**EXTRACTION METHODS OF BIOACTIVE COMPOUNDS FROM THE PLANTS**

Sudipto Debnath\*1, Deepak Kumar1, Manosi Das1, Gajji Babu1

1Central Ayurveda Research Institute (Kolkata), CCRAS, Ministry of Ayush, Govt. of India

**Introduction**

Uses of medicinal plants in traditional medicine have drawn immense attention from time immemorial. It is essential to look into the matter from a modern perspective. Hence, the mode and method of experimental procedures should be well-established and documented. Extraction is the first step to isolate and purify [bioactive compounds](https://www.sciencedirect.com/topics/food-science/bioactive-compound) from plant material. In the analysis or study of herbal or medicinal plants, extraction plays the most crucial role because it is the extraction procedure that can ensure the presence of desired chemical components in the fraction of plant extraction for subsequent chemical analysis, like isolation of bioactive markers and their characterisation. Before going to extraction, there are some basic steps that one needs to follow, which include pre-washing of the plant material followed by drying of the plant part, chopping, and grinding to yield a homogenous matrix of samples which improves the extraction kinetics by increasing the solvent contact with the sample surface [1,2]. Next comes the solvent selection part. Indeed, extracting solvent is not selected arbitrarily; the specific nature of the targeted bioactive compound(s) to be extracted guides the solvent selection. Plenty of various different solvents or mixtures/combinations of solvent systems are available to extract the desired bio-active entities from medicinal plants. Polar solvents such as acetone (CH3COCH3), ethyl acetate (EtOAc), methanol (MeOH), ethanol (EtOH) or water can be used to extract hydrophilic (water-loving) compounds; on the other hand, chloroform (CHCl3), dichloromethane (DCM) or a mixture of MeOH/DCM in the ratio of 1:1 is used for the extraction of lipophilic (fat loving) compounds. The insoluble nature of phenolic acids and flavonoids makes their extraction troublesome. Traditional extraction techniques include reflux, Soxhlet, percolation, maceration etc., which are well-known procedures for the extraction of [bioactive entities](https://www.sciencedirect.com/topics/food-science/bioactive-compound) and the equipment entangled in these techniques are dissimilar from one another. Now one question may arise. What is an appropriate extraction technique? A suitable extraction method is one that balances the quality of the product, efficiency of the process, costs of production, and environmentally benign methods that should be used to extract bioactive markers from herbal plants. Besides these, various new methods, including greener approaches for sustainable and nontoxic techniques of extraction, have also been adopted in recent times. In the green method of extraction use of hazardous chemicals is avoided. Technologies like ultrasound (US), high [hydrostatic pressure](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/hydrostatic-pressure) (HHP), [supercritical fluid](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/supercritical-fluid) (SF), [pulsed electric field](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/pulsed-electric-field) (PEF) etc., are rapidly replacing the traditional techniques. The use of unconventional new techniques increases the extent of extraction, thereby increasing yields along with increased extraction rates. This extraction accounts for fewer impurities in the resultant extract, safeguards thermo-labile compounds, uses various inorganic solvents, and consumes less energy. In this review, our main purpose will be to discuss different conventional and novel new technologies entangled in the extraction process of bioactive motifs from medicinal plants [3,4].

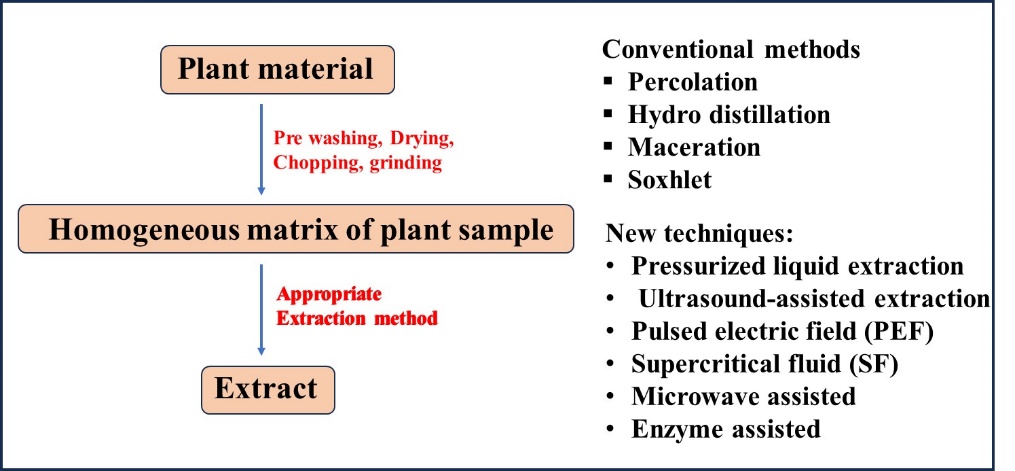


Fig. 1: Schematic representation of steps involved in extraction protocol

**Bioactive entities present in plants**

The biological system of the plant is composed of primary and secondary metabolites. The primary metabolites are mainly saccharides (carbohydrates), amino acids, and proteins and are essential for the development and maturation of plant tissues [3]. The secondary metabolites are created in the course of development cycle to help plants survive and overcome natural impediment. Bioactive compounds are found in a wide range of plant products and can be categorized into different classes such as terpenoids, flavonoids, alkaloids, nitrogen containing compounds; organosulphur compounds; and phenolic compounds [1]. The most well-known class of terpenoids are tocotrienols, which include: Atropine, Colchicine, Morphine, Strychnine, Ephedrine, Acridine, Quinine, Nicotine, Carotenoids, Pyrrolidine, Isoquinoline, Quinoline, Lycopodium, Quinolizid, Phytosterols etc. [5]. The bioactive compounds mentioned above have various health benefits, such as anti-inflammatory properties, anti-cancer properties, anti-diabetes properties, blood circulation properties, digestion properties, etc. [6].

**Selection of appropriate extraction protocol**

Bioactive motifs can not only be derived from plant sources, it is present in every living organism, be it a microorganism or animal/marine organism [7]. But in this review, we will restrict our discussion to plants only. The quantity of bioactive entities present in plant parts is low enough and all plant parts, such as barks, leaves, stems, roots, tuber roots, gums/oleoresin, exudates, fruits, stolon, flowers, rhizomes etc. produces bioactive chemicals in fairly low quantities and at different concentrations. And for this selection of proper extraction technique is very much essential to augment the extract from plants [8]. The extractability is dependent on various elements like extraction technique, plant component, matrix composition of plant materials, extracting solvent, pressure, temperature and time [9]. In the last couple of decades, researchers have focused more towards novel extraction techniques that are environment-friendly [10]. They have also tried to minimize the use of organic solvents, shortening the operational time and obtaining superior-quality extract. Because of these advantages, novel extraction techniques are gaining much attention over conventional techniques. Conventional extraction procedures have certain drawbacks which can easily be circumvented by these novel extraction strategies [11].

