ADVANCES IN SUPER CRITICAL FLUID EXTRACTION

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Abstract:

In this chapter, Super-Critical fluid Extraction a new separation technique developed in recent years is discussed. It is used to extract and separate substances using a supercritical fluid as a solvent. This chapter focuses on difference between super critical fluid extraction and other extraction techniques; Terminology related to Super critical fluid extraction; General principles of Extraction; Fundamentals of super critical fluid extraction; Super critical fluid; Properties of Super Critical Fluid; Parameters Affecting the Extraction Process**;** Advantages and drawbacks of supercritical fluid Extraction; Special applications of supercritical fluids to food processing; Recent advances and applications of supercritical fluid extraction

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1. INTRODUCTION

One area in food and chemical processing industries that is receiving increasing attention is extraction. Extraction or solvent extraction is the process of separating a component substance (the solute) from a solid or liquid mixture by dissolving it in a liquid solvent. This separation process involves two phases. The solvent is the material added to form a phase different from that where the material to be separated originally was present. Separation is achieved when the compound to be separated dissolves in the solvent while the rest of the components remain where they were originally. The two phases may be solid and liquid, immiscible liquid phases, or solid and gas. Depending on the phase of the mixture and the extraction agent, extraction can be divided into the following types:

- liquid - liquid extraction,
- solid - liquid extraction,
- supercritical 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. It is therefore, a solution process and depends on the mass transfer phenomena. The controlling factor in the rate of extraction is normally the rate of diffusion of the solute through the liquid boundary layer at the interface.

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. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. So, extraction continues to be of considerable interest in order to obtain improved yields of drug derived from plant and animal sources.

Extraction of spice oils and natural flavour extracts has also been practiced in the flavour industry. Interest in functional food additives used to fortify formulated food products has led to the development of extraction systems to separate useful ingredients from food processing waste and medicinal plants. Extraction is also used in the beet sugar industry to separate sugar from sugar beets. Sugar from sugar cane is separated by multistage mechanical expression with water added between stages. This process may also be considered a form of extraction.

Roller mills used for mechanical expression of sugar cane juice is capital intensive and when breakdowns occur, the down time is usually very lengthy. It is also an energy intensive process, therefore, modern cane sugar processing plants are installing diffusers, a water extraction process, instead of the multiple roller mills previously used. Extraction has been practiced in the vegetable oil industry for a long time. Oil from soybean, corn, and rice bran cannot be separated by mechanical pressing, therefore, solvent extraction is used for their recovery.

In other areas of the food industry, water extraction is used to remove caffeine from coffee beans, and water extraction is used to prepare coffee and tea solubles for freeze or spray drying. Supercritical fluid extraction has been found to be effective for decaffeinating coffee and tea and for preparing unique flavour extracts from fruit and leaves of plants.

2. TERMINOLOGY:

Extraction: Extraction is a contact equilibrium separation process where a solid material containing a solute of interest is brought into contact with a liquid solvent, time is allowed for the equilibrium to be reached and the target component to be transferred from the solid phase to the liquid phase and finally, the solid and liquid phases are separated by physical means. This also applies to liquid/liquid extractions

Supercritical: The term "supercritical" refers to a substance in a non-condensing and single-phase fluid when brought above its critical temperature (T_c) and critical pressure (P_c). Beyond this point, there is a supercritical region where the substance shows some typical physicochemical properties of gases or liquids, such as high density, intermediate diffusivity and low viscosity and surface tension.[63]

Supercritical Fluid: A fluid at temperature and pressure conditions above its critical point is referred to as a supercritical fluid, which is a dense fluid with interesting properties in between those of a gas and a liquid. Its density is similar to that of a liquid, while its viscosity and diffusivity are similar to those of a gas. Therefore, a supercritical fluid can act as a solvent similar to a liquid, but with enhanced mass transfer kinetics.

Supercritical fluid Extraction (SFE) principle: Supercritical carbon dioxide technology (SC-CO_2 technology) utilizes pressure in combination with carbon dioxide to destroy microorganisms without affecting the nutritional content, organoleptic attributes, being a promising alternative for pasteurization of bioactive compounds in food and medicine in which compounds would be destroyed by conventional thermal processes. [32] The driving force for any extraction process is the solubility of the target compound in the selected solvent, which depends on the interactions between the solvent and solute. Supercritical fluid extraction (SFE) has emerged as a superior alternative technique for extraction of bioactive species from natural produces, because of its reduction of extraction time, less consumption of organic solvents, being suitable for thermo-sensitive substance; production of cleaner extracts a environmental benignity. [68] SFE is based on the solvating properties of supercritical fluid (SF), which can be obtained by employing pressure and temperature above the critical point of a compound, mixture or element. By proper controlling of SFE parameters, the extractability of supercritical fluid can also be modified which enable this process to find its field from food to pesticide researches. Even though CO_2 is the preferred extraction solvent (for extracting non-polar compounds), the polarity of supercritical SC-CO_2 can be increased by the addition of a miscible polar compound (such as ethanol) as modifier. [65] Due to the selectivity involved in the SFE process, the extracts obtained by this technique possess low concentration of undesired compounds. [57] Besides, SC- CO_2 becomes gaseous after depressurization and can be easily eliminated from a flow system.

3. GENERAL PRINCIPLES OF EXTRACTION

1. **Diffusion:** Diffusion is the transport of molecules of a compound through a continuum in one phase, or through an interface between phases. In solid liquid extraction, the solvent must diffuse into the solid in order for the solute to dissolve in the solvent, and the solute must diffuse out of the solvent saturated solid into the solvent phase.

The rate of diffusion determines the length of time needed to achieve equilibrium between phases. The time required for diffusion to occur in order to reach equilibrium, is inversely proportional to the square of the diffusion path. Thus, in solvent extraction, the smaller the particle size, the shorter the residence time for the solids to remain within an extraction stage. Particle size, however, must be balanced by the need for the solvent to percolate through the bed of solids. Very small particle size will result in very slow movement of the solvent through the bed of solids, and increases the probability that fines will go with the solvent phase interfering with subsequent solute and solvent recovery.

