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Supercritical fluid extraction as a novel technology for extraction of bioactive compounds: A review

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Abstract

Supercritical fluid extraction is the most effective and efficient way to extract valuable constituent botanicals. Supercritical Fluid Extraction (SFE) is the process of separating one component (extractant) from another (matrix) using supercritical fluids. Supercritical fluids are highly compressed gases, which have combined properties of gases and liquids in an intriguing manner. Supercritical fluids can lead to reactions, which are difficult or even impossible to achieve in conventional solvents and the process can be completed within 10 to 60 minutes, supercritical fluid can be separated from analyte by simply releasing pressure, leaving almost no trace and yields a pure residue. CO₂ is the leader of extraction solvents for botanicals. The extraction conditions for supercritical CO₂ are above the critical temperature of 31 °C and critical pressure of 74 bar. Supercritical fluid extraction (SFE) has been successively applied in different industries such as food, pharmaceutical, cosmetic, where a sustainable and “green” extraction is desired. The selectivity, low applied extraction temperatures, and in many cases short processing times, have led to the extensively use of this technique in extraction process. The objective of this review is to critically analyze traditional and new directions in the research on natural matter separation by supercritical fluids extraction.

Keywords: Supercritical fluid, extraction, CO₂, bioactive compounds, fluid

1. Introduction

The recovery of high-value biomolecules necessitates efficient, inexpensive and environmental-friendly processing of plant sources. The bioactive ingredients from food and agro-industrial sources possess appealing therapeutic properties and exhibit several health benefits (Hamed *et al.*, 2015; Liu, 2013; Singh *et al.*, 2016). Amongst the different classes of bioactives, polyphenolic bioactives have various applications in food, flavoring industries, cosmetics, and pharmaceutical sectors (Chhikara *et al.*, 2018). Their multiple biological functional activities can be efficiently employed in the health sector and medical sciences (Cot *e et al.*, 2010; Rengasamy *et al.*, 2020; Wijesinghe & Jeon, 2011).

Conventionally, organic solvent-based extraction (hydro-thermal) processes are predominantly being employed in the food industry to extract nutritionally important food supplements. Although conventional methods have been performing reasonably well, solvent-based are time consuming and result in low extraction yield with poor extract quality due to the inevitable presence of traces of organic solvents in them (Ameer *et al.*, 2017; Azmir *et al.*, 2013; Barba *et al.*, 2016). Therefore, over the last few decades, concerted efforts have been made to develop novel techniques that can overcome these limitations while improving extraction efficiency. Various innovative approaches have been developed, comprising EAE, SFE, microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) (Bagade & Patil, 2019; Dzah *et al.*, 2020; Essien *et al.*, 2020; Gligor *et al.*, 2019; Patil and Yadav, 2018). Most of these non-conventional extraction techniques facilitate the disruption of a complex polysaccharide network made up of pectin, cellulose, lignin, proteins, and hemicelluloses in order to release the bioactive molecules entrapped within it, which ultimately improves the extraction yield (Garcia *et al.*, 2020). However, individual operation of these methodologies requires consideration of opportunities that they provide while addressing the obstacles that hamper their progress.

UAE and MAE are ineffective in extracting the thermo-sensitive and oxidation-sensitive bioactive compounds due to the generation of local heat and internal pressure. On the other hand, in SFE, supercritical carbon dioxide (SC-CO₂) is not easily penetrated through the

complex cell wall structure, which drastically affects the overall extraction efficiency. Although other experimental parameters (particle size, temperature, pressure, exposure time, etc.) can be tuned to enhance the overall extraction process, it is still a challenge to obtain the enhanced yield considering the biochemistry of cell wall and binding between bio active compounds and cell matrix. Therefore, there is a need for integral approaches that could lay an innovative and efficient platform for the extraction technology (Bezerra *et al.*, 2020; del Pilar *et al.*, 2017). The integration of advanced non-conventional methods will open new avenues while enhancing the efficiency of the overall extraction methodology. In this context, the extraction of bioactive compounds by a supercritical system (SCS) can be a highly effective system capable of attaining the extraction of bioactive compounds without affecting their overall biological properties (Wen *et al.*, 2019). This approach offers several advantages: Improves the breakdown of polysaccharide networks around the bioactive molecules followed by non-polar based SFE considerably with enhanced extraction yield, operates in mild conditions, which help to retain the biological properties of extracted compounds, results in relatively pure extracted bioactive and safer, greener, and suitable system for the extraction of oxidation-sensitive plant material. The present review provides an insight into the implementation of a combined/sequential approach of enzyme and SCS to extract various bioactive from plant sources.

2. History or background of SFE

In 1822, the initial discovery of the supercritical phase was realized by Baron Charles Cagniard de la Tour, who noticed changes in solvent behavior at a particular value of pressure and temperature. The term "critical point" was then coined by Thomas Andrews in 1869 as a result of his experiments on the effect of temperature and pressure on sealed glass tube of partly liquefied carbonic acid. He described it as the endpoint of the phase equilibrium curve which the critical temperature (T_c) and critical pressure (P_c) reached when the existence of two phases disappeared. A few years later, Hannay and Hogarth discovered SFE method application and the fundamentals of this technology using CO_2 in the supercritical state were developed in 1960. The earliest practical application of supercritical fluids was the decaffeination of green coffee beans started in Germany; after a few years, the extraction of oils from hops using liquid CO_2 was developed in Australia. By the 1980s, industrial applications of both technologies were developed and effectively adopted in different countries. Currently, various products are being produced using the technology and are accepted all over the world (Sapkale *et al.*, 2010) [25].