The extractability of any traditional technique mainly depends on the selection of solvents [12]. The solvent is chosen in such a way that it matches the polarity of the compound of interest. In addition to this, the molecular affinity between solvent and solute, role of co-solvent, transfer of mass, issues related to environment, toxicity to human and financial viability should also be considered while selecting the solvent for bioactive compound extraction. Following table documents some examples of bioactive motif extraction using the suitable solvents in the traditional way.

|  |  |
| --- | --- |
| **Solvents** | **Class of compounds extracted using suitable solvent** |
| Water (H2O) | Tannins, Anthocyanins, Carbohydrates, Terpenoids, Saponins |
| EtOH | Terpenoids, Tannins, Polyphenols, Alkaloids, Flavonol |
| MeOH | Anthocyanin, Tannins, Terpenoids, Polyphenols, Saponins, Flavones, |
| CHCl3 | Flavonoids, Terpenoids |
| DCM | Terpenoids |
| Et-O-Et | Terpenoids, Alkaloids |
| CH3COCH3 | Flavonoids |

**Properties of some common extracting solvents**

(i) Water. With a polarity index of 1.000 and being the most polar solvent, it is utilized to extract a wide range of polar stuff. Water's key benefit is that it dissolves a wide variety of compounds and is also affordable, nontoxic, non-flammable, and highly polar. Although it has certain drawbacks, such as the fact that it encourages bacterial and mould growth and might cause ester bond hydrolysis. [13,14]

(ii) Alcohol. Alcohol has a polar character (Polarity index of methanol is 0.762 and ethanol is 0.654). Since it is fully miscible with water is utilized for the extraction of polar secondary metabolites. To act as a self-preservative, the alcohol concentration should be greater than 20%. Alcohols in low concentrations are harmless, and because of their low boiling point, extracts can be concentrated with a low flame easily. Its disadvantage is that it is volatile and combustible, and it does not dissolve wax, gums, or fats. [13,14]

(iii) Chloroform. It works as a nonpolar solvent (Polarity index is 0.259) for extracting substances like terpenoids, flavonoids, oils, and lipids. Advantages. It is colourless having pleasant smell and miscible with alcohols. Main disadvantage is that both sedative and carcinogenic. [14,15]

(iv) Ether. It works as a nonpolar solvent (polarity index is 0.117) that can be used to extract substances including fatty acids, terpenoids, coumarins, and alkaloids. Ether is a low boiling solvent, is miscible with water, and has no taste. Additionally, ether is stable enough not to react with metals, acids, or bases. Its disadvantage is that it has a significant degree of volatility and flammability. [14,15]

(v) n-hexane. With a polarity index of 0.009, n-hexane is the most non-polar solvent and is used to remove wax, gums etc., present in the plant material. Hexane finds its wide use as an oil extraction material due to its ease of oil recovery, low b.p. (63-69 °C), and high solubility. [16]

(vi) Ionic liquid (green solvent). This particular extraction solvent is very polar and incredibly heat-stable. Even at 3,000°C, it can maintain a liquid condition and is suitable in high-temperature applications. It is exceedingly miscible with water and other solvents and works well for polar chemical extraction. The main advantage is that it is convenient for microwave-mediated extraction because it contains superior solvent that draws and transmits microwaves. It is extremely polar, incombustible, and appropriate for liquid-liquid extraction. [17]

Apart from these solvents, acetone (polarity index: 0.355), ethyl acetate (polarity index: 0.228) and DCM (Polarity index: 0.309) are the other solvents which researchers frequently use for the extraction purpose.

**Conventional extraction methods**

Conventional extraction techniques are utilized to extract bioactive motifs from plant sources. Extraction power of various solvents as well as the use of heat, mixing are the key factors of these approaches. The known traditional techniques for the extraction of bioactive compounds from plants are i) Soxhlet extraction, ii) Maceration, iii) Hydro distillation, and iv) Percolation.

**Soxhlet extraction**

The Soxhlet extractor was invented by Franz Ritter von Soxhlet, a German scientist, in 1879 [18]. It was mainly aimed at Lipid extraction; although, in recent times, it has widespread application. Soxhlet extraction has been widely utilized to extract important bioactive chemicals from various natural sources. It is referred to as a model for comparing novel extraction methods. A thimble is usually filled with an appropriate amount of dry material. The thimble is then put in the siphon-containing apparatus with a distillation flask containing the solvent of interest below. A siphon is used to aspirate the thimble-holder solution when it has reached an overflow level. Siphon returns the solution back to the distillation flask. In this way, extracted solutes are carried back into the bulk liquid by this solution. The solute remains in the distillation flask while the solvent returns to the plant's solid bed to complete the extraction cycle again. The procedure is repeated until the extraction is completed.

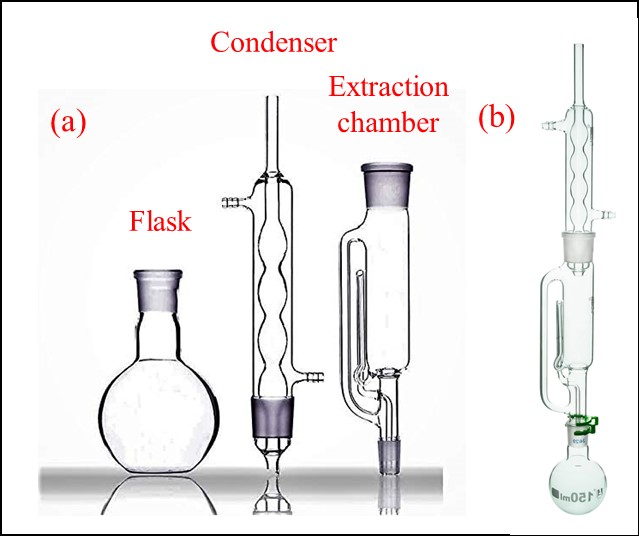


Fig. 2: (a) Different parts, (b) Full set up of Soxhlet apparatus

**Maceration**

Maceration has traditionally been utilised in the mass production of tonic. In a very short period of time, this process became a well-liked and cost-effective method for isolating essential oils and bioactive components. Small scale maceration process generally consists of multiple phases. Plant materials are ground into tiny particles to increase the surface area in order to maximize the solvent mixing. Second, an appropriate solvent known as menstruum is added to a closed vessel in the maceration technique. Finally, the liquid is strained, and the solid residue of this extraction process, known as marc, is pressed to recover many occluded solutions. Strained liquid is filtered to remove contaminants and to squeeze out liquids from them. Occasional shaking in the maceration process increases its extraction efficacy in two ways: (i) by increasing diffusion and (ii) by removing concentrated solution from the sample surface, facilitating the additional solvent to enter the menstruum and boost extraction yield.

**Hydro distillation**

The extraction of bioactive components and essential oils from plants by hydrodistillation is a conventional process. Organic solvents are not required for this process and can be carried out without dehydrating the plant materials. Hydro distillation process can be carried out in three ways i.e., i) Water distillation, ii) water and steam distillation, and iii) direct steam distillation [19]. At first, the plant materials are packed in a separate chamber; secondly, the required amount of water is added and heated to a boil. The introduction of direct steam into the plant sample is also an alternative way of extraction. Steam and hot water are the key factors which accelerate the release of bioactive compounds from plant parts. Water condenses the vapour combination of oil and water during indirect cooling. The mixture so condensed passes from the condenser to the separator, and bioactive chemicals and oils separate automatically from the water [20]. Hydro-diffusion, hydrolysis, and heat degradation are the three major physicochemical processes of Hydro distillation. Some volatile compounds may be lost at high temperatures during the extraction process, which limits their use in chemical extraction of thermolabile components.

