebiotics, probiotics, and postbiotics

Sr. no.	Plant seed	Bioactive component	Function	Reference
1.	Hemp seeds (*Cannabis sativa*) Probiotics used for fermentation are (*Lactobacillus fermentum, Lb. plantarum, and Bifidobacterium bifidum*)	Prebiotic potential	Ability to support probiotics growth was observed. Increase in the content of some bioactive compounds like presence of different terpenes that inhibit the growth of enteropathogens and high levels of short chain fatty acids like acetate, propionate and butyrate produced during fermentation that support the growth of probiotics	[107]
2.	*Artocarpus integer's* seed Probiotics used for fermentation are *Lactobacillus acidophilus* DSM 20079, *Lactobacillus casei* DSM 20011, and *Escherichia coli* DSM 1103	Prebiotic potential	*A. integer* extract was found to support the growth of probiotics such as *L. acidophilus* and *L. casei*. The results of the present study indicated that *A. integer* extract was comparable to the commercial prebiotics inulin	[108]
3.	Red and white rice seeds	Probiotic: Lactic acid bacteria (LAB)	Gut flora heath booster stimulate cell-mediated immunity	[109]
4.	Fermented finger millet flour: Three varieties namely Ravi, Raavana, and Oshadha	Five potential probiotic LAB strains (lactic acid bacteria) were isolated: R17 (*L. plantarum*), RV02 (*L. fermentum*) and RV19 (*L. lactis sub species lactis*), RV28 (*E. faecium*), and O24 (*P. acidilactici*)	Potential in terms of health benefits	[110]
5.	Fermented beverage using red rice (*Oryza sativa* var. Indica, Tapol), barley (*Hordeum vulgare* L.), and buckwheat (*Fagopyrum esculentum*), fermentation culture: Lactic acid bacteria	Postbiotics: Decrease in antinutrient phytic acid, increase in phosphorous, increase in fibers (prebiotics), etc.	Enhance the bioavailability of minerals, digestibility, and sensory properties of the final products	[111]

(continued)

Table 3
(continued)

Sr. no.	Plant seed	Bioactive component	Function	Reference
6.	Fermented soybean meal using bacillus strains (*Bacillus subtilis* TP6 strain)	Postbiotics: Product reach in probiotics, digestible peptides, polyglutamic acid, short chain fatty acids like lactic acid, non-reducing oligosacchrides like Raffinose, Stachyose, etc., isoflavones, lipopeptides, protein hydrolysates, and enzymes	Acts as probiotic supplement as gut flora booster and help to improve innate immunity, protein content is high, and digestion and absorption rates are also improved by low-molecularization of the proteins. Polyglutamic acid helps to reduce body fat	[112, 113]

5.2.1 Materials Used

Probiotic supplement—*Bacillus coagulans SNZ 1969*, (Sporlac sachets, Sanzyme Biologics Private Limited) Ragi Seeds. The herbal character of plant specimen of ragi was affirmed by a taxonomist at the Department of Botany, Botanical Survey of India, Pune. It was validated to be *Eleusine coracana (L.)* Gaertn. belonging to family Poaceae. A voucher specimen number No. BSI/WRC/100-1/Tech./2020 was obtained.

5.2.2 Sprouting Procedure

A total of 250 g of ragi seeds was taken and cleaned to eliminate unfamiliar particles from it. The cleaned seeds were then soaked into adequate measure of water for 8 h at room temperature. After 8 h, the excess of water from seeds was eliminated utilizing filtrations and the seeds were half dried. The seeds were then kept for germination for around 24 h. The developed seeds were further kept for drying. The sprouts on the seeds were shed and cleaned physically. The seeds were then crushed to get the ragi powder, passed through sieve number #100 to get uniform particle size.

5.2.3 Fermentation Procedure

Eleusine coracana sprouted seed powder (30 g) obtained as per the above-mentioned procedure was taken, and to it was added to 1 g of *Bacillus coagulans SNZ 1969* powder and 1 g of citric acid powder. Forty milliliters of water was added to the mixture and the mixture was placed for 24 h at room temperature. After 24 h of fermentation, wet mass was extruded through the extruder. The extrudates are shaped into small spherical granules. The wet granules were allowed to dry. *Methodology*: Probiotic proliferation study of sprouted, non-sprouted, and fermented products was performed as reported in our own research publication [95].

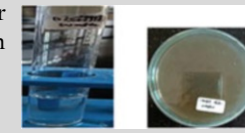
5.2.4 Research Highlights

Sprouted ragi seed powder has been proved to have excellent prebiotic activity in comparison to non-sprouted powder. Sprouted and non-sprouted ragi seed powder shows self-probiotic potential. Sprouted powder support the probiotic spore and culture growth in the presence of antibiotic as compare to non-sprouted powder. Similarly, combined run real time simulated biorelevant media study with and without enzymes and antibiotic (Azithromycin) also depicted the prominent growth of the probiotic spore powders. The study concludes that germination of seed increases prebiotic and probiotic potential of seed. Overall results indicated that ragi seed sprouting and fermentation increase the extraction of prebiotic components and probiotic endophyte activation. Results helps to conclude that sprouting and fermentation are the natural in situ green extraction techniques which could be explored well for health benefits. Table 4 indicates some representative images for the ragi seed sprouted powder prebiotic as well as probiotic potentials.

6 Conclusion

Understanding this interaction and developing ways to model the outcome is essential for developing effective crop establishment practices as well as beneficial utilization of the plant seeds components for maintenance of human health. Nature takes care of ecological homeostasis through utilization of prebiotics, probiotics, and postbiotics. The simplest lesson to be taken from nature is degraded seeds when they are in soil, in moist conditions, ferment, gets converted to postbiotics which flourish the soil microflora. Seeds, the complete package of defense system containing antimicrobial biopeptides. The AMP's get activated during germination and fights against the pathogens in the zone around the seeds in the soil. Seeds endophytes and epiphytes take care of vertical and horizontal innate immune systems throughout the plant life cycle. These are nature's unique in situ extraction procedures to activate the components at the respective required locations. Activation and utilization of enzymes, vitamins, minerals, prebiotics, postbiotics, probiotics, etc., occur in nature without harming the ecosystem balance. Nature makes use of these processes using safe parameters, solvent like water, temperature, humidity, etc. Enzymes act as a catalyst for the completion of biochemical reactions. In situ generation of acidic and basic components acts as buffering agents to provide respective pH for the completion of the process. Interactions of prebiotics and probiotics during fermentation lead to the formation of metabolites like short chain fatty acids and alcohol, which act as natural solvent systems for activation and extraction of the required components and completion of many reactions. The

Table 4
Prebiotic and probiotic potentials of Ragi seed (*Eleusine coracana*) sprouted powder

Sr. no.	Observations	Images
1.	*Environmental negative controls*: No growth observed in MRS broth tube as well as MRS agar plate maintained throughout the study, it indicates maintenance of strict sterile conditions during the experimentation	
2.	*Negative growth controls with sterile distilled water*: No growth after 24 h incubation in tubes as well as after plating and incubation on MRS agar media (indicates that water does not act carrier for contamination during studies)	
3.	*Positive growth control*: Both MRS broth and MRS agar media support the growth of *Bacillus coagulans* probiotic spores considered for studies	
4.	*Viability of probiotic spores in sterile distilled water*: Sterile water with probiotic spores does not support growth in water but spores remain viable after 24 h as growth is observed after plating and incubation further on MRS agar media	
5.	*Prebiotic potentials of sprouted powder*: Probiotic spore in the presence of ragi sprouted powder shows comparatively increased growth, indicates the supportive prebiotic nature of the ragi seed sprouted powder(without addition of MRS broth for first 24 h media)	
6.	*Probiotic potentials of sprouted powder*: Sterile water with sprouted ragi seed powder (with no prebiotic spores added externally) after incubation for 24 h and plating on MRS agar media showed the growth indicating the activation of the endophytic microflora associated with seeds	

beneficial interaction of these components during fermentation gives rise to many health-benefiting constituents. To date many plant seeds are unexplore for their potential as prebiotics, endophytes, epiphytes, postbiotics, antimicrobial biopeptides, synbiotics, nutritional comonents and much more. Further it is imrpotant to learn and understand what and how nature do these processes. Nature maintains ecological homeostasis in an optimized manner. Let us learn to optimize the procedures the way nature does.

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Chapter 15

Essential Oils: Sustainable Extraction Techniques and Nutraceuticals Perspectives

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Abstract

Essential oils in their unadulterated form, can be primarily classified into two fundamental chemical constituents, namely: hydrocarbons, oxygenated and terpenoidal bioactive compounds. The biochemical characteristics of essential oils exhibit significant variations contingent upon the specific extraction methods employed. While traditional techniques such as cold pressing, hydro-distillation, and maceration have long been prevalent, they are not without their drawbacks, such as lower yield, the potential for degradation of thermolabile compounds, and concerns regarding the environmental impact of solvent usage. In the pursuit of sustainable and effective extraction, modern methodologies have risen to prominence, including microwave-assisted, supercritical, and ultrasonic extraction techniques. These innovative approaches have circumvented the inherent limitations of conventional methods, offering novel possibilities for harnessing the full potential of essential oils. This chapter offers a brief review of both classical and contemporary extraction techniques, shedding light on their influence over the biochemical properties of essential oils. Furthermore, it delves into the promising perspectives of utilizing these oils in for nutraceutical applications, underscoring their potential for enhancing human well-being.

Key words Essential oil, Extraction, Biochemical activities, Conventional, Classical

1 Introduction

Essential oils can dissolve well in polar solvents including benzene, toluene, acetone, ethanol, and methanol, despite being volatile hydrophobic liquids with a comparatively lower density than water [1]. Due to the existence of a complex mixture of bioactive substances, the bioactivity, flavor, smell, and components of essential oils are well known [2]. They often originate from plants like leaves, exfoliate, twigs, flowers, petals, and pods. There are around 3000 essential oils recognized today, with over 300 of them being commercially important, primarily in the pharmaceutical, culinary, domestic, and cosmetic industries [3]. The increasing interest in the study of essential oils is attributed to their diverse biological

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functions, which encompass anti-inflammatory, antibacterial, antifungal, anticarcinogenic, antioxidant, antiviral, and antimutagenic properties [4]. The most extensively explored biological activity in essential oil research is the antioxidant activity considering that several biological molecules are affected by oxidation and thereby prompting many diseases such as cardiovascular and neurological disorders [5].

In recent times, many studies have elucidated the antioxidant properties of various essential oils in the hope of discovering safe, natural antioxidants. One such study was established by Shaaban et al., [6], who investigated the antioxidant activities of the essential oil from 25 spices including thyme, chamomile, clove leaf, eucalyptus cinnamon leaf, and basil. Tian et al. [7] reported that the antioxidant activities of essential oils from Egyptian corn silk and *Curcuma zedoaria*. Some group of bioactive compounds such as terpenoids, terpinene, terpinolene, 1,8-cineole, and terpenes has also been studied and found to boost essential oil's antioxidant activities [8]. Also, a wide variety of these plants oils has been found to exhibit antibacterial properties; as different spices and herbs, for example, have been traditionally employed as preservatives in food to kill bacteria [9]. Take, for instance, *Nandina Domestica Thunb*, which has been reported to be an effective preservative for different food products [10]. Bioactive constituents with antibacterial activities against the *H. pylori* from most essential oils include carvacrol, sabinene, nerol, and isoeugenol [11, 12]. Badekova et al. [13] reported the antibacterial activities of thymol and carvacrol against *E. coli*, which suppressed pathogenic bacterial strains: key components of essential oils from oregano and thyme.

In addition, synthetic antiviral drugs have been largely used for the treatment of viral diseases in humans such as the HSV (herpes simplex virus) [14]. The use of synthetic drugs is not without their attentive side effects, which necessitates the application of plant-based essential oil for the treatment of many viral diseases. Reichling [15] investigated the effectiveness of lemongrass essential oils which was reported to provide a significant anti-HSV-1 effect more than all the drugs that have been previously used. Furthermore, essential oils have a long history of use against inflammation because of their strong anti-inflammatory bioactive constituents. Inflammation has generally been linked to various diseases such as hypertension, cancer, and stroke [16]. Ogidi et al. [17] investigated the anti-inflammatory qualities of a pale, clear, cold-pressed *Aloe vera* essential oil with great potential as a carrier medium in aromatherapy. Sánchez et al. [18] experimented on diabetic rats and cases of cutaneous ischemia with *Aloe vera* oil reported to promote wound healing. In another study, oil extracted from *Aloe vera* plant has been used to treat carrageenan-induced edema in rat paw, which had anti-inflammatory characteristics and suppressed

cyclooxygenase activity [19]. The result obtained shows that *Aloe vera* oil demonstrated the strongest lipoxygenase inhibitory activity (up to 96%) with a concentration of 0.5 g/mL. Other oils such as thyme oil (86%) and bergamot oil (85%) were not lesser in their effectiveness [19]. Chandel et al. [20] also reported that chamomile oil had modest lipoxygenase inhibitory action at 0.5 g/mL, while it had significant lipoxygenase stimulating activity at 5 g/mL (123%).

Also, some essential oils have been proven to have a wide reach of fungicidal activities on post-harvest infections. The antifungal characteristics of essential oils are often maximized in the vapor phase for the storage of food [21]. However, because the food item may still decay in the vapor phase, more investigation is needed [21]. Hossain et al. [22] identified the resistance of carvacrol and thymol against food-borne fungi such as *Aspergillus flavus, Aspergillus parasiticus, and Aspergillus niger*. Essential oils from plant-based materials reduce free radicals, activates antioxidant enzymatic cells, and prevent the permeation of mutagens [23]. An aromatic plant-derived compound such as terpinene, and terpineol, was reported with such activity. Only a few studies have been conducted on the antimutagenicity of DNA repair by phenolic and terpenic compounds found in essential oils [24]. The quality characteristics and biological activities of essential oil are largely dependent on the extraction technique utilized.

2 Effects of Conventional Extraction on Biochemical Activities of Essential Oil

Due to the obvious wide range of extraction processes, the biochemical activities of essential oil components are highly diverse. The most common extraction procedures are cold pressing, hydrodistillation, and maceration, each with advantages and disadvantages. Take instance, cold pressing (expression) is solely employed in the extraction of citrus oils as it is primarily utilized to recover essential oils from citrus fruits. Expression is a four-step procedure that includes scarification, pressing, centrifugation, and filtration. In this process, the citrus skins are scrubbed (scarified) to rupture the microcellular structure holding the essential oils, which is thereafter pressed to extract the oily substance from the sample [25] (Fig. 1). The essential oil on the sample surface is then separated into layers using centrifugal pump and thereafter filtered to get pure essential oil.

One significant advantage that cold pressing has over other methods is their high degree of purity and higher biological activities of the oily extracts. In addition, the cold-pressed oil keeps the oil's original flavor and color while also preserving the oil's biologically active components. However, the drawback of cold pressing (expression) is that the sample is not generally extracted at optimal conditions, resulting in a low extraction yield. Furthermore, most

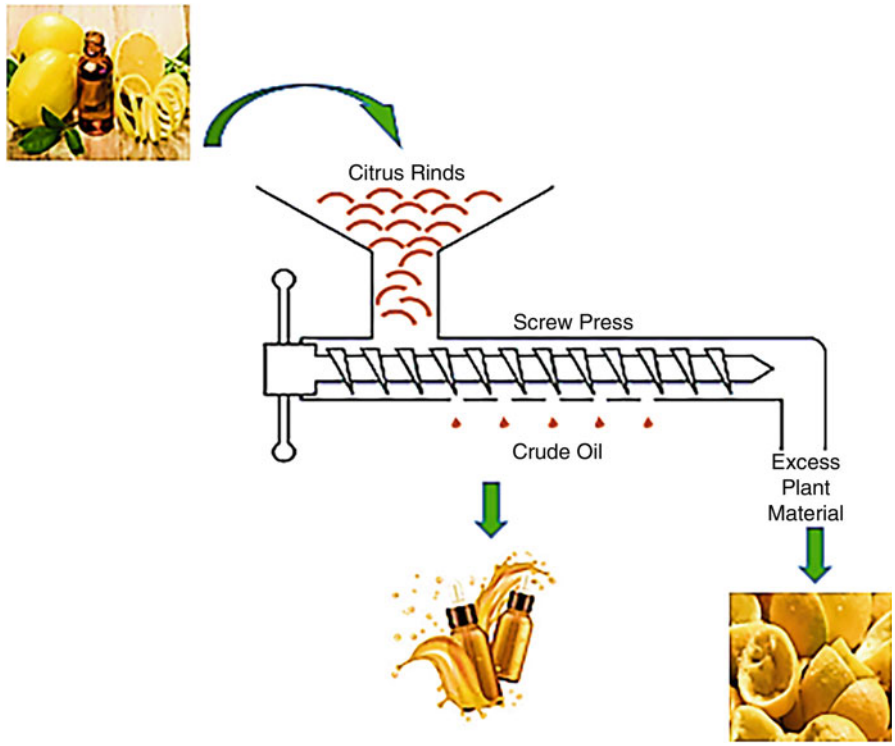

Fig. 1 Schematic diagram of a typical cold press extraction of citrus peel [26]

plant samples are unsuitable for cold pressing since they cannot sustain high mechanical pressure [25]. Furthermore, there is fluctuation or inconsistent moisture content in cold pressing, which might affect the biochemical activities of the essential oil as reported by Çakaloğlu et al. [25].

Furthermore, the earliest and easiest technique of extracting essential oils is the hydro-distillation technique, which begins with the immersion of the plant sample straight into the extracting solvent (water) within the reactor and then boiling the entire mixture. This extraction technique is said to be a one-of-a-kind approach for extracting oil from plant parts such as tough nuts, wood, seeds, and hard surface powders. It is commonly utilized for the extraction of oil that contains hydrophobic matter with a high boiling point. Because the oils are covered in water, this technique allows essential oils to be extracted at a controlled temperature without being overheated [27]. The capacity to separate plant components under 100 °C is the major benefit of this extraction process [28]. The hydro-distillation set-up consists of a heater source, a reactor, a condensation chamber that converts vapor from the reactor into liquid, and a decanter to capture the condensate and separate the water and essential oils mixture (Fig. 2).

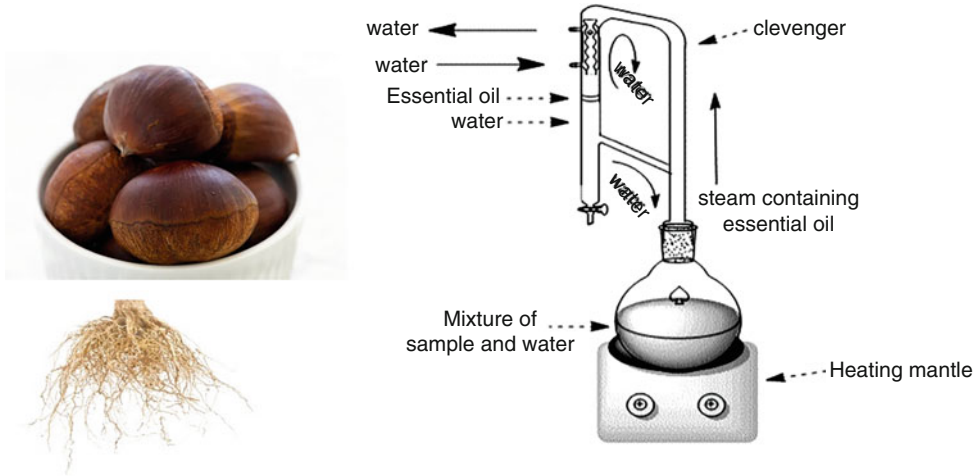

Fig. 2 Schematic diagram of a hydro-distillation apparatus [29]

To recover essential oils by hydro-distillation method, the plant sample is usually loaded and a considerable amount of extracting solvent (water) is poured and heated to a boiling temperature; conversely, steam is normally introduced. The essential oil is released from the cellulosic oil glands by the impact of high pressure. The water-oil vapor combination is condensed by evaporative cooling water. Distillate runs from the condensing compartment into a splitter, where the essential oil separates efficiently from the condensate. The advantage of this approach is the utilization of the extracting solvent (water) and the ease with which it can be set up [28]. This is put up before the dehydration of the plant material and works best when the material is dry. Compared to other extraction methods, it is a superior alternative due to the ease of use and accessibility of its ancillary equipment [30]. Unfortunately, there are some inherent disadvantages to using this approach. There are a number of drawbacks associated with extracting biological components, including excessive solvent use, poor output yield, liquid contamination, costly extraction, and an extended process time.

Maceration is another traditional method of essential oil extraction in which various carrier oils are used as solvents to extract the bioactive essential oil. The essential oil obtained through the maceration process is also called infused or macerated oils [31]. This technique is superior to distillation because it recovers higher molecular compounds from the plant samples [31–33]. The procedure involved loading into a closed vessel of a finely divided plant material and solvents (menstruum). The mixture of the plant sample and the solvent are kept for 7 days and intermittently stirred using a magnetic stirrer. The mixture is then pressurized to collect the fluid from the waste of the plant (marc). Then, filtering of the resulting liquid mixture is carried out to remove the infused oil as presented in Fig. 3.

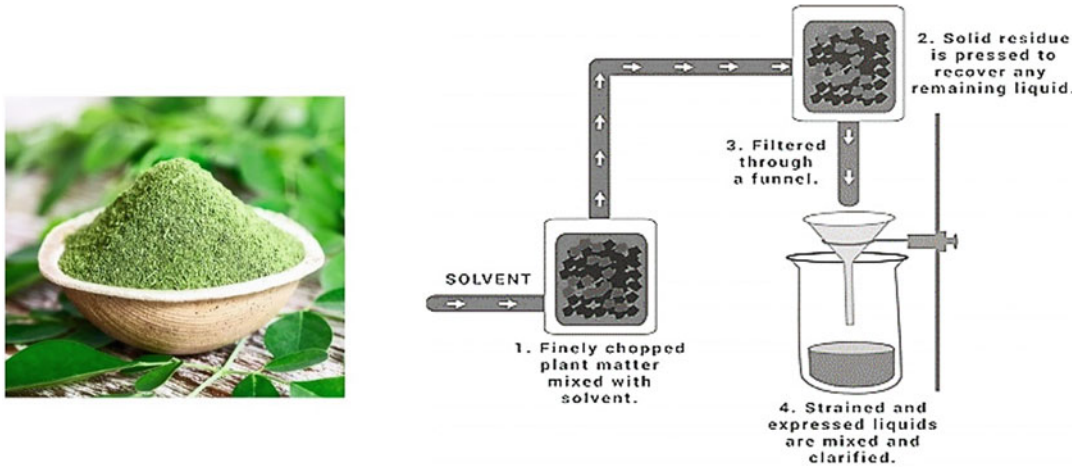

Fig. 3 Schematic diagram of maceration extraction of infused oils [34]

Past and recent studies had modified the use of the maceration method for the recovery of essential oil from plant sources. Notable among them are Kowalskia and Wawrzykowskib [35] who employed an ultrasound-assisted maceration technique to extract essential oil from thyme (*Thymus vulgaris L.*) dried leaves. Kowalski et al. [36] reported the use of maceration techniques as a preliminary extraction process before ultrasonic processing of essential oil from peppermint leaves, marjoram herb, and chamomile flowers. Mariane et al. [37] investigated the recovery of olive oil from Brazilian pink pepper using different stages of the maceration process. Soares et al. [38] incorporated the ultrasonic and maceration process for the extraction of enhancing flavoring of rosemary and basil extra virgin olive oil. Unfortunately, the use of maceration for extraction of essential oil has many limitations which include a longer duration for extraction which could take days for completions [39]. Higher solvent consumption and a low degree of other drawbacks affect the effectiveness of maceration extraction of essential oil [39].These limitations reduce the quality characteristics and hence the biochemical activities of their essential oils. Traditional extraction methods often take a long time, which means that some of the plant material's bioactive components will inevitably degrade. Examples of these conventional approaches are listed in Table 1.