In soybean oil extraction, the soy is tempered to a certain moisture content in order that they can be passed through flaking rolls to produce thin flakes without disintegration into fine particles. The thin flakes have very short diffusion path for the oil, resulting in short equilibrium time in each extraction stage, and solvent introduced at the top of the bed of flakes percolates unhindered through the bed. The presence of small particle solids is not desirable in this system because the fine solids are not easily removed from the solvent going to the solvent/oil recovery system. The high temperature needed to drive off the solvent will result in a dark coloured oil if there is a large concentration of fine particles.

Some raw materials may contain lipoxygenase, which catalyzes the oxidation of the oil. Extraction of oil from rice bran involves the use of an extruder to heat the bran prior to extraction to inactivate lipoxygenase. The extruder produces small pellets which facilitates the extraction process by minimizing the amount of fines that goes with the solvent phase.

In cane sugar diffusers, hammer mills are used to disintegrate the cane such that the thickness of each particle is not more than twice the size of the juice cells. Thus, equilibrium is almost instantaneous upon contact of the particles with water. The cane may be pre-pressed through a roller mill to crush the cane and produce very finely shredded solids for the extraction battery.

2. **Solubility:** The highest possible solute concentration in the final extract leaving an extraction system is the saturation concentration. Thus, solvent to solids ratio must be high enough such that, when fresh solvent contacts fresh solids, the resulting solution on equilibrium, will be below the saturation concentration of solute.

In systems where the solids are repeatedly extracted with recycled solvent (e.g., supercritical fluid extraction), a high solute solubility will reduce the number of solvents recycles needed to obtain the desired degree of solute removal.

3. **Equilibrium:** When the solvent to solid ratio is adequate to satisfy the solubility of the solute, equilibrium is a condition where the solute concentration in both the solid and the solvent phases are equal. Thus, the solution adhering to the solids will have the same solute concentration as the liquid or solvent phase. When the amount of solvent is inadequate to dissolve all the solute present, equilibrium is considered as a condition where no further changes in solute concentration in either phase will occur with prolonged contact time. In order for equilibrium to occur, enough contact time must be allowed for the solid and solvent phases. The extent to which the equilibrium concentration of solute in the solvent phase is reached in an extraction stage is expressed as a stage efficiency. If equilibrium is reached in an extraction stage, the stage is 100% efficient and is designated an “ideal stage.”

4. TYPES OF EXTRACTION:

1. Liquid - liquid extraction, where a solvent extracts a solute from a liquid phase;
2. Solid - liquid extraction, or leaching, where a solvent extracts a solute from a solid phase;
3. Supercritical extraction, where a fluid under supercritical conditions is used as the solvent

Solvent extraction or Liquid-Liquid extraction: Solvent extraction also known as liquid-liquid extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. It is a basic technique in chemical laboratories, where it is performed using a separatory funnel. In other words, this is the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. Solvent extraction may be made use analytically for concentrating or rejecting a particular substance, or for the separation of mixtures. This process usually separates a soluble compound from an insoluble compound. Solvent extraction is used in nuclear processing, ore processing, production of fine organic compounds, processing of perfumes and other industries. Thus, although the method can be used for extraction and preconcentration of a wide range of non-volatile or semi volatile species from water using only routine laboratory equipments, its use is decreasing in most situations because solvents of the required purity tend to be expensive, and can also cause problems with proper disposal after use.

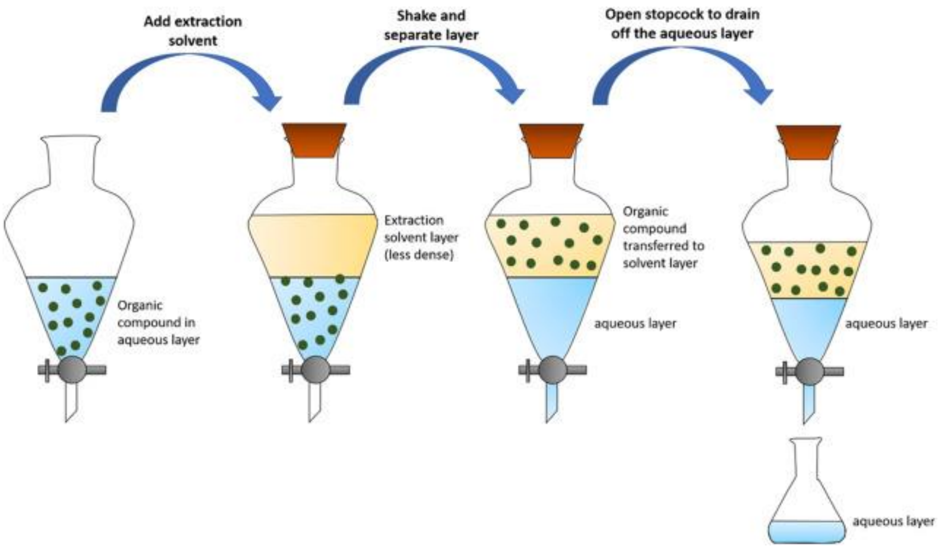


Figure: 1.1 The solvent–solvent extraction technique using separation funnel.

Leaching (Solid-Liquid Extraction): Most extractions in the food industry involve solid-liquid extraction. Solid – liquid extraction (SLE) is the removal of a soluble component A from a solid C by contact with a liquid solvent B. It is also called leaching, although this term is sometimes reserved for situations when the dissolution of A is caused or accompanied by a chemical reaction. Most extractions in the food industry involve solid-liquid extraction. An everyday example is the leaching of coffee from ground coffee beans with hot water. The desired product of leaching may be the solute (which will have to be separated from the solvent in the extract liquid by other means), the liquid extract (i.e., solute – solvent solution) or the depleted solid. Osmo-dehydration is the extraction of water using a low water activity solution (such as a concentrated sugar solution), accompanied by diffusion of other solutes into the solid. SLE is a very widely used process in the food industry and the number of applications is still growing.