**Percolation**

Percolation is a process in which a liquid is slowly filtered through a filter, similar to the way coffee is typically made. The term "percolation" originates from the Latin phrase "percolare", which translates to "strain through". Unlike maceration, percolation is an ongoing process in which a fresh solvent continually replaces a saturated solvent. In a study conducted by Zhang and co-workers, refluxing was compared with percolation and other extraction techniques for the extraction of *Undaria pinnatifid*. The percolation method yielded a higher content of the main component (fuxanthin) than the other extraction method (refluxing), while extract yield was more or less similar in either method. The study was conducted in the context of a compound Chinese medicinal product, Goupil Patch, which is a combination of 29 Chinese medicinal products [21]. Fu and co-workers employed the total alkaloid content as the index, with the EtOH percolating technique being optimized by soaking Goupil Patch for 24 hours with 55% alcohol, then percolating 12 times the same amount with alcohol (55 %) [22]. Gao further optimized the percolation method for sinomenine hydrochloride and ephedrine hydrochloride by using the extracting rate as the index. This method involved soaking the medicine for an additional 24 hours with 70 % EtOH, followed by percolating 20 times the amount with 70 % EtOH; the transfer rates for these two substances were nearly similar (78.23 % for sinomenine hydrochloride and 76.92 % ephedrine hydrochloride) [23].

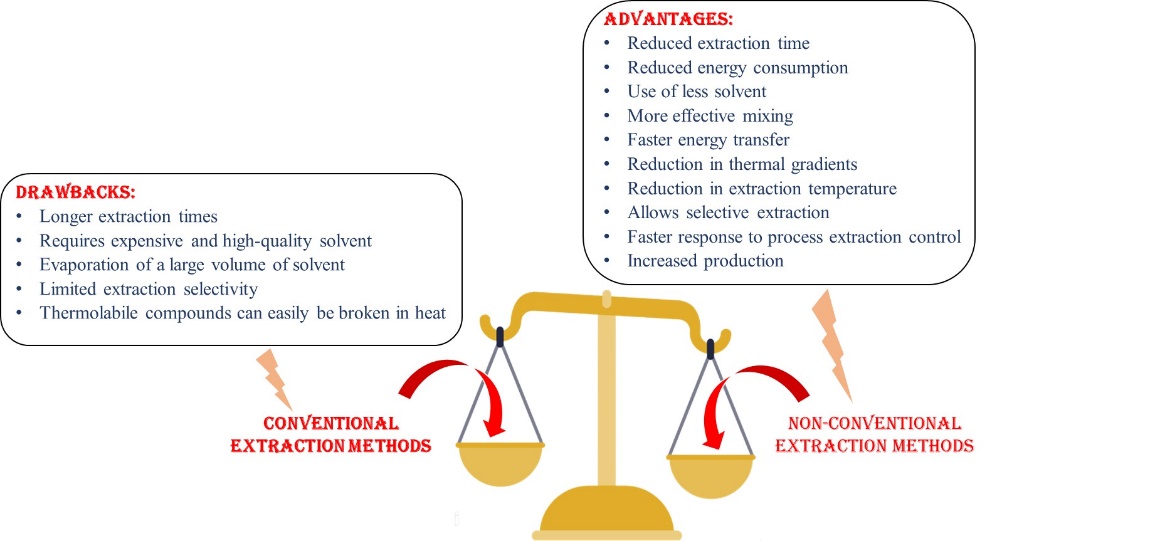


Fig. 3: A schematic comparison between two extraction protocols

**Non-conventional extraction methods**

Conventional extraction procedure has the following major drawbacks [24]:

1. Longer extraction times
2. Requires expensive and high-quality solvent
3. Evaporation of a large volume of solvent
4. Limited extraction selectivity
5. Thermolabile compound can easily be broken in heat

New, novel and promising extraction strategies are developed to address the limitations of conventional extraction methods. Under the umbrella of these new techniques, namely non-conventional extraction methods, following are some of the most promising approaches:

1. Ultrasound aided extraction (UAE)
2. Pulsed electric field-assisted extraction
3. Enzyme-assisted extraction
4. Microwave-assisted extraction (MAE)
5. Pressurized liquid extraction (PLE)
6. Supercritical fluid extraction

Some of these approaches are termed ''green processes,'' since they meet requirements established by the EPA (Environmental Protection Agency) in the US. Less hazardous chemical synthesis requires proper planning and methodology which includes [25]:

1. Designing safer chemicals
2. Use of safe solvent auxiliaries
3. Designing for energy efficiency
4. Use of less derivatives
5. Use of renewable feedstocks
6. Catalysis
7. Atom economy
8. Proper designing to prevent degradation
9. Time analysis for pollution prevention
10. Inherently safer chemistry for accident prevention

**Ultrasound assisted extraction (UAE)**

Ultrasound is a form of sound wave that is above the range of human hearing ranging usually in between 20 kHz and 100 MHz. It is a transverse wave, travels through a material by compressing and expanding and cannot pass through vacuum. This process causes cavitation, which is the formation, growth, and collapse of bubbles. The transfer of kinetic energy (K.E) of motion into heat energy the contents of the bubble provide a huge amount of energy. Bubbles, according to Suslick and Doktycz, have a temperature of ̴ 5000 K, a pressure of 1000 atm, and a heating and cooling rate ˃ 1010 K/s [26]. UAE was created on the basis of this premise. Cavitation occurs only in liquids and liquids containing solids. The primary advantage of UAE may be seen in solid plant samples because ultrasonic energy accounts for the increase of the leaching of organic and inorganic chemicals from plant tissues [27]. The most likely mechanism is ultrasound-mediated mass transfer and quicker solvent access to plant tissues. The ultrasonic extraction technique incorporates two major physical phenomena: i) diffusion over the cell wall and ii) washing the contents of the cell after shattering the walls [28]. Sample moisture content, extent of grinding, solvent, and particle size are all critical aspects in achieving efficient and successful extraction. Besides these, the controlling parameters for ultrasonic action include pressure, temperature, frequency, and sonication time. UAE has also been used in conjunction with conventional processes to leverage the efficiency of a traditional system. An ultrasonic unit is positioned in an appropriate position in a solvent extraction unit to improve extraction efficacy [29]. The following are the benefits of UAE [30]:

1. Reduced extraction time
2. Reduced energy consumption
3. Use of less solvent
4. More effective mixing
5. Faster energy transfer
6. Reduction in thermal gradients
7. Reduction in extraction temperature
8. Allows selective extraction
9. Smaller equipment size
10. Faster response to process extraction control
11. Quick start-up
12. Increased production
13. Elimination of process step

UAE is proved to be an efficient extraction process for extracting bioactive compounds from medicinal plants. Rostagno *et al.* demonstrated the extraction efficacy of four (04) isoflavone derivatives from soybean using a mix-stirring technique with varying extraction periods and solvents, namely genistin, daidzin, glycitin, and malonyl genistin [31]. The authors discovered that depending on the solvent used, ultrasound can boost extraction yield. Herrera *et. al.* developed a semiautomatic technique based on ultrasounds to extract phenolic chemicals such as naringin, rutin, naringenin, ellagic acid, quercetin, and kaempferol from straw berries at 0.8 s duty cycle for 30 seconds [32]. Li *et al.* discovered that UAE, under optimal conditions (70% MeOH, 20:1 solvent, sample ratio, and 30 min duration) recovered more chlorogenic acid from fresh leaves and bark, and dried bark of *Eucommia ulmodies* Oliv. than traditional extraction procedures [33]. Yang and co-workers extracted bioactive chemicals termed rutin and quercetin from *Euonymus alatus* (Thund.) Sieb using optimum sonication conditions and determined that the ultrasonic approach was more efficient than conventional methods [34]. Ionic liquid-based UAE has been shown to be particularly successful in extracting three alkaloids from Catharanthus roseus (vinblastine, vindoline, and catharanthine) [35]. Anthocyanins and phenolic compounds were extracted from grape peel utilizing UAE, and the extraction method was adjusted in terms of extraction temperature, solvent, and extraction duration [36,37]. Phenolcarboxylic acids, carnosic acid, and rosmarinic acid were extracted from *Rosmarinus officinalis* utilizing an Ionic liquid-based UAE methodology that showed to be more effective and faster than conventional ones [38].