3 Effects of Classical Extraction on Biochemical Activities of Essential Oil

Conventional procedures have been enhanced by new techniques such as microwave-assisted, supercritical, and ultrasonic essential oil extractions. Microwave-assisted extraction for example has more capabilities than the conventional solvent extraction mentioned in

Table 1
Conventional methods of extracting essential oils from various plant sources

Sample	Parts of plant	Conventional extraction methods	References
Pequi (*Caryocar brasiliense*)	Fruit	Cold pressing	[40]
Rice bran	Husk	Cold pressing	[41]
Cannabis sativa L. hemp	Leaves	Cold pressing	[42]
Fennel	Leaves	Cold pressing	[43]
Prunus serotine	Seeds	Cold pressing	[44]
Clove	Buds	Cold pressing	[45]
Hemp	Seeds	Cold pressing	[46]
Rosmarinus officinalis and *Origanum compactum*	Whole plant	Hydrodistillation	[47]
Lamiaceae (Mint)	Leaves	Hydrodistillation	[48]
Kumquat	Peels	Hydrodistillation, ultrasonic, microwave extraction	[49]
Schinus molle	Leaves and fruits	Hydrodistillation, fractional hydrodistillation, and steam distillation	[27]
Bitter orange	Peel wastes	Hydro-distillation	[50]
O. basilicum L.	Leaves	Hydrodistillation	[51]
O. vulgare L. subspecies *hirtum*	Aerial parts	Hydro-distillation	[52]
Litsea cubeba (Lour.) Pers.	Fruits	Hydro-distillation	[53]
Aquilaria malaccensis	Leaves	Hydro-distillation	[29]
Thymus serpyllum L. herb	Leaves	Maceration	[54]
Brazilian pink pepper	Fruit	Maceration	[37]
Rosemary and basil	Leaves	Maceration	[38]
Orange peels	Peels	Maceration	[55]

the previous section. In microwave-assisted extraction, conduction and convection occur at a pace that is so fast that it is frequently neglected or thought to be inconsequential since it occurs in a matter of seconds [56]. Microwave heating is usually through electromagnetic radiation with heat and mass transfer rate uniformly spread throughout the heating reactor; unlike the conventional approach where the heat transfer is not homogenous from the elevated temperatures to the lowest part [57]. The ability to extract pure essential oils free of undesirable impurities utilizing

microwaves has been shown in several research studies [58]. This opens up the possibility of shortening the extraction process, reducing energy consumption, using less solvent, increasing bioactive selectivity, and improving extraction yields [33, 59]. Ionic conduction and dipole rotation are the fundamental mechanisms at work in microwave extraction [60]. These two characteristics may coexist, with ionic conduction acting as a formidable obstruction to ion transport when present. This causes a temperature differential in the extraction media and also creates impedance. Meanwhile, the biological dipole moment is readjusted into the electric field by dipolar rotation [61]. Even as dipole revolves around its axis, an ionic flux is consequently present. In the microwave cavity, the electrical field produces an ionic current in the medium, which initiates the separation process. Because of their electromagnetic characteristics, microwaves' electric field is an orthogonal orientation to the magnetic fields [62]. So under the effect of the highly dynamic electrical field, the solvent provides resistance for its ion flux. Desai and Parikh [63] argued that the amount of turbulence in the passage of solvent ions into the plant tissue diminishes as the dipole rotation lowers, thereby reducing the thermal energy created in the media [64]. The pressure gradient is subsequently created, culminating in the transference of mass and energy into the reactor. This implies that solvents migrate from one zone to another to cause resistance in the media and hence to isolate bioactive chemicals from the constituents from the plant material [65]. However, uneven pressure gradients in the reacting vessels are typical of traditional extraction methods [66]. The appropriate selection of optimized extraction parameters is critical to the essential oil yield via electromagnetic-based microwave technology. One such factor is the microwave irradiation time. When the compounds of interest are stable to heat, a prolonged extraction time is necessary for the material with a greater dielectric constant such as ethanol, methanol, and water [67]. The shorter duration of extraction is one of the merits of microwave technology over the conventional methods of extraction. This helps in the preservation of thermo-labile constituents and hence a better biochemical quality of the essential oil extracted.

Above the critical temperature and pressure for a liquid or gas, another conventional extraction process known as supercritical fluid is constantly in use. Liquid and gas phases blend into one another and disappear altogether in the supercritical region. The diffusivity and density of supercritical fluids (SFs) are intermediate between those of liquids and gases. Different SFs have different solvating powers because its density varies with pressure and temperature, unlike liquids [68]. To isolate individual substances from a complex combination, this phenomenon is relied upon. Several different kinds of hydrocarbons, including those with four or more carbon atoms, nitrous oxide, sulphur hexafluoride, and fluorinated

hydrocarbons, have been studied as potential SFE solvents. Carbon dioxide (CO_2) is one of the most used SFE solvents since it is non-toxic, abundant, and cheap. In this way, supercritical processes may be carried out at pressures as low as 1 bar and temperatures as low as 20 °C. By use of these phenomena, it is possible to separate individual substances from a complex combination. Alkanes with four or more atoms, nitrous oxide, fluorinated gases, and fluorinated hydrocarbons have all been tested for in SFE solvents. On the other hand, carbon dioxide (CO_2) is the most often used SFE solvent since it is non-toxic, readily available, and inexpensive [69]. It paves the way for supercritical operations to take place at ambient or almost ambient pressures and temperatures. Density, diffusivity, depressurization, viscosity, and critical temperature are all crucial characteristics of supercritical fluids. The solubility of a supercritical fluid is proportional to its density, which in turn depends on its pressure and temperature. Once the density of the fluid is known, its solvating ability may be calculated with ease. Because the diffusion rates are so high, the extraction times are much shorter than they would be with liquid solvents. This is crucial because extraction rates are ultimately limited by the rate at which analyte molecules diffuse from the solid phase into the liquid phase [68]. Moreover, depressurization can be used to remove SFEs like carbon dioxide (CO_2) and nitrous oxide (N_2O) from analytes because they are gaseous at ambient temperature and pressure. Supercritical fluids have far lower viscosities than liquids (often by an order of magnitude), resulting in better flow properties [68]. This allows supercritical fluids to have direct access into the plant matrix quite more rapidly than traditional solvents. The majority of chemicals employed in analytical supercritical fluid extraction are non-toxic, inert, and generally affordable. Fluids with low critical temperatures such as CO_2 and N_2O can be used to supercritically extract thermally sensitive compounds.

Essential oil from aromatic plants with strong biochemical activity may now be extracted using the supercritical fluid extraction (SFE) technique rather than the time-consuming and tedious traditional methods. Effective and rapid extraction may be achieved using this technique without the need of high heat, tedious cleanup, or potentially harmful organic solvents. Most studies on the SFE of EOs look at how varying factors like temperature, pressure, fluid flow rate, sample size, modifiers, and fractionation affect extraction yield. The extraction yield, the amount of time and resources saved, and the accuracy of the data from future studies may all be greatly improved by adjusting these settings. Typical studies on the SFE of EOs look at how changing factors like temperature, pressure, fluid flow rate, sample size, modifiers, and fractionation affect the amount of extract that can be extracted. Estimating how temperature affects individual EOs may be challenging. The greater the temperature, the less dense the fluid,

which enhances the solubility of the EO, and hence explains the observed occurrence. So, the extraction yield is determined by the equilibrium between the density of SC-CO_2 and the volatility of the EOs at the specified circumstances, and this equilibrium shifts at various temperatures. Furthermore, as many EO constituents are thermo-labile, higher temperatures may accelerate their degradation. SFE has the advantage of producing a high yield at low pressure; this makes it a viable option for extracting EOs; however, in order to fully comprehend a solute's behavioral patterns in SFE, it is important to take into account four characteristics: the solute's cutoff point pressure; the solute's optimum solubility pressure; the solute's fractionation pressure; and the solute's physicochemical properties[68]. When greater pressures are applied, however, mass transfer and EO release are both enhanced from the plant matrix. Therefore, increasing the solvent power results in a decline in extraction selectivity and an increase in pressure. Separating the EO from the other co-extracted components requires a fractionation system with at least two separators when high pressures are applied.

The particle size, surface area, shape, and porosity of the plant matrix all have a significant role in the SFE yield, and all have an effect on the quality of the extracts. Limiting the particle size of a solid matrix increases surface area, decreases resistance to mass transfer, and improves extraction efficiency, all of which contribute to a shorter processing time. If the plant matrix is reduced too much, the solute may be re-adsorbed on its surface, so delaying the extraction process, and the pressure in the extractor may decrease [68]. Extraction efficiency is also affected by the rate at which the SF moves through the plant cells. By decreasing the flow rate, we may lower the linear velocity. Mass transfer resistance restricts the injection of analytes into a fluid at low flow rates, and unsaturated SC-CO_2 is introduced to the extraction vessel. As the flow rate of the fluid rises, the mass transfer resistance decreases until the fluid being removed is saturated, at which point equilibrium is established and maximum yield is achieved. Due to a decrease in residence time as flow rate rises, the system deviates from equilibrium and unsaturated fluid exits the extraction vessel, even while the mass transfer rate remains constant. This is because the leaves that can be extracted are skipped through if too much moisture enters their cells [70].

Furthermore, ultrasonic-assisted extraction (UAE) is increasing in popularity in the food and pharmaceutical sectors as a non-thermal extraction method for its many benefits [71]. This innovation was made to fix issues with both older and newer extraction methods. In comparison to conventional methods, UAE yields a more desirable essential oil quality profile, shorter extraction times, lower energy usage, fewer contaminants, and fewer or no solvent requirements [49]. UAE is a simple, effective,

and cheap technology when contrasted to other innovative extraction methods for essential oil recovery, such as supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE) [71]. The flow of ultrasonic waves causes cell disturbances and a large contact surface area between the extracting solvent and sample material, enhancing mass and heat transfer, which is believed to be how UAE achieves its great efficacy in essential oil extraction [72]. The ultrasonic parameters (such as frequency and amplitude), product parameters (including viscosity and surface tension), and environmental factors (including temperature and pressure) all play significant roles in the formation of cavitation during UAE [73]. The classification of UAE technologies in food industries include low intensity-high frequency ($f > 100$ kHz) and high intensity-low frequency (20 kHz $< f <$ 100 kHz) ultrasound [74]. In the laboratory, UAE can be performed by immersing the plant material in water or other solvents (e.g., methanol or ethanol) and allowed to receive ultrasonic treatment at the same time [75].

Generally, process variables of significant importance contributing to the extractability of essential oil from plant materials include ultrasonic intensity/energy, solvent type, temperature, ultrasonic time, sample—solvent ratio, and ultrasonic power. Cavitation intensity is determined by the amount of ultrasonic energy supplied per unit volume of the plant samples. Moreover, there are lesser cavitation effects when an elevated ultrasonic wave is delivered to a bigger sample volume. Addressing both intensity and power density can be explained by considering the amount of energy applied to a given volume of sample (in joules per milliliter or watts per gram). Most studies investigate described this in terms of intensity (W/cm^2), but many additionally reported it in terms of power density (W/mL) as well. These variables are sometimes underestimated when replicating results from ultrasound, even though they're crucial for replicating sonication results from ultrasound. Moreover, ultrasonic cavitation and its thresholds are affected by the solvent's viscosity, surface tension, and vapor pressure. These three variables raise the cavitation threshold and sample resistance to device displacement when they are increased. It follows that to form cavities, the intensity of the oscillations must be increased. Due to decreased solvent vapor pressure, cavitation bubbles rupture more abruptly. This depends on the kind and strength of the interactions between a solute and its solvent as well as between a solvent and its solute. Oils being non-polar, non-polar solvents are the most effective at extracting oil. The effectiveness of oil extraction gradually decreases the polarity of a solvent. Solvents such as n-hexane and petroleum ether are both non-polar with the maximum oil extraction rate. Van der Waals forces are indeed very weak in such solvents, with inherent lower volatility and boiling temperatures. Van der Waals forces are also the only forces that exist within the non-polar solute molecules. In addition, due to

cavitation energy, as extraction time increases, cellular membranes are ruptured, thereby increasing the contact area between the ruptured cell walls and the extracting solvent, allowing for a greater amount of oil to permeate into the solvent. A 40-min increase in the UAE time can increase oil extraction yield from 23.46 to 26.71% at a fixed solvent-to-solid ratio [76]. Extraction efficiency declines with increasing extraction time because of equilibrium between the solid sample and solvents. Temperature is one of the most important factors in the ultrasonic extraction of essential oil. Cavitation bubbles increase with temperature rise and this results in a rise in contact surface area between extracting solvent and the cellulosic cell walls, as well as a corresponding drop in viscosity. Mass transfer improves extraction efficiency because the solvent with lower viscosity is better able to penetrate easily into the sample matrix. Marhamati et al. [76] reported that an increase in temperature from 40 to 50 °C, even when the solvent-to-solid ratio was kept the same, increased the oil extraction from 18 to 20%.

In summary, classical extraction methods, including steam distillation, solvent extraction, microwave-assisted extraction, and ultrasonic extraction, play a pivotal role in obtaining essential oils. However, it's essential to recognize that these methods can exert significant influences on the biochemical activities of essential oils, leading to several noteworthy consequences. Firstly, these extraction techniques have the potential to bring about alterations in the chemical composition of essential oils. The application of heat or solvents during the extraction process can induce modifications in the oil's chemical profile, consequently affecting its aroma and therapeutic attributes. This change in composition can be both beneficial or detrimental, depending on the specific compounds involved. Furthermore, the classical extraction methods can impact the yield and purity of essential oils. Some valuable constituents may be lost or degraded during the extraction process, while unwanted contaminants or solvent residues may be introduced, potentially compromising the oil's overall quality. Additionally, the changes in the chemical profile can have a direct bearing on the therapeutic efficacy of essential oils. Certain bioactive compounds responsible for the oils' beneficial properties may be either diminished or enhanced, which, in turn, affects their potential health benefits. Moreover, the stability of essential oils may be jeopardized during classical extraction. Exposure to heat or chemical solvents can lead to oxidative degradation, thereby reducing the oil's shelf life and diminishing its overall shelf-stability. The aroma and flavor of essential oils, integral to their various applications, are not exempt from the impacts of classical extraction. Some compounds contributing to the characteristic scent and taste of the oil may undergo alterations or loss during the extraction process, potentially affecting the sensory qualities of the oil. Lastly, the use of chemical solvents in classical extraction methods can raise safety

concerns. Residues of these solvents must be diligently removed to ensure the oil's suitability for therapeutic, culinary, or other applications. Failure to do so can render the oil unsafe for use. To address these concerns and mitigate the potential drawbacks, alternative extraction techniques, such as CO_2 extraction or cold pressing, are increasingly employed. These methods are known to preserve the biochemical activities and overall quality of essential oils to a greater extent, ensuring that the end-product maintains its intended attributes and benefits.

4 Conclusion

The extraction of essential oils (EOs) is gaining more attention than ever before, because of their numerous benefits. Moreover, due to the sensitivity of several of their biochemical constituents; extraction in extreme conditions is not feasible. Classical extraction methods which allow for lower temperatures and shorter processing periods have been described as enhanced technologies for extracting high-quality essential oils with minimal component losses. These methods have significant advantages over other conventional extraction methods in terms of preserving the main biochemical constituents of the oil. Many of essential oils' qualities, like antibacterial, antioxidant, and anti-inflammatory action, are determined by their constituents and the method of extracting them. This review therefore critically examined the mechanism and advantages of conventional and non-conventional extraction methods. This would surely help academics and business professionals choose the best extraction techniques to get the most out of their materials while maintaining the highest grade biochemical properties.

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Chapter 16

Green and Clean Extraction Technologies for Novel Nutraceuticals

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Abstract

Phytochemicals extracted from leaves, fruits, roots, and seeds of plants exhibit high nutraceutical, antioxidant, and antimicrobial potential. Such valuable components can be obtained from natural materials via extraction, an important parameter in analytical chemistry. Since conventional extractions methods pose severe environmental threats and have a negative impact on energy economy, moving toward green strategies is important. Green technologies are ecofriendly and give maximum yield, and the product obtained are pure and less toxic. Ultrasound, microwave, supercritical fluid, subcritical fluid, pressurized liquid, enzymatic hydrolysis, radio frequency, electroosmotic dewatering, cold plasma treatment, high-pressure processing, electrotechnology, ionic liquid, accelerated solvent extraction, and hydrotropic extraction are some of the methods used in clean and green technologies to extract biologically active components from plants. This chapter also discusses nonthermal extraction technologies . This chapter on clean and green technologies, processes, and protocols will provide collective and advanced knowledge to research community in the food and nutraceutical sectors.

Key words Extraction, Nutraceuticals, Conventional techniques, Toxicity, Green technology

1 Introduction

It is possible to consider extraction as initial step in the development of analytical procedure and creation of subsequent products. The phenomenal development of green technology has had a profound effect on the recovery of natural compounds intended to be used in food industry. Deep eutectic solvents and ionic solvents are examples of green solvents that may be used in conjunction with green technology to recover natural components without producing toxic effluents and effects. In addition to the ecologically advantageous features, health and safety concerns of green procedures are taken into account [1]. A high level of selec-

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tivity is required to extract high concentrations of certain compounds or to prevent the extraction of unwanted compounds during plant extraction process [2].

Technologies that support sustainable development and assist in the reduction of negative environmental impact are collectively referred to as "green technologies." The main advantages of green technology are sustainability, economic viability, and social equity. Today, the planet earth and its environment have been permanently and irreparably harmed. The globe is being moved towards an ecological landslide by our current course of conduct, and if this occurs, disaster will be unavoidable. The use of green technologies is a strategy toward protecting the planet. So, both the advantages and disadvantages have to be taken into account to further develop these technologies. Green technology makes use of naturally replenishable resources. One of the newest green technologies is green nanotechnology, which employs green chemistry and engineering. One of the main factors contributing to environmental pollution is the disposal of chemical wastes, which can be overcome by using green technology. This method is environmentally friendly and, therefore, can successfully change trash production and pattern. The anticipated sector poised for growth, driven by advancement, encompass green energy, organic farming, eco-friendly textile, green building construction, and the production of related goods and materials to sustain environmentally conscious business. According to Soni (2015), they have no harmful environmental consequences. The majority of classical methods for solvent extraction of nutraceuticals depend on the right solvents coupled with heat and/or agitation to make the target chemicals more extractable to enhance mass transfer [3]. The majority of phytoconstituents are susceptible to heat degradation, as traditional extraction methods frequently involve prolonged extraction time [4].

2 Supercritical Fluid Extraction (SFE)

A state of matter of a substance at a temperature and pressure above its critical point, where neither a liquid nor a gas phase exists, is referred to as a supercritical fluid (SFE) [5]. In supercritical fluid extraction, fluids with both liquid and gaseous characteristics at pressures and temperatures over their critical points are used is based on the solvating properties of supercritical fluid (SF), which can be produced by using pressure and temperature above a chemical, mixture, or element's critical point [6]. SFE provides substantial operating advantages due to a variety of physicochemical characteristics of supercritical solvents such as density, diffusivity, viscosity, and dielectric constant. Supercritical fluids have greater diffusion properties than any other liquids due to their low viscosity

and relatively high pressure, and they may diffuse swiftly through solid materials, resulting in faster extraction rates. The capacity of a supercritical fluid to modify its density by altering its pressure and/or temperature is one of its most crucial characteristics. Changing the extraction pressure might alter the solvent strength of the fluid, given that density and solubility are related [7]. This technology's expanding industrial utilization is primarily attributable to its selectivity, facility, and separation capacity. According to Ahmad, Masoodi, Rather, Wani, and Gull (2019), the SFE enables the extraction of a large number of nutraceutical compounds, many of which are impossible or impractical to extract using conventional methods or whose purification requires high resolution columns, which are not always available on the national market, thereby making their use very expensive [8]. Supercritical extraction is often used to separate nonpolar bioactive components (carotenoids and lipids) since the solvents used in this technique are nonpolar. One alternative is the extraction of polar compounds like flavonoids using modifiers like ethanol, methanol, water, and acetone [9, 10]. Less viscosity and a shorter extraction time lead to an improvement in the diffusion and mass transfer of the crucial fluid [1, 11]. Table 1 shows the extraction of various bioactive compounds by supercritical extraction. Factors such as temperature, pressure, and other inherently changeable features, as well as some extrinsic factors like the properties of the supercritical fluid, all affect SFE: interactions with specific analyzers, the sample matrix, and various environmental factors [6].

Table 1
Extraction of phytocompounds via supercritical fluid extraction methods

Food plant	Compositions	Conditions	References
Rosemarinus officinalis L.	Carnosic acid, rosmarinic acid, carotenoids, and chlorophylls	CO_2, ethanol: water (50:50 v/v), 25 °C	[2]
Castanea sativa	Antioxidant and polyphenols	15% ethanol, 60 °C, 350 bar	[12]
Rubus idaeus L.	Oil	0.4 kg CO_2/h, 40 °C, 35 MPa	[13]
Beta vulgaris subsp. Saccharifera	Amino acids	CO_2, ethanol, 184 bar, 43 °C, 76 min	[14]
Terminalia catappa	Oil	CO_2, 60 °C, 300 bar	[15]
Daucus carota	Oil	CO_2, ethanol, 70 °C, 400 bar	[16]
Saccharum L.	Amino acid	CO_2, methanol, 316 bar, 50 °C, 76 min	[17]

2.1 Working Principle

SFE comprises a solvent pump or compressor and a modifier (or co-solvent) pump, which requires an organic solvent or water. An extraction chamber or reactor with a heat jacket holds the feed material. When materials ranging from micrograms to grams are to be extracted, the capacity of the extraction reactor of an SFE system is designed to be 50–300 mL, especially for SFE systems in laboratory setting. However, when larger volumes of extracts are required, the capacity of the reactor is increased to hundreds of liters, especially in industrial-scale SFE operations [18]. It is necessary to have a pressure regulator and a fractionation/collection vessel (or) flash tank. In this method, dried and ground feed material is added to the extraction chamber to produce a stable bed. A cylinder of liquid CO_2 (purity 99.998%) is compressed to working pressure and then cooked to supercritical temperatures in a temperature-controlled environment. The SC-CO_2 is injected into the extraction reactor and then allowed to diffuse across the feed material's fixed bed. The solubilized components are then removed from the extraction chamber and placed in a separator at a lower pressure. After that, in the flash tank, the SC-CO_2 and solute precipitate are separated. The SC-CO_2 may then be cooled, compacted, recycled, or released into the atmosphere [18]. Figure 1 shows that the extraction vessel contains a high-pressure chamber where the reaction takes place and a pumping system that pressurizes the solvent and delivers to the extraction vessel. The next is a reliable source of high-purity carbon dioxide. The temperature control system and pressure control system maintain precise control over solubility of target compounds [18].