To accelerate the diffusion of solutes out of the solid, leaching is often preceded by some form of size reduction, such as grinding, breaking, cutting or flaking. It will be seen later that the required extraction time is proportional to the square of the particle size. Furthermore, grinding helps in breaking down the cell wall structure of many foods, which facilitates the diffusion process.

5. EQUIPMENT

Due to the difficulty of circulating solids, leaching is often carried out in batch fashion. Therefore, leaching equipment can be classified into batch extractors and continuous extractors.

Batch Extractors: Agitated vessels are often used for batch leaching of small particles that can be easily suspended in the liquid. Various types of impellers, propellers or paddles may be used. The leaching time depends on the size of the particles, the diffusivity of the solute in the solid matrix, and the mass transfer coefficient. The latter depends on the flow pattern and mechanical energy input to the mixer. When the desired residence time has been reached, agitation is stopped and the solid allowed to settle out of the liquid. The liquid is then decanted or filtered.

Percolators are another possibility, especially when the particle sizes are large or dense and difficult to keep in a suspended state. The solid is held in a vessel while the solvent is fed at the top and percolates down the bed, possibly under pressure to increase the flow rate. A well - known example is the espresso coffee machine.

Counter-current Extractors: Batch extraction is not very efficient as the most that can be achieved in a batch unit is one equilibrium stage. As with Liquid–Liquid Extraction (LLE), higher extraction efficiencies require a counter-current cascade with solid and solvent flowing in opposite directions. Batch percolating extractors can be operated as a counter-current cascade in a semi – continuous manner. Several vessels are connected in a series and the solvent flows through the vessels sequentially, say from left to right. When the solid in the first (leftmost) vessel becomes depleted, it is emptied, filled with fresh solid, and shifted to the end of the cascade, while the second tank receives the fresh solvent. In practice, this rearrangement can be achieved simply by rerouting the fluid flow with a system of valves (Fig. 1.2). A counter-current cascade can also be assembled from continuous mixers and separators, similar to the mixer - settlers used for LLE. Separators may include gravity settlers (clarifiers and thickeners), filters, hydro-cyclones or centrifugal separators. Dedicated counter-current leaching units contain several counter-current stages within the same vessel. They differ mainly in the arrangement used to convey the solid from one stage to another. Two examples are shown in Fig.1.2(a) Fig.1.2(b) Some commercial solid liquid extractors.

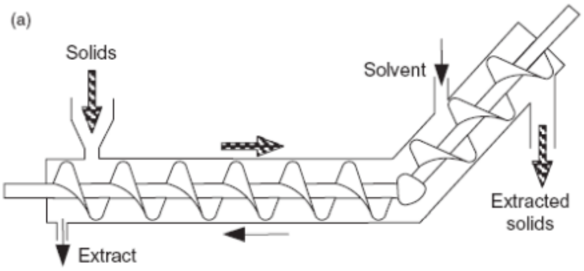


Fig:1.2 (a) Hildebrandt screw extractor

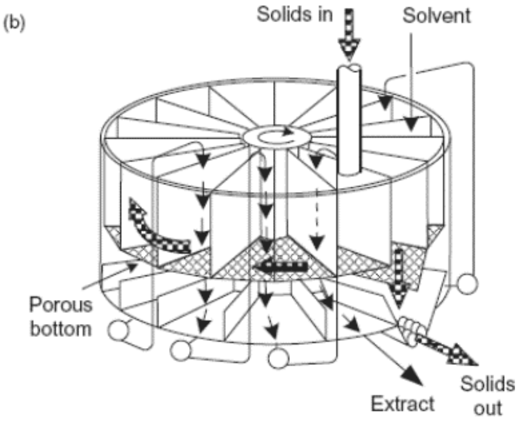


Fig:1.2 (b) Rotocel extractor.

Belt and screw conveyors are readily converted to leaching equipment simply by adding a liquid circulation system (pump or gravity). In the perforated - belt extractor, a horizontal perforated belt conveys the solid from left to right. Solvent is introduced as a spray at the right end, collected under the belt, pumped to the next spray nozzle to the left, and so on, creating counter-current contact. In screw extractors, the screw conveys the solid up the slope while the solvent percolates down the slope (Fig.1.2).

6. SUPERCRITICAL FLUID EXTRACTION:

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but it can also be from liquids. SFE can be 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). Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical CO_2 are above the critical temperature of 31°C and critical pressure of 74 bars. Addition of modifiers may slightly alter this. Supercritical extraction mostly uses carbon dioxide at high pressure to extract the high value products from natural materials. Unlike other processes, the extraction process leaves no solvent residue behind. Moreover, the CO_2 is non-toxic, non-flammable, odourless, tasteless, inert, and inexpensive. Due to its low critical temperature 31°C, carbon dioxide is known to be perfectly adapted in food, aromas, essential oils and nutraceutical industries.

Supercritical fluid extraction may be done on solids or liquids. The solvent is a dense gas at conditions that exceed the critical temperature and pressure where further increase in pressure or a reduction in temperature will not result in a phase change from gas to liquid. The density of a supercritical fluid, however, is almost that of a liquid, but it is not a liquid. Extraction with supercritical fluids (SCFs) is based on the experimental observation that many gases become good solvents for solids and liquids when compressed to conditions above the critical point. The solubility of solutes in a supercritical fluid approaches the solubility in a liquid. Thus, the principle of solute extraction from solids using a supercritical fluid is very similar to that for solid-liquid extractions.