**Pulses electric field extraction**

Pulsed electric field treatment has been identified in the last 10 years as a useful process for enhancing the press, dry, extract, and diffusion of plant material. The principle of the PEF treatment is to destroy the matrix of the cell wall/membrane to facilitate the extraction. During the suspension of the living cell in the electric field, the electric potential traverses through the cell membrane of the cell [39-43]. Due to the dipolar character of the membrane molecules, the electric potential separates the molecules according to the charge of the membrane molecules. After reaching a critical value (about 1 V of transmembrane potential) of transmembrane potential, the repulsion of charge-carrying molecules causes the formation of pores in weak regions of the membrane, causing a radical increase in permeability [44]. PEF treatment for plant materials is usually performed using a simple circuit of exponential decay pulses. The treatment chamber consists of two electrodes, and the plant material is placed in the treatment chamber. Depending upon the design of the treatment chamber, the PEF process may operate in continuous mode or batch mode [45]. The efficiency of PEF treatment is highly dependent on process parameters such as field strength, specific power input, pulse number and treatment temperature, and properties of the material to be treated [46]. PEF can enhance mass transfer during the extraction by destroying the membrane structure of the plant material for enhanced extraction and decreased extraction time. PEF has been used to improve the release of intracellular compounds from plant tissue by increasing the cell membrane permeability. PEF treatment at a medium electric field (500-1000 V/cm; 104-102s) is shown to damage the cell membrane of plant material with little temperature increase. Therefore, PEF can minimise the degradation of heat-sensitive compounds. PEF is also used on plant material as a pretreatment procedure before conventional extraction to reduce the extraction effort [47]. Pulsed electric field (PEF) treatment (1 kV/cm, low energy consumption = 7 kJ/ kg) in the solid-liquid extraction process of beetroots to extract betanin showed the highest degree of extraction when compared to freezing and mechanical pressing. Guderjan *et al.* showed that phytosterols recovered by 32.4 % from maize increased by PEF treatment. Isoflavonoids recovered by 20–21 % from soybeans when PEF treatment was used as a pretreatment process [48]. Corralesa *et al.* extracted bioactive compound(s) like anthocyanins(s) from grape by-products using several techniques [49]. They found better extraction of Anthocyanin Mono Glucosides by using PEF [50]. The application of PEF on grape skin prior to the maceration step reduces the maceration time and improves bioactive stability (anthocyanins, polyphenols, etc.) during vinification. Pulsed electric field treatment of Merlot skin improves the permeability of Merlot skin [51].

**Enzyme assisted extraction**

Enzyme-assisted extraction is a process of extracting organic compounds from plant matrices through seed cell wall hydrolysis. This method is known as Enzymatic Pre-treatment and has been found to be a novel and efficacious way to release bound compounds, thus increasing overall yield [52]. EAAE (Enzyme-Assisted Aqueous Extraction) and Enzymatic Cold Pressing (EACP) are two approaches to this process and are typically used to extract oils from a variety of seeds. In EAAE, enzymes are used to break down the cell wall, which is not possible in EAAE due to the lack of Polysaccharide-Protein Colloid in this system. Adding certain enzymes such as Cellulase, Pectinase, and **-amylase during the extraction process further enhances recovery by hydrolysing the Structural Polysaccharides and Lipid Bodies [53]. The key determinants of enzymic hydrolysis are the composition and concentration of the enzymic material, the particle size of the plant material, the solubility ratio of the material to water, and the time required for the hydrolysis process [54]. According to the research conducted by Dominguez and colleagues, the moisture content of the plant material is also a significant factor in the hydrolysis of the material. In the study of EACP, it was found that the oil extracted from oilseed oil by enzymic-assisted methods had a higher percentage of FFA and Phosphorous content than the oil extracted from traditional hexane-extracted oil [55]. The EAE process is recognised as an environmentally friendly technology for the extraction of organic compounds and oil, as it utilises water as a solvent rather than organic chemicals [56]. Meyer *et al.* demonstrated the efficacy of enzyme-based exfoliation (EAE) of phenolic anti-oxidants in wine production. This study showed a relationship between the yield of the total phenols obtained and the degree of the breakdown of the plant cell wall by the enzyme used [57]. Landbo *et al.* demonstrated an improvement in the exfoliating power of various enzymes when using EAAE to extract phenolic compounds (pectinyl acid, non-antichyanoid flavonoids, and phenocyanins) [58]. Li *et al.* extracted the total phenolic content of five Citrus Peels (Yen Ben Lemon, Meyer Lemon, Grapefruit, Mandarin and Orange) using various EAAE enzymes, with the highest recovery rate being achieved with the use of Celluzyme MX (Cellulolytic enzyme) [59]. Another important finding from this study was that the extraction of the phenolic antioxidants was significantly improved with higher enzyme concentrations [60]. Finally, Laroze *et al.* demonstrated an increase in the extraction of Phenolic Antioxidant from raspberry Solid Waste by utilization of Enzyme in Hydro-alcoholic Extraction, compared to non-enzymatic control. Enzymes may be used as an alternate source to bioactive compounds to extract phenolic compounds from agri-industrial byproducts [61]. In 2012, Gómez-Garcia *et al.* demonstrated that phenolic compounds can be extracted from grape waste using a variety of enzymes, including celluclast and pectinex, as well as novoferm, in EAE. Novoferm was found to have the most significant effect on releasing phenolic compounds from grape waste [62].

**Microwave assisted extraction (MAE)**

Microwave-assisted extraction (MAE) is an unique technique for the extraction of soluble products from a broad range of materials by the use of microwave energy [63]. It is based on the principle that electromagnetic fields, which are oscillating fields of two perpendicular lengths, such as electric and magnetic fields, can be induced by the direct impact of microwaves on polar molecules [64]. Heat is generated by the conversion of electromagnetic energy to heat, which is achieved through the use of ionic and dipole rotational mechanisms [65]. During the ionic process, heat is generated due to the resistance of the medium-to-flow ion. Conversely, ions maintain their direction along the field signs, which are frequently altered. Microwave-assisted extraction (MAE) is a selective extraction technique that involves the frequent changing of directions of molecules, resulting in collisions between them and the generation of heat. The mechanism of MAE extraction is proposed to involve three steps: first, solute separation from the active sites of the sample matrix under high temperature and pressure; second, solvent diffusion across the sample matrix; and third, solute release from the sample matrix to the solvent [66]. Several benefits of MAE extraction have been identified, such as the ability to heat bioactive substances more quickly, the reduction of thermal gradients, the reduction of equipment size, and improved extract yield [67]. Additionally, MAE is a green technology as it reduces the consumption of organic solvents. For the extraction of polyphenols, caffeine, and other organic compounds, MAE yielded a higher extraction yield in 4 minutes than any other extraction method over 20 hours at room temperature. Ginsenosides can be extracted from ginseng roots in 15 minutes using a focused MAE technique, which is better than regular solvent extraction over 10 hours. Dhobi *et al.* showed that MAE is more efficient than conventional extraction methods like Soxhlet and maceration when it comes to extracting flavolignins, silybinins, and other bioactive compounds [68]. Hui *et al.* for example, used MAE to extract flavonoids, phenolics, and cinnamaldehyde from various plants under optimal conditions, and showed that it's faster and easier than other extraction methods [69]. Chiremba *et al.* used MAE for releasing bound phenolic acids in sorghum and corn fractions of different hardness [70]. MAE process is also used to extract some bioactive compounds, including cinnamaldehyde, tannin, and flavonoids, from Chinese quince. They also used designed experiments to maximize the recoveries of the extracts, as well as enhance their electron donating ability [69].