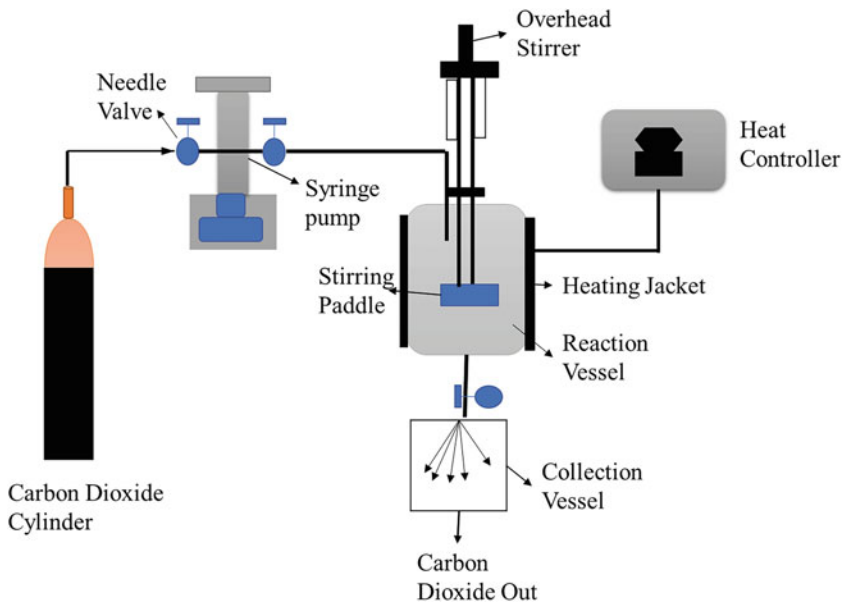

Fig. 1 Flow design of supercritical fluid extraction technique

2.2 Subcritical Fluid Extraction

Subcritical fluid extraction is an ecologically friendly and perfect method that is used in selective extraction operations, in the treatment of agro-food waste, and in industries to manufacture safe and high-quality products [19]. According to Cravotto et al. (2022) subcritical, near-critical, or pressurized hot water are all terms used to describe this technique, in which water is subjected to adequate pressures (generally 10 to 100 bar) between 100 °C and a critical temperature of 374 °C (often between 100 and 250 °C) [20]. Researchers have documented the utilization of supercritical fluid and their liquid counterparts as appropriate solvents. This approch not only yields the highest amount of value added nutraceuticals but also represent a sustainable and environmentally friendly processing method. It serves as a substitution for hazardous organic solvents with more ecologically sound alternative [21]. In contrast to "supercritical" fluids, "subcritical" fluids, also known as pressurized liquids like water and ethanol above their boiling points but below critical temperatures, show good solvency properties for the extraction of a variety of biological moieties present in matrices made from agricultural sources. Some of the drawbacks of supercritical fluids, such as poor affinity of carbon dioxide for polar solutes and high capitalization costs of the process, are alleviated by the use of subcritical fluids. Subcritical water has been used to extract essential oils with effectiveness [22]. The use of water being ecologically friendly, the ability of extracting more rapidly with less amount of solvents, and the ability to produce higher yields make subcritical water superior to standard organic solvents (Fig. 2) [19]. Solubility and mass transfer are the two most important variables in subcritical solvent extraction [23].

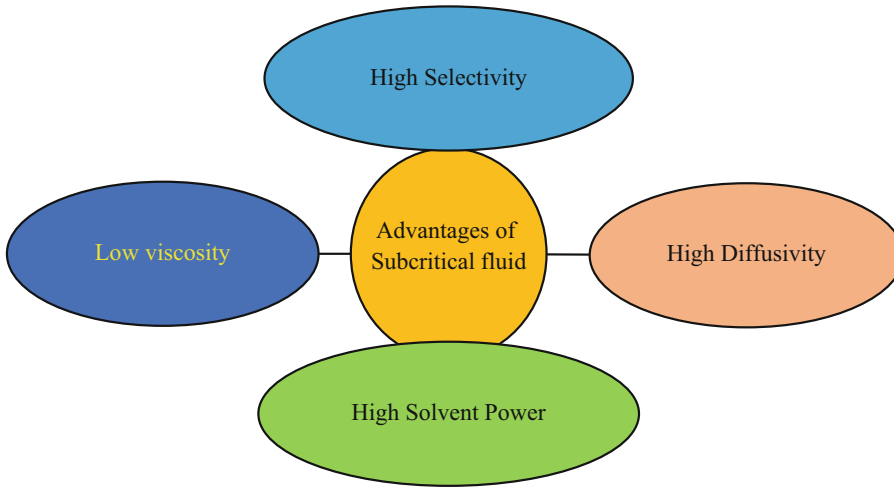

Fig. 2 Different major advantages of subcritical extraction technique

With values that fall between methanol and ethanol, the dielectric constant of water decreases from 80 to about 30 due to the weakening of hydrogen bonds, which makes the efficient extraction of moderately polar and nonpolar target compounds possible. Some advantages of subcritical fluids are summarized in Fig. 2 [20].

2.3 Working Principle and Mechanism

Strong hydrogen bonds of water gives it a unique characteristics. The characteristics of water are affected by variations in temperature and pressure [24]. Under the required pressure, water will boil and reach its critical temperature at 100 and 374 °C, respectively. Hot water that is sufficiently under pressure to maintain liquid condition at this critical temperature is referred to as subcritical water [25]. Subcritical water extraction (SWE) has an amazing ability to change dielectric constants across a broad range by varying the temperature and pressure. SWE also results in mass transfer by convection and diffusion. The energy delivered by subcritical water can disrupt the relationship between cohesive and dispersive forces by reducing the temperature. While subcritical water can disrupt cohesive (solute-solute) and adhesive (solute-matrix) interactions by lowering the activation energy required for the desorption process, elevated pressure can aid in extraction by forcing water to enter the matrix (pores), which is impossible under normal pressure [25]. In general, the SWE extraction technique comprises four steps that follow one another. In the first stage, the solute is first extracted from the surface at various active sites in the sample matrix under highly increased conditions of temperature and pressure. The main goal of the second phase is to spread the extracts throughout the matrix. The sample matrix dictates the third step, and solutes may be partitioned into the extraction fluid from the sample matrix. The sample solution is then eluted and extracted from the extractor using chromatography. Table 2 shows the extraction of nutraceuticals by using subcritical fluid extraction in different conditions. The extraction mechanism was modeled thermodynamically by Holgate and Tester in 1994. In this paradigm, chemicals are extracted in two steps from their matrix. Similar to front-elution chromatography, the compounds must first be desorbed from their original binding site in the sample matrix before being ejected from the sample. Enhancing surface equilibrium damage and solubility and mass transfer effects might increase the effectiveness of subcritical water extraction [25, 26].

2.4 Modes of Extraction

This extraction method has two primary modes: static extraction and dynamic extraction. In static extraction, to retain water in a liquid form, subcritical water is combined with the sample to be extracted in an extraction vessel and heated to an appropriate temperature under light pressure. Once extraction is completed, the extractant is collected for chromatographic examination. This extraction method is comparable to rapid solvent extraction and its efficiency typically falls short of dynamic extraction's efficiency.

Table 2
Extraction of different compounds by using subcritical fluid extraction technique

Food plants	Compositions	Conditions	References
Aquilaria malaccensis	Essential oils	100–175 °C, 1–3 mL/min, 2–9 Mpa, 30 min	[28]
Camellia oleifera	Free fatty acids	60–160 °C, 2–7 Mpa, 5–60 min, 1:3–1:25 g/mL	[29]
Terminalia chebula	Polyphenols	120–220 °C, 2–4 mL/min, 4 Mpa	[30]
Piper betle	Essential oil	50–250 °C, 2 Mpa, 10–90 min, 0.25–1 mm, 1–4 mL/min	[31]
Nelumbo nucifera	Polyphenols	100–180 °C, 5–25 min, 1:20–1:60 g/mL, 1–5‰ $NaHSO_3$	[32]
Citrus hystrix	Essential oil	120–180 °C, 5–20 g/mL, 5–30 min	[33]
Camellia sinensis	Flavonoid	40–160 °C, 5 min, 5 Mpa, 1–10 mm, pH 3.0–7.0	[34]

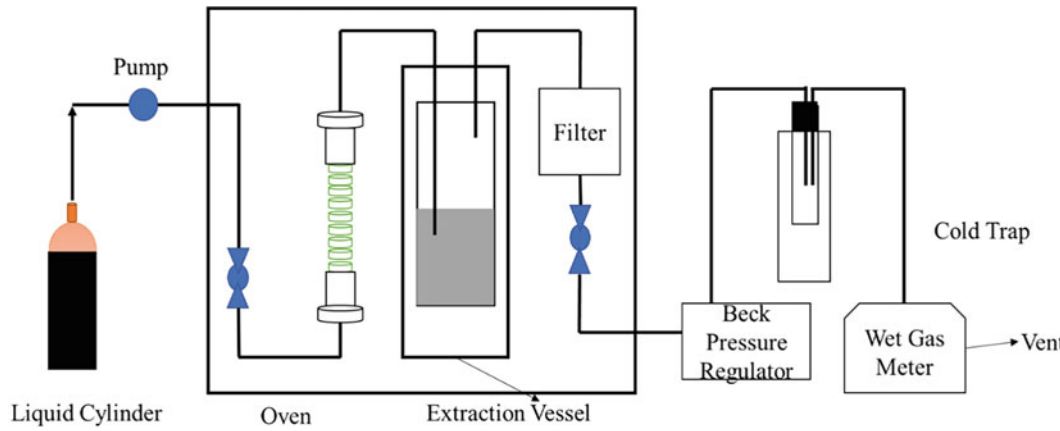

Fig. 3 Flow design of subcritical extraction technique

Following the placement of the samples in the extraction vessel, a pump continually pumps water into the extractor while the extraction is carried out at a fixed or gradient temperature. Constant flow or pressure can both be set for the pump. In addition to accelerating mass transfer and reducing extraction time, dynamic SWE allows for customizable continuous extraction. The SBWE system might get blocked as a result of dynamic SBWE, on the other hand [27].

Figure 3 shows the subcritical fluid extraction – its extraction vessel is similar to that of supercritical fluid extraction; pump is used to deliver solvent; and pressure ranges from 2 to 20 psi. Temperature and pressure control system maintain the temperature and pressure below the critical point providing the flexiblity in choosing the solvents [26].

3 Pressurized Liquid Extraction (PLE)

It is considered one of the advanced extraction technologies over the conventional methods [35]. This technique was first introduced in 1995 by Dionex Corporation at Picton conference and described it as an accelerated solvent extraction [36]. In this method, liquid solvents are used at increased temperature and pressure [35] and below their critical point. High pressure maintains the solvents in their liquid state, which increases the output of the process as compared to the atmospheric temperature [36], also known as "accelerated solvent extraction [37], pressurized fluid extraction (PFE), pressurized liquid extraction (PLE), pressurized hot solvent extraction (PHSE), high-pressure solvent extraction (HPSE), high-pressure, high-temperature solvent extraction (HPHTSE), and sub critical solvent extraction" [5, 38]. The increase in condition correspond to changes in mass transfer and solubilities compared to normal condition [36] due to increase in the dielectric constant (used to evaluate the interaction between solute and solvent) of the water in the extraction method [38].

Water is readily nontoxic, recyclable food-grade solvent and is easily available. This method is rapid as compared to the other conventional methods [39] and yields is pure extracts [40]. Due to high pressure, it reduces the effect of light- and air-induced degradation [39], and organic or aqueous solvents either alone or in combination are used by static or dynamic mode. In the dynamic mode, the flow of solvent through the sample is continuous, and in static method, the flow is controlled for a specified time at constant temperature and pressure [38]. Because of the need for rapid, less hazardous, and more environmentally friendly extraction technologies, PLE has gained popularity, particularly in the pharmaceutical and food sectors. PLE applications for the extraction of pollutants from various foods have been developed [35].

3.1 Working Principle

Handling liquid sample requires their conversion into the solid due to the absence of commercial kits, achieved by adding adsorbent or adsorbents. The efficiency of the system is influenced by thermodynamic and kinetic parameters, with three interconnected processes playing a crucial role: mass transfer, matrix effect and solubility [35].

3.2 Factors

Factors such as the duration of extraction, temperature, solvent utilized, and pressure all affect the performance of PLE process. However, the nature of the matrix, the unique characteristics of the target molecules, and their positioning within the matrix all affect the effectiveness of the process. Therefore, it is essential to comprehend and establish the influence of these factors on the extraction process in order to obtain high yields and highly pure extracts

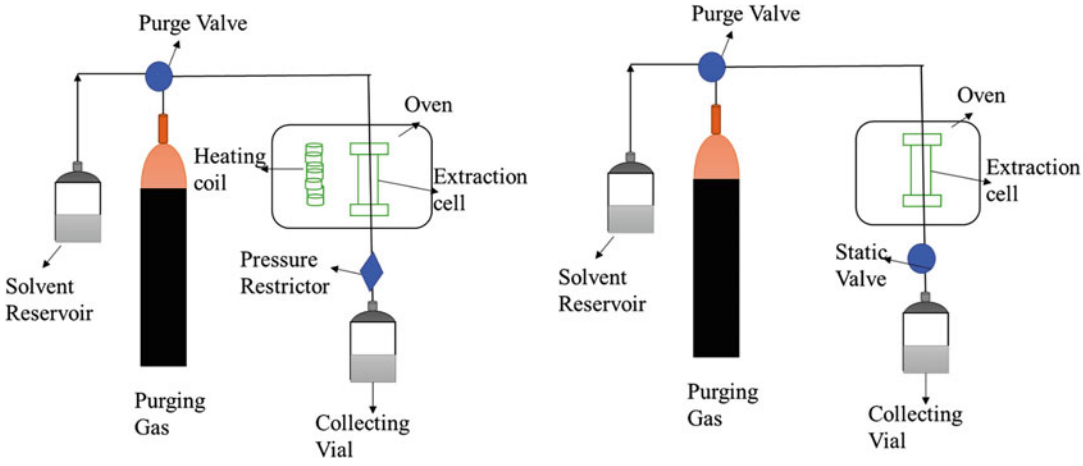

Fig. 4 Flow designs of pressurized liquid extraction (**a** dynamic; **b** static)

[41]. This process is constrained by the amount of time, flow rate, temperature, and pressure management [35]. Hydromatrix is used to adsorb water from the matrix since moisture in the sample matrix lowers extraction efficiency, which ultimately increases the extraction.

3.3 Instrumentation

The instrumentation depends upon whether it is dynamic or static mode. The basic instruments of the pressurized liquid extraction are reservoirs containing solvent, purging gas source, valves to restrict the flow, pump, oven in which the extraction coil is placed, and a collecting vial.

Figure 4 shows the flow design of PLE. Extraction cell is where the sample and solvent are combined, which is made up of stainless steel or other materials that can withstand high pressure and temperature. Pressure control system maintains pressure of several thousand psi and temperature of up to 200 °C or higher. The solvent delivery system typically includes pumps or syringe driver that delivers the solvent at a controlled flow rate. The sample center design may vary and the choice depends upon the nature of sample [35].

The high-pressure pump is then linked to the solvent reservoir. After the operation is finished, the pump helps with the extraction by introducing the solvent into the system. Extraction takes place in cell, where sample is added to the stainless cell first, then a filter paper is added, and if necessary, a dispersion agent as well. The cell is then mechanically or manually put inside the oven. Various valves and restrictors are needed to control the extraction pressure. Finally, the endpoint of the extraction system is connected to the collecting vial. The instrumentation may be more or less sophisticated depending on the process requirements. For example, a solvent controller may be necessary when there are several solvent

Table 3
Extraction of different compounds via pressurized fluid extraction

Food plant	Compounds recovered	Extraction condition	References
Vaccinium vitisidaea L.	Phenolic compounds	Ethanol, 10.3 MPa, 50 °C, 45 min	[39]
Vaccinium sect. Cyanococcus Rydb.	Anthocyanins, polyphenols, flavonoids	Acetone/water/acetic acid (70:29.5:0.5), 20 °C, 500 MPa	[42]
Capsicum annum L.	Oil	n-hexane, 370 MPa, 50 °C, 5.7 min	[43]
Solanum lycopersicum	Polyphenols	Methanol 50%, hydrochloric acid, 45 °C, 5 min, 600 MPa	[40]
Allium sativum	Melanoidins	Water, 300 MPa, 25 °C, 5 min	[40]
Satureja montana	Bioactive phenolic compounds	Ethanol (35%), 348 MPa, 20 min, 20–25 °C	[44]
Ficus carica	Bioactive phenolic compounds	Water, ethanol 600 MPa, 18–19 min	[45]

reservoirs accessible, or an inert gas (often nitrogen) circuit may help flush out solvent from the lines after extraction. Additionally, the collecting vial can be placed in a cooling bath to reduce the temperature of the extracting matter and decrease thermal damage. Dynamic PLE also involves solvent preheating coils, a pressure restrictor (back pressure regulator), or a micro metering valve rather than a static open/close valve as in static PLE [35]. Additionally, it requires a little more complex high-pressure pump to control the solvent flow rate. The static extraction process depends heavily on the temperature and extraction duration. The solubility of the analyte in the extraction solvent and the distribution of the target component between water and the extraction solvent both affect the extraction efficiency. Due to the restricted supply of the extraction fluid, full extraction may not be feasible in the static extraction mode [36]. In Table 3, extraction of different nutraceuticals by pressurized liquid extraction is elaborated, in which extraction conditions vary in each of extraction [1].

4 Ultrasound-Assisted Extraction

The range of an ultrasound frequency falls between 20 kHz and 100 MHz. Cavitation is an extraction method aided by ultrasound that involves the development, growth, and deflation of bubbles [46]. Ultrasound-assisted extraction (UAE) is a rapid and "easy-to-use" technique that can quickly and consistently extract numerous classes of food components from a variety of plant and food

matrices [47]. This "green" form of processing also conserves water and energy, enables by-product recycling through bio-refining, and yields a product that is both safe and of high quality [48]. Yet, process variables, e.g., time, temperature, ultrasonic power, and solvent, affect how effectively these processes work. To determine the ideal extraction conditions that produce the best results in terms of target chemical recovery, proper experimental designs and optimization approaches are necessary [49]. Additionally, since mechanical stirrers cannot be utilized in supercritical fluid extraction, this method offers a novel approach to provide agitation [50].

4.1 Working Principle

The primary technique employed in sonication is acoustic turbulence. The molecules of the medium undergo a series of contraction and coalescence as ultrasound passes through them. In a liquid medium, such alternating pressure changes lead to the production and eventual rupture of bubbles. Acoustic cavitation is a phenomenon that occurs when micro bubbles in ultrasonic liquids develop, grow, and then implosively collapse [51].

Figure 5 shows the ultrasound-assisted extraction. Ultrasonic transducer is the key component that generates high frequency sound waves and moves through the extraction solvent and sample; the power control system controls the intensity of the waves applied to the sample; the sample holder holds the sample; and the temperature and pressure control maintain the temporary size, temperature and pressure [4].

The primary technique employed in ultrasound-assisted extraction is acoustic cavitation. The collapsing cavitation bubbles and sound waves may be responsible for the fragmentation, localized erosion, pore formation, shear force, enhanced absorption, and swelling index in the plant's cellular matrix. Shockwaves are formed by cavitation bubbles that rupture, and cellular structure is disturbed by fast particle collisions. Due to rapid fragmentation, the

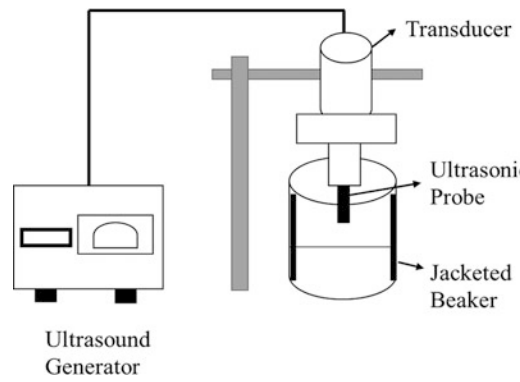

Fig. 5 Operational design of ultrasound-assisted extraction

bioactive component dissolves in the solvent due to an increase in surface area, a decrease in particle size, and rapid mass transfer rates in the solid matrix's border layer. Erosion occurs in plant tissues as a result of the localized damage caused by ultrasonography. The implosion of cavitation bubbles on the tissues of plants may be the source of this damage. More solvent comes into touch with the eroded region, thereby increasing extraction yield. Bioactive substances that are already present in the cell are released as a result of sonoporation, a phenomenon brought on by the creation of holes in the cellular membrane during cavitation. Additionally, the formation and dissolution of cavitation bubbles cause turbulence and shear stress in the fluid, which help break down cell walls and release the bioactive material. Ultrasound improves the pomace's capacity to absorb water, as well as the bioactive compounds' diffusivity and accessibility to the solvent being employed for separation. Additionally, ultrasound boosts the extraction rate and elevates the swelling index of the plant tissue matrix, which encourages solute desorption and diffusion. The greater extraction yield in the UAE is the result of a combination of variables rather than one method [52]. Direct application is accomplished by immersing ultrasonic probes in the sample and executing direct ultrasonication over the solution with no additional barrier than the solution itself. Indirect application is often carried out using an ultra-sonication bath, in which the ultrasonic wave must first cross the liquid within the ultrasonic instrument and then the sample container's wall. Different examples of nutraceuticals extracted by UAE are shown in Table 4 [4].

Table 4
Extraction of different compounds via ultrasound-assisted extraction

Food plants	Compounds	Extraction condition	References
Beta vulgaris	Betanin and phenolic compounds	B-cyclodextrin (5%) solution, ultrasonic bath, 28 kHz, 80 W, 30 min	[53]
Hibiscus sabdariffa calyx	Anthocyanins natural food colorant	Ethanol (39.1%), 26.1 min, 296.6 W, 20 kHz, 30–35 °C, probe model	[49]
Rubia sylvatica	Natural food colorant, anthocyanins, and phenolics	Ethanol (30%), 55 °C, 400 W, 20 min, bath model	[54]
Curcuma longa L.	Curcuminoids	Deep eutectic solvent choline chloride: lactic acid, 1:1	[55]
Pisum sativum	Protein isolates	Water at pH 9.6, 750 W, 13.5 min, 33.7% amplitude, probe model	[50]

4.2 Factors Affecting the Extraction

Factors that impact extraction efficiency alone or in combination were investigated. They are as follows:

I. The type of tissue being removed and where in respect to tissue structures the components are to be removed.
II. The tissue is prepped before being extracted.
III. The characteristics of the components being extracted.
IV. Ultrasonic impacts, which primarily cause superficial tissue disruption increasing surface mass transfer, enhancing intra-particle diffusion, loading substrate into the extraction chamber; improved extractable component yield.
V. Higher extraction rates, particularly early in the extraction cycle, result in considerable time savings and higher processing throughput [4].

5 Microwave-Assisted Extraction (MAE)

The low-impact approach known as microwave-assisted extraction (MAE) employs microwave wavelengths between 1 mm and 1 m and frequencies between 0.3 GHz (1 m) and 300 GHz (1 mm). The sample can be penetrated by microwaves, which can then interact with polar components [56]. According to Silva et al. (2021) and Alara, Abdurahman, Ali, and Zain (2021), MAE is a straightforward, rapid, and inexpensive method with certain key benefits including quick heating, low solvent requirements, and clean operations [57, 58]. A target chemical can be extracted from a number of source materials using the MAE process. Due to its natural ability to swiftly heat the sample-solvent mixture, MAE is a user-friendly technique that shortens extraction times and speeds up the extraction process. MAE provides a number of benefits over conventional techniques, one of which is a drastically shortened extraction time [59]. According to T. Wu et al. (2012), MAE heating is based on the direct interaction of microwaves with molecules through ionic conduction and dipole rotation [60]. Both are in charge of the simultaneous heating of several items. Ionic conduction [4] is the electrophoretic movement of ions caused by a fluctuating electric field. At less aggressive extraction conditions, an electromagnetic field is applied directly to the sample, increasing cell breakdowns and the release of chemicals into the solvent while minimizing harm to sensitive components [61]. Microwave heating only works on liquids or dielectric materials with persistent dipoles. Different solvents heat up in microwaves in a manner that is strongly influenced by the dissipation factor, a measurement of the solvent's capacity to absorb microwave energy and transmit it on to the surrounding molecules as heat [4].