7. FUNDAMENTALS OF SUPERCRITICAL FLUID EXTRACTION

Supercritical fluid extraction was first introduced in 1879 by Hannay and Hogarth. Despite the advantages associated to the use of supercritical fluids as extracting agents, it was not until around 1960 that this technique started to be thoroughly investigated as an alternative to conventional extraction methods such as solid–liquid extraction (SLE) and liquid–liquid extraction (LLE), both requiring large amounts of hazardous chemicals such as chlorinated solvents

Supercritical fluids have been investigated since last century, with the strongest commercial interest initially focusing on the use of supercritical toluene in petroleum and shale oil refining during the 1970s. Supercritical water is also being investigated as a means of destroying toxic wastes, and as an unusual synthesis medium. The biggest interest for the last decade has been the applications of supercritical carbon dioxide, because it has a near ambient critical temperature (31^0C), thus biological materials can be processed at temperatures around 35^0C. The density of the supercritical CO_2 at around 200 bar pressure is close to that of hexane, and the solvation characteristics are also similar to hexane; thus, it acts as a non-polar solvent.

Around the supercritical region, CO_2 can dissolve triglycerides at concentrations up to 1% mass. The major advantage is that a small reduction in temperature, or a slightly larger reduction in pressure, will result in almost the entire solute precipitating out as the supercritical conditions are changed or made sub critical. Supercritical fluids can produce a product with no solvent residues. Examples of pilot and production scale products include decaffeinated coffee, cholesterol-free butter, low-fat meat, evening primrose oil, squalene from shark liver oil, etc. The solvation characteristics of supercritical CO_2 can be modified by the addition of an entrainer, such as ethanol, however some entrainer remains as a solvent residue in the product, negating some of the advantages of the "residue-free” extraction.

Supercritical Fluid:

A supercritical fluid is any substance at a temperature and pressure above its critical point. It can diffuse through solids like a gas, and dissolve materials like a liquid. Additionally, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties of a supercritical fluid to be "fine-tuned". Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Carbon dioxide and water are the most commonly used supercritical fluids, being used for decaffeination and power generation, respectively. CO_2 is the kind of extraction solvents for botanicals. It leaves no toxic residue behind. Its extraction properties can be widely and precisely manipulated with subtle changes in pressure and temperature.

8. PROPERTIES OF SUPERCRITICAL FLUIDS USED IN FOODS

Carbon dioxide is by far the most widely used supercritical fluid because it has the desirable properties of being nontoxic, non-flammable, readily available in high purity and inexpensive. The critical point of carbon dioxide is 31.1ºC and 7.39 MPa (74 Bars). Ethanol may also be added in small amounts to supercritical carbon dioxide to change its polarity in some extractions. The critical point of ethanol is 243ºC and 6.38 MPa (64 Bars). The relative density of a supercritical fluid is in the range 0.1 to 1 s compared with a density of 1for liquids and 0.001 for gases. The relative viscosity is 0.1 to 1 compared with 1 for liquids and 0.01 for gases. The relative diffusivity is 10 to 100 compared with 1 for liquids and 104 for gases.

Properties of Supercritical Fluid

I. Supercritical fluids have highly compressed gases, which combine properties of gases and liquids in an intriguing manner

II. Supercritical fluids can lead to reactions, which are difficult or even impossible to achieve in conventional solvents.

III. Supercritical fluids have solvent power similar to light hydrocarbons for most of the solutes. However, fluorinated compounds are often more soluble in supercritical CO_2 than in hydrocarbons; this increased solubility is important for polymerization.

IV. Solubility increases with increasing density (that is with increasing pressure). Rapid expansion of supercritical solutions leads to precipitation of a finely divided solid. This is a key feature of flow reactors.

V. The fluids are commonly miscible with permanent gases (e.g., N_2 or H_2) and this leads to much higher concentrations of dissolved gases than can be achieved in conventional solvents.

Physical Properties of Supercritical Fluids: As the substance approaches its critical temperature, the properties of its gas and liquid phases converge, resulting in only one phase at the critical point: a homogeneous supercritical fluid. The heat of vaporization is zero at and beyond this critical point, and so no distinction exists between the two phases. On the pressure-temperature diagram (Figure:1.3 A), the point at which critical temperature and critical pressure meet is called the critical point of the substance. Above the critical temperature, a liquid cannot be obtained by increasing the pressure, even though a solid may be formed under sufficient pressure. The critical pressure is the vapor pressure at the critical temperature. In the vicinity of the critical point, a small increase in pressure causes large increases in the density of the supercritical phase (Figure:1.3 B).

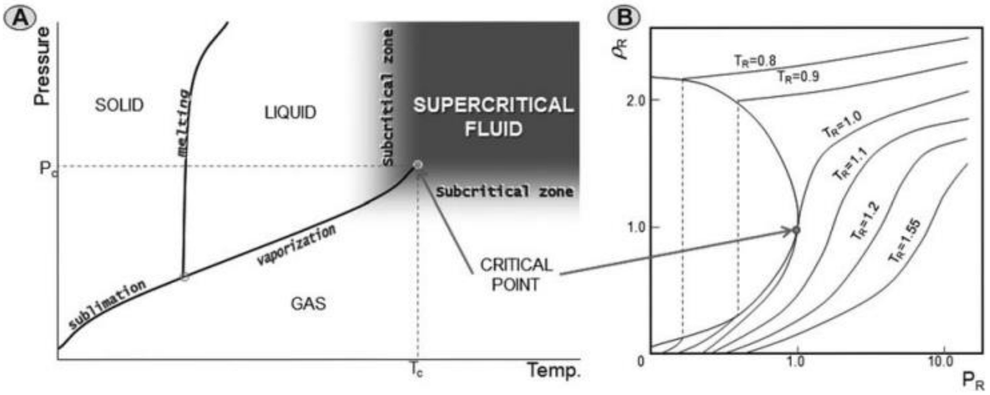


Figure:1.3 Carbon dioxide pressure–temperature phase diagram (A) and density–pressure phase diagram at different temperatures (B) considering reduced variables (TR = T/T_C, PR= P/P_C and $\rho_R = \rho / \rho_C$).