**Pressurized liquid extraction (PLE)**

Richter et al., 1996, coined the term "Pressurized Liquid Extraction" (PLE) [71]. This method has since been referred to by various names, including Pressurized Fluid Extraction (PFE), Accelerated Liquid Extraction (ASE), Enhanced Solvent Extraction (ESE), and High-Pressure Solvent Extracting (HSPE) [72]. The principle of PLE is to apply high pressure to a solvent liquid beyond its normal boiling point, which accelerates the extraction of the solvent. Automation techniques have been the primary driving force for the development of PLE techniques, as they reduce the extraction time and solvent requirements. PLE technique necessitates the use of small quantities of solvents due to the combination of the high pressure and temperatures, which results in a more rapid extraction. Furthermore, the higher extraction temperature can improve the solubility of analytes by elevating both the solubility and the mass transfer rate and decreasing the viscosity of solvents and surface tension, thereby enhancing the extraction rate [73].

Compared to the conventional Soxhlet method, PLE is cost effective in terms of time and solvent consumption (Richter et Al., 1996). Nowadays, PLE is used to extract polar com pounds, and is also seen as an alternative to supercritical fluid extractions [74]. PLE is also used to extract organic pollutants from environmental matrixes that are stable at high temperature [75]. PLE has been used to extract bioactive compounds from marine sponge. Plant-Based Extractions (PLES) Plant-based extraction (PLES) is a widely used technique for extracting natural products. Plant Based Extraction (PLES) has been widely reorganised as a Green Extraction Technique (PLES) due to the use of small quantity of organic solvents [73].

The use of PLE has been demonstrated to be effective in the extraction of bioactive motifs from a variety of plant materials. Utilizing optimized conditions, isoflavones have been extracted from soybeans that have been frozen-dried without being degraded by PLE [76]. Shen *et al.* compared ASE for the extraction of Terpenoids and Sterols from tobacco with the use of Soxhlet extraction, as well as Ultrasonically Assisted Extraction (SAE) [77]. PLE has been proposed as an alternative to conventional methods, due to its faster process and reduced solvent consumption. For example, flavonoids derived from spinach using a PLE-based mixture of ethanol and a 70:30 solvent at a temperature range of 50–150 °C was more effective than a 50–130 ºC water solvent [78]. The results of Luthria's (2008) study demonstrated that the temperature of the solution, the pressure, the size of the particles, the flush volume, the duration of the reaction, and the solubility ratio of the solution all have an effect on the ability of PLE to extract phenolic compounds. The optimized method of PLE extraction was particularly effective in extracting lycorine, galanthamine, and alkaloids from *Narcis sujonquilla*. Additionally, the optimized method was more efficient than hot-sourced extraction, Methylene ether (MAE), and United States of America (U.S.A.); individual phenolic compounds (GCT, Epicatechin, Catechin, Gallate, Chlorogenic Acid, Caffeic Acid, and Myricetin), as well as total phenolic content, were recovered from different parts of the genus *Anastasia propolis* at optimal conditions (40 °C, 1500 psi, 15min) [79].

**Supercritical fluid extraction**

The use of supercritical fluid in extraction applications began with the discovery of Hannay in 1879 by Hogarth and Hannay-Hogarth. However, Zosel (1964) was the first to patent a technique for decaffeinating coffee using SFE. Since that time, the technique has generated widespread scientific interest and has been utilised in environmental, pharmaceutical, polymer, and food analysis applications [80]. Several industrial sectors have been utilizing this method for a long time, particularly in the decaffeinated coffee production industries [81].

The three basic states of all earthly substances are Solid, Liquid and Gas. A supercritical phase/state is a distinct state that can only be achieved when a substance is exposed to a temperature and pressure greater than its critical point. A critical point is defined as a temperature (Tc) or pressure (Pc) threshold above which distinct gas and liquid phases cease to exist [82]. In a supercritical state, the particular properties of the gas or liquid become undetectable, thus preventing the liquefaction of the supercritical fluid by changes in temperature and pressure. The supercritical fluid has gas-like properties, such as diffusion and viscosity, as well as solvation power and surface tension, making it suitable for the extraction of compounds in a short period of time with higher yields. A typical SFE system is composed of a tank containing a mobile phase, typically CO2, a pressurised gas pump, a co-solvent pump and an extraction vessel. Other types of meters may also be connected to the system, such as flow meter and dry/wet gas meter. Finally, a controller is used to ensure the high pressure within the system is maintained [83].

Carbon dioxide has been identified as an optimal solvent for the synthesis of SFE. Its critical temperature (Tc) of 31ºC is comparable to that of room temperature, while its low critical pressure of 74 bars provides the opportunity to operate at moderate pressures (generally ranging from 100 to 450 bar) [84]. Its only disadvantage is its lack of polarity, which makes it suitable for lipids, fats and non-porous substances, but not suitable for most pharmaceutical or drug samples. To overcome this limitation, a chemical modifier has been used [85], and a small amount is usually considered sufficient to significantly increase carbon dioxide polarity. For instance, the addition of 0.5 mL of DCM can significantly improve the extraction, which is equivalent to 4 hours of hydro distillation [86]. The characteristics of the sample and the targeted compounds, as well as the prior experimental result, are the primary criteria for selecting the most suitable modifier.

The success of bioactive compounds extraction from plant materials depends on various parameters of SFE, and the most important are the parameters which can be tuned [87]. Precise control over these parameters is needed in order to maximize the advantages of this method. The main parameters that influence the extraction efficiency are the temperature, the pressure, the size of the particles and the moisture content of the feed material, the extraction time, the flow rate of the CO2, and the ratio of the solvent to the feed material [84].

The following are some of the advantages of using a supercritical fluid for bioactive compound extraction: Supercritical fluid has higher diffusion coefficient compared to a liquid solvent. This allows for more penetration to the sample matrix and better mass transfer. This reduces the extraction time significantly compared to conventional methods. Supercritical fluid can be repeated to the sample for complete extraction. This improves selectivity of the fluid compared to liquid solvent. The solvation power of the supercritical fluid can be fine-tuned either by changing the temperature or/or the pressure. The process of separating the solute from the solvent in a conventional extraction method can be easily bypassed by the use of a depressurizing supercritical fluid. The supercritical fluid operates at room temperature. This makes the supercritical fluid ideal for thermolabile compound extraction. Small amount of sample is extracted in SFE compared to solvent extraction methods. This saves time for the overall experiment. The use of SFE does not require large amounts of organic solvent. This is considered environment friendly. It's possible to connect SFE to chromatographic processes online, which is great for volatile compounds. Plus, you can recycle and reuse supercritical fluid, so you don't have to worry about waste. You can also set up a scale for SFE, from a few milligrams in the lab to kilos to tons of samples in industries. Finally, the SFE process gives idea about the extraction process and how it works, so one can adjust it to make it as efficient as possible [88].