3. Differences between SFE and other extraction methods

Commercial large-scale extraction of seed oils is typically based on solvent extraction or mechanical extraction. Mechanical pressing is commonly employed to extract oil from oilseeds and is dependent on pretreatment of raw seed material (Mildner *et al.*, 2019) [17]. However, mechanical pressing processes have low extraction efficiency (<70% oil is extracted). Solvent extraction involves using organic solvents such as hexane on a commercial scale. Analytical scale SFE uses a variety of organic co-solvents such as petroleum ether, chloroform, acetone, etc. due to the possibility of extraction of certain compounds, which are more soluble in such solvents

than CO_2 . The choice of solvent media influences the relative amounts of nonpolar and polar lipids extracted from the seeds. The Soxhlet extraction method employed on an analytical scale is a standard analysis sample preparation method used in the laboratory. However, Soxhlet extraction has serious drawbacks including, long extraction times (Fakhfakh *et al.*, 2019). To remove these disadvantages, there has been an increasing tendency recent decade to utilize alternative extraction techniques for the extraction of seed oils (Al Juhaimi *et al.*, 2019) [1]. Aside from the previously-mentioned hemp (*Cannabis sativa*) flower extraction, hemp seed oil is a has been extracted for its unique ratio of omega-6/omega-3 fatty acid triglyceride content. In order to evaluate potential extraction methods for hemp seed oil for the oleochemical industry, supercritical fluid extraction, Soxhlet, percolation, coupled with ultrasonication and pyrolysis, pretreatment processes were studied by Devi & Khanam (2019) [10]. A central composite design was employed to optimize the parameters for the supercritical fluid extraction and ultrasonication processes. Quadratic models were developed with satisfactory R^2 (>0.93). A maximum yield of 37.30% was attained through the ultrasonication combined with Soxhlet extraction. However, economic analyses for different processes revealed the supercritical fluid extraction as the best choice followed by liquid Soxhlet solvent extraction, and then ultrasonication combined with other modes of extraction.

3.1 Supercritical fluids

Supercritical fluids are chemical solvents that can be compressed above their critical point, are generally considered environmentally friendly, and are commonly used in the extraction process because they provide excellent results due to their unique characteristics. SCF is used as a replacement for organic solvent in laboratory processes and various industries such as food, pharmaceuticals, agriculture, and cosmetics.

A fluid is regarded as supercritical when its pressure and temperature are beyond its critical points, that is, critical pressure (T_c) and critical temperature (P_c) (Fig.1.). In this state, the fluid is represented by both its gas and liquid phase properties in an advantageous way. Their actions are close to that of gas in some ways, but close to liquid in others (Gopaliya *et al.*, 2014) [14].

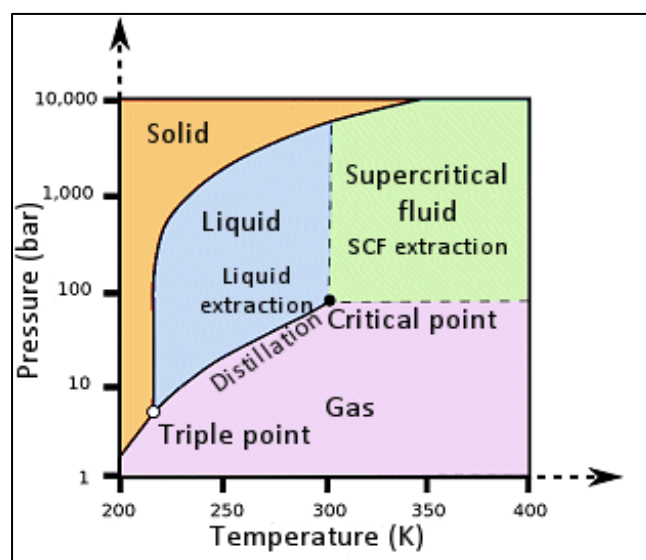


Fig 1: Phase Diagram showing supercritical fluid region

4. Formation of Supercritical Fluids

The formation of a supercritical fluid is the result of a dynamic equilibrium. When a material is heated until its specific critical temperature in a closed system, at constant pressure, a dynamic equilibrium is generated and this equilibrium includes the same number of molecules coming out of liquid phase to gas phase by gaining energy and going in to liquid phase from gas phase by losing energy. At this particular point, the phase curve between liquid and gas phases disappears and supercritical material appears (Fig.1.). In theory, supercritical region can be reached in two ways, by increasing the pressure above P_C value of the material by keeping temperature stable and then increasing the temperature above T_C value at a stable pressure value. The other way is increasing the temperature first above T_C value and then increasing the pressure above P_C value (Rodrigues *et al.*, 2003) [23]. Supercritical fluids have gas like transport properties and their diffusivities are one or two orders of higher magnitude than that of liquids. Therefore, extractions from solids can be fast.

- They have very high “liquid like” densities which increases their dissolving power to those approaching liquids.
- They have viscosities as low as gases, and they have essentially zero surface tension which allows them to easily penetrate into microporous structures. After the extraction, their high volatility at regular pressures enables them to be readily separated from the solute with little or no residue.
- They are energy and safety efficient, particularly with foods.
- Another powerful aspect to supercritical fluid extraction (SFE) is the ability to precisely control which component(s) in a complex matrix are to be extracted and which ones should be left behind. This is accomplished through precise control of several key parameters such as temperature, pressure, flow rates and processing time (Rizvi *et al.*, 1998).

5. Supercritical fluid extraction

Extraction can be defined as the removal of soluble material from an insoluble residue, either liquid or solid, by treatment with a liquid solvent. Extraction involves the separation of medicinally active portions of plant or animal tissue from the inactive or inert components using selective solvents in standard extraction procedures. Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. It is used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or

collect a desired product (e.g. essential oils). A simple SFE consists basically of two major steps:

- (1) Extraction of the soluble substances from the solid substratum by the SCF solvent
- (2) Separation of these compounds from the supercritical solvent after the expansion (Rizvi *et al.*, 1998).