Physical properties of supercritical fluids are between those of a gas and those of a liquid, as can be observed in Table1.1, in which some data taken from Pereda, Bottini, and Brignole has been included. For instance, the density of a supercritical fluid is similar to a liquid while its viscosity is similar to a gas and its diffusivity is placed between gas and liquid. Thermal conductivities are relatively high in supercritical fluids and have large values near the critical point. Surface tension is close to zero in the critical point, being similar to gases and much smaller than for liquids. Many other physical properties such as relative permittivity, solvent strength, etc., highly related to density, show large gradients with pressure above the critical point. Changes in those properties are crucial when dealing with extraction since they are related to changes in solubility and mass transfer ratios, and, therefore, related to changes in the selectively of the solvent.

TABLE:1.1 COMPARISON OF GASES, SUPER CRITICAL FLUIDS AND LIQUIDS

	Density (kg/m^3)	Viscosity (µPa-s)	Diffusivity (mm²/s)
Gases	1	10	1-10
Supercritical fluids	100-1000	50-100	0.01-0.1
Liquids	1000	500-1000	0.001

The solvent strength of a supercritical fluid can be characterized, among others, by the Hildebrand solubility parameter, d, which relates to the density of the solvent as follows:

$$\delta = 1.25 P_c^{1/2} \left[\frac{\rho}{\rho_{liq}} \right]$$

where P_c is the critical pressure, ρ is the gas density, and ρ_{liq} is the liquid density. At low pressures, the density of a gas is low, so the solvating power is rather low; at near critical conditions, the density increases rapidly approaching that of a liquid and thus the solubility parameter increases as the critical pressure is approached.

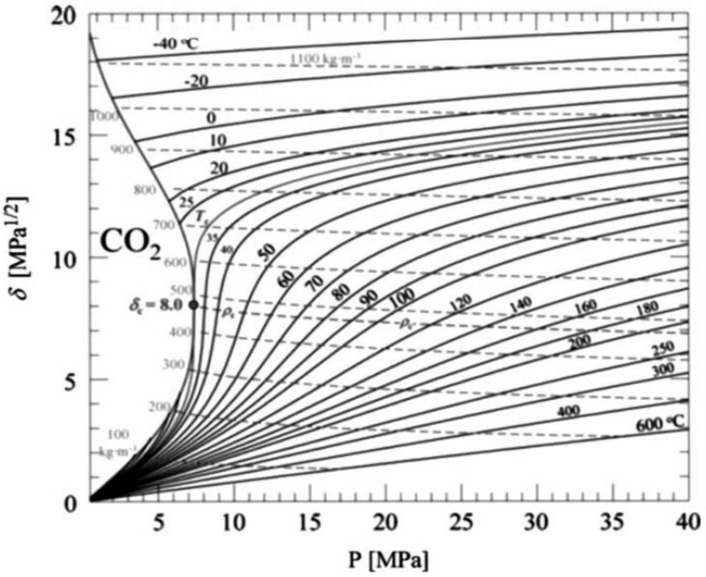
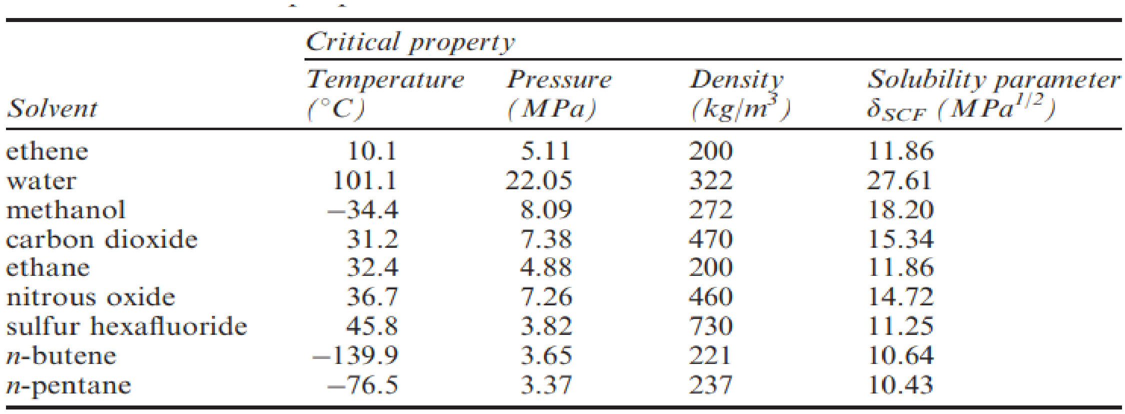


Figure: 1.4 Solubility parameter of carbon dioxide. Reprinted from Machida *et al.*, The Journal of Supercritical Fluids, Vol. 60, December 2011, pp. 2–15, copyright 2011, with permission from Elsevier.

This effect can be seen graphically in Figure 1.4 in which the Hildebrand solubility parameter for CO_2 is represented as a function of the pressure for different temperatures.[38] This is one of the key features of SFE since the solvating power of the fluid can be strongly influenced by small changes in pressure and temperature either favouring the extraction of the target compounds or the precipitation of the solutes dissolved in the supercritical fluid.

Supercritical Solvents: Although there is a wide range of compounds that can be used as supercritical fluids (see Table 1.2 in which the critical properties of several solvents used in SFE are given), it is true that after the Montreal Protocol, introduced in 1987 to restrict or eliminate the manufacture and use of particularly damaging ozone depleting solvents (at present signed by 170 nations), there is a worldwide pressure for the industry to adopt new sustainable processes that do not require the use of environmentally damaging organic solvents.7

TABLE:1.2 CRITICAL PROPERTIES OF SOME SOLVENTS USED IN SFE



	Critical property			
Solvent	*Temperature* (°C)	*Pressure* (MPa)	*Density* (kg/m^3)	*Solubility parameter* δ_{SCF} ($MPa^{1/2}$)
ethene	10.1	5.11	200	11.86
water	101.1	22.05	322	27.61
methanol	−34.4	8.09	272	18.20
carbon dioxide	31.2	7.38	470	15.34
ethane	32.4	4.88	200	11.86
nitrous oxide	36.7	7.26	460	14.72
sulfur hexafluoride	45.8	3.82	730	11.25
n-butene	−139.9	3.65	221	10.64
n-pentane	−76.5	3.37	237	10.43