The study of Saldaña and Verma demonstrated that SFE can be used to extract purine alkaloid substances (caffeine and theobromine) from the leaves of the herbal maté tea *Ilex Paraguaryensis* at a temperature and pressure of 313–343 K and 14 to 24 MPa respectively [89,90]. Additionally, the study of Supercritical CO2 (15 Wt.%) modified with ethanol yielded higher extraction yields of the flavonoid naringin (from *Citrus paradise*) than the pure supercritical CO2 (9.5 MPa) at a temperature of 58.6 C [91]. Similarly, in 2004, Khorassani and Taylor found that SFE could be used to extract polyphenols and protcyanidins from grape seeds, with methanol as a modifier and CO2 as a modifier (40%). Furthermore, the study showed that SFE was able to release more than 79 percent of catechin (from *Citrus Paradise*) and epicatechin (from grape seed) using 6.6 percent methanol for 40 minutes [92].

**Conclusion**

The ever-increasing demand for plant bioactive substances drives the ongoing thirst for searching of more simple extraction techniques. The improvement in chromatographic techniques and environmental consciousness are two major drivers towards non-conventional extraction techniques. Although, knowing every part of the non-conventional extraction procedure is critical since most of these approaches are contingent with distinct mechanisms and improvement in the extraction is the consequence of it. Hybrid approaches should also be incorporated and developed keeping the plant material properties in mind and chemical selection should be in line to that. As of now many of the new approaches lacks sufficient experimental data, so research should be carried out to overcome those gaps. We anticipate that with the growing scientific as well as economic importance of bioactive motifs and bioactive compound-rich entities will definitely lead to the evolution of more complex extraction technologies in the future.

**References**

[1] Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants. 2017; 6(4): 42-64. <https://doi.org/10.3390/plants6040042>

[2] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha YL. 2011. Extraction, isolation and characterization of bioactive compounds from plants’ extracts. *African Journal of Traditional Complementary and Alternative Medicines*. 2011; 8(1): 1-10.

[3] Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F et al. Techniques for extraction of bioactive compounds from plant materials: A review. [*Journal of Food Engineering*](https://www.sciencedirect.com/journal/journal-of-food-engineering). 2013; 117(4):426-436.

[4] Jah AK. Sit N. Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. [*Trends in Food Science & Technology*](https://www.sciencedirect.com/journal/trends-in-food-science-and-technology). 2022; 119: 579-591

[5] [Dillard](https://onlinelibrary.wiley.com/authored-by/Dillard/Cora+J) CJ, [German](https://onlinelibrary.wiley.com/authored-by/German/J+Bruce) JB. Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture.* 2000;  [80(12](https://onlinelibrary.wiley.com/toc/10970010/2000/80/12)): 1744-1756.

[6] Zhang QW, Lin LG, Ye CW. Techniques for extraction and isolation of natural products: a comprehensive review [Chin Med.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5905184/) 2018; 13: 20. https://doi.org/[10.1186/s13020-018-0177-x](https://doi.org/10.1186%2Fs13020-018-0177-x)

[7] Swamy MK, Akhtar MS. Natural Bioactive Compounds: Volume 2 Chemistry, Pharmacology, and Health Care Practices. Springer Nature Singapore Pte Ltd, 2019, Hardcover, ISBN: 978-981-13-7204-9.

[8] Gil-Chávez GJ, Villa JA, Ayala-Zavala JF, Heredia JB, Sepulveda D, Yahia EM et al. Technologies for Extraction and Production of Bioactive Compounds to be Used as Nutraceuticals and Food Ingredients: An Overview. *Comprehensive Reviews in Food Science and Food Safety*. 2013; 12(1): 5-23.

[9] Drosou C, Kyriakopoulou K, Bimpilas A, Tsimogiannis D, Krokida M. A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. *Industrial Crops and Products*. 2015; 75(30): Part B, 141-149.

[10] Belwel T, Chemet F, Venskutonis R, Cravotto G, Jaiswal DK et al. Recent advances in scaling-up of non-conventional extraction techniques: Learning from successes and failures. TrAC Trends in Analytical Chemistry. 2020; <https://doi.org/10.1016/j.trac.2020.115895>

[11] Putnik P, Lorenzo J, Barba F, Roohinejad S, Rezek Jambrak A et al. Novel food processing and extraction technologies of high-added value compounds from plant materials. Foods, 2018; 7(7): 106. <https://doi.org/10.3390/foods7070106>

[12] Cowan, MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews.* 1999; 12(4): 564-582.

[13] Das K, Tiwari RK, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends. *J Med Plants Res.*2010; 4(2):104-111.

[14] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. *Int Pharm Sci.*2011; 1:98–106.

[15] Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem.*2014; 2(5):115-119.

[16] Liu SX, Mamidipally PK. Quality comparison of rice bran oil extracted with d-limonene and hexane. *Cereal Chem.*2005; 82: 209-215. <https://doi.org/10.1094/CC-82-0209>

[17] Bhan M. Ionic liquids as green solvents in herbal extraction. *Int J Adv Res Dev.*2017; 2:10-12.

[18] Soxhlet F. Die gewichtsanalytische Bestimmung des Milchfettes. *Dinglers Polytechnisches Journal.* 1879; 232: 461-465.

[19] Vankar PS. Essential oils and fragrances from natural sources. *Resonance*. 2004; 9(4), 30-41.

[20] Silva LV, Nelson DL, Drummond MFB, Dufossé L, Glória MBA. Comparison of hydro distillation methods for the deodorization of turmeric. *Food Research International*. 2005; 38 (8-9): 1087-1096.

[21] Zhang H, Wang W, Fu ZM, Han CC, Song Y. Study on comparison of extracting fucoxanthin from Undaria pinnatifid with percolation extraction and refluxing methods. *Zhongguo Shipin Tianjiaji.* 2014; 9: 919-95.

[22] Fu M, Zhang L, Han J, Li J. Optimization of the technology of ethanol extraction for Goupi patch by orthogonal design test. *Zhongguo Yaoshi (Wuhan, China).* 2008; 11(1): 75-76.

[23] Gao X, Han J, Dai H, Xiang L. Study on optimizing the technological condition of ethanol percolating extraction for Goupi patch. *Zhongguo Yaoshi.* 2009; 12(10):1395–1397

[24] Luque de Castro MD, Garcia-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Analytica Chimica Acta* 1998; 369 (1-2), 1-10.

[25] Awad AM, Kumar P, Ismail-Fitry MR, Jusoh S, Ab Aziz MF, Sazili AQ. Green Extraction of Bioactive Compounds from Plant Biomass and Their Application in Meat as Natural Antioxidant. Antioxidants 2021; 10*(*9): 1465-1503. <https://doi.org/10.3390/antiox10091465>.