5.1 Types of MAE

MAE vessels are classified into two types, closed and open. Both systems have four common components, which are listed below: a microwave generator that works by using magnetron to generate microwave radiation; a waveguide through which microwave spreads to microwave cavity; a circulator that allows the microwave to change ahead; and an applicator to perform the test [62].

5.2 Instrumentation and Mechanism of Microwave Extraction

Mono- or multimode microwave oven cavities are created. The monomode cavity, which stimulates just one mode of resonance, can produce a frequency. The sample may be positioned at the electrical field's maximum since it is known how the field is distributed. The size of the multimode cavity raises the possibility that the incident wave will affect several modes of resonance. The field can be homogenized by the superimposition of modes. Systems such as rotating plates are utilized for homogenization [63]. Modern techniques like MAE warm the solvent with microwave energy and release the plant extractable compositions into an aqueous phase. Although mass transfer for convention and MAE takes place in the same direction, they are different because energy is lost volumetrically during the MAE process inside the illumination medium. The power of the microwave and the material's dielectric loss factor both affect how quickly temperature rises during microwave heating. Veggi et al. (2013) state that numerous interactions must occur during the solid-solvent extraction phase of the MAE process [64]:

- The solid matrix's contact with the solvent
- Component breakdown or solubilization
- Moving the solute away from the solid matrix
- The solute's transition from the solid's surface to the bulk solution after being extracted
- The extract's motion in relation to the solid
- Disposal of the extract and solids after separation

As a result, the solvent effectively diffuses into the solid matrix and the solute disintegrates up until it reaches a concentration that is constrained by the solid's physical features [65]. Stability, transformation, and dissemination are the three steps that help compensate the extraction process. Solubilization and partition control the way the substrate is taken from the outer surface of the subatomic particle during the equilibrium phase. There are transitional stages before dissemination. At the solid-liquid interface, mass transfer encounters resistance; at this point, convection and diffusion are the dominant modes of mass transfer. The solute must get past the interactions holding it to the matrix in the final stage in order to enter the extraction solvent [64, 66]. The closed MAE system is widely employed for extraction under harsh conditions, such as

high extraction temperatures. Today, the operator has a variety of options for controlling the extraction process with safe-to-use MAE equipment intended for laboratory usage. A magnetron tube, an oven with the extraction vessels set on a turntable, monitoring devices for temperature and pressure management, and a number of electrical power components are used in commercial systems for closed-vessel MAE. The extraction vessel is filled with the sample first, then the solvent is added, and lastly the extraction vessel is sealed. A pre-extraction procedure is started after heating the solvent using microwave radiation. Heating typically lasts around 2 min. After that, the sample is subjected to radiation and extracted for a predetermined period of time often between 10 min 30 min (static extraction stage). After the extraction is finished, the samples are allowed to cool to a temperature that can be controlled. Before analysis, there could have been a need for an internal standard and/or a clean step. Because it can achieve a higher heat without destroying the volatile component and the fumes remain in the vessel, less solvent is required [67, 68].

5.2.1 *Focused Microwave-Assisted Extraction System (FMAE)*

A Soxhlet apparatus with variable heating power was used to perform FMAE at 2450 MHz and atmospheric pressure. A quartz extraction tank containing solvent is filled with powdered and air-dried sample matrix. Once the container had reached room temperature, the extracts were centrifuged, and the supernatant is collected and dried by vacuum evaporation. This can manage a large amount of sample material and an additional reagent may be added at any time during the process of extraction [67, 68]. Table 5 shows the extraction of various nutraceuticals by MAE at different conditions. Figure 6 shows the microwave-assisted extraction's instruments; the power control system and frequency control system adjust the intensity of microwave energy applied to the sample. The sample holder material should be transparent and distant to thermal and chemical degradation and should be transparent [67].

5.2.2 *Dynamic MAE*

A dynamic MAE system developed by Ericsson and Colmsjo in 2000 generated extract with yields comparable to those of Soxhlet extraction but in a lot less time. The dynamic microwave extractor comprises the solvent supply system, microwave oven, extraction cell, temperature set point controller with type K thermocouple, fluorescence detector, and fused-silica restrictor [67].

6 Hydrotropic Extraction

There are many techniques for extracting nutraceuticals from plants. However, the utilization of hydrotropic extraction for obtaining natural products for medicinal use is currently becoming popular due to its potential applications. Both the solubility and

Table 5
Extraction of different compounds via microwave-assisted extraction

Food plants	Compounds	Extraction conditions	References
Humulus lupulus L.	Polyphenols	Ethanol, 75 °C, 400 W, 1 min	[69]
Arthrospira platensis Gomont	Natural pigments, phycobiliproteins	Protic ionic liquids (hydroxyethyl ammonium acetate and 2-hydroxyethylammonium formate), 62 W, pH 7.0, 2.0 min	[70]
Daucus carota L.	Carotenoids	Flaxseed oil, 165 W, 9.39 min	[71]
Ficus racemose	Phenolic components	Water 3.5 pH, 360.55 W, 30 s	[72]
Garcinia mangostana	Antioxidant-rich xanthone	Ethanol (71%), 300 W, 2.24 min	[73]
Plukenetia volubilis	Phenolic compounds	Ethanol (63.0%), 1500 W, 110 s	[74]
Origanum vulgare	Essential oil	Water, 600 W, 20 min	[75]

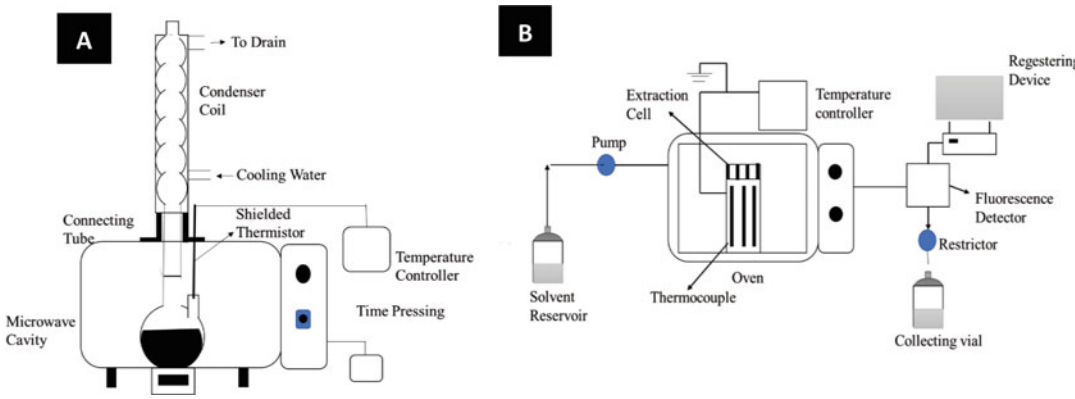

Fig. 6 Flow designs of focused (**a**) and dynamic (**b**) microwave-assisted extraction

surface permeability of the solvent are important factors in the extraction of phytoconstituents. Because of solubility consideration, many phytoconstituents are often not extracted in the standard extraction process. Hydrotropic extraction is used for the recovery of naturally occurring secondary metabolites [76]. In simple terms, hydrotropic extraction involves chemical extraction through the solubilization of plant matrix with hydrotropic agent. The term hydrotropy is the solubilization of organic compounds in water by using hydrotropes or hydrotropic agents. The hydrotropes

are basically amphiphilic molecules which increase the solubility of organic molecules in aqueous solutions by meditating the interactions between hydrophilic and hydrophobic molecules. Hydrotropes can take on a variety of shapes, but their two most prevalent molecular features are an ionic moiety and a saturated hydrocarbon ring [77]. It is a molecular phenomenon caused by poorly soluble solutes becoming much more miscible in an aqueous solution when a hydrotropic agent is added. This extraction process is very feasible, and it follows the principles of "green extractions" being nontoxic and reusable.

6.1 Hydrotropic Agents

The term "hydrotropy" was first utilized by Carl A. Neuberg in 1916 [79]. He used the term "hydrotropic agents" for anionic organic salts, which increase the miscibility of less-soluble solutes in aqueous solutions at high concentrations. These compounds are amphiphilic molecules with a short or branched alkyl chains attached to ionic or polar groups for hydrophilicity while sulfate, sulfonate and carbonate group confer hydrophobic characteristics [78]. Most commonly used hydrophytes are xylene, polyhydroxy benzene, toluene sodium salts of lower alkanols, aromatic acid derivatives, sodium alkyl benzene sulfonates, etc. Some neutral or cationic aromatic derivatives are also used as hydrotropic agents, but these are rarely available [80]. As hydrophytes have both hydrophobic and hydrophilic moieties, they are compared to surfactants, but the presence of short chain hydrophobic moiety distinguishes them from surfactants. Therefore, they are sometimes called short chain surfactants or salting agents. Some hydrotropic agents are used for selective extraction of nonpolar phytofragments, which are water insoluble by cell permeabilization [81]. The structures of some hydrotropes like sodium toluene sulfonate, sodium xylene sulfonate (SXS), sodium benzene sulfonate, sodium cymene sulfonate, and sodium cumene sulfonate are shown in Fig. 7.

6.2 General Mechanism of Action

Many theories have been proposed for describing the mechanism of action of hydrotropic agents. The extensive study led to various perspectives, and their finding were summarized in three approaches regarding the mechanism.

1. In the first approach, hydrophyte and solutes forcefully interact to form hydrophyte-solute complexes [80]. The phospholipid bilayer of plant cell wall is destructed by hydrophytes, after which the hydrophytes penetrate the inner structure of the plant cell. When immersed in aqueous medium, the effect of hydrophytes on cork cells is very minimal. The cork cell wall contains cellulose and suberine lamellas. Suberine lamellas make cork cells impermeable to water. Hydrotropic solution opens these water-impermeable suberine lamellae and then breaks down the mature cork cells. The cork cell layer is disturbed by hydrotropy, and the aqueous solution penetrates the

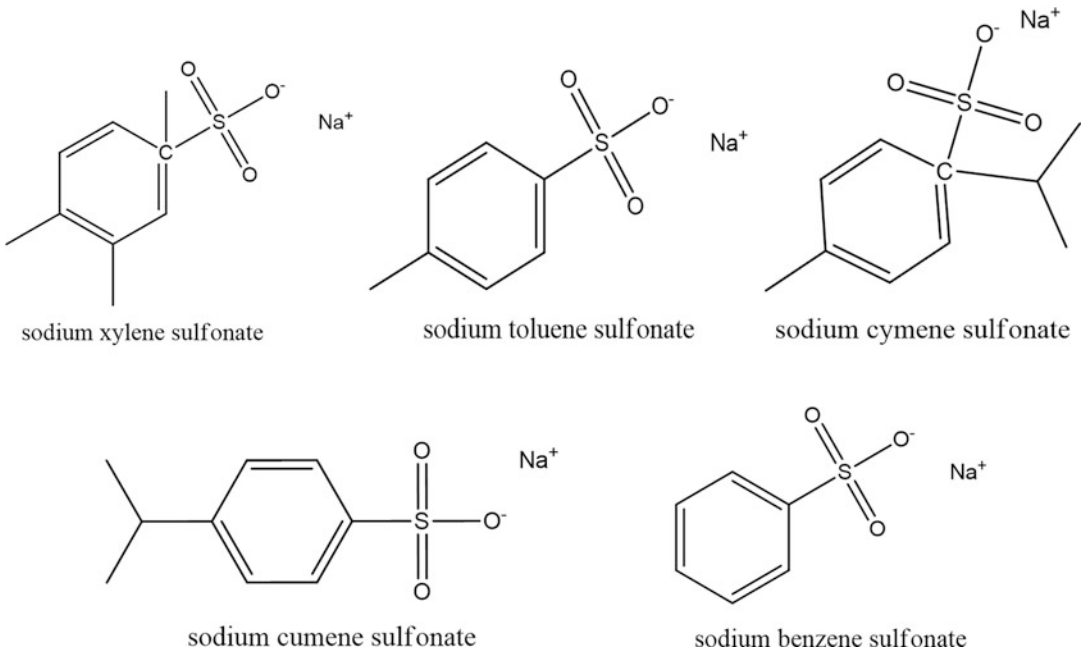

Fig. 7 Structures of different hydrotropes

cell wall. When the hydrotropes solution enters the cell cytoplasm, the cells swell, releasing them from closely coupled structures and hydrotropic solution cause precipitation of solute.. Therefore, the instantaneous recovery of dissolved solutes from solutions diluted with aqueous solution is viable [82].

2. In the second mode of action, hydrophytes are considered as "structure breakers and structure makers" as they alter the solute structure by inserting themselves into water structure [83].
3. The most widely accepted mechanism for hydrotropic extractions is the ability of hydrophytes to act as micelle structures at a particular concentration. When diluted with distilled water, the hydrotropic solutions cause the bioactive chemicals to precipitate out of the solution, making it possible to obtain the extracted solute with ease [84].

6.3 Advantage and Disadvantages

Advantages of hydrotropic extraction are its nontoxicity and easy accessibility. Hydrotropic solvents have some characteristics that would appeal to modern researchers' tastes. Hydrotropic solvents have made a noteworthy mark as a smart choice for phytochemical extraction, thanks to their toxic-free, chemically inert, affordable, accessible, temperature- and pH-independent, and high selectivity properties. In addition to having a high extraction efficiency, pureness, and quality of the crystalline product, this environmentally friendly solvent can be recycled numerous times with a comparable extraction efficiency [78]. Some disadvantages regarding this

technique are its poor recycling ability. Water dilution during recovery and reuse creates a large amount of aqueous hydrotropic solution that needs to be reconcentrated for recycling [85]. Additionally, the majority of the investigations concluded that high hydrotropes concentrations are necessary to accomplish high phytochemical solubilization, which necessitates the consumption of a sizable quantity of hydrotropes salts in order to create highly concentrated hydrotropic solvents. Most importantly, there is uncertainty over how efficiently a hydrotropic agent will interact with the intended solute.

6.4 Extractions

Some food materials extracted by using hydrotropic extraction process, which are used in pharmaceuticals, are described in the following sections.

1. *Vanillin from Vanilla*

Vanillin ($C_8H_8O_3$) from vanilla beans is extracted hydrochemically using hydrotropic solvents. One of the best-known flavor components in vanilla beans is the phenol vanillin (4-hydroxy-3-methoxybenzaldehyde) [86]. By employing organic solvents such as ethanol, methanol, acetonitrile, acetone, chloroform, and hexane, vanillin is traditionally extracted from vanilla beans [87]. Since vanilla is widely used in the food, beverage, cosmetics, and pharmaceutical industries, it is crucial to determine the amount of vanillin present in vanilla extract [88]. Nicotinamide, a non aromatic hydrotropic compound frequently used in pharmaceutical industry, follow other hydrotropes such as resorcinol and citric acid in terms of its ability to enhance vanillin solubility in water [77, 89]. Vanillin exhibits increased solubilty up to 2.6mol/L of nicotinamide, representing the maximum hydrotropes concentration. The primary mechanism based on which nicotinamide works is stacking aggregation, which involves the formation of complex molecules with the solute by binding to its hydrophobic area. But according to a different theory, nicotinamide causes solubility by dismantling water's self-associated structure, which is known to function as a chaotropic [83].

2. *Piperine from Black Pepper*

Piperine is selectively extracted from *Piper nigrum* (Black Peppers) by cell permeable mobility using hydrotropes like sodium alkylbenzene sulfonates and sodium butyl mono-glycol-sulfate. Increased extraction rates of aqueous hydrotropes solutions were suggested to be due to hydrotropic molecules entering the cellular structures, thereby resulting in cellular permeable mobility. In order to speed up the extraction of piperine, hydrotrope molecules adhere to a cell wall and disrupt its structure as well as the bilayer cell membrane. The recovered piperine is 90% pure and largely free of oleoresins [90, 91].

3. *Limonene from Citrus*

Bioactive limonenes are extracted from *Citrus aurantium* [78] seeds hydrotopically. Potentially bioactive substances called limonoids are found only in citrus fruits and vegetables. Research works have focused on utilizing aqueous hydrotropic solutions, a novel method, for removing limonoid aglycones from the seeds of sour orange (*Citrus auratium* L.). Hydrotropic concentration, extraction temperature, and percentage of input material loaded all had an impact on extraction efficiency. The Box-Behnken experiment design is used to study two hydrotropes, sodium salicylate (Na-Sal) and sodium cumene sulfonate (Na-CuS). Data is subjected to response surface analysis [63] to examine how parameters affected the effectiveness of the extraction process. Limonene, a prominent limonoid aglycone, was isolated and measured for process improvement. Maximum limonene yield was produced by both hydrotropes at 2.0 M concentration, 45 °C extraction temperature, and 10% solid loading. Na-CuS produced a maximum limonene output of 0.65 mg/g seeds, whereas Na-Sal produced only 0.46 mg/g seeds. When bioactive substances are extracted with this method, the amount of organic solvents used can be drastically reduced while maintaining environmental friendliness.

4. *Curcuminoids from Turmeric*

Turmeric (*Curcuma longa*), which belongs to Zingiberaceae family, has powerful anticancer properties and is well known for its many medical advantages such as antibacterial, anti-inflammatory, antiparasitic, and antifungal properties [92]. Hydrotropes have been shown to directly impact cell structure, increasing the accessibility of curcuminoids either by breaking the cell wall or by dissolving components of the cell membrane or wall. The most effective hydrotrope for extracting curcuminoids is NaCS [82]. Due to presence of both solubilized curcuminoids and hydrotropic in the recovered precipitate, it exhibited a gradually decreasing concentration of solubilized curcuminoids at concentration exceeding 1.0 mol/dm3, akin to piperine.

5. *Citral from Lemongrass*

The major element of lemongrass oil is citral (3,7-dimethyl-2,6-octadienal), which is extracted from the leaves of *Cymbopogon flexuosus* (Steud.) Wats. It possesses sedative, antidepressant, antiviral, antifungal, and antitumor properties and is commonly used as a lemon flavoring and scenting ingredient. Maximum citral yields were reported in a 0.25-mm piece of plant material at 1.75 M hydrotropic concentration, 5% solid loading, and 30 °C using a hydrotropy solubilization technique combining NaSal and NaCS (sodium cumenesulfonate). Citral is extracted more efficiently by using NaSal than by NaCS.

6. *Electro-osmotic Dewatering*

In recent era, there is a great interest in electro-regulated food-processing techniques and their use in the extraction of nutraceuticals. In electro-osmotic dewatering, the moisture of food is removed at low electrical field and is controlled by the electrical resistance present in the system to maintain specific conditions. Electro-osmotic dewatering is basically a drying method and has recently become quite popular in the nutraceutical business. To extract moisture from plant materials and food, electro-osmotic technique employs an electric field of approximately 5–30 volts. This process is better than the traditional heat drying methods since it can reduce carbon emissions by about 80%. Furthermore, energy consumption is reduced up to two-thirds in this technique as compared to traditional thermal processes. The basic principle followed by this technique is osmotic dehydration, as it is a drying process, and enhanced mass transfer. An electrochemical double layer is formed on the interface of aqueous suspension due to the applied mechanical pressure. This mechanical dewatering leads to the drying of substrate [93]. Since electro-osmotic dehydration is frequently used to concentrate fruit fragments and achieve improved jam properties on difficult-to-dewater substrates, it would be excellent to employ it to treat high-sugar fruits and vegetable by-products that contain labile antioxidant and surface colorants. Additionally, if the substance is elastic and well grained, it is too delicate to treat and too viscous to pump, making water removal more difficult. Prior to industrial deployment, this issue of electro-osmotic dewatering should be overcome [94].

6.5 Design and Working Principle

Direct current (DC) is used to impart an external electrical field to a semisolid material sandwiched between two electrodes, resulting in electrical dehydration (ED). When dewatering proceeds downhill in a bed of semisolid material where the initial water content is uniform across the bed, the water content in a section of the material close to the top electrode opposing the drainage surface decreases regionally. The higher electrode and the dewatered material now have a greater electrical contact resistance. To effectively apply electro-osmotic dewatering to various types of materials, it is essential to increase the dewatering rate, decrease the final water content, and utilize the least amount of energy feasible to remove the water. Electrical dehydration (ED) differs from mechanical dewatering in that it does not employ fluid pressure, compressive forces, or centrifugal forces. The process of electro-osmotic dewatering is elaborated in Fig. 8. It offers several advantages over mechanical procedures, particularly in effectively dewatering colloidal particles, gelatinous substances and solid-liquid mixture based on biological components, which mechanical techniques may struggle to adequately adress.

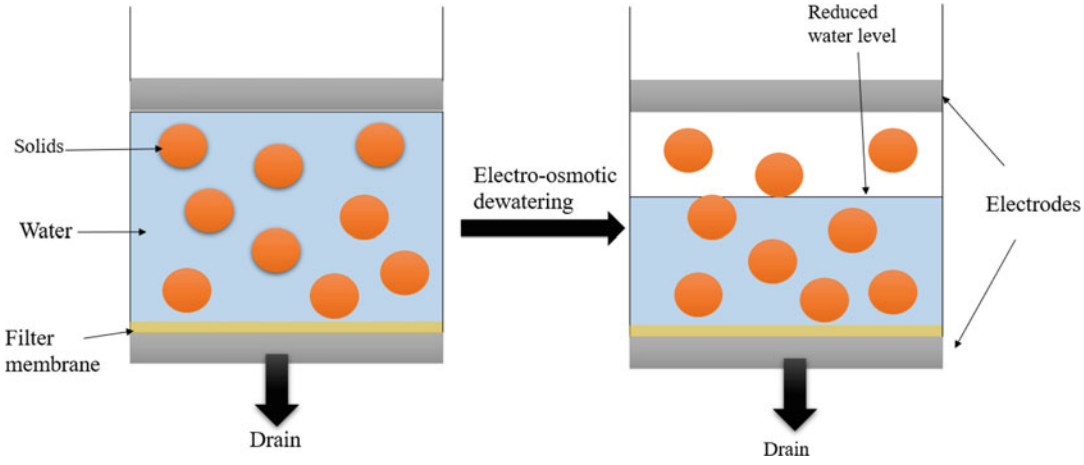

Fig. 8 Process of electro-osmotic dewatering [95]

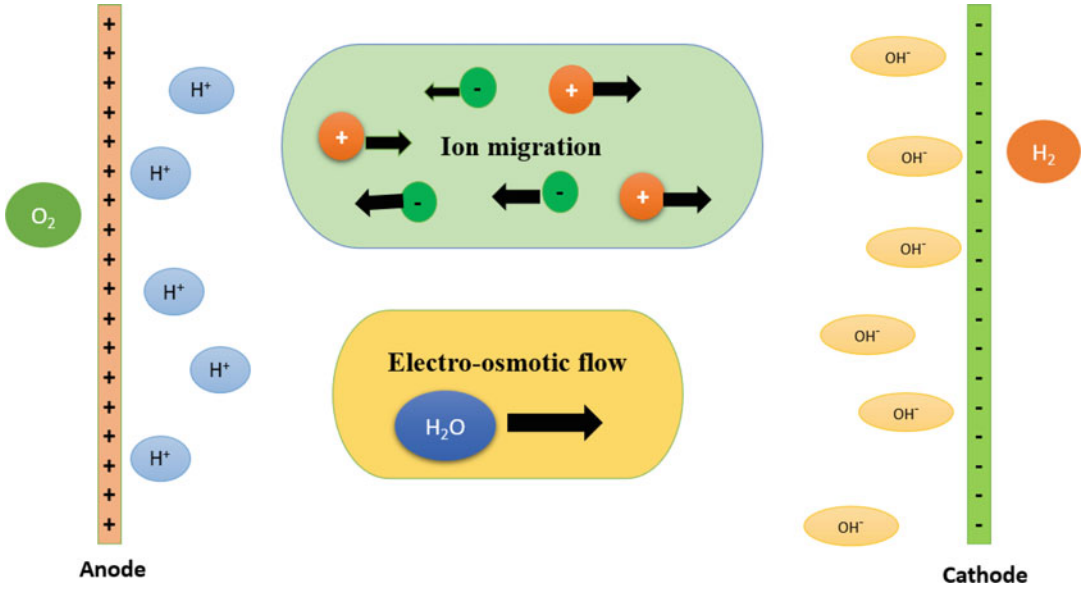

Fig. 9 Systematic diagram of electro-osmotic process [97]

The application of an electric field causes physical and electrochemical reactions in addition to the movement of water molecules that have a negative impact on the effectiveness of the electro-osmotic dewatering (Fig. 9) [78, 96].