In this sense, SFE using green solvents has been suggested as a clean alternative to hazardous processes and thus, SFE has found its growing niche. Among the green solvents used in SFE, carbon dioxide (critical conditions= 31.2 1ºC and 7.38 MPa) is undoubtedly the most commonly employed. CO_2 is inexpensive, environmentally friendly and generally recognized as safe (GRAS). Supercritical CO_2 (SC-CO_2) is also attractive because of its high diffusivity combined with its easily tunable solvent strength. Another advantage is that CO_2 is gaseous at room temperature and pressure, which makes extract recovery very simple and provides solvent-free extracts. Also important for food and natural products is the ability of SFE using CO_2 to be operated at low temperatures using a non-oxidant medium, which allows the extraction of thermally labile or easily oxidized compounds. [60] As can also be seen in Table 1.2, supercritical CO_2 has a low polarity (with a low solubility parameter, around 15 $MPa^{1/2}$), and therefore, its efficiency to extract polar compounds from natural matrices is quite limited. To overcome this problem, polar co-solvents (methanol, ethanol, water) are commonly used in small amounts to increase the solubility of polar compounds in the supercritical mixture.

The widest application of supercritical fluids is extraction, especially with carbon dioxide. The first patent dealing with supercritical fluid extraction was filed by Messmore in 1943, although the first industrial application was developed by Zosel in 1978. Since then, supercritical fluids have been used to isolate natural products, but for a long-time applications relied only on a few of them. The development of processes and equipment is beginning to pay off and industries are getting more and more interested in supercritical techniques. This interest is also observed in the high amount of scientific papers dealing with supercritical fluid extraction (SFE) published in recent years. Moreover, industrial applications of SFE have experienced a strong development since the 1990s in terms of patents. [60, 28]SFE has been used in different fields such as the food, pharmaceutical, chemical, and fuel industries. Due to the absence of toxic residue in the final product, among other advantages, supercritical fluids are especially useful for extraction in two situations: (a) extracting valuable bioactive compounds such as flavours, colorants, and other biomolecules or (b) removing undesirable compounds such as organic pollutants, toxins, and pesticides. [49] In this chapter we will focus on the use of supercritical fluids to extract valuable compounds from vegetal and marine sources and by-products from the food industry.

Extraction Principles:

Supercritical fluid extraction is done in a single-stage contractor with or without recycling of the solvent. When recycling is used, the process involves a reduction of pressure to allow the supercritical fluid to lose its ability to dissolve the solute, after which the solid is allowed to separate by gravity, and the gas at low pressure is compressed back to the supercritical conditions and recycled. Temperature reduction may also be used to drop the solute and the solvent is reheated for recycling without the need for recompression. Fig. 1.5 shows a schematic diagram of a supercritical fluid extraction system

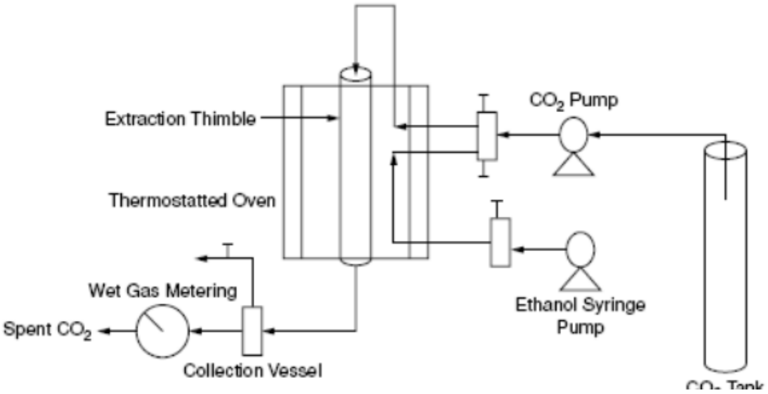


Figure 1.5 Schematic diagram of a supercritical fluid extraction system using entrained ethanol in supercritical carbon dioxide

The basic components are an extractor tank and an expansion tank. Supercritical fluid conditions are maintained in the extractor. Temperature is usually maintained under controlled conditions in both tanks. Charging and emptying the extractor is a batch operation. The pressure of the supercritical fluid is reduced by throttling through a needle valve or orifice after which it enters an expansion tank where the supercritical fluid becomes a gas. Because solute solubility in the gas is much less than in the supercritical fluid, solute separates from the gas in the expansion tank. The spent gas is then recompressed and recycled. Heat exchanges are needed to maintain temperatures and prevent excessive cooling at the throttling valve due to the Joule-Kelvin effect.

Two of the major problems of supercritical fluid extraction are channelling of solvent flow through the bed of solids, and entrainment of the non-extractable component by the solvent. Time of solidsolvent contact is the quotient of extraction vessel volume divided by the solvent volumetric flow rate. The volume is calculated at the temperature and pressure inside the extraction vessel. Normally, volume of the solvent is measured at atmospheric pressure after the gas exited the expansion tank. From this measured volume, the number of moles of gas is calculated and the volume of the supercritical fluid in the extraction vessel is then calculated using the equations of state for gases. The contact time should be adequate to permit solvent to penetrate solid particles and permit diffusion of solute from inside the solid particles into the solvent phase. To achieve equilibrium between the solution inside solid particles and the solvent phase, solvent flow must be adjusted to achieve the necessary contact time and to provide enough solvent such that concentration of dissolved solutes in the solvent phase will be below the solubility of solute in the solvent. A large quantity of solute to be extracted would require a larger rate of solvent flow to permit thorough solute extraction within a reasonable length of time. Supercritical fluid penetration into the interior of a solid is rapid, but solute diffusion from the solid into the supercritical fluid may be slow thus requiring prolonged contact time in the extraction vessel. Solvent flow rate, pressure, and temperature in the extraction vessel are the major supercritical fluid extraction process parameters.