6. Supercritical fluid extraction process

First, the solvent is fed into heat exchanger to maintain its liquid form and feed/ sample after pre-treatment is directly added to the extractor to form a fixed bed of solid substratum. Supercritical fluid (solvent) from heat exchanger passes to the pump, where it is exposed to high pressure and is pumped into the oven to heat it at higher temperatures. Solvent is then uniformly distributed throughout the fixed bed formed by the solid substratum. During the extraction, the solvent flows through the fixed bed and dissolves the soluble compounds. The solute–solvent mixture is separated in the flash tanks by rapidly reducing the pressure, increasing the temperature, or both. The solvent is cooled (cooler) and recompressed (compressor) and returns to the extractor.

For all of them, mass transfer resistance should be avoided or reduced to increase the extraction rate. The extraction phenomenon can be described as follows:

- 1) The solid substratum absorbs the supercritical solvent, promoting the dilatation of the cellular structures. This step facilitates the solvent flow by decreasing the mass transfer resistance.
- 2) Concurrently, the soluble compounds are dissolved by the solvent.
- 3) The dissolved compounds are transferred by diffusion to the surface of the solid.
- 4) The compounds are transported by the solvent and then removed from the extractor.

The knowledge of the best conditions is essential for efficient extraction of desired compound. This involves the study of global yield and/or solubility and kinetic parameters. The global yield is defined as the maximum amount of solute that can be extracted from specific solid substratum under given conditions of temperature and pressure. It is the yield obtained in an exhaustive process. Global yield isotherms provide a framework for analyzing the effects of temperature and pressure on the extraction. This analysis can be made in terms of the global yield of the extraction as a whole or of the global yield of a specific compound.

7. Components of supercritical fluid extraction apparatus

The system must contain a pump for the CO_2 , a pressure cell to contain the sample, a means of maintaining pressure in the system and a collecting vessel.

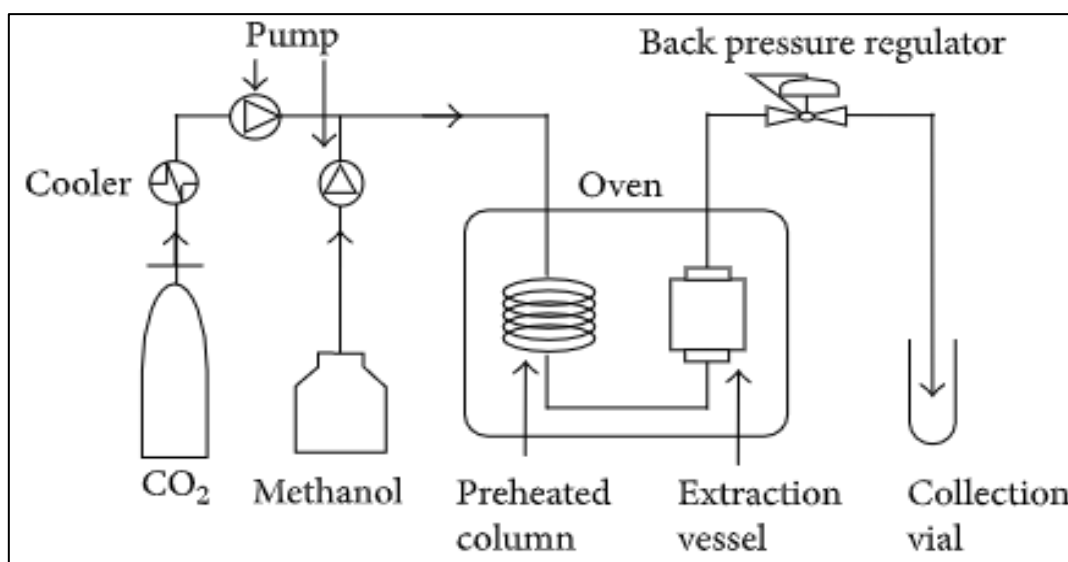
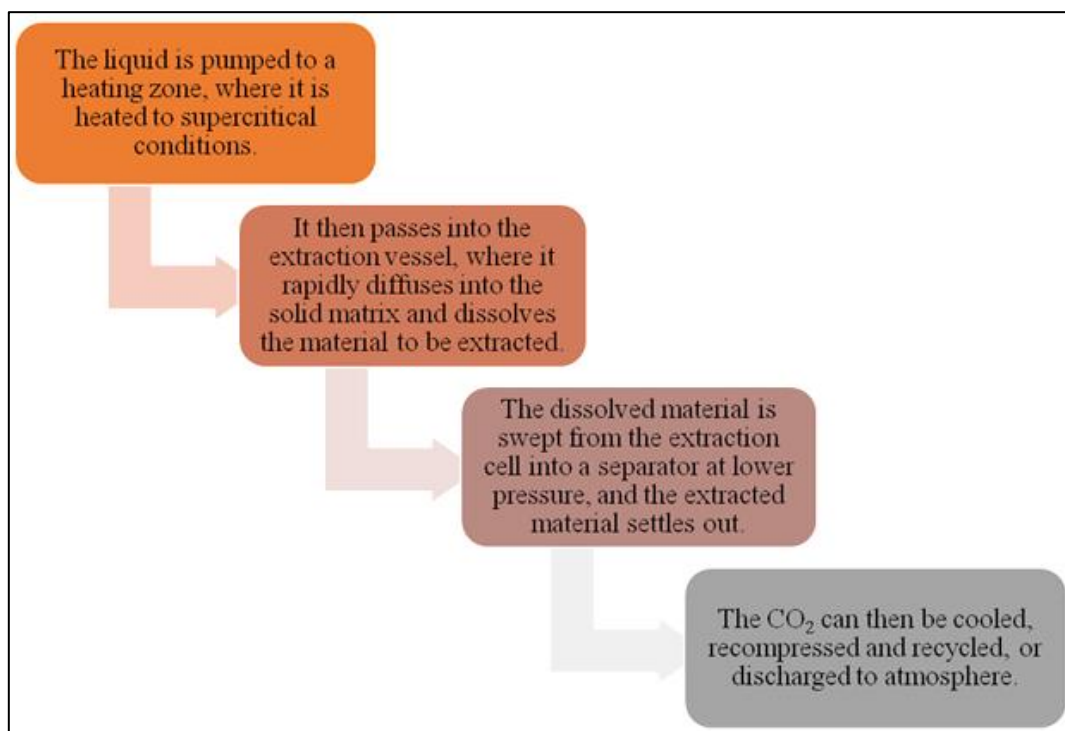