1. Development of crack as a result of negative pore water pressure and severe drying at the anode
2. Severe variations of pH at both electrodes
3. Development of bubbles at the electrodes as a result of water electrolysis, which reduces solute-electrode contact

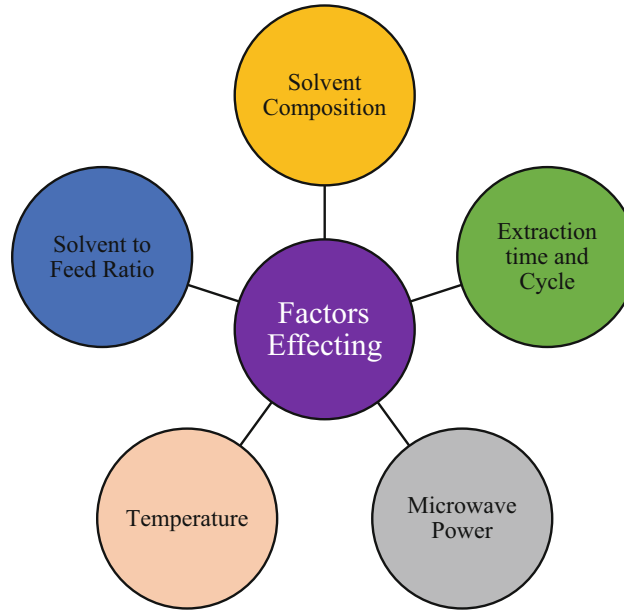

Fig. 10 Factors effecting the extraction of nutraceuticals

6.6 Factors

Some of the factors affecting electro-osmotic dewatering of substrates are given below and also described in Fig. 10 [98]:

1. The amount of material that needs to be dewatered
2. The mixture of substrate and zeta potential of water
3. The pressure used (pressure and electro-osmosis combined have been shown to be more cost-effective than electro-osmosis alone)
4. The kind of membrane employed

After retting process, electro-osmosis proves to be a rapid method for dewatering fibrous plant with high moisture level. Another post-retting procedure that uses less energy than lengthy hot air drying is electro-osmosis followed by microwave drying. Electrochemical based method have been employed in various sludge treatments, ranging from laboratory to pilot and full scale experiment [99]. The advantages of electro-osmotic technique have led to its commercialization since it concurrently achieves sludge dewatering, odor control, and pathogen removal. Additional field study and research in the extraction processes would provide technical input on how to lower startup and operating costs and set up minimal processing.

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Chapter 17

Optimization of Nutraceuticals Extraction

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Abstract

Optimization is the selection of the most efficient system that can resolve and help in attaining the objective functions by designing or operating various optimized procedures. In this chapter, optimized extraction of nutraceuticals is discussed. With an increasing demand for nutraceuticals, there is now greater focus on nutrapharmaceutical industry that produces safer natural products/extracts in a sustainable manner by cost-effective and eco-friendly green extraction routes. So the strategies that need to be devised for this purpose include the evaluation and optimization of various extraction variables (particle size, material/solvent ratio, extraction time and cycle, type of extraction technique, extraction conditions like temperature, agitation rate, etc.) that aim to control the stability of the bioactives and achieve the sustainable quality of the end-use nutraceutical products. In this chapter, we have focused on the optimized extraction of nutraceuticals along with the recent technological trends that are applicable toward the recovering of best possible levels of such high-value components.

Key words Optimized extraction, High-value components, Bioactives stability, Green extraction, Sustainable quality, Healthy products, Nutrapharmaceuticals

1 Introduction

Plants have always served human beings as a source of food and folk medicine, as well as they offer a platform for isolation of lead molecules for development of modern drugs. However, in consistent with the notion "*let your food be your medicine*," currently there is a renewed interest on the use of plants as a source of food and medicine. In line with the concept of optimal nutrition, currently, the science of functional food and nutraceuticals is an emerging area in food science [1–3]. In fact, a huge number and kind of plants, especially herbs, spices, food crops, and medicinal species have been explored for isolation of a broad range of bioactives and high-value phytochemicals with biological and nutraceutical potential [2–5].

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Nutraceuticals are products isolated and/or made from various food sources that offer additional health advantages beyond their basic nutritional benefits. They might consist of dietary supplements, nutritious foods, and drinks supplemented with bioactive ingredients like probiotics, vitamins, minerals, antioxidants, and herbal extracts. The ability of nutraceuticals to promote general health and treat and/or prevent specific health disorders underlies their significance.

In terms of developing and sustaining excellent health, nutraceuticals can be extremely helpful. They offer vital nutrients and bioactive substances that support numerous biological processes and aid in preventing nutritional deficits. They aid in the improvement of general health by providing particular beneficial components [6].

A long list of nutraceuticals with therapeutic effects and disease-preventing qualities have been developed that may help lower the chance of chronic diseases. For instance, the antioxidants in fruits and vegetables work to combat dangerous free radicals, potentially lowering the prevalence of oxidative stress–related diseases like cancer, aging, inflammation, heart disease, and neurological disorders [7].

By providing nutrient support, particularly when specific needs for nutrients cannot be satisfied through regular diet alone, nutraceuticals can supplement or enhance standard dietary practices. They fill in nutritional gaps by assuring proper intake of minerals, vitamins, and other crucial nutrients. They are practical and efficient. In line with the development of nutrigenomics, nutraceuticals also offer the advantage of personalized nutrition by addressing individual health needs and genetic variations. [8] As scientific advancements continue, the development of tailored nutraceutical products based on genetic profiling and biomarker analysis may become more prevalent, providing customized health solutions. While nutraceuticals hold promise, it's essential to note that their efficacy and safety can vary. It's important to choose reputable products to ensure optimal benefits and minimize potential risks [8, 9].

There are various nutraceuticals that are being used as dietary supplements (as shown in Fig. 1) and provide a significant amount of nutrition along with their medicinal benefits. Nutraceuticals are frequently utilized in medicine as a complimentary treatment to traditional medicine. To boost treatment outcomes and enhance patient well-being generally, they may be used in combination with approved medications or therapies. For example, omega-3 fatty acid supplements are regularly utilized as an additional therapy for the control of cardiovascular disease [6, 10].

Nutraceuticals' ability to encourage optimal aging and lengthen lifespan is being recognized more and more in the aging and longevity fields. Resveratrol, a substance found in red grapes,

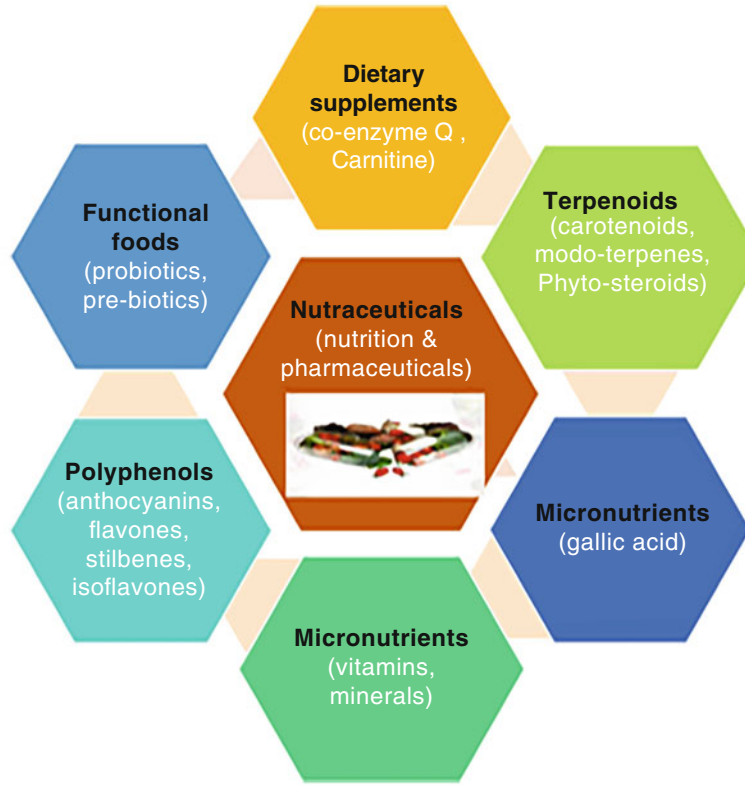


Fig. 1 Application of nutraceuticals as dietary fibers [6]

has shown antiaging properties and is thought to activate genes related to lifespan, possibly postponing age-related degenerative processes. The process of getting bioactive substances from sources that are natural, such as trees, fruits, herbal remedies, and marine life, for use as food supplements or food products that function is referred to as nutraceutical extraction. There are numerous recognized techniques for extracting nutraceuticals, each with unique risks and advantages [11].

In order to extract high-quality and bioactive chemicals from a variety of natural sources, nutraceutical extraction methods must be optimized. To improve the extraction effectiveness, production yield, as well as preservation of the nutraceuticals, several approaches have been developed. Solvent extraction is one of the often-used technique that uses water, ethanol, or methanol to extract bioactive chemicals. Utilizing cutting-edge green technologies like microwave-assisted extraction [12] and ultrasound-assisted extraction (UAE), which make use of the physical and thermal impacts to increase extraction efficiency, is another strategy. Additionally, for the extraction of sensitive chemicals and its environmental friendliness have made SFE (supercritical fluid extraction) employing carbon dioxide as the solvent popular.

Furthermore, enzyme-assisted extraction (EAE), a potential method that uses enzymes to dissolve cell walls and release valuable substances, has also come to light. To achieve highest extraction efficiency, parameters such as solvent concentration, extraction duration, temperature, or particle size need to be optimized. In this regard, statistical techniques like response surface methodology (RSM) and modeling based on artificial intelligence can help the optimization process [13].

These are only a few methods utilized for the extraction of nutraceuticals. The type of target chemicals, the attributes of the source material, and the intended extract properties all influence the selection of extraction process. For choosing the best extraction technique, it's important to consider parameters like efficiency, selection of solvent and material, cost effectiveness, and the impact on environment into account.

2 Importance of Optimizing the Extraction Process of Nutraceuticals

Nutraceutical extraction procedure needs to be optimized for a number of reasons, including the potential to improve product quality. The quality and chemical structure of nutraceutical goods are directly impacted by the extraction process. Higher quantities of bioactive substances can be obtained by reducing the presence of undesirable components and by improving the extraction process. As a result, the finished product has enhanced uniformity, purity, and potency. The pace and degree at which a nutrient or bioactive chemical is taken up and used by the human system is referred to as improved bioavailability. Because of their complex structures and poor solubility when dissolved in water or lipids, several nutraceuticals can exhibit low bioavailability. By enhancing the release of active chemicals and enhancing their solubility, optimization of the extraction process can increase bioavailability and maximize the absorption and bioactivity in the body [14].

The cost of producing a nutraceutical can be considerably impacted by the extraction method. Optimization enables more effective use of raw materials, lower energy use, and less waste production. Nutraceutical producers can achieve better yields, lower manufacturing costs, and eventually deliver the products at a more affordable price by expediting the extraction procedure [8]. Extraction processes that use organic solvents or consume a lot of energy have the potential to harm the environment. In the concept of green extraction includes, less hazardous solvents, less energy, and ecologically friendly alternatives are the goals of optimization strategies [15]. Ecofriendly extraction methods can help the nutraceutical industry promote sustainability initiatives and reduce its environmental impact [16].

By streamlining the extraction process, scalability and consistent product quality are guaranteed. Without compromising the efficacy or purity of the nutraceuticals, it enables the development of extraction procedures that are standardized and repeatable on a larger scale. Consistency is necessary for clinical studies, complying with regulations and meeting customer expectations.

By making improvements in extraction procedures promotes continued investigation and development in the nutraceutical industry. Researchers might discover new opportunities for extracting bioactive molecules from various sources by investigating novel extraction strategies like enzyme-assisted extraction, supercritical fluid extraction, and the microwave-assisted extraction. This upgrades the variety in health-promoting items, consumers can choose from and results in the discovery of new nutraceutical ingredients.

Therefore, it is crucial to optimize the extraction procedure for nutraceuticals to ensure product quality, improve bioavailability, cut costs, promote sustainability, ensure consistency, and spur innovation in the industry. Together, these advantages help the nutraceutical business develop and grow, which eventually benefits both producers and customers [17].

2.1 Selection of Source Material

Choosing the source material for nutraceutical extraction is quite challenging and critical. The yield and makeup of bioactive chemicals can be influenced by elements like plant species, the portion of the plant that is used (leaves, roots, seeds, etc.), and the maturity stage. The main source materials used for nutraceutical extraction are shown in Fig. 2.

Additionally, the geographical origin of the starting material and the variation in chemical structure within the identical species can have an impact on the success of the extraction. Numerous aspects, including the presence of bioactive chemicals, accessibility, sustainability, safety, and legal and regulatory considerations, should be taken into account when choosing the material sources for extracting nutraceuticals [10].

Here are some typical sources that are frequently utilized to extract nutraceuticals:

(a) *Plants*: Numerous dietary supplements, such as vegetables, fruits, herbal remedies, and medicinal plants, are obtained from plants. Examples include *Ginkgo biloba* extract, curcumin from turmeric, resveratrol from grapes, and the caffeine in green tea extracts derived from *Camellia sinensis*.

(b) *Marine sources*: Marine life like algae, seaweed, and some fish can be great sources of dietary supplements. Fish oil–derived fatty acids known as omega-3 (eicosapentaenoic acid/EPA and docosahexaenoic acid/DHA) are frequently used as nutraceutical supplements.

Fig. 2 The main sources used for nutraceutical extractions [18]

(c) *Fungi*: Some varieties of mushrooms, like shiitake (*Lentinula edodes*) and reishi (*Ganoderma lucidum*), are recognized for their potential as nutraceuticals. They include bioactive substances like triterpenoids and polysaccharides.

(d) *Animal sources*: Several nutraceuticals can be made using materials that come from animals. Collagen peptides from bovine or fish sources, for instance, are utilized for healthy skin, joint support, among other advantages.

(e) *Microorganisms*: Some microorganisms, including yeast and probiotic bacteria, are sources of nutraceuticals. Active yeast or bacterial cultures are known as probiotics, and they have several health advantages, especially for the gut.

(f) *By-products and waste products*: By-products and waste products from agriculture can potentially be used as valuable sources for nutraceuticals. For instance, winemaking leftovers can be used to make grape seed extract [19].

Make sure that the source materials you use are of high quality and pure. The amount of bioactive compounds and overall standard of raw material can be affected by cultivation or sourcing

practices, processing procedures, and storage conditions. In order to guarantee the safety and effectiveness of the extracted nutraceuticals, compliance with regulatory requirements and good manufacturing procedures (GMP) are also essential.

3 Factors Involved in Nutraceuticals Extraction

The process of separation and isolation of bioactive components from various natural sources such as trees, marine life, or microorganisms is known as nutraceutical extraction. Extraction of bioactive compounds involves stable circumstances and conditions. Hence, various factors affect the extraction process and yield of nutraceuticals, and some factors are described below and shown in Fig. 3. These factors affect the efficiency of any extraction process as these parameters are involved in almost all of the extraction techniques. So in order to obtain better yield of nutraceuticals, we need to optimize the extraction processes and minimize the effect of these factors on the process.

(i) *Solvent Selection*

The selection, efficacy, and safety level of the extraction process can be affected by the solvent choice, which is crucial. Water, methanol, ethanol, and their mixtures are employed as common solvents. The effectiveness of the extraction process and the kinds of phytochemicals and other bioactives extracted can be influenced by the solvent's polarity, boiling point, and toxicity. Additionally, cosolvents' presence, pH changes, and temperature changes can affect the yield or stability of the isolated bioactive components [18].

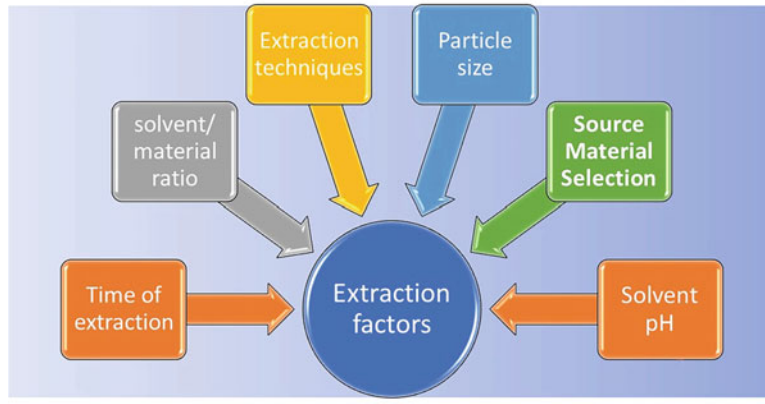


Fig. 3 Factors affecting the extraction efficiency of plant bioactive compounds [20]

The kind of nutraceutical ingredient, its ability to dissolve, and the desired effectiveness of extraction all play a role in the solvent choice for nutraceutical extraction. Here are some typical solvents and their properties for extracting nutraceuticals:

(a) *Water*: Water is the universal solvent and is frequently used to extract water-soluble nutraceuticals, including certain polyphenols, vitamins, and minerals. Although it is a risk-free and economical choice, it might not be appropriate for extracting a lipophilic (fat-soluble) chemicals and many other organic compounds.

(b) *Ethanol*: Due to its adaptability and capacity to separate both hydrophilic and lipophilic molecules, ethanol is a commonly utilized solvent for nutraceutical extraction. Extracting flavonoids, polyphenols, and certain oils that are essential is where it succeeds. Although ethanol is generally safe and enlisted in biosolvents, nevertheless, a larger quantity of such solvents should be handled carefully as it is flammable.

(c) *Methanol*: Particularly for compounds derived from plants, methanol is a typical solvent. It is efficiently used for extracting a variety of nutraceuticals and possesses solvency characteristics that are similar to those of ethanol. Methanol must be handled carefully and under ventilated conditions because it is highly flammable and poisonous.

(d) *Hexane*: It is a nonpolar solvent and is typically employed for the extraction of lipophilic compounds such as fats, carotenoids, and other lipid-soluble nutraceuticals. Although it has good extraction efficiency, due to its combustibility and potential health risks, it needs to be used with caution.

(e) *Supercritical fluids*: As a matter of green extractions, supercritical fluids such as supercritical carbon dioxide (CO_2) are gaining popularity in the extraction of nutraceuticals,. They have the benefit of being risk-free, ecofriendly, and very selective in extracting particular molecules. For extracting delicate substances including essential oils and heat-sensitive nutraceuticals, supercritical CO_2 is especially successful.

It's crucial to remember that while choosing solvent, factors such as the particular nutraceuticals of concern, their solubility traits, safety issues, and legal requirements should be taken into account. To maximize extraction efficiency and reduce nutraceutical degradation, the extraction parameters (temperature, pressure, and extraction time) are to be adjusted [21].

(ii) *Selection of Extraction Techniques*

Nutraceutical extraction uses both traditional procedures (like maceration and Soxhlet extraction) and cutting-edge technologies (like ultrasound-assisted extraction and supercritical fluid

extraction). The type of bioactive chemicals, their physical and chemical characteristics, and the desired extracting parameters (yield, selectivity, consumption of energy, etc.) all influence the technique chosen. With regard to cost, selectivity, and efficiency, each method has pros and cons. Pressure, temperature, extraction duration, and agitation are additional variables that affect the extraction process [22].

(iii) *Process Parameters*

The efficacy and purity of nutraceutical extraction can be affected by a number of process variables, including extraction duration, temperatures, solid-to-liquid ratio, and agitation. To optimize the extraction yield and maintain the integrity and biological activity of the target molecules, these parameters must be optimized.

(iv) *Particle Size*

The extraction efficiency is influenced by the raw material's particle size. Increased surface areas during solvent interactions are provided by smaller particle sizes, which boost extraction rates. Therefore, prior to extraction, the raw material is frequently pulverized or ground into smaller pieces.

(v) *Extraction Conditions*

Parameters like temperature, pressure, time, and solvent-to-solid ratio influence the extraction process. Optimal conditions need to be determined for each specific raw material and target compound. These conditions should promote efficient extraction while minimizing the degradation of sensitive bioactive compounds.

(vi) *pH and Ionic Strength*

The pH and ionic strength of the extraction medium can affect the solubility and stability of bioactive compounds. Adjusting the pH or adding salts can enhance or inhibit the extraction of certain compounds.

(vii) *Pretreatment Techniques*

Preprocessing techniques like blanching, drying, freeze-drying, or enzymatic treatments may be applied to the raw material to enhance extraction efficiency by breaking down cell walls, deactivating enzymes, or concentrating the target compounds [21].

(viii) *Pre- and Post-treatment*

The effectiveness and stability of bioactive ingredient extraction can be impacted by pre- and post-treatment procedures such as drying, crushing, particle size reduction, and storage conditions.

The efficacy and biological activity of the isolated nutraceuticals must be maintained by proper handling and processing of the source material [23].

(ix) *Retention and Storage*

The durability and bioactivity of these isolated chemicals must be maintained by appropriate storage practices and preservation procedures. To avoid deterioration and loss of potency, factors like light exposure, temperature, oxygen, or moisture content must be taken into account.

(x) *Quality Assurance*

To maintain constant product quality and safety throughout the extraction process, regular quality control procedures should be put in place. This entails performing contaminant detection tests, calculating the amount of bioactive substances, and evaluating the overall purity of the product [24].

4 Techniques for Nutraceutical Extraction

In order to improve the production and quality of the required bioactive components, it is necessary to optimize the process of extraction of nutraceutical substances. Following are a few popular optimal extraction techniques for nutraceuticals, along with reading recommendations [15]:

(a) *Solvent Extraction*: One popular technique for extracting nutraceuticals is solvent extraction. To extract particular molecules, a variety of solvents like methanol, ethanol, and water can be utilized. Solvent concentration, extraction duration, temperature, and solvent to sample ratio are all optimization parameters.

(b) *Supercritical Fluid Extraction (SFE)*: This method extracts nutraceuticals using supercritical fluids like carbon dioxide (CO_2). In order to create supercritical conditions, pressure and temperature must be controlled. Selectivity, gentle extraction conditions, and little solvent residue are just a few benefits of SFE [25, 26]:

(c) *Microwave-Assisted Extraction (MAE)*: MAE method boosts the extraction process by using microwave energy. It delivers quick extraction, lower solvent use, and better extraction effectiveness. Extraction time, power, liquid or solvent type, and sample-to-solvent ratio are variables that should be optimized [27].

(d) *Ultrasound-Assisted Extraction (UAE)*: UAE breaks down cell walls and improves mass transfer during extraction by using

high-frequency ultrasound pulses. It is a safe and effective process that can speed up the extraction process and increase the amount of nutraceuticals produced [28].

(e) *Pressurized Liquid Extraction* [29]: PLE, often referred to as accelerated solvent extraction (ASE), increases extraction efficiency by using high temperatures and pressures. It increases yield while decreasing extracting time and solvent usage.

