# 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.

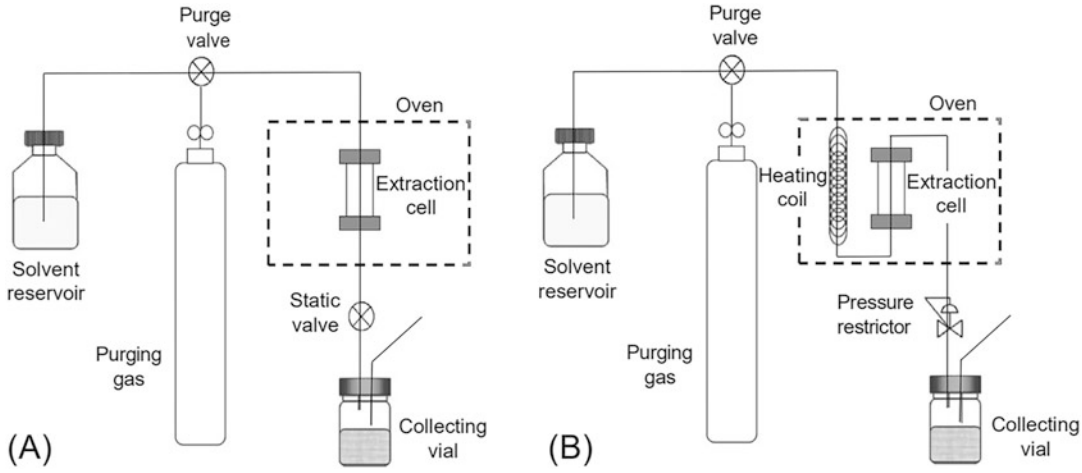


**Fig. 1** Representative diagram of a pressurized liquid extraction (PLE) system showing configurations for the development of static system (**a**) and a dynamic system (**b**)

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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