Fig 2: Supercritical Fluid Extraction Apparatus

- Solvent Reservoir:** It is generally in the form of cylinder when CO₂ is used as the extracting solvent.
- Cooler/ Heat Exchanger:** The fluid is cooled before pumping to maintain liquid conditions. This is generally done using a heat exchanger or a cooler.
- Pumps:** Carbon dioxide is usually pumped as a liquid, usually below 5 °C with a pressure of about 50 bars. The solvent is pumped as a liquid as it is almost incompressible in liquid form. As a supercritical fluid, much of the pump stroke will be "used up" in compressing the fluid, rather than pumping it. For small-scale extractions (up to a few grams/ minute), reciprocating CO₂ pumps or syringe pumps are often used. For larger scale extractions, diaphragm pumps are most common. The pump heads will usually require cooling, and the CO₂ will also be cooled before entering the pump.
- Co-solvent reservoir and an additional pump:** There is an additional reservoir. Additionally, there is another pump which holds co-solvent or modifier that is sometimes needed to extend the solvent capabilities, allowing the extraction and recovery of more polar compounds.
- Pressure vessels and pre heating column:** Pressure vessels can range from simple tubing to more sophisticated purpose-built vessels with quick release fittings. The pressure requirement is at least 74 bars, and most extractions are conducted at less than 350 bar. However, sometimes, higher pressures will be needed, such as extraction of vegetable oils, where pressures of 800 bars are sometimes required for complete miscibility of the two phases. The vessel must be equipped with a means of heating. It can be placed inside an oven for small vessels, or oil or electrically heated jacket for larger vessels.
- Back Pressure Regulator:** The pressure in the system

must be maintained from the pump right through the pressure vessel. In smaller systems (up to about 10 mL/min), a simple restrictor can be used. This can be either a capillary tube cut to length, or a needle valve, which can be adjusted to maintain pressure at different flow rates. In larger systems a back-pressure regulator will be used, which maintains pressure upstream of the regulator by means of a spring, compressed air, or electronically driven valve. Whichever is used, heating must be supplied, as the adiabatic expansion of the CO₂ results in significant cooling. This is problematic if water or other extracted material is present in the sample, as this may freeze in the restrictor or valve and cause blockages.

- 7. Collection Vessel/ Vial:** The supercritical solvent is passed into a vessel at lower pressure than the extraction vessel. The density and dissolving power, of supercritical fluids varies sharply with pressure, and hence, the solubility in the lower density CO₂ is much lower, and the material precipitates for collection. It is possible to fractionate the dissolved material using a series of vessels at reducing pressure. The CO₂ can be recycled or depressurized to atmospheric pressure and vented. For analytical SFE, the pressure is usually dropped to atmospheric level, and now the gaseous carbon dioxide is bubbled through the solvent to trap the precipitated components.

8. Supercritical Fluid Extraction Modes

8.1 Supercritical fluid extraction can be carried via three different modes namely, static, dynamic and combined

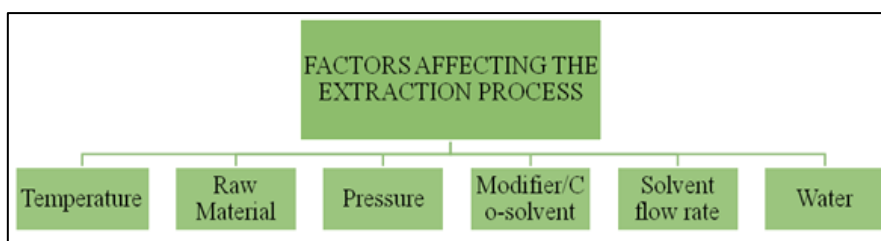
Under static conditions, the supercritical fluid is introduced in the extraction vessel and is kept in contact with the sample for a given extraction time. Once the desired time is achieved, the extract is released through the pressure restrictor to the trapping vessel.

On the other hand, in a dynamic process, the supercritical fluid continuously enters the extraction vessel and flows through the sample to the separators for a cascade fractionation.

In the combined mode, a static extraction is performed for a period of time, and subsequently a dynamic extraction is carried out. Medium and large scale SFE are generally carried out in dynamic conditions: the supercritical solvent flows through the solid material extracting the target compounds until the substrate is depleted. On the other hand, liquid samples, according to the design of the extractors, are commonly extracted in a continuous mode (Sovilj *et al.* 2011).

9. Factors affecting the extraction process

The most important parameters for the SFE are temperature, pressure, CO₂ flow rate, the seed particle size, co-solvent addition, and extraction time. A summary of the influence of these parameters on the extract yield in SFE.