[26] Suslick KS, Doktycz SJ. The effects of ultrasound on solids. In: Mason TJ. (Ed.), Advances in Sonochemistry. 1990. vol. 1. JAI Press, New York, pp. 197–230.

[27] Herrera MC, Luque de Castro MD. Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. *Journal of Chromatography*. 2005; 1100(1): 1-7.

[28] Mason TJ, Paniwnyk L, Lorimer JP. The uses of ultrasound in food technology. *Ultrasonics Sonochemistry*. 1996; 3(3): 253-260.

[29] Vinatoru M, Toma M, Filip P, Achim T, Stan N, Mason TJ et al. Ultrasonic Reactor Dedicated to the Extraction of Active Principles from Plants. *Romanian Patent.* 1998. Nr. 98-01014.

[30] Chemat F, Tomao V, Virot M. 2008. In: Otles S. (Ed.), Handbook of Food Analysis Instruments. Ultrasound-Assisted Extraction in Food Analysis. CRC Press, 2008, pp. 85-94.

[31] Rostagno MA, Palma M, Barroso CG. Ultrasound-assisted extraction of soy isoflavones. *Journal of Chromatography A*. 2003; 1012(2): 119-128.

[32] Herrera MC, Luque de Castro MD. Ultrasound-assisted extraction for the analysis of phenolic compounds in strawberries. *Analytical and Bioanalytical Chemistry.* 2004; 379(7-8): 1106-1112.

[33] Li H, Chen B, Yao S. Application of ultrasonic technique for extracting chlorogenic acid from Eucommia ulmodies Oliv. (*E. ulmodies*). *Ultrasonics Sonochemistry*. 2005; 12(4): 295-300.

[34] Yang Y, Zhang F. Ultrasound-assisted extraction of rutin and quercetin from Euonymus alatus (Thunb.) Sieb. *Ultrasonics Sonochemistry*. 2008; 15(4): 308-313.

[35] Yang L, Wang H, Yuan-gang Zu, Zhao C, Zhang L, Chen X et al. Ultrasound-assisted extraction of the three terpenoid indole alkaloids vindoline, catharanthine and vinblastine from Catharanthus roseus using ionic liquid aqueous solutions. *Chemical Engineering Journal.* 2011; 172 (2-3): 705-712.

[36] Ghafoor K, Choi YH, Jeon JY, Jo IH. Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants and anthocyanins from grape (Vitisvinifera) seeds. *Journal of Agricultural and Food Chemistry.* 2009; 57(11): 4988-4994.

[37] Ghafoor K, Hui T, Choi YH. Optimization of ultrasound-assisted extraction of total anthocyanins from grape peel. *Journal of Food Biochemistry.* 2011; 35: 735-746.

[38] Zu G, Zhang R, Yang L, Ma C, Zu Y, Wang W, Zhao C. Ultrasound-assisted extraction of carnosic acid and rosmarinic acid using ionic liquid solution from Rosmarinus officinalis. *Int J Mol Sci.* 2012; 13(9): 11027-11043. <https://doi.org/10.3390/ijms130911027>

[39] Barsotti L, Cheftel JC. Traitement des aliments par champs electriques pulses. *Science des Aliments.* 1998; 18: 584-601.

[40] Angersbach A, Heinz V, Knorr D. Effects of pulsed electric fields on cell membranes in real food systems. *Innovative Food Science and Emerging Technologies*. 2000; 1(2):135-149.

[41] Vorobiev E, Lebovka NI. Extraction of intercellular components by pulsed electric fields. In: Raso, J., Heinz, V. (Eds.), Pulsed Electric Field Technology for the Food Industry: Fundamentals and Applications. Springer, New York, 2006, pp. 153-194.

[42] Vorobiev E, Jemai AB, Bouzrara H, Lebovka NI, Bazhal MI. Pulsed electric field assisted extraction of juice from food plants. In: Barbosa-Canovas, G., Tapia, M.S., Cano, M.P. (Eds.), Novel Food Processing Technologies. CRC Press, New York, 2005, pp. 105-130.

[43] Lebovka NI, Bazhal MI, Vorobiev E. Estimation of characteristic damage time of food materials in pulsed-electric fields. Journal of Food Engineering. 2002; 54(4): 337-346.

[44] Bryant G, Wolfe J. Electromechanical stress produced in the plasma membranes of suspended cells by applied electrical fields. *Journal of Membrane Biology*. 1987; 96(2): 129-139.

[45] Puértolas E, López N, Saldaña G, Álvarez I, Raso J. Evaluation of phenolic extraction during fermentation of red grapes treated by a continuous pulsed electric fields process at pilot-plant scale. *Journal of Food Engineering.* 2010; 119(3): 1063-1070.

[46] Heinz V, Toepfl S, Knorr D. Impact of temperature on lethality and energy efficiency of apple juice pasteurization by pulsed electric fields treatment. *Innovative Food Science and Emerging Technologies.* 2003; 4(2): 167-175.

[47] López N, Puértolas E, Condón S, Raso J, Álvarez I. Enhancement of the extraction of betanine from red beetroot by pulsed electric fields. *Journal of Food Engineering*. 2009; 90(1): 60-66.

[48] Guderjan M, Töpfl S, Angersbach A, Knorr D. Impact of pulsed electric field treatment on the recovery and quality of plant oils. *Journal of Food Engineering.* 2005; 67(3): 281-287.

[49] Corralesa M, Toepflb S, Butza P, Knorrc D, Tauschera B. Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. *Innovative Food Science and Emerging Technologies.* 2008; 9(1): 85-91.

[50] López N, Puértolas E, Condón S, Álvarez I, Raso J. Effects of pulsed electric fields on the extraction of phenolic compounds during the fermentation of must of Tempranillo grapes. *Innovative Food Science and Emerging Technologies.* 2008; 9(4): 477-482.

[51] Delsart C, Ghidossi R, Poupot C, Cholet C, Grimi N, Vorobiev E et al. 2012. Enhanced extraction of phenolic compounds from merlot grapes by pulsed electric field treatment. *American Journal of Enology and Viticulture*. 2012; 63(2): 205-211.

[52] Rosenthal A, Pyle DL, Niranjan K. Aqueous and enzymatic processes for edible oil extraction. *Enzyme Microbial Technology*. 1996; 19(6): 402-420.

[53] Singh RK, Sarker BC, Kumbhar BK, Agrawal YC, Kulshreshtha, MK. Response surface analysis of enzyme-assisted oil extraction factors for sesame, groundnut, and sunflower seeds. *Journal of Food Science and Technology*. 1999; 36(6): 511-514.

[54] Latif S, Anwar F. Physicochemical studies of hemp (Cannabis sativa) seed oil using enzyme-assisted cold-pressing. *European Journal of Lipid Science and Technology.* 2009; 111(10): 1042-1048.

[55] Dominguez H, Ntiiiez MJ, Lema JM. Enzyme-assisted hexane extraction of soybean oil. *Food Chemistry*. 1995; 54(2): 223-231.

[56] Puri M, Sharma D, Barrow CJ. Enzyme-assisted extraction of bioactives from plants. *Trends in Biotechnology.* 2012; 30(1): 37-44.

[57] Meyer AS, Jepsen SM, Sørensen NS. Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. Journal of Agricultural and Food Chemistry. 1998; 46(7): 2439-2446.

[58] Landbo AK, Meyer AS. Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (Ribes nigrum). *Journal of Agricultural and Food Chemistry.* 2001; 49(7): 3169-3177.