Instrumentation:

Supercritical fluid extraction is commonly carried out considering two basic steps:

1. Extraction of soluble substances from the matrix by the supercritical fluid and
2. Separation or fractionation of the extracted compounds from the supercritical solvent after the expansion.

The basic instrumentation to carry out supercritical fluid extractions should be composed of materials that are capable to withstand high pressures, typically as high as 50 MPa (although systems requiring extractions pressures as high as 70 MPa have also been used). The equipment needed is different depending if the application deals with solid or liquid samples. Figure 1.6 shows the two schemes corresponding to a SFE extractor for solid and liquid samples. As can be observed, the main differences are related to the extraction cell itself. While the solid samples equipment has an extraction vessel of a given internal volume (see, Figure 1.6 A), the liquid samples extraction plant uses an extraction column in which the extraction is performed in counter-current mode (Figure 1.6 B). Counter-current extraction (CC-SFE) is performed introducing the sample in the system from the top of the column and the pressurized solvent from the bottom; in this process, the components distribute between the solvent and the liquid sample which flows counter-current through the separation column. Depending on the separation factor between components to be extracted, the desired contact time between the solvent and the sample can be reached by adjusting the height of the sample introduction into the extraction column. It can also be adjusted by modifying the performance of the separation column, in terms of height and diameter, or of the packing material (structured/random, packing dimensions, surface area, etc.). Factors such as solvent-to-feed ratio are of crucial importance in this type of extraction.

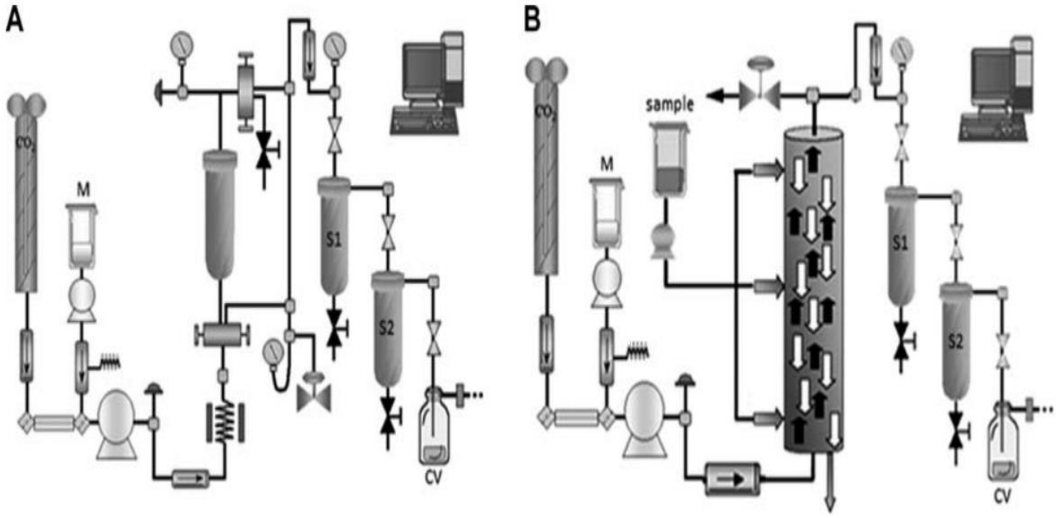


Figure 1.6 (A) Scheme of a typical SFE instrument for the extraction of solid samples. (B) Scheme of a typical SFE plant for the extraction of liquid samples. M= modifier reservoir, S1= separator 1, S2= separator 2, CV = collection vessel.

As can be seen in Figure 1.6, both systems are composed by a tank for the extracting solvent, usually CO_2, a pump to pressurize the gas to the desired extraction pressure, a restrictor or valve to maintain the high pressure inside the system, and a trapping vessel (or separation cell, also called fractionation cell) for the recovery of the extracts. Different factors should be optimized in order to avoid losses of extracted compounds. One of these factors is the trapping method, selection of which should be done considering the extract volatility and polarity, the volatility of the extracting agent, the volatility of the modifier (if used), and the solvent flow rate, among other parameters. Also, different trapping methods are available, such as solid trapping, liquid trapping, cool trapping, etc. In pilot or industrial systems, collection of the extracted solutes is done by rapidly reducing the pressure, increasing the temperature, or both. In this case, depressurization after the extraction can be performed in cascade considering that each separation vessel can have a particular temperature and pressure in order to have some of the extracted compounds precipitated and separated.

Additionally, the system may include another pump to introduce an organic modifier (co-solvent) that is sometimes needed to extend the solvent capabilities of, for instance, supercritical CO_2, allowing the extraction and recovery of more polar compounds.

Regarding the extraction mode, at small scale, solid samples can be extracted in dynamic or static modes or even in a combination of both. 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.

Figure 1.7 displays an example of a process flow diagram of a SFE process at industrial scale showing the different components needed for large-scale operations;15 in this particular case, prebiotic carbohydrates were extracted from a complex mixture using a mixture of carbon dioxide and ethanol: water. In this process, carbon dioxide (stream 1) and a co-solvent mixture (stream 2) are pumped (pumps 1 and 2) and mixed into a heat exchanger (HE1) used to guarantee that the solvent reaches the extraction cell at the target temperature. After the extraction cell, a valve is placed to control the extraction cell pressure. Carbon dioxide depressurizes through this valve and is removed as a gas (at low pressures) in stream 9; after that, carbon dioxide is recompressed up to 4–5 MPa, condensed in the heat exchanger 3 (HE3) to be pumped again as a liquid and recirculated into the system. Extracted solutes remain dissolved in the co-solvent and are withdrawn from the collection vessel in stream 11. Other operations needed to collect and purify the extracts can be performed afterwards.