- 1. Temperature:** The control of the temperature in SFE is accomplished utilizing a thermostatted bath or chamber or by the use of concentric fluid heat exchange tubing. Control of temperature is important in the extraction of seed oils, since their extraction is very sensitive to temperature variations, i.e., temperature influences the solubility of the seed oils as well as minimize their oxidation which is otherwise induced by higher temperatures. Typical temperature ranges used for many seed oil extractions range from 40 °C to 80 °C. For example, increasing of temperature just from 50 °C to 60 °C increase the oil recovery on a mass basis from 7.6% to 12.2% (w/w) (Gustinelli *et al.*, 2018). Temperature does not always have a direct relationship with SFE efficiency. In fact, temperature has a dual impact on the yield of SFE, depending on the type of seed, targeted compounds and pressure. Increasing the temperature decreases the CO₂ density, resulting in the decrease of the solubility of oil in SC-CO₂ up to the cross-over point of the seed oil yield versus extraction pressure as various isotherms, due to raise of the vapor pressure of the oil (Montan[~]es *et al.*, 2018). Increasing the extraction pressure can have an augmenting effect with temperature on the SFE efficiency. Increasing the temperature, at constant pressure, promotes two opposite effects: it reduces the solvent

power of CO₂ by a decrease of the density, and, on the other hand, it increases the vapor pressure of solutes which can be more easily transferred to the supercritical phase.

- 2. Raw Material (Particle Size, Porosity):** Despite the raw material normally being imposed on the process, there are several factors to take into account. The influence of the physical state of the sample (solid, liquid) on the outcome of the extraction is well known, when dealing with solid samples, other factors such as particle size, shape, and porosity of the solid material are of crucial importance since they have direct effects on the mass transfer rate of the process. In order to increase the extraction rate, the solid matrix must be comminuted to increase the mass transfer area. On the other hand, too small particles must be avoided as their use can compact the bed, increasing the internal mass transfer resistance and causing channeling inside the extraction bed. As a result, the extraction rate decreases due to a non-homogeneous extraction.

In the case of liquid samples two main strategies are used, to trap the liquid on a solid support (e.g. sepiolite) and to treat it like a solid and to perform column countercurrent extractions. The first strategy is mainly used at a small scale since the employment of solid supports can increase the extraction costs. During,

countercurrent extraction, the liquid sample is continuously added on a column by the top or the middle point, while the supercritical phase is supplied by the bottom point. This strategy has been very useful for oil refining.

3. **Pressure:** Increase in pressure of supercritical medium, increases the density of the medium. Higher the density, higher the solubility of solute. Thus, given extraction can be performed using small volumes of fluid.
4. **Modifier/ co-solvent:** CO₂ is largely the most used solvent to perform SFE. From the point of view of natural products extraction, its main drawback is its low polarity, which seriously limits its ability to extract polar components from the raw material. As for many other substances, its dielectric constant may change with density, but even at high densities, CO₂ has a limited ability to dissolve high-polarity compounds. To address this problem, small amounts of co-solvents (modifiers) are added to the CO₂ stream. The addition of modifiers to CO₂ can improve the extraction efficiency by raising the solubility of the solutes. Two mechanisms have been proposed by Pereira and Meireles to explain the effects:
 1. Solute-co solvent interactions, caused by increase in solvent polarity;
 2. Matrix swelling that facilitates the contact of the solute by the solvent.

The effect is not only dependent on the nature of the modifier used, but also on the type of matrix, and the target solutes. As a general rule, the amount of modifier used is lower than 10–15%. The most used modifiers are methanol, ethanol, and water.

5. **Solvent Flow Rate (Solvent-to-Feed Ratio):** Solvent to feed ratio (S/F) is the most important parameter for supercritical fluid extraction, once the extraction pressure and temperature have been selected. Solvent flow rate must be high enough to provide a good extraction yield in short time, but it should also grant enough contact time among solvent and solutes. Moreover, it must be considered that higher solvent flow rate promotes an elevation of the operational and capital costs, which should be carefully studied for industrial applications. In

general, it is common to use S/F ratios around 25-100:1 for analytical and 5-15:1 for large-scale processes.

6. **Water:** Water content is one of the key aspects in determining the quality of the output of SFE processes. Water is considered to be soluble at approximately 0.3% v/v in supercritical CO₂. The presence of water, however, may either assist in or be an impediment to the diffusion of supercritical carbon dioxide; what is necessary for effective extractions depends on the type of compounds targeted. It serves to increase the polarity of the fluid and enable higher recoveries of relatively polar species. However, if excess water remains in the vessel, a highly water-soluble analyte will prefer to partition into the aqueous phase and its SFE recovery will be low. For example, the moisture content of paprika is reported to be as high as 85% and SFE extractions result in extremely low yields (Mendiola *et al.*, 2013) [16].

10. Solvents used in supercritical fluid extraction

1. **Carbon Dioxide:** Carbon dioxide is the most commonly used solvent in SFE because it is safe, non-toxic, and generally available at a reasonable cost. It has low critical temperature (31 °C), thus is efficient in extraction of components that are sensitive to extreme conditions. In the supercritical state, carbon dioxide behaves as a lipophilic solvent and so, is able to extract most non-polar solutes. It is generally used for separating antioxidants, pigments, flavors, fragrances, fatty acids, and essential oils from plant and animal materials.
2. **High Pressure water:** High pressure water comes under the category of 'green solvents'. It exploits the physicochemical property changes of water to extract the desirable solutes. As, increase in temperature destroy the structure of water, this phenomenon reduces their dielectric permittivity's and makes them less polar and tight, and facilitates the dissolution of non-polar substances in them. It is generally used for the extraction of compounds like pectins, lignin's, proteins, carbohydrates, saponins, cyclopeptides, and anthraquinones (Budisa & Schulze-Makuch, 2014) [3].

Table 2: Different solvents used in SFE and their critical properties

Solvent	Critical Temperature (K)	Critical Pressure (MPa)	Critical Volume (cm ³ / mol)
Ammonia	405.4	11.35	72.5
CO ₂	304.1	7.37	94.1
Dimethyl ether	400.1	5.27	171
Ethane	305.3	4.87	145.5
Ethylene	282.3	5.04	131
Methanol	512.6	8.09	118
n-Hexane	507.5	3.02	368
Propane	369.8	4.25	200
Water	647.1	22.06	55.9

11. Supercritical fluid fractionation

Fractionation refers to the separation process in which one or more components of a mixture (gas, solid, liquid, enzymes, suspension, or isotope) is divided during a phase transition, into a number of smaller quantities (fractions) with the employment of a miscible or immiscible solvent. In the

process of fractionation, after exiting the batch extractor, the mixture goes through a series of separator vessels in a multistage operation allowing selective and sequential fluid extraction. Each of the separators is set at a very specific pressure, allowing only one component of the mixture to drop out.