5 Approaches for Optimized Nutraceutical Extractions

The optimization of general extraction mechanisms takes into account a number of variables, including picking the best extraction technique, fine-tuning the extraction's variables, and improving the yield as well as the purity of the molecules being extracted. The following are some essential steps for optimizing nutraceutical extractions.

(a) *Selection of Extraction Method*

With the growing importance of natural medicine, it is very important to select the most efficient and economical technique. As the bioactive components are present in a meagre amount in natural medicines, it is obligatory to increase their yield by developing or choosing methods that provide optimized yield under optimized conditions [28]. Therefore, the selection of extraction technique is a very important step. Ultrasound-assisted extraction, solvent extraction, microwave-assisted extraction, and supercritical fluid extraction are a few extraction methods that can be applied. Most of these techniques are successfully applied for extracting various nutraceuticals at laboratory level, and among these, supercritical fluid extraction is also being used at industrial scale [13, 15].

The method chosen will rely on elements like the source material and the type of target chemicals.

(b) *Optimization of Extraction Parameters*

The effectiveness of the extraction process and the quality and bioactivity of the extracted materials can be greatly impacted by different variables such as extraction duration, solvent type, temperature, pH, agitation solvent-to-sample ratio, and nature of extracts. To get the desired result, these factors have to be optimized using an experimental approach and statistical methods. For instance, the response surface technique methodology [30] involves the use of experimental data and statistical analysis to determine the optimal conditions and yield. This technique works for experimental design and test its validity by ANOVA weather the design is feasible and adequate for desired extraction process [15, 30].

(c) *Selection of Target Species*

Nutraceuticals are used as both nutritious agents and medicines treating wide range of diseases such as allergy, inflammation, cancer, and diabetes. To extract these compounds from their natural sources, we need to first define the type of nutraceutical that is to be extracted and then identify and optimize the method of extraction. The type of technique utilized depends upon the compound to be extracted; for instance, tocopherols and free fatty acids are sensitive to heat [13], so ultrasound-assisted or sonication-assisted extractions could be used to optimize the product yield.

(d) *Choice of Solvents*

The effectiveness and selectivity of a specific molecule extraction can be considerably impacted by the solvent chosen. To find the most effective solvent for extraction the desired bioactive chemicals, a variety of solvents with various polarity and characteristics can be tried. To increase the extraction efficiency, solvent combinations or modified solvents can be utilized. For example, for extracting nutraceuticals from milk thistle, various solvents such as acetone, ethanol, acetonitrile, or methanol can be used [31]. The yield can be optimized by knowing which solvent gives the optimum results for the desired extracts.

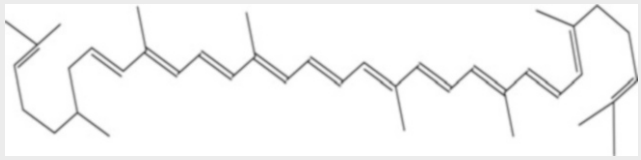
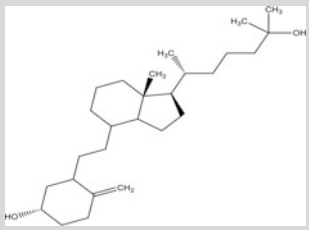
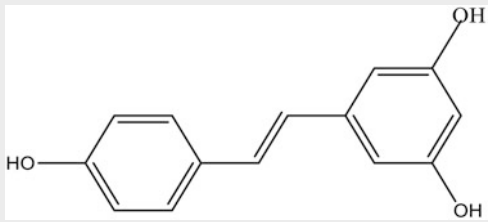
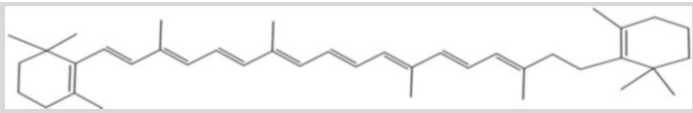
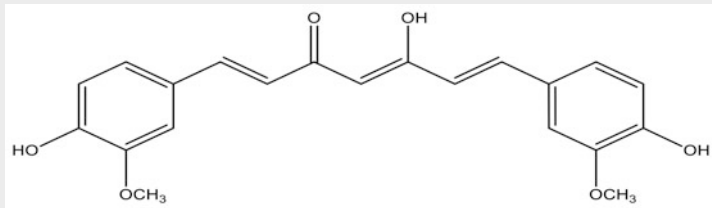
(e) *Quality Control and Standardization*

To ensure consistent quality of the nutraceuticals obtained, it is important to establish quality control measures and standardize the extraction process and nutraceuticals doses and usages. This includes testing the extracts for their bioactive compound content, purity, stability, and potential contaminants. These controls provide much more assurance to the product obtained. Qualitative as well as quantitative evaluations of the composition's overall profile and primary distinctive chemicals should be included in quality control. Because the purity levels of samples used in animal tests and clinical studies directly affect the accuracy of the research results, it is crucial to both ensure the safety of herbal products for consumers and accurately analyze the efficacy of nutraceutical extracts [32]. The structural formulas and natural sources of commonly known nutraceuticals are given in Table 1.

(f) *Innovative Extraction Techniques*

Researchers are continually experimenting with new methods to improve the extraction of nutraceuticals [5, 15, 26]. This may entail developing extraction technology, such as using sophisticated reactors, membranes, or enzymes, along with investigating environmentally friendly and sustainable extraction techniques.

Table 1
Different types of nutraceuticals and their sources [29]

Nutraceutical compounds	Chemical structure	Sources
Lycopene		Plant sources, tomato
Vitamin D3		Marine organism, fishes
Resveratrol		Fruits, grapes
B-carotene		Plants, carrots
Curcumin		Turmeric

Numerous novel techniques including supercritical fluid extraction, accelerated solvent extraction, microwave-assisted extraction ultrasound-assisted extraction, and sonication-assisted extraction have been established for effective extraction of nutraceuticals as these techniques play a role in shortening the extraction time, increasing the yield of extraction, diminishing the solvent intake, and enriching the quality of products [13].

6 Methods for Optimizing Nutraceutical Extractions

Nutraceutical extraction yield can be optimized using several methods. Some of these commonly employed techniques are described here briefly.

1. *Response Surface Methodology*

RSM involves the use of statistical analysis and mathematical techniques for the optimization of experimental designs. This technique is utilized to determine the optimized parameters for extracting nutraceuticals from natural sources, especially food sources. In the context of nutraceutical extractions, RSM helps in determining the relationship between multiple input variables (such as extraction time, temperature, solvent ratio, and pH) and the desired output response (such as yield, concentration of target compounds, antioxidant activity). Researchers can effectively investigate how these variables affect the extraction technique and pinpoint the ideal set of conditions that optimizes the intended result by using RSM. The flowchart of response surface methodology is illustrated in Fig. 4 [30, 33, 34].

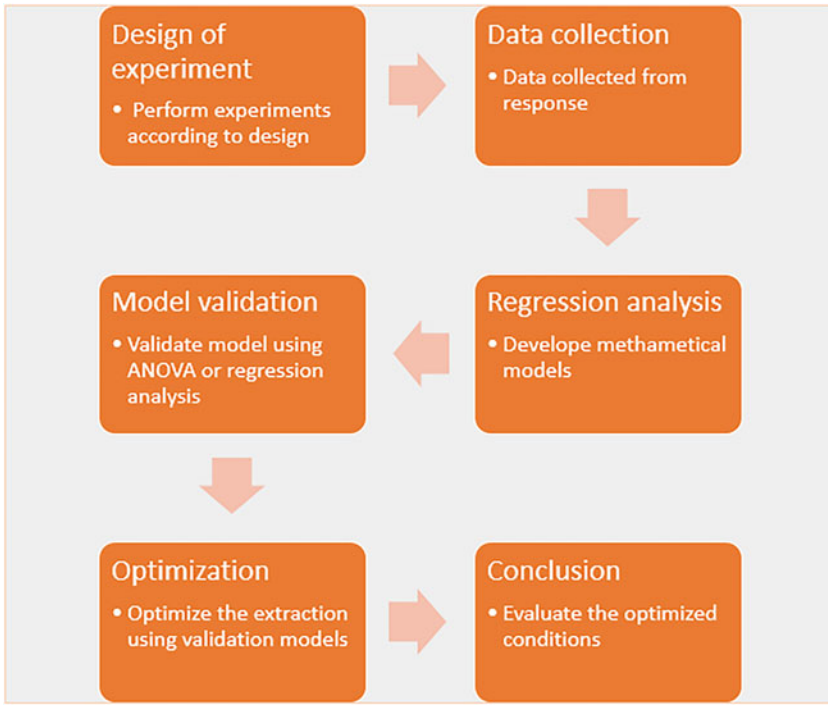


Fig. 4 Flowchart of RSM scheme [35]

The steps to optimize the extraction conditions are as follows:

I. *Design of experiment*: RSM calls for the planning and execution of a series of tests based on a particular experimental design, for instance the central composite design (CCD) and a Box-Behnken design. By using these approaches, the parameter space can be explored effectively while requiring fewer tests.

II. *Data collection*: The experiments are performed according to the designed plan, and data is collected on the responses of interest. The responses could be the yield of bioactive compounds, the concentration of specific compounds, or any other relevant parameters.

III. *Regression analysis*: The collected data is then subjected to regression analysis to fit mathematical models that describe the relationship between the input variables and the response [35]. The models can be linear, quadratic, or higher-order polynomials, depending on the complexity of the system and the experimental design.

IV. *Model validation*: Models must be validated after development using additional experiments or statistical methods like analysis of variance (ANOVA). By doing this, the models are made sure to accurately describe the system and be suitable for optimization.

V. *Optimization*: The ideal conditions for the extraction procedure are then chosen using the verified models. Numerical optimization techniques that seek to maximize the response or reach particular goal values can be used to undertake optimization.

Researchers can learn more about the effects of different extraction parameters and improve the extraction process to increase the yield, concentration, or bioactivity of the compounds of interest by applying RSM to nutraceutical extractions. With this strategy, resources can be used more effectively, and the need for costly experimentation is diminished.

Various techniques make use of response surface optimization to optimize the yield of extraction. In recent studies, this technique was used to optimize the antioxidant extractions from the roasted rice germ–flavored herbal tea. This type of herbal tea is very commonly used in Asia as these are known for their gentle aroma and fragrances along with many health benefits. These herbal teas contain bioactive compounds that possess anti-inflammatory, antibiotic, antiaging, anticarcinogenic, and antidepressant effects [36].

As response surface methodology (RSM) used to study the ideal process parameters while analyzing the antioxidant capacity, total polyphenol content, and attributed like in roasted rice germ

flavored herbal tea. Five central point replicates of a full factorial approach on three distinct levels with two variables [37] were used to evaluate the impact of time and temperature on the extraction process [38].

2. *Ultrasound-Assisted Extraction*

This technique, also termed sonication-assisted extraction, uses sound waves that expand and compress while traveling through medium unlike electromagnetic radiations. One of the promising techniques for obtaining plant bioactive chemicals is ultrasound-assisted extraction. This has great performance low solvent and time consumption, suitability for thermos-sensitive chemicals, and acceptance as a green extraction process, and ultrasonic-assisted extraction [37]. The time, energy, and solvent requirements for conventional extraction have some restrictions. Bioactive components can be extracted using ultrasound-aided extraction (UAE) in a remarkably short duration of time at low temperature with little need for energy or solvent. UAE is a non-thermal extraction method and is more effective in maintaining the functionality of bioactive chemicals [35].

There are two main types of ultrasound-assisted extractions which are closed ultrasonic built-in with an ultrasonic horn transducer or baths extractors. To achieve an efficient and successful ultrasound-assisted extraction, it is vital to consider plant parameters such as moisture content, size of particles, and the extraction solvent [39]. Additionally, a number of variables, including frequency, pressure, temperature, and sonication time, affect how well ultrasound works [13].

Procedure

The general steps involved in this procedure are briefly described below and shown in Fig. 5 [40]:

I. Preparation of sample: The first step is the preparation of the sample material. It involves cleaning and drying the raw material to remove impurities and moisture. The sample may also need to be ground or chopped into smaller pieces to increase the surface area for better extraction.

II. Solvent selection: Next, an appropriate solvent is selected based on the target compounds to be extracted. The solvent should have good solubility for the bioactive components and be safe for human consumption. Common solvents used in UAE include water, ethanol, methanol, and their mixtures.

III. Sample-solvent mixing: The prepared sample material is then mixed with the selected solvent to create a homogenous mixture. The ratio of sample to solvent can vary

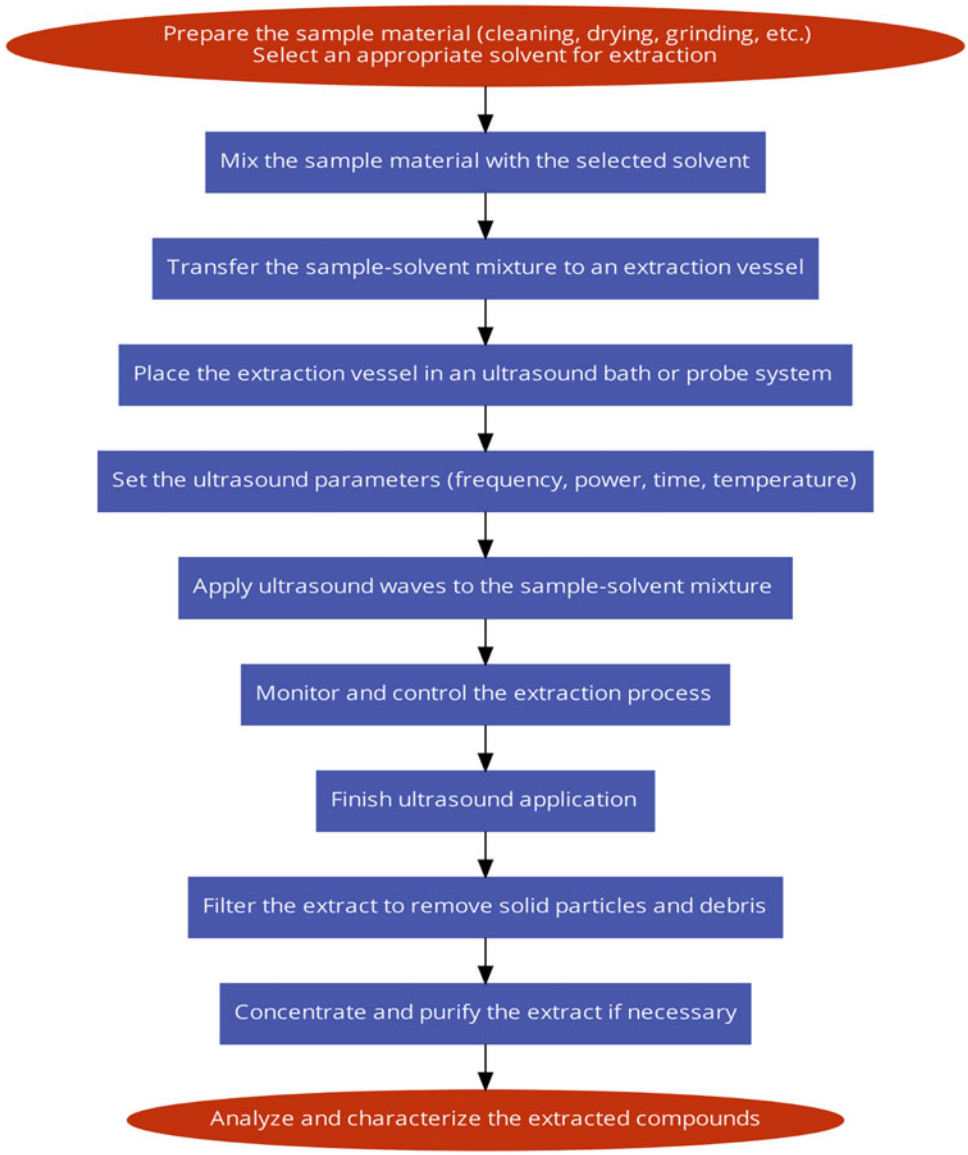


Fig. 5 Flowchart of ultrasound-assisted extraction

depending on the characteristics of the sample and the desired extraction efficiency. It is important to ensure good contact between the solvent and the sample material.

IV. Ultrasound extraction: The sample-solvent mixture is transferred to an extraction vessel or container. The extraction vessel is usually made of a material that can withstand the ultrasound waves, such as glass or stainless steel. The vessel is then placed in an ultrasound bath or probe system.

V. Ultrasound application: Ultrasound waves are applied to the extraction vessel containing the sample-solvent mixture, which create cavitation bubbles in the solvent, leading to the formation and collapse of microbubbles. These microbubbles generate localized high temperatures and pressures, causing the cell walls of the sample material to rupture and facilitating the release of bioactive compounds.

VI. Extraction parameters: Several parameters need to be optimized to achieve efficient extraction. These include the ultrasound frequency, power, extraction time, and temperature. The parameters may vary depending on the characteristics of the sample and the target compounds. Generally, a frequency range of 20–100 kHz is used, with power levels between 20 and 500 W.

VII. Filtration and separation: After the ultrasound treatment, the extract is filtered to remove solid particles and plant debris. Filtration methods such as vacuum or gravity filtration are commonly used. The filtrate contains the desired bioactive compounds extracted from the sample.

VIII. Concentration and purification: The obtained extract may undergo further concentration and purification steps, such as solvent evaporation, liquid-liquid extraction, or chromatographic techniques, depending on the nature of the target compounds and the desired purity level.

IX. Analysis and characterization: The final step involves the analysis and characterization of the extracted compounds to determine their quantity, purity, and potential nutraceutical properties. Analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry [36], and nuclear magnetic resonance (NMR) can be employed for compound identification and quantification.

6.1 Optimization of Ultrasound-Assisted Extraction

The optimization of ultrasound-assisted extraction (UAE) involves adjusting various parameters to maximize the extraction efficiency and yield of target compounds. Here are some key factors to consider when optimizing UAE:

(a) *Ultrasound frequency*: The frequency of ultrasound waves can significantly impact the extraction efficiency. Different frequencies, typically ranging from 20 kHz to 100 kHz, may have different effects on specific compounds or sample matrices. Experimentation with different frequencies can help identify the optimal frequency for a particular extraction.

(b) *Ultrasound power*: The power level of ultrasound waves affects the intensity of cavitation and, therefore, the extraction efficiency. Higher power levels generally lead to more cavitation

and potentially higher yields, but excessively high power can cause degradation or denaturation of heat-sensitive compounds. Optimizing the power level is crucial to strike a balance between extraction efficiency and compound integrity.

(c) *Extraction time*: The duration of ultrasound application impacts the extraction efficiency. Longer extraction times can enhance the release of target compounds from the sample matrix. However, there is an optimal extraction time beyond which additional extraction does not significantly increase the yield. It is essential to determine the appropriate extraction time through experimentation and monitoring of compound extraction kinetics.

(d) *Temperature control*: Ultrasound-assisted extraction can generate heat due to the cavitation process. Controlling and monitoring the extraction temperature are crucial to prevent thermal degradation of the target compounds. Cooling systems or periodic cooling intervals during extraction can help maintain the desired temperature range.

The extraction of important molecules is now frequently done by using ultrasonography. As the biological activity of the substances obtained using this green chemistry strategy is evaluated in the current paper, and the results showed that of the examined plants, *S. nigra* flowers and *A. linearis* leaf extracts exhibited good total polyphenolic quantity and antioxidant capabilities. The extracts may serve as powerful sources of physiologically active compounds [41].

3. *Microwave-Assisted Extraction*

This technique, one of the novel and optimized procedures used for extraction purposes, uses microwaves ranging from 0.3 to 300 GHz, and these waves could easily penetrate through biomaterials and produces heat by interacting with polar molecules such as water present within the natural compounds [13]. The shorter duration of extraction is one of the key benefits of this method. This is mostly due to the superior heating performance of this method compared to conventional heating technique. Microwave heating directly heats the solution, whereas conventional heating requires a certain amount of time to warm the vessel before the heat is delivered to the solution. This minimizes the temperature gradient and quickens the rate of heating. Additionally, MAE enables the potential of running several samples and a large reduction in the consumption of organic solvent [42].

The first step in the extraction procedure is to load the sample inside the extraction vessel. The solvent is then added and the vessel is then sealed. Microwave radiation is used for heating the solvent to the desired temperatures, followed by a preextraction procedure.

Usually, the heating process lasts below 2 min. After receiving more radiation, the sample is removed for a certain period of time, often between 10 and 30 min. The samples are given time to cool back to a temperature that is safe to handle while the extraction is finished [42, 43].

6.2 Optimization of MAE

The optimization depends on different variables on which extraction yield depends. The results could be improved greatly by using experimental designs and by using response surface methodology for statistical analysis. For instance, in Microwave-assisted extraction (MAE) variables could be optimized by the use of a face-centered central composite design a experimental design used for optimization of response surface methodology for optimum recoveries of total flavonoid content (TFC) and antioxidants (DPPH and ABTS) of the extracts obtained from leaf *Vernonia amygdalina* [44].

Similarly another example containing optimization of microwave assisted extraction is to extract phenolic compounds found in licorice roots using response surface methodology [12]. RSM reduces the number of unnecessary experiments while enabling the user to pinpoint the ideal circumstances for a chosen answer. The relation between various variables (independent variables) and one or more of the responses (dependent variables) can be studied using RSM, which is a set of statistical and mathematical techniques [45]. With great repeatability, microwave-aided extraction can be finished in minutes as opposed to hours, using less energy and solvent.

There are some extraction parameters by arranging them through proper experimental design we could obtain optimized conditions, these parameters are Extraction Parameters:

(a) *Microwave power*: This power level determines the intensity of the microwave energy applied. Higher power levels can lead to faster extraction, but excessive power may cause degradation or loss of heat-sensitive compounds. Optimization is required to find the balance.

(b) *Extraction time*: The duration of microwave exposure affects the extraction efficiency. Longer extraction times may increase the yield, but there is a limit beyond which further extraction becomes insignificant or detrimental.

(c) *Microwave frequency*: The most common frequency used for MAE is 2.45 GHz, as it is readily absorbed by polar molecules. However, different frequencies may be utilized for specific applications.

(d) *Sample-to-solvent ratio*: The ratio of sample to solvent can impact the extraction efficiency. It should be optimized to ensure sufficient contact between the sample and the solvent for effective extraction.

Recently, optimized microwave-assisted extraction has emerged as an effective extraction technique due to the applications of response surface methodology. As such this MAE technique has been in use for about a century but recently the optimization of this procedure leads to open up new windows of Especially, the short time of extraction and improvement in yields are major benefits that makes this technique a novel and fitting in the principle of green extraction. Reduced solvent usage, shorter extraction periods, and higher sample throughput are the main advantages. There is a need for further cleanup when the extraction is finished, even though careful process development it may produce some extraction selectivity [42]. The future of this technique could be even brighter if more suitable experimental design is sought along with providing optimal conditions.

4. *Enzyme-Assisted Extraction*

Enzyme-assisted extraction is one of the optimized techniques that lead to enhanced release of bioactive components [26, 33, 34]. By disrupting plant cells and extracting bioactive compound through the cell wall, it is possible to maximize the release of these compounds by utilizing enzyme preparations either alone or in combinations. However, food industry is not currently taking advantage of biotechnological applications of enzymes to the fullest extent [46].