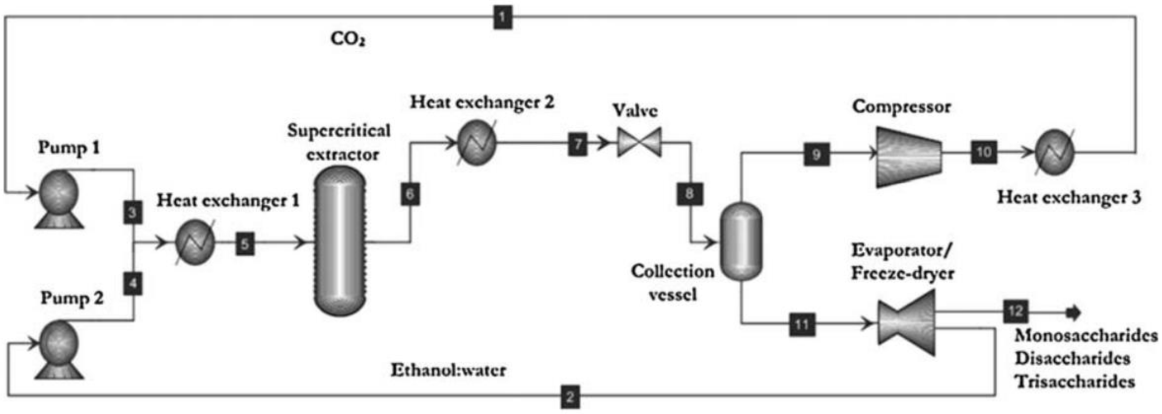


Figure 1.7 Flow diagram of SFE process at industrial scale. Reprinted from Montanes *et al.*, *Journal of Chromatography A*, Vol. 1250, 10 August 2012, pp. 92–98, copyright 2012, with permission from Elsevier.

9. PARAMETERS AFFECTING THE EXTRACTION PROCESS

The extraction of the soluble substances from the matrix can be described by considering several steps, each one influenced by several factors that should be optimized. When dealing with solid samples, there is, at the beginning of the extraction process, diffusion of the solvent into the matrix leading to absorption of the supercritical solvent and therefore to decrease of the mass transfer resistance; after this step, soluble compounds are dissolved into the supercritical fluid and are further transferred by diffusion first into the surface of the solid and later to the bulk of the fluid phase. The extraction process ends with the transport of the solute and the bulk fluid phase and their removal from the extractor. The kinetics of the extraction process can be followed by determining the amount of extract (mass of extract or yield) as a function of process time (or solvent consumption), providing an overall extraction curve (OEC), such as the one shown in Figure 1.8 Although this figure refers to the extraction rate of artemisin,52 it shows the typical behaviour of SFE. A typical extraction curve can be divided into three periods.49,40

1. A constant extraction rate period (CER), characterized by the extraction of the solute contained in the surface of the particles, that is, easily accessible. The mass transfer in this step is controlled by convection.
2. A falling extraction rate period (FER), in which most of the easily accessible solute has been extracted and mass transfer starts to be controlled by diffusion.
3. A diffusion-controlled rate period (DCR), in which the easily extractable solute has been completely removed and the extraction process is controlled by the diffusion of the solvent inside the particles and the diffusion of solute þ solvent to the surface.

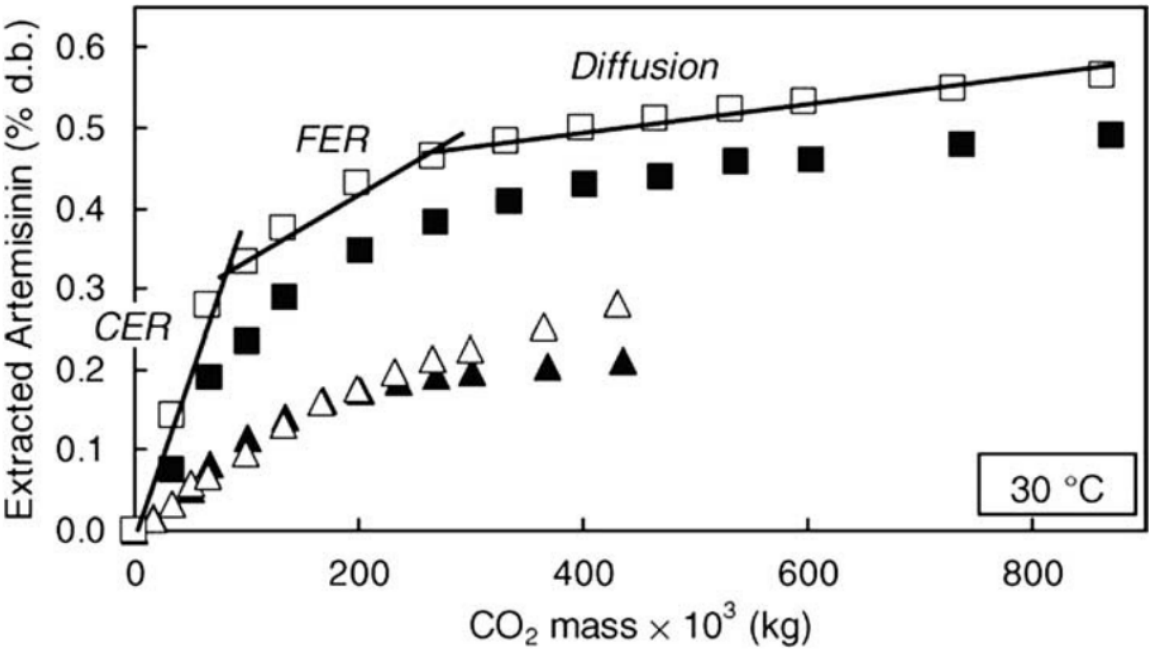


Figure 1.8 Overall extraction curves for artemisinin obtained in different conditions of SFE: (m, n) 5.5×10^{-5} kg CO_2/s; (’, &) 1.1 ×10^{-5} kg CO_2/s (full symbols: 40MPa and empty symbols: 20MPa), reprinted from Quispe-Condori et al., 52 Journal of Supercritical Fluids, Vol. 36, Issue 1, 2005, pp. 40–48, copyright 2005, with permission from Elsevier.