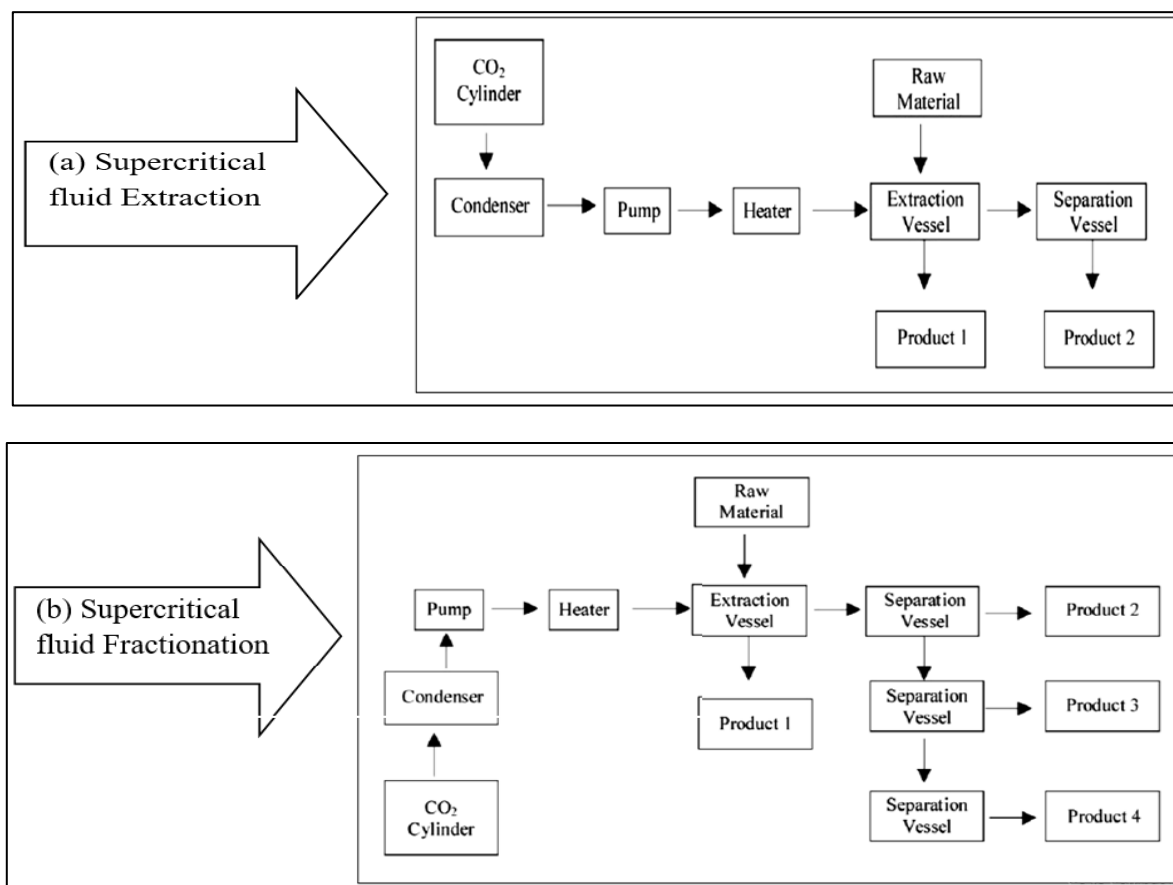


Fig 3: Supercritical fluid extraction (a) v/s Supercritical fluid fractionation (b)

12. Applications of supercritical fluid extraction in food processing industry

Supercritical carbon dioxide has attractive properties (it is nontoxic, inexpensive, odorless, colorless, non-flammable and has near ambient critical temperature, low viscosity and high diffusivity compared to liquids) that it has become the preferred solvent in the processing of essential oils and oils in food industry. Further, the extracts color, composition, odor, texture are controllable and extraction by carbon dioxide retains the aroma of the product. Supercritical fluid extraction is used as a replacement for hexane in extracting soybean-oil and has been tested for extraction from corn, sunflower and peanuts. Supercritical fluid extraction provides a distinct

advantage not only in the replacement but also extracts oils that are lower in iron and free fatty acid. Another application is removal of fat from food (Perrut, 2000) [21]. The process has been fully designed for commercial application, using the aforementioned standard design. The removal of fat process has the advantage of producing fat-free or fat reduced potato chips. According to the expected taste the amount of remaining fat in the potato chips can easily be controlled by SFE. A large amount of research has been concentrated on the decaffeination of coffee by supercritical carbon dioxide. Thus, it is not surprising to note that this was the first process to be commercialized (Dunford *et al.*, 2000) [11].