Irrespective of the extraction techniques used, there are always some barriers that hinder the optimal yield of the extract, which includes the structure of the cells from which the bioactive compound is to be extracted. Cell components that provide cell support hinder the extraction of the target metabolites. Therefore, enzyme-assisted extractions provide lesser resis tance caused by these natural barriers in extraction [47, 48]. As enzymes are specific in nature, they target the specific portion, and during these extractions, conditions such as temperature, cell structure, and pH are highly maintained. The enzymes used in EAE catalyzes the C-H bond present in molecules such as water, which in result disintegrates the cell structure and allows the permeability of the materials through cell. This treatment is also used as pretreatment for other techniques [49].

6.3 Optimization of EAE

Enzyme-assisted extractions are known for their optimized results and conditions. Several studies have shown recently that EAE provides optimal yield when used with response surface methodology with different experimental designs [26, 33, 34]. For instance, by using EAE with response surface methodology and by optimizing variables such as temperature, pH, and solvents, a maximum of proteins was extracted from sugar beets as enzymes are very specific in nature [50].

One of the bioactive chemicals now available that has considerable potential for use in pharmaceutical and medical treatments for humans is chondroitin sulfate, which is derived from various natural materials. But commercialization of chondroitin sulfate is challenging because it is present in very low levels in raw materials and the raw materials are expensive. In a recent study, this compound was extracted from *Bohadschia argus* through enzyme-assisted extraction. Experimental conditions were optimized with response surface methodology using Box–Behnken design (BBD). And by using this model, the experimental yield was noticeably increased [51].

Following are some of the factors to be monitored to optimize the experimental yield of extracts obtained through enzyme-assisted extraction:

(a) *Enzyme selection*: Different enzymes have different specificities and activities toward different types of compounds. Optimization involves selecting the most suitable enzyme for the target nutraceuticals. For example, cellulases may be used for the extraction of cellulose-based compounds, while proteases can be employed for protein extraction.

(b) *Enzyme concentration*: The concentration of the enzyme used in the extraction process significantly impacts its activity. Optimization involves determining the optimal enzyme concentration by testing various concentrations and measuring the extraction yield. Too low enzyme concentration may result in incomplete extraction, while too high concentration may lead to unnecessary enzyme costs.

(c) *Pretreatment techniques*: Pretreatment of the sample material can enhance the accessibility of target compounds to the enzymes. Optimization involves exploring different pretreatment techniques such as grinding, blanching, freezing, or steam treatment to disrupt the sample matrix and facilitate enzyme penetration and contact with the compounds of interest.

(d) *Enzyme immobilization*: Enzyme immobilization techniques can improve the stability and reusability of enzymes during extraction. Optimization involves selecting the appropriate immobilization method and optimizing the immobilization conditions to enhance enzyme activity and prolong its lifespan.

Enzyme-assisted nutraceutical extraction can be optimized to maximize extraction efficiency, experimental yield, and quality by paying close attention to these parameters and using optimization approaches, leading to improved nutraceutical extraction and improved extraction procedures.

An emerging method that appears to hold promise for more effective exploitation of natural resources is enzyme-assisted extraction. Being a sustainable and environmentally friendly technology, it offers an appealing substitute for traditional extraction methods [49]. Therefore, it has been concluded that EAE is a quick and effective choice for extraction that offers a number of noteworthy advantages that may be stated as follows: moderate circumstances that not only retain the bioactive components of interest but also use less energy and solvent while being more effective than other standard extraction techniques [52].

A comparative analysis of different extraction techniques for nutraceuticals extractions is given in Table 2. This table depicts the overview of techniques along with their advantages and disadvantages.

7 Case Studies

7.1 Extraction of Saffron

The red stigmas of *Crocus sativus* L. are used to make saffron, the most expensive spice in the entire world. Saffron contains roughly 10% moisture, 12% protein, 5% fat, 5% minerals, 5% crude fiber, and 63% carbohydrates, containing starch, reducing sugars, gum, pectin, pentosans, and dextrin (w/w%). Saffron has also been shown to contain traces of thiamin, riboflavin, and fatty acids such as palmitic, linoleic, stearic, oleic, and linolenic acids [54].

The three main bioactives of saffron—crocin, picrocrocin, and safranal—are extracted in an optimized way by using response surface process. The extraction process variables include the temperature (5–85 °C), extraction time (2–7 h), and ethanol concentration (0–100%). The three bioactives (picrocrocin, safranal, and crocin) were detected spectrophotometrically with highest absorbance values at 257, 330, and 440 nm, respectively. The final data were fitted using four models: linear, linear squares, linear interactions, and full quadratic. As anticipated, the whole quadratic model had the highest R^2 values for the picrocrocin, safranal, and crocin concentrations, respectively, at 83.91%, 86.60%, and 92.42%. Our findings showed that short durations, high temperatures, and moderate ethanol concentrations had the greatest influence on the chemical extraction efficiency. An ethanol content of 33.33%, extraction period of 2 h, and extraction temperature of 85.0 °C, respectively, were observed. Under these conditions, the observed empirical values E1 for picrocrocin, safranal, and crocin were 1190.47, 474.02, and 2311.68 , whereas the theoretical values were 1237.27, 652.08, and 2821.23 [55].

7.1.1 Application of Response Surface Methodology

Bee pollen, a product produced by honeybees, is a blend of plant pollen, nectar, and substances secreted by bees. It consists of various bioactive components, including proteins, amino acids,

Table 2
Comparison of different extraction processes [53]

Techniques	Description	Extraction time (min)	Sample size (g)	Solvent vol. (mL)	Cost	Advantages	Disadvantages
Solvent extraction	Solvent is heated in conventional oven and passed by sample	6–8	1–20	10–100	Moderate	Rapid and easy handling	High solvent consumption, thermal degradation, long treatment time
Microwave-assisted extraction	Immersion of the sample in solvent and microwave energy is submitted	3–30	1–10	10–40	Moderate	Rapid, easy to handle moderate solvent consumption	Extraction solvent must absorb microwave energy Filtration step required
Supercritical fluid extraction	A high-pressure vessel is filled with sample and crossed continuously by the supercritical fluid	10–60	1–5	30–60	High	Rapid, low solvent consumption, concentration of extracts, no filtration necessary, possible high selectivity	Many parameters to optimize
Ultrasound-assisted extraction	Immersion of the sample in solvent and submission to ultrasound using a US probe or US bath	10–60	1–30	50–200	Low	Easy to use	Large amount of solvent consumption, filtration step required
Pulsed electric field extraction	Pulses of high electric voltages are applied to the sample placed in between two electrodes	5–10	10–50	10–100	High	Rapid and non-thermal process	Mechanism not well known and process intensification is difficult
Pressurized solvent extraction	Heat of the sample by a conventional oven and crossed by the extraction solvent under pressure	10–20	1–30	15–60	High	Rapid, no filtration necessary, low solvent consumption	Possible degradation of thermolabile analytes
High hydrostatic pressure extraction	Sample is pressurized (100–1000 MPa) through a pressure transmitter liquid	1–30	10–20	10–50	High	Rapid, green technology, high selectivity, high extraction yield, no degradation of target molecules	High cost equipment

enzymes, coenzymes, carbohydrates, lipids, fatty acids, phenolic compounds, essential vitamins, and essential minerals. On average, bee pollen contains 22.7% protein, which includes vital amino acids like tryptophan, phenylalanine, methionine, leucine, lysine, threonine, histidine, isoleucine, and valine [56]. Moreover, it contains a significant amount of beneficial phytochemicals, such as rutin, resveratrol, quercetin, protocatechuic acid, phlorizin, p-coumaric acid, myricetin, luteolin, kaempferol, isorhamnetin, gallic acid, ethyl gallate, chlorogenic acid, catechin, caffeic acid, 2,5-dihydroxybenzoic acid, trans ferulic acid, and salicylic acid [57]. Additionally, bee pollen is a source of organic acids like oxalic acid, tartaric acid, malic acid, citric acid, succinic acid, acetic acid, lactic acid, and gluconic acid. Due to its rich nutritional profile, bee pollen has been consumed as a functional food or food supplement for centuries and is utilized in various products. With the growing demand for nutritious foods, it is important to assess the quality and safety of bee pollen to ensure consumer well-being.

7.1.2 Extraction Parameters

Due to their many benefits, green extraction techniques and solvents have recently attracted growing interest. This study looked at how different bioactive compounds may be extracted from bee pollen by using ultrasonic methodology and polar aprotic solvents (DESs). In this regard, response surface methodology was used to examine the overall effects of process variables on individual amino acids, organic acids, and phenolic compounds. These variables include molar ratio of the DES (1, 1.5, and 2), sonication duration (15, 30, and 45 min), and ultrasonic power (90, 135, and 180 W) (RSM). A molar ratio of 2, a sonication duration of 45 minutes, and an ultrasonic power of 180 were discovered to be the ideal parameters. The control group was composed of extracts produced by the maceration process using ethanol as a solvent. The total individual amino acid and total individual organic acid levels utilizing DESs were greater than those in the control group. Additionally, when utilizing DESs as opposed to controls, substances like myricetin, kaempferol, and quercetin were extracted at higher amounts. Antimicrobial activity testing revealed that the DES groups had a wide range of antibacterial activities against each and every one of the bacterial species tested. However, this inhibitory effect was incredibly weak in yeast-like fungi samples. The study for the extraction of saffron is the first to assess how DESs affect bee pollen's ability to extract beneficial compounds. The outcomes demonstrate the applicability of this novel and environmentally friendly extraction method and solvent (ultrasonic extraction/DES) [58].

7.2 Extraction from *Fruit of* Ziziphus lotus

Ziziphus lotus are pulpy fruits that are valued for their particular flavor, nutritional value, and therapeutic uses and are consumed as food all over the world. The abundance of bioactive chemicals in this fruit is thought to be responsible for its useful characteristics. Unfortunately, despite ideal extraction conditions, the extraction of these chemicals and their underlying phytochemical characterization has been rarely studied. In this study, *Z. lotus* fruit pulp extracts were obtained employing heat-assisted extraction method and response methodology. These extracts obtained were rich in beneficial biocompounds in terms of their compositional and nutraceutical potential [59].

7.2.1 Extraction Parameters

The optimal conditions were noted to be as follows: time, 71 min; temperature, about 50 °C; solid to solvent ratio, 1:60 (g/mL); and ethanol concentration, 50%. This gave 48.62% output, including 106.64 milligrams of Gallic acid equiv [31]/gram dry matter of reducing ability with the Folin-Ciocalteu (FCR) reagent, and 49.65 mg of quercetin equiv (QE)/g DM of total flavonoid (TAA). A total of 38 substances were discovered utilizing LC-ESI-MS/MS analysis using these parameters. Results also revealed that the optimized pulp extracts from *Z. lotus* fruit exhibited good antibacterial activity. The pulp can be utilized to extract bioactive chemicals that can be employed as ingredients in functional foods and nutraceuticals, and this study offers crucial information on their potential application [60].

7.3 Extraction from Rubus ellipticus

The *Rubus* genus has an extensive record of medicinal use with notable therapeutic effects in treating the liver and kidney meridians. In China, its roots and bark are used to relieve lower back pain, enhance vision, and prevent uterine, cervical, and colon cancer. The genus has a number of species that are employed as antimicrobials, anticonvulsants, muscle relaxants, radical scavengers and used in the treatments of ulcers, gastrointestinal issues, diabetes, and inflammation. Although the potential of nutraceutical and functional food derived from *Rubus ellipticus* fruit is well established, there are no comprehensive research works on the optimization of extraction procedures for increasing yield. Plackett-Burman (PBD) and Central Composite Design were used in the current work to extract bioactive chemicals (CCD).

7.3.1 Extraction Parameters

The extraction of bioactive chemicals was strongly affected by factors including the solvent to sample ratio, concentration of methanol, and extraction temperature under linear, quadratic, and interaction effects (p 0.05 and p 0.001, respectively). The bioactive compound and antioxidant predicted values were found to be close to the experimental value with the lowest coefficient of variation. Additionally, a non-significant lack of fit and a high coefficient of determination (R^2) were identified in the regression analysis.

Under ideal extraction conditions, high-performance liquid chromatography with a photodiode detector (HPLC- PDA) examination found seven bioactive chemicals, with catechin having the highest concentration (27.67 mg/g DW). In contrast to previous studies and past publications on the species, the results demonstrated a 35–99% improvement in yield. The improved procedure can be scaled up further to maximize the species advantages in the manufacture of nutraceuticals and energy supplements on an industrial scale [61].

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Chapter 18

Computational Approach and Its Application in the Nutraceutical Industry

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Abstract

In recent years, application of the computational approach, a predictive tool, in food and nutritional sciences has outweighed classical analytical methods. The sophisticated informatics approaches such as chemo-informatics and bioinformatics methods are integrated with the food industry for the extraction and identification of bioactive compounds. Model simulation of the molecule and the assessment of the dynamics are frequently used to study the structure and function of food carbohydrates, lipids, and other small molecules. Beyond the simulation, current advancement in algorithms has solidified machine learning's ability to predict the availability of the functional component and its biomolecular activity. The chapter summarizes the joint applications of bioinformatics and simulation methods in the extraction and discovery of bioactive and nutraceutical components, in particular, the selection of analytical procedure, activity prediction, docking, and physicochemical properties.

Key words Bioactive compounds, Nutraceuticals, Chemo-informatics, Bioinformatics

1 Introduction

The number of studies on nutraceuticals, cosmeceuticals, and functional foods has grown exponentially. This research shows that nutraceuticals and functional foods provide anti-oxidative, reproductive, renal, gastrointestinal, and many other health benefits. These health benefits are attributed to several bioactive compounds, such as peptides, phytochemicals, essential oils, polysaccharides, and other bioactive metabolites. However, the effectiveness of food that confers health benefits has always been speculated with skeptical remarks. This has resulted from inconsistencies in the evidence of their beneficial effects. The inconsistency with the evidence on its effectiveness could be due to a gap between its clinically controlled and aftermarket effectiveness. In this case, the main constituent of the gap is the variables acting upon the

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biological mode of action, which must be subdued with a computational approach. This approach not only delivers the probable biological confounding factors for the mode of action, but also applies to discovering significant novel bioactive compounds or novel functions of the existing ones [1].

The combined application of bioinformatics and chemoinformatic can amplify the observation of fundamental mechanisms on the mode of action, target dynamics, and possible toxicity of the bioactive compounds. Bioinformatics and cheminformatics, which utilize computer applications, exploit databases to identify and interpret chemical and biological data. Furthermore, the interpretation of chemical and biological data is made more accessible by the development of an artificial intelligence (AI) approach that can efficiently extract information regarding large-scale molecular interactions. In this chapter, we will discuss the use of different food science databases that assist in the chemoinformatic, bioinformatics, and artificial intelligence processes. Additionally, we will elaborate on the application of chemoinformatic, bioinformatics, and AI in food science and nutraceutical research.

2 Database in Food Science

Chemical space is a framework for studying the influence of structure on the biological behavior of a compound. Different types of molecular and visual representations of chemical spaces aid in exploring the structure-property and structure-activity relationships (SPR and SAR) and linking the character to the specific spaces, which can help predict the intersection of specific chemical and biological spaces [2]. The framework places molecules in the mathematical dimension based on their physicochemical and chemoinformatic descriptors. Descriptors are the numerical linear notation that represents the space. Maximizing the descriptor representing biological activity can build a qualitative/quantitative structure-activity relationship (QSAR) matrix and enable complex multivariate analysis, which is the basis for the computational approach [3]. The chemical space's reliability is mostly dependent on the qualitative and quantitative aspects of the descriptors used to define the mathematical dimension. Chemical space has been reported to contain at least 10^6 organic molecules below 500 Da. These molecules could be explored by constructing a chemical space as a database and then navigating the database using molecular descriptors [4].

Databases are fundamental to discovering new bioactive compounds and pursuing their mode of action, relevancy of therapeutic claims, and other impact variables. The in silico approach requires data set representing structure, qualitative information, activity, physicochemical properties, functional molecular fragment,

toxicity, and other physiological interaction to cast out the algorithms [5]. The major benefit of the open-source data/program remains to include researchers from a wide economic background, which is crucial in fueling the progression of open science. The other advantages can be noted for its ability to harbor data from all spectrums and act as a source of data to cross evaluate and aid in strengthening the finding's validity and reliability [6]. The virtual chemical database has been advanced to harboring millions of compounds which has significantly accelerated the pursuit of novel organic molecules for the betterment of health [7]. The natural bioactive compounds, which are the product of plants, animals, and microorganisms' resilience towards harsh ambiance and other hostile elements, have maximum biologically relevant scaffolds [8]. Hence, they have bioactivity against the diverse target, and chemical space is massive to accommodate all the diverse biologically relevant information—as opposed to synthetic compounds [9].

Modern medicine constantly pursues novel bioactive compounds from natural sources, be it plant, animal, or microorganisms. The exploration benefits from the advancement in silico capability, providing direct estimation and additional support to the in vitro assay. The virtual databases contain structural information of the compound, which is the basis for the application of cheminformatics. The chemical space of the nature-based compound comprises massive information on its bioactivity [10]. The data library gives an edge to discover the unexplored action of the compound or open the door of modifying the compound by stereoselective transformation or adhesion of a new functional group [11].

The computational approach (docking, QSAR modeling, and pharmacophore-based modeling) eases the identification of comparatively high bioactive compounds and prediction of an efficient process for extraction, purification, and estimate ADME (absorption, distribution, metabolism, excretion) along with toxicological properties [12]. In addition, computational analysis's complexity is stacked upon the nature-based bioactive compound's complex chirality [10]. The undefined chiral centers require vigorous screening of all the probable stereochemical configurations from the plethora of complex configurations. Thus, to render reliable prediction outcomes, the computational approach must recruit datasets from the different databases (Tables 1 and 2) with precise structures with well-defined stereochemistry [12].

Generated Databases (GDB) is the largest database featuring 166.4 billion molecules, including both bioactive (aromatic and planar) and non-aromatic 3D-shaped molecules, which are significant for drug discovery. Unlike other databases-which focus on the combination of the building blocks in novel drugs-like compound discovery, it prioritizes by lensing into the chemical diversity and

Table 1
Database for chemicals

Database	Availability	No. of compound	Link	Reference
Generated databases-17	Free	166.4 billion	https://gdb.unibe.ch/downloads/	[13]
Zinc 20	Free	1.4 billion	https://zinc20.docking.org/	[14]
PubChem	Free	112 million	https://pubchem.ncbi.nlm.nih.gov/	[15]
ChemIDplus	Free	4,00,000	https://chem.nlm.nih.gov/chemidplus/	[16]

Table 2
Database for bioactive compound

Database	Specification	No. of molecules	Link	Reference
Carotenoids database	Carotenoid structure, structural isomers and stereoisomers, chemical fingerprint	1204	http://carotenoiddb.jp/	[20]
ChEMBL	Bioactive compounds with drug-like properties	2.3 million	https://www.ebi.ac.uk/chembl/	[21]
PADFrag	Biological-functional molecular fragments	5919	http://chemyang.ccnu.edu.cn/ccb/database/PADFrag/	[22]
FooDB	Physicochemical data, sensory information (color, flavor, aroma), its concentration effects on other attributes of food, physiological effect, and impacts on human health	28,000	https://foodb.ca/	[23]
Ambinter	Structure, biological target	38 million	https://www.ambinter.com/	[24]
BIOFACQUIM	Compounds from the plants, fungi, and propolis of Mexican origin	400	https://biofacquim.herokuapp.com/	[25]
NPASS	Connects natural products with the biological targets	35,032	https://bidd.group/NPASS/	[5]
BioPhytMol	Anti-mycobacterial phytochemical information	2582	https://ab-openlab.csir.res.in/biophytmol/	[26]
BIOPEP-UWM	Bioactive peptides, quantitative parameters of bioactive fragments, SMILES code	4540	https://biochemia.uwm.edu.pl/biopep-uwm/	[27]
AHTPDB	Antihypertensive peptides	6000	https://webs.iiitd.edu.in/raghava/ahtpdb/	[28]

deep into the first principle of organic chemistry. GDB has subsets and focuses on the specific spectrum of the compound, such as *FDB17b* on the fragment, *GDBMedChem* on medicinal chemistry [17], *GDBChEMBL* on ChEMBL-like molecule [18], and *GDB4c* on novel 3D-shaped molecules with quaternary centers [13].

ZINC 20 is a free database hosted by Irwin and Shoichet laboratories of the University of California, which harbors information (docking, 2D, and 3D models) of more than 509 million purchasable compounds and analogs of over 750 million compounds with a constant update of billions of new molecules [14].

PubChem is the largest chemical database comprising information such as 3D structures, biological assay descriptions, and results of more than 112 million compounds, 298 million substances, and 301 million bioactivities. Compared to other databases, it is more user-friendly due to its simple and alternative views via dedicated web pages and presents data related to genes and diseases associated with the pathway, biological activity, protein, and patent [15].

ChemIDplus is a free web-based search system with more than 4,00,000 chemical records containing over 3,00,000 chemical structures. This chemical record aids in the identification of the compounds by providing structure and nomenclature authority files from the National Library of Medicine (NLM), US states, and its federal agencies, along with other scientific sites. ChemIDplus have two versions, ChemIDplus Lite and ChemIDplus Advanced, on which the latter has additional information regarding molecular formula, classification code, chemical structure, physical property, toxicity, and locator code searching [16]. It has been scheduled to move to PubChem in December 2022 [19].

Carotenoids Database is a repository for 1204 natural carotenoids from 722 sources of organisms. It contains data regarding the classification of carotenoid structure, structural isomers, and stereoisomers, chemical fingerprint which describes the carotenoids' chemical substructure, and the details regarding modification and eases prediction of the biological function of provitamin A, membrane stabilizers, allelochemicals, odorous substances, antiproliferative activity against the cancerous cell, and reverses multidrug resistance (MDR) activity against cancer cell [20].

ChEMBL is a database that harbors information curated from the primary medicinal chemistry research literature and multiple other sources. In addition, it contains information regarding the compound's bioactivity, molecules, target, and other drug data. Its web services are built on a RESTful architecture which allows users to access data programmatically and is also available via other sources, such as PubChem BioAssay and BindingDB [21].

PADFrag database, which is for a bioactive molecular fragment, holds 1652 FDA-approved drugs, 1259 agricultural chemicals, and 5919 molecules generated from the mentioned drugs and chemicals. This database consists of physicochemical properties, 3D

structures, and target information of the specific molecules which has two versions, drug2fragment mode and fragment 2drug mode. The first mode contains information about the FDA-approved drug/pesticide and all the fragments. In contrast, the latter mode contains information about the fragments from FDA drugs/pesticides with their respective FDA-approved drugs/pesticides [22].

FooDB is a class of database comprising more than 28,000 compounds in over 1000 unprocessed food's elaborative information about the biochemical, compositional, and physiological makeup. It is classified into Food Browse and Compound Browse; in the first classification, foods are classified as per their chemical composition, whereas chemicals are grouped as per their food source in the latter. This includes data regarding the compound's nomenclature, structural data, physicochemical data, sensory information (color, flavor, aroma), its concentration effects on other food attributes, physiological effects, and impacts on the human health database [23].

Ambinter comprises over 38 million compounds' information for QSAR, docking, and virtual screening. It is a paid database managed by Greenpharma, which assists in custom synthesis, chemoinformatic, and molecular modeling of the compound [24].

BIOFAQUIM contains data on more than 400 compounds from plants, fungi, and propolis of Mexican origin. The species are selected by taking into account their geographical location and the information is curated accordingly [25].

NPASS is a database that provides 446,552 quantitative activity records, 222,092 target pairs and 288,002 species pairs, 35,032 natural products with 5863 targets (2946 proteins, 1352 microbial species, and 1227 cell lines) from 25,041 species [5].

BioPhytMol database contains more than 2582 Phyto molecules from 188 plant families, which are primarily directed towards the anti-mycobacterial against 25 target mycobacteria. It is experimentally verified and curated with experimental assay preparation, including details of the compound's bioactivity, and assists in generating analogous compounds for target-specific inhibitors [26].