[59] Li BB, Smith B, Hossain MM. Separation and purification in the food industry extraction of phenolics from citrus peels: II. Enzyme-assisted extraction method. *Separation and Purification Technology.* 2006; 48 (2): 189–196.

[60] Maier T, Göppert A, Kammerer DR, Schieber A, Carle R. Optimization of a process for enzyme-assisted pigment extraction from grape (Vitis vinifera L.) pomace. *European Food Research and Technology*. 2008; 227(1): 267-275.

[61] Laroze L, Soto C, Zúñiga ME. Phenolic antioxidants extraction from raspberry wastes assisted by-enzymes. *Electronic Journal of Biotechnology.* 2010; 13(6): 0-0. <http://dx.doi.org/10.2225/vol13-issue6-fulltext-12>.

[62] Gómez-García R, Martínez-Ávila GCG, Aguilar CN. Enzyme-assisted extraction of antioxidative phenolics from grape (Vitis vinifera L.) residues. *3 Biotech*. 2012; 2: 297-300. <http://dx.doi.org/10.1007/s13205-012-0055-7>.

[63] Paré JJR, Bélanger JMR, Stafford SS. 1994. Microwave-assisted process (MAP™): a new tool for the analytical laboratory. *TrAC Trends in Analytical Chemistry.* 1994; 13(4): 176–184.

[64] Letellier M, Budzinski H. Microwave assisted extraction of organic compounds. *Analusis.* 1999; 27(3): 259-270.

[65] Jain T, Jain V, Pandey R, Vyas A, Shukla SS. Microwave assisted extraction for phytoconstituents-an overview. *Asian Journal of Research in Chemistry*. 2009; 2(1): 19–25.

[66] Alupului A, Călinescu L, Lavric V. Microwave extraction of active principles from medicinal plants. *U. P. B. Science Bulletin*, Series B. 2012; 74 (2): 129-142.

[67] Cravottoa G, Boffaa L, Mantegnaa S, Peregob P, Avogadrob M, Cintasc P. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. *Ultrasonics Sonochemistry*. 2008; 15(5): 898-902.

[68] Dhobi M, Mandal V, Hemalatha S. Optimization of microwave assisted extraction of bioactive flavolignan–silybinin. *Journal of Chemical Metrology*. 2009; 3(1): 13-23.

[69] Hui T, Ghafoor K, Choi YH. Optimization of microwave-assisted extraction of active components from Chinese quince using response surface methodology. *Journal of Korean Society of Applied and Biological Chemistry*. 2009; 52(6): 694-701.

[70] Chiremba C, Rooney LW, Trust BJ. 2012. Microwave-assisted extraction of bound phenolic acids in bran and flour fractions from sorghum and maize cultivars varying in hardness. Journal of Chromatography A. 1012(2), 119–128.

[71] Richter BE, Jones BA, Ezzell JL, Porter NL. Accelerated solvent extraction: A technique for sample preparation. *Analytical Chemistry*. 1996; 68(6): 1033- 1039.

[72] Nieto A, Borrull F, Pocurull E, Marcé RM. Pressurized liquid extraction: a useful technique to extract pharmaceuticals and personal-care products from sewage sludge. *TrAC Trends in Analytical Chemistry.* 2010; 29(7): 752–764.

[73] Ibañez E, Herrero M, Mendiola JA, Castro-Puyana M. Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In: Hayes, M. (Ed.), Marine Bioactive Compounds: Sources, Characterization and Applications. Springer, 2012, pp. 55-98.

[74] Kaufmann B, Christen P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurized solvent extraction. *Phytochemical Analysis*. 2002; 13(2): 105-113.

[75] Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*. 2006; 17(6): 300-312.

[76] Rostagno MA, Palma M, Barroso CG. Pressurized liquid extraction of isoflavones from soybeans. *Analytica Chimica Acta*. 2004; 522(2): 169-177.

[77] Shen J, Shao X. A comparison of accelerated solvent extraction, Soxhlet extraction, and ultrasonic-assisted extraction for analysis of terpenoids and sterols in tobacco. *Analytical and Bioanalytical Chemistry*. 2005; 383(6): 1003-1008.

[78] Howard L, Pandjaitan N. Pressurized liquid extraction of flavonoids from spinach. *Journal of Food Science.* 2008; 73(3): C151–157.

[79] Erdogan S, Ates B, Durmaz G, Yilmaz I, Seckin T. Pressurized liquid extraction of phenolic compounds from Anatolia propolis and their radical scavenging capacities. *Food and Chemical Toxicology.* 2011; 49(7): 1592-1597.

[80] Zougagh M, Valcárcel M, Ríos A. 2004. Supercritical fluid extraction: a critical review of its analytical usefulness. *Trends in Analytical Chemistry*. 2004; 23(5): 399-405.

[81] Ndiomu DP, Simpson CF. Some applications of supercritical fluid extraction. *Analytica Chimica Acta*. 1988; 213: 237-243.

[82] Inczedy J, Lengyel T, Ure AM. 1998. Supercritical Fluid Chromatography and Extraction. Compendium of Analytical Nomenclature (Definitive Rules 1997), third ed. Blackwell Science.

[83] Sihvonen M, Järvenpää E, Hietaniemi V, Huopalahti R. Advances in supercritical carbon dioxide technologies. *Trends in Food Science and Technology*. 1999; 10(6-7): 217-222.

[84] Temelli F, Güçlü-Üstündag Ö. 2005. Supercritical Technologies for Further Processing of Edible Oils. Bailey’s Industrial Oil and Fat Products. John Wiley & Sons, Inc. <http://dx.doi.org/10.1002/047167849X>.

[85] Lang Q, Wai CM. Supercritical fluid extraction in herbal and natural product studies—a practical review. *Talanta*. 2001; 53(4): 771-782.

[86] Hawthorne SB, Yang Y, Miller DJ. 1994. Extraction of organic pollutants from environmental solids with sub- and supercritical water. *Analytical Chemistry*. 1994; 66(18): 2912-2920.

[87] Raverchon E, Marco ID. Review: supercritical fluid extraction and fractionation of natural matter. *Journal of Supercritical Fluids*. 2006; 38(2): 146-166.

[88] Lang Q, Wai CM. Supercritical fluid extraction in herbal and natural product studies- a practical review. *Talanta*. 2001; 53(4): 771-782.

[89] Saldaña MDA, Mohamed RS, Baer MG, Mazzafera P. 1999. Extraction of purine alkaloids from maté (Ilex paraguariensis) using supercritical CO2. *Journal of Agricultural and Food Chemistry.* 1991; 47(9): 3804-3808.

[90] Verma A, Hartonen K, Riekkola ML. Optimisation of supercritical fluid extraction of indole alkaloids from Catharanthus roseus using experimental design methodology- comparison with other extraction techniques. *Phytochemical Analysis*. 2008; 19(1): 52-63.

[91] Giannuzzo AN, Boggetti HJ, Nazareno MA, Mishima HT. Supercritical fluid extraction of naringin from the peel of citrus paradise. *Phytochemical Analysis*. 2003; 14(4): 221-223.

[92] Khorassani MA, Taylor LT. Sequential fractionation of grape seeds into oils, polyphenols, and procyanidins via a single system employing CO2-based fluids. *Journal of Agricultural and Food Chemistry*. 2004; 52(9): 2440-2444.