Table 3: Industrial applications of SFE

Natural sources	Temperature (°C)	Pressure (bar)	Identified molecule	Remarks
Tea leaves	35-38	248	Caffeine	Decaffeination of tea leaves
Tomato skin and seeds	60	300	E-lycopene	86% recovery of E-lycopene
Agricultural by-product	35	140	Lignin derived bioactive compounds (catechins)	Relatively poor extractions yields of flavonoids
Orange Peel	35	131	Oxygenated compounds	Used as a second step to recover flavor compounds
Passion fruit bagasse	50-60	170-260	Tocopherols, unsaturated fatty acids and carotenoids	Increased retrieval of bioactives
Olive husk	40-60	205-250	Tocopherols, carotenoids & chlorophylls	Increase the recovery of bioactive compounds 2-4 times
<i>Coffea arabica</i>	35.9	331	Palmitic, linoleic, oleic, stearic and arachidic acid, furans and pyrazine	Recovery of volatile compounds from coffee beans

12.1 Advantages of SFE

1. Supercritical fluids have a higher diffusion coefficient and lower viscosity than liquids.
2. Absence of surface tension allows for their rapid penetration into the pores of heterogeneous matrices, which helps enhancing their extraction efficiencies.
3. Selectivity during extraction may be manipulated by varying the conditions of temperature and pressure affecting the solubility of the various components in the supercritical fluid.
4. Supercritical fluid extraction does not leave a chemical residue.
5. Supercritical fluid extractions can use carbon dioxide gas, which can be recycled and used again as part of the unit operation.

12.2 Disadvantages of SFE

- Elevated pressure required.
- Compression of solvent requires elaborate recycling measures to reduce energy costs.
- High capital investment for equipment.
- Technical knowledge of SCF properties required.
- Lower extraction yield of non-polar solutes.

13. Advances in supercritical fluid extraction

13.1 Ultrasound-assisted Supercritical Fluid extraction:

Ultrasonic waves are able to generate mechanical deformation in solid, liquid and gaseous media and are characterised by a frequency range from 20 kHz to 10 MHz. It is considered to be an environmentally sustainable addition to existing extraction methods by providing cleaner extracts while using less solvent and shorter extraction times when compared to conventional methods. Ultrasound techniques have been employed in SFE as a sample pre-treatment step and also during the SFE process (Rombaut *et al.* 2014). Waterbath-based systems are a means of using ultrasound during SFE (placing the extraction vessel without heating wires directly into a sonicating bath), while the use of an ultrasonic probe would be used as a pre-treatment process. There are SFE systems that are capable of housing a sonotrode, however, there does not appear to be literature available regarding its use in SFE. Both ultrasound processes, either used as a pre-treatment or by using a waterbath, were reported to enhance the extraction rate and yield through mechanical stirring and the main driving force may be attributed to cavitation phenomena (Chemat *et al.* 2011).

Applications of Ultrasound-assisted SFE

Description of study	Process conditions	Major findings	References
Extraction of oil from particulate almonds	Pressure:28MPa; flow rate:20kg/h, temperature: 55 °C, power: 50W	Enhancement of the extraction kinetics and yields with an average extraction time of 8.5 h	Riera <i>et al.</i> , 2004 [22].
Oil extraction from passion fruit (<i>Passiflora edulis</i> sp.) seeds	Temperature: 40 °C, pressure: 16 MPa, power: 160W	Increase of the oil extraction yield by 29%.	Barrales <i>et al.</i> , 2015 [2].
Extraction and removal of caffeine from green tea.	Pressure :30 MPa, temperature: 55 °C, extraction duration: 4 h, moisture content: 30%, and ultrasound power of 100W	The removal of caffeine from green tea without damaging the structure of the active ingredients.	Tang <i>et al.</i> , 2010.
Extraction of antioxidant compounds from blackberry (<i>Rubus sp.</i>) bagasse (industrial by-product).	Temperature (40, 50 and 60 °C), pressure (15, 20 and 25 MPa), and ultrasound power (0, 200 and 400 W).	Extraction rate increased at the beginning of the process. Higher antioxidant activity and phenolic contents were obtained with higher temperatures.	Reategui <i>et al.</i> , 2014.
Pasteurization of fresh-cut coconut spiked with <i>Salmonella enterica</i> Typhimurium.	Pressure: 12 MPa and US power: 10 W, delivered every 2 min of treatment, as a function of temperature (from 24 to 50 °C) and treatment time (from 5 up to 30 min).	Microbial inactivation was more efficient using US-SFI, compared to CO ₂ applied alone.	Ferrentino <i>et al.</i> , 2015 [13].
Inactivation of <i>E. coli</i> in nutrient broth, apple and orange juices.	Pressures of 10, 22.5, 29 and 35 MPa; 36 °C, and temperatures of 31 °C, 36 °C and 41 °C; 22.5 MPa.	When combining ultrasound with sc-CO ₂ , the time needed to reach the same inactivation level (than sc-CO ₂ alone) was reduced by 95%.	Ortuno <i>et al.</i> , 2012 [20].

13.2 Membrane-assisted Supercritical Fluid extraction:

The membrane-based dense gas extraction process is a commercial supercritical fluid extraction (SFE) that uses a hollow fiber membrane contactor (HFMC). This system uses a macroporous membrane (porous size over 50 nm), which allows the contact between two fluids: a liquid solution on the lumenside and, a dense gas at the outside of the fiber or shellside. Apparatus consists of a hollow fiber contactor, housed in stainless-steel tubing operating in countercurrent. Sample solution as the liquid feed phase flows inside the

fiber, and SCF as the solvent extraction phase, flowed in countercurrent outside the fiber (see Figure 4). As the process proceeds, desired compound is then recovered by expansion using a valve from the extracting stream and raffinate is collected for analysis once the system reached the steady-state condition. This system is firstly loaded with SCF, and then the equalizing vessel is loaded with the aqueous feed solution. The role of the equalizing vessel is to ensure that both sides of the membrane have the same pressure (Cabezas *et al.* 2020) [4].

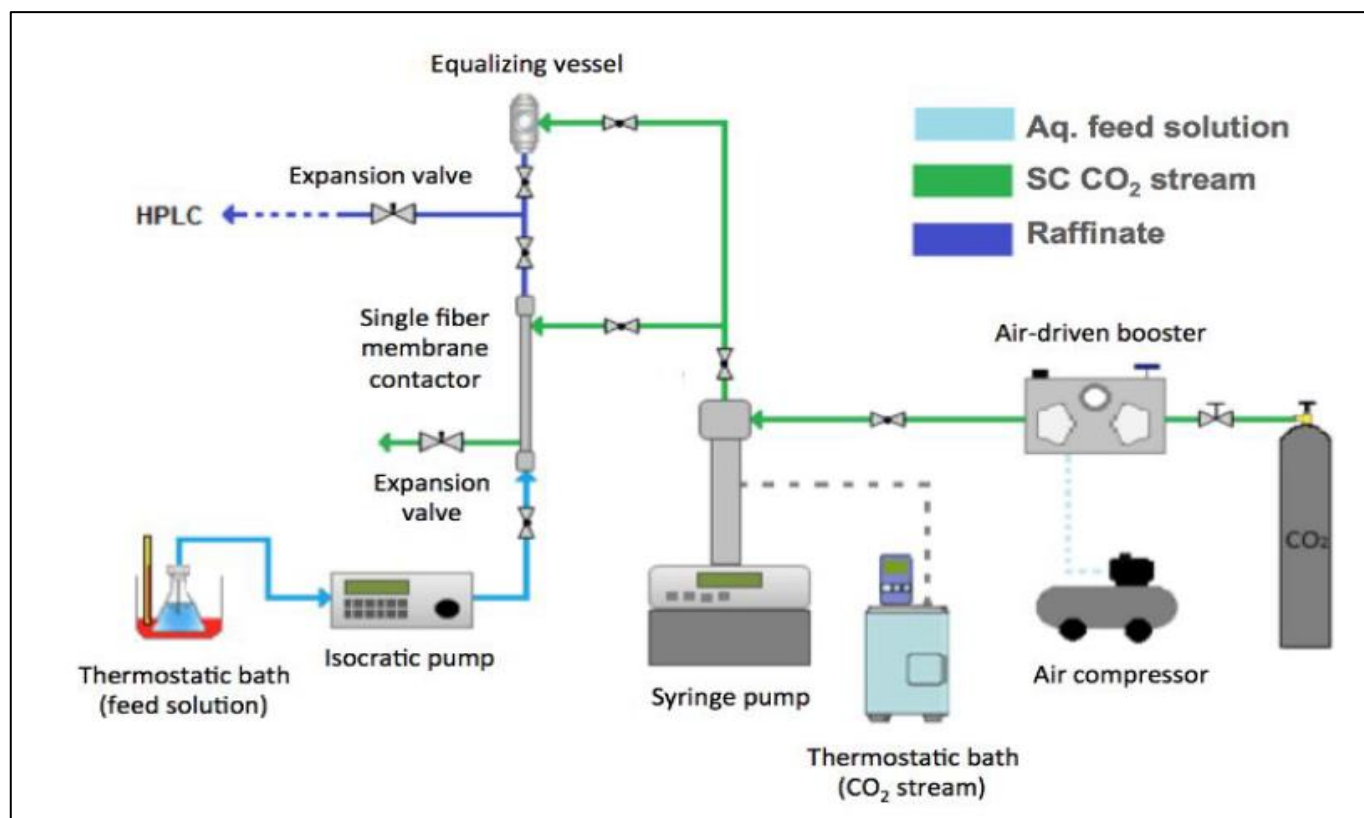


Fig. 4. Membrane-assisted Supercritical fluid extraction system

13.3 Enzyme-assisted supercritical fluid extraction:

Enzyme assisted extraction of biomolecules from plants is a potential alternative to conventional solvent extraction methods and is gaining more attention because of being an efficient, sustainable and eco-friendly extraction technology. Enzyme-based extraction depends on the characteristic property of enzymes to carry forward reactions with accurate specificity, region-selectivity and their ability to conduct reactions under mild conditions with the retention of their biological potentials of bioactive compounds (Puri *et al.*, 2012). The basic principle of enzyme assisted extraction is the disruption of plant cell wall by hydrolysing it using enzyme as a catalyst under optimum experimental conditions, in order to release the intracellular components. The plant cell wall binds to the active site of the enzyme. This causes the enzyme to change its shape so that the substrate fits onto its active site, thus causing maximum interaction between the two. Change in the shape of enzyme leads to breakage of bonds of the cell wall, thereby releasing the active constituents out of it (Sheldon and Van Pelt, 2013). These green extraction methods not only reduce the requirement of hazardous solvents but also require short extraction time. Additionally, as this extraction is carried out under controlled temperature condition, it is very useful for extraction of thermosensitive molecules such as flavours, pigments, oil, etc. A wide range of carbohydrate hydrolysing enzymes is generally used during enzyme assisted extraction. Recently, enzymes such as cellulases, pectinases, and hemicellulases have also been used to assist supercritical fluid extraction processes as they degrade the structural integrity of the plant cell wall through hydrolysis. For example, the use of α -amylase in pre-treatment of black pepper prior to supercritical fluid extraction increased the yield of extract and piperine-rich extract by 53% and 46%, respectively (Mushtaq *et al.*, 2017).

14. Conclusion

SFE is nowadays one of the most popular alternative methods for extracting valuable compounds from different natural raw materials such as plants, marine products, and agricultural by-products. Advantages of the use of such technology have been underlined as well as the parameters that can be modified to optimize the process in terms of yields and/or purity of the target compounds. Recent applications have been summarized, allowing us to identify both the target compounds and the key raw materials that have been studied lately. SFE has been recognized as an advantageous process from an environmental point of view, sustainability and eco-friendliness. Supercritical fluid extraction is favored due to its high selectivity, high efficiency, and short extraction time. Thus, in this review, factors influencing the supercritical fluid extraction process (temperature, pressure, and co-solvent) were discussed using examples. We assume that this technology will experience continued growth in the coming years and will be beneficial to sustainable development, given its green credentials, and will help to reduce the use of organic chemical.

15. References

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