BIOPEP-UWM is a free database comprising proteins, biologically active peptides derived from food, sensory peptides, amino acids, and allergenic proteins. The data are also crowd-sourced from the user. The users can submit new peptide sequences in the database, and the curators verify and upload them for the public. It currently holds 739 proteins, 4540 bioactive peptides, 136 allergenic proteins with epitopes, and 533 sensory peptides and amino acids [27].

AHTPDB is a manually curated database from several research articles and peptide repositories of antihypertensive peptides (AHTPs) that have been experimentally validated. The database comprises 1700 unique peptide sequences with 6000 sequences originating from 35 types of plants (soybean) and animal sources

(pork, fish, chicken, milk, egg, etc). Besides these, the database withholds information regarding structure (tertiary and secondary), inhibitory concentration (IC50), toxicity, bitterness value, source, purification method, and log value of inhibitory concentration (pIC50) information [28].

A number of deserted databases are not maintained anymore or might be maintained poorly. In some cases, the wrong interpretation might occur due to the lack of a definite standard for stereochemistry and aromaticity, leading to new versions of the same molecules. The current misrepresentation of "Publish or perish" has adversely flourished among researchers, guild-leading to the spawn of databases that have a dire chance of getting maintained after a certain time of publication.

3 Biomedical Informatics

3.1 Chemoinformatic

Chemoinformatic is an in silico technology employed in research relating to chemistry. It adopts an integrated approach to study and understand the function of chemical systems using available ligand resources such as pharmacophore modeling, quantitative structure-activity relationship (QSAR), docking, and molecular dynamics (MD) simulations. Cheminformatics has found several significant uses in the pharmaceutical industry, including agricultural, environmental, and food chemistries [29].

3.1.1 Quantitative Structure-Activity Relationship (QSAR)

QSAR is a chemoinformatic technique that links the relationship between the chemical structure and biological activity of the compounds. It plays a role in designing and screening new molecules and predicting the activity and mechanism of biologically active compounds. Compared to conventional techniques, QSAR screening is more feasible and convenient for identifying various chemical structures [30]. Moreover, it contributes to quantifying the stability, potency, and toxicity of known bioactive compounds [30].

3.1.2 Application of QSAR

The application of the QSAR technique involves the development of a QSAR model, where a set of numerical descriptors related to the structure of interest serves as independent variables, while the targeted biological activities are the dependent variables. Then, the relationship between the dependent and independent variable is built using multiple linear regression (MLR), partial least square (PLS) regression, support vector machine (SVC), artificial neural network (ANN), etc. [31, 32]. For instance, there are many amino acid descriptors, such as hydrophobicity, bulkiness/molecular size and electronic property of amino acids, isotropic surface area, and electronic charge index [32, 33]. However, the properties of amino acids described by a single parameter are more complicated as they have less explanatory power and neglect the relationship between

different descriptors [34]. Therefore, studies use the integration of different amino acid descriptors to predict different properties of the target peptides [35]. To date, many QSAR studies have investigated ACE inhibitory or antimicrobial peptides (AMPs), antioxidant, antimicrobial, renin, and DPP-IV inhibition, as well as the organoleptic properties of the peptides [36].

3.1.3 Antioxidants

Antioxidant peptides play a significant role in preventing and treating various diseases due to their capacity to scavenge free radicals and prevent oxidative stress [37]. It is essential to determine the dynamics between human physiology and bioactivity of antioxidants. QSAR has the potential to explore the dynamics and forecast the interaction between food-derived antioxidant and physiology. Specifically, QSAR study is focused on dipeptides and tripeptides as they are absorbed intact from the intestinal lumen to produce biological effects at the tissue level [38]. Dent et al. 2019 conducted a QSAR study on two datasets of antioxidant tripeptides where the first dataset contained 214 artificially designed tripeptides, and the second dataset contained 72 Beta-lactoglobulin tripeptides. The study used 16 amino acid descriptors to conduct model population analysis (MPA), which improves prediction ability and interpretability by forming multi-model clusters [39] with higher cross validated coefficient of determination.

3.1.4 ACE-Inhibitory Peptides

Several studies have been conducted on identifying and isolating several ACE-inhibitory peptides derived from food. Application of QSAR modeling has identified the novel ACE inhibitory peptides derived from Qula casein hydrolysates using a two-enzyme combination. The QSAR model involved amino acid descriptors as predictors and log transformed IC50 values as the dependent variable. Amino acid descriptors include five z-scale descriptors where z1 represented lipophilic properties, z2 represented steric properties, z3 represents electronic properties, and z4 and z5 were related to other properties like electronegativity, the heat of formation, and electrophilicity. Finally, the QSAR model was analyzed by SIMCA-P software (Umetrics, Umeå, Sweden) using partial least squares regression (PLS). The study also concluded that the peptides produced by utilizing thermolysin + alcalase exhibited stronger ACE inhibitory function [40].

3.1.5 Phytochemical Peptides

Phytochemical peptides can be defined as a chemical peptide produced by plants that may provide favorable health benefits to reduce the risk of major chronic diseases. Urease inhibitor is one of the phytochemical peptides studied for its ability to treat the Helicobacter pylori infection. Chopdar et al., developed a QSAR model using CORAL SEA 17 software to predict the urease inhibitors. The model utilized SMILES and GRAPH descriptors as the

independent variable to predict pIC50 from the NPACT database. The utilization of both SMILES and molecular graphs descriptors is known to result in a hybrid descriptor developing a QSAR model with better statistical quality [40].

3.1.6 Molecular Docking

The molecular docking process includes predicting the molecular orientation of a ligand within a receptor and calculating their complementarity interaction (binding affinity) using a scoring function. Molecular docking allows for studying the behavior of peptides in the binding site of target proteins. It is also called a structure-based method that allows it to determine the structure-activity relationship of peptides. For example, once the peptides have been sequenced and their bioactivity has been determined through in vitro and in vivo assays, they undergo structural preparation for docking. Next, the receptor-ligand complex structures are prepared using docking simulation software. The final analysis is done to predict the binding modes and affinities of a ligand, i.e., bioactive peptides [41].

3.1.7 Application of Molecular Docking

Molecular Interactions

Studying food enzyme interaction and toxins is essential in food processing and producing novel foods. Studies have utilized molecular docking to study the binding mechanism between a commonly consumed protein, ovalbumin (OVA), and a malachite (MG-dye). They found that there are hydrophobic and van der Waals interactions between ovalbumin and a food additive, indicating that the mixture of these two components may lead to the formation of a toxic compound (OVA-MG) [42]. Another study showed the health-promoting properties of broccoli through the interaction of an enzyme myrosinase with glucosinolates to produce isothiocyanates at a neutral pH. The study also identified competitive inhibitors (amygdaline and arbutin) of an enzyme myrosinase that prevents the formation of undesirable compounds at low pH [43]. Besides, the effect of different nutrients and enzymes on trypsin and alpha-amylase inhibitors was determined based on the molecular docking technique [44].

Angiotensin-I-Converting Enzyme (ACE) Inhibitory Peptides

A molecular docking technique has been utilized to determine the molecular mechanism of novel ACE-inhibitory peptides generated from *Larimichthys crocea* titin. Firstly, titin protein was hydrolyzed using the ExPASy PeptideCutter program to identify peptides. This program predicts potential cleavage sites in titin protein sequences. The generated peptides were then compared with known ACE-inhibitory peptides of the BIOPEP-UWM database and the antihypertensive inhibiting peptide database (AHTPDB). Furthermore, molecular docking was done to identify the potential

molecular mechanism of the identified tripeptides (WAR and WQR) using the SwissDock webserver. SwissDock webserver predicted their binding site and confirmed their interaction with the ACE active sites [45].

Molecular docking methods have some limitations, such as using mathematical scoring functions, which might not correlate with actual experimental binding affinities, and the limited sampling of both ligand and receptor conformations [46]. Molecular dynamic simulation is another in silico technique that calculates the motion and equilibrium of each molecule and provides protein-ligand information at excellent temporal resolution. MDS can be applied to limit the number of possible ligands obtained with molecular docking and filter the false-positive molecule that shows high affinity during the complex formation [47]. In contrast to molecular docking, MDS can precisely control the experiment's physical conditions, from the temperature and ions around the peptides to the properties of the solvent [41].

3.2 Bioinformatics

Bioinformatics is a broad scientific domain mainly concerned with applying computational resources to biological data. Due to its low cost and high throughput, bioinformatics has been used in the food industry to improve the quality and functionality of food sources. For example, bioinformatics technologies provide information on the conformation of bioactive peptides (BAPs), predict potential activities, illustrate molecular interaction mechanisms, and improve peptide properties, which helps study BAPs for human medicinal use [48].

3.2.1 Allergens

The use of bioinformatics ranges from developing novel nutraceuticals to detecting the toxic compounds in food, including allergens. Regarding the detection of allergens, bioinformatics facilitates the efficient detection of allergens from the massive amount of data, predicting information about the allergen, and can validate the traditional strategies by guaranteeing the reliability of the conclusions. The bioinformatics approach can determine cross-reactive allergens by determining the degree of homology of different allergens within the epitome to maintain immunoglobulin E (IgE) binding [49]. Bioinformatics was utilized in identifying latent allergic proteins from chickpeas by collecting known allergen sequences in the Fabaceae family from the database WHO/IUIS (http://allergen.org) and Basic Local Alignment Search Tool (BLAST). The study found that out of seven potential allergens from chickpeas, four had cross-reactivity with the allergens in the databases [50]. Another study utilized the proteomics and METLIN database to identify hazardous allergens (glutaredoxin and oleosin-B2) in bee pollen. Bee pollen is utilized for its

nutritional properties. Therefore, the presence of hazardous allergens within bee pollen indicates the need for developing the process to remove those allergens [51].

3.2.2 Bioactive Peptides

In silico approach, prior to wet laboratory analysis, hydrolysis of proteins allows focusing on a small number of peptides for predicting the types and potency of peptides with the selected combination of protein and enzyme(s) [52]. Bioinformatic approaches narrow down the number of enzyme combinations for protein hydrolysis and predict bioactivity or interaction with specific molecules and receptors by homology-based searches [53]. The co-ordinate of the cleavage point in the sequence could be based on the specialized databases such as UniProt Knowledgebase [52]. Furthermore, with the large set of databases, it is feasible to characterize peptides for their theoretical physicochemical, specific bioactivity, and sensory properties [54], and reject peptides with undesirable properties.

For example, Panjaitan, Gomez [55] did a study on optimization of enzyme combination using in silico in order to render tryptic peptides and determined their bioactive property. The resultant peptides sequence was identified using mass spectrometry and a sequence similarity search was done with BLAST, which revealed that the aligned with tryptic peptide sequences from *Epinephelus coioides* sharing 70% identity to the protein sequences from *Epinephelus lanceolatus* (database). Further, the BIOPEP analysis revealed that the pepsin and mixed proteases (pepsin, trypsin, and chymotrypsin A; pepsin, trypsin, and chymotrypsin C) exhibited better production of ACEI peptides compared to other proteases. In this way, the computational method is convenient for biotechnologists as it can efficiently identify peptide types and determine appropriate protease combinations that can theoretically produce optimal bioactive peptides.

3.2.3 By-products

The utilization of food by-products is another growing field in the food industry, which is blooming due to the development of bioinformatics tools. The combined application of in silico and ex vivo has demonstrated the potential bioactivity (ACE inhibition, renin inhibition, ACE inhibition,) of the peptides from by-products like pigeon peas waste [56]. Senadheera et al. did a study on sea cucumber where they utilized in silico techniques for generating antioxidant and ACEI peptides by processing the by-products of sea cucumber. The study utilized mass spectrometry to identified peptides and was virtually screened by the PepRank tool. Then, in silico, proteolysis was simulated with digestive enzymes using the BIOPEP-UWMTM database tool. After simulated digestion, ToxinPred software evaluated the peptides for toxicity and found that peptides resistant to the in silico digestion were non-toxic [57].

4 Artificial Intelligence (AI) Approaches in Bioactive Extraction and Nutraceuticals

The term "AI" refers to a computer program in which a machine is programmed to think and behave as a human. Big data from different databases is extracted, patterns are developed with the help of different algorithms, which enables AI to do as humans do. In an AI-based computational approach, machines can predict using various algorithms and AI models. Prior to diving into AI, it's important to understand machine learning (ML). ML is a subset of AI that leverages data to generate knowledge for AI systems on its own. For ML, raw data are encoded into the machine along with numerous algorithms, such as SVM, regression, decision trees, and so on, which support machine learning without explicit programming. However, in AI approach, the big data sets are extracted and utilized to form patterns for AI systems with the help of neural networks (the heart of deep learning algorithms). With a neural network, machines could categorize and arrange coded data in a way that resembled the functioning of the human brain [58].

The application of computational approaches in the field of applied life-science research, such as protein sequence prediction, target identification, molecular dynamic simulation, modeling, and redesign, dates back a long time. However, as AI, ML, and deep learning have advanced, there has been a significant increase in the accuracy and precision of their results when compared to the traditional approach. AI-based molecular and/or deep docking, for example, has enabled structural-based virtual screening of large molecular libraries, understanding molecular interaction, and molecular design with unprecedent accuracy [59, 60]. In the food and nutraceutical industries, they have adopted AI-guided models for modeling for optimal extraction, qualitative and quantitative prediction, process simulation, and release modeling of bioactive and functional compounds. Integrated systems, such as the adaptive-neurofuzzy inference system (ANFIS), stepwise linear regression (SWLR), and multilayer perceptron (MLP) models that consist of neural networks, are employed to predict the outputs (nutrients, bioactive molecules, and ligands). In respect of accuracy and efficiency, the application of these AI-based models for the prediction of bioactive compounds is superior to that of a traditional predictive approach (a regression model) [61].

4.1 Machine Learning (ML)

ML is data-driven and performs on the basis of various numerical and statistical models, based on which predictive models can be developed in in silico applications for extraction scale-up, optimized processing, and understanding the physiological mechanisms of the bioactive compounds. Previously standardized statistical techniques for determining factors such as solute-to-solvent ratio, physical properties, and structural complexity in the

chemical composition are still in practice in the food and nutraceutical industries. These traditional statistical tools are tedious and of low efficiency in the age of automation, so industries are attracted towards machine learning and other computational approaches to produce more efficient and rigorous results. Particularly, the accuracy of applying machine learning and conventional statistical techniques in classifying samples (fermented and unfermented rooibos) based on the phenolic content and antioxidant activities revealed that ML yields data results nearly identical to those of conventional statistical analytical techniques [62]. When ML is applied for bioactive extraction, the selection of the right machine learning algorithm is crucial and depends on several different criteria.

Different sophisticated analytical machines are guided by a number of machine learning algorithms since decades. Broadly, ML algorithms are categorized into four different types: supervised, semi-supervised, reinforcement, and unsupervised. In the case of supervised methods, datasets are labeled prior employing new data sets to analyze. The most common supervised algorithms in food science applications are the naive bayes classifier algorithm, SVM, regression (linear and logistic), and random forest. These algorithms can be employed to analyze data that is predictable, recurring, or that falls within the range of the training data sets [63]. For instance, to predict the relationship between the input parameters (ethanol concentration, solvent/solid ratio, and temperature) and the output response (total flavonoids and extraction yield) for optimal extraction of flavonoids from celery seed using response surface methodology governed by different algorithms, a supervised algorithm (a multiple linear regression algorithm) was the best model for predictive optimization [64]. Contrarily, the unsupervised approach groups the data into clusters based on its characteristics and then uses dimensionality reduction to determine the most dominantly featured data. The large, unclassified data sets are more appropriate to analyze using unsupervised methods. For example, the demonstration of the extracting reaction kinetics and degradation mechanisms of the phenolic resin under different conditions and the reduction in the variability of the molecular dynamic simulation were extracted using unsupervised ML. When the data was subjected to unsupervised ML techniques such as non-negative matrix factorization (NMF), principal component analysis (PCA), and non-negative tensor factorization (NTF), the dimensionality of the data was reduced in order to determine dominantly featured data [65].

4.1.1 ML Supported Virtual Screening (VS)

In the development of drugs or bioactive compounds, the most time-consuming steps are identification of the most potent compound, parameter optimization for extraction, appropriate delivery, and validation of the effectiveness of those bioactive compounds in a clinical setting. Virtual screening has recently been discovered to

be the best alternative to traditional screening. VS is an in silico approach used in bioactive compounds identification, toxicity evaluation, and understanding pharmacokinetics. Virtual screening employs the use of a database to evaluate how closely an object and a specific molecule resemble one another to discover novel, physiologically active compounds with high efficiency and high throughput. Virtual screening in protein-based nutraceutical industries could be applied to screen the 3D structure of the target protein and bioactive peptide via docking, identification of novel bioactive peptides, and interactions of peptides and ligands [66]. When the bioactive peptide and bioactivity were screened using VS, it was discovered that the major flavonoids were 3,4-di-OMe luteolin and acacetin, while salicylic acid and melilotic acid were the key phenolic acids contributing to the observed antidiabetic effect. QSAR, used to identify the critical functional groups required for protein-phenolic molecular interactions, validates the role of pearl millet phenolics in inhibiting carbolytic enzymes and regulating GLUT [67].

Furthermore, structural-based virtual screening (SBVS) predicts the best interaction mode between two molecules to form a stable complex. It uses scoring functions to estimate the force of non-covalent interactions between a ligand and molecular target [68]. Researchers have employed machine learning approach in scoring functions to improve SBVS algorithms. To find a new bioactive peptide, numerical descriptive vectors (NDVs) for peptide sequences were developed. These techniques use quantitative structure-activity relationship (QSAR) analysis to predict angiotensin-converting enzyme (ACE) inhibitor dipeptides, bitter-tasting dipeptides, and nonameric binding peptides of the human leukocyte antigens [69]. Lastly, the effectiveness of the VS can be justified by the research outcomes of Wang, Niu [70], where VS of a food-derived antihypertensive peptide using a ML strategy with the eXtreme Gradient Boosting (XGBoost) algorithm predicts antihypertensive peptide with high efficiency and throughput.

4.2 Artificial Neural Network (ANNs) and Deep Learning

Deep learning is a subset of machine learning that depends on a common algorithm with the important function of automatic information extraction from raw data. Deep learning is a data-hungry algorithm, and supervised learning datasets require a large amount of labeled data. The use of more complex network architectures in DL expands on the use of deep artificial neural networks (ANN) in ML. It utilizes the deep ANN made up of several layers of nonlinear modules to enhance multilayer representation [71]. Deep learning algorithms perform with accuracy and precision in ADMET property prediction, target prediction, virtual screening, and chemical synthesis. Deep learning has been used for high-performance test screening (HTS), quantitative structural analysis (QSR), and other purposes. In recent years, de novo

molecule synthesis, which uses sequence data to generate molecules with desired attributes, has indeed made significant use of deep learning. Therefore, deep learning is useful for comprehending the variety and chemistry of natural components in the nutraceuticals and bioactive industries [63]. In analytical chemistry, ANN-based modeling is more accurate compared to RSM for extraction optimization because of excellent representation of the nonlinear, including quadratic equations, relationships. This can be observed from the study conducted by [72], who utilized the back propagation (BP) algorithm and a feed-forward multilayer perceptron (MLP) type architecture of an ANN model to construct the predictive mathematical model with the conditions of the four parameters (water content, time, temperature, and solid-liquid ratio) and alkaloids' yields as outputs. The ANN model provided more precise predictions because it had a lower MSE value and a higher R^2 value.

The utilization and interaction of numerous fully connected or convolutional hidden layers defines platforms, like deep neural networks (DNNs) and convolutional deep neural networks (CNNs), which have shown potential application in the nutraceutical industry. DNNs are artificial neural networks that include numerous hidden layers and the ability to simulate complex non-linear interactions. DNNs can still perform even in the absence of a large training data set. In biological and pharmaceutical chemistry, neural networks are recently being used to predict ligand binding sites and optimize molecular properties and structure generation. Ligand-binding activity from QSAR modeling, predicting binding sites, and ADMET properties of small molecules can be obtained accurately using DNN [73]. This indicates the possible application in nutraceutical and bioactive compounds too. For example, prediction of the flavonoid level within the leaves of the velvet apple using the DNN algorithm was carried out by Qasthari and Saputro [74]. Velvet apple leaf hyperspectral images were taken between 400 and 1000 nm wavelengths. Laboratory data on flavonoids was labeled in the cropped leaf image, where flavonoids compound was prediction with an R^2 performance of 70.47% using an unoptimized and shallow DL model. Moreover, deep neural networks have been used to classify Caco-2 binary penetrability. For instance, Shin, Jang [75] used 663 chemical substances to construct an in vitro Caco-2-specific data model, where the overfitting and nonlinear activation issues were resolved via dropout regularization. The results show that high-level DNN features outperform handwrought features in predicting structurally varied chemical compound cell permeability in Caco-2 cellular lines. CNN, on the other hand, is being applied to improve the secondary metabolites of plant. Non-invasive hyperspectral imaging can identify changes in secondary metabolism, and relevant wavelengths of imaging can be reduced by integrating deep learning [76]. Those secondary plant metabolites are connected to spectral information that is

significant for the classification of healthy food sources. The development of a deep learning-based regression approach can be applied to determine the chemical compositions of the fruits. Zhang 2020 designed convolutional neural networks (CNN) to predict the chemical composition in dry black goji berries and determined it using hyperspectral imaging. In terms of modeling and feature extraction, a deep learning-based regression model like partial least squares and least-squares support vector machines for modeling and principal component analysis and the wavelength transform for feature determination were used, respectively [77].

Lastly, integrated applications of the AI, DL, and ML can aid in molecular recognition, identification of bioactivity of phytochemicals, proteins, and peptides, molecular docking, and interaction between bioactive compounds and other molecules [78–80], which can enhance efficiency in the food and nutraceuticals industries. It also improves functional activity by accurately predicting active components and target interaction, target validation, and the active site of the target. Application of AI, ML, and DL assists in production maximization of bioactive and nutraceuticals by providing insight into important factors that influence the multifaceted process of extraction and processing [80, 81]. Beside these, ML and DL help in the prediction of the toxicity of different phytochemicals and peptides with high precision using either deep neural networks, ANNs, or other algorithms to establish a QSAR model [68, 82]. ML, DL, and AI are now applied in the signal processing and analysis of nano- and macromolecules for automatic structural verification and prediction using high-resolution analytical equipment like NMR [83].

5 Future Perspectives

In the last decade, application of the computational approach has led to robust food and nutrition research. This advancement can be observed in the current application of specialized "in silico" groups supporting the hit and lead identification processes concurrently in large nutraceutical companies. They are being used to predict novel bioactives such as peptides, as well as discovery procedures in conjunction with a wide range of experimental techniques to validate properties such as bioactivity evaluation, toxicity, and so on. The foundation of a computational approach includes virtual screening, the design of the screening collection, data extraction and analysis from databases with different algorithms, and data profiling. In the bioactive compound and nutraceutical industries, AI approaches such as ML and DL are being significantly utilized. Several computational researchers concluded that AI performed better than conventional statistical techniques. Regardless of a number of limitations and obstacles in AI, the technology will

soon revolutionize research and innovations in the fields of medical and food science. AI and data science may enable the development of the "virtual human" in the near future. If this is the case, bioactive and phytochemical clinical trials will be more extensive and effective. In a relatively short period of time, the wide variety of compounds can be evaluated for potential bioactivity under optimum conditions with increased efficiency, omitting the possible toxicity. Thus, the use of various computational approaches aids in the prediction and elucidation of molecule interactions, structure-property relationships, potential bioactivity, and many other phenomena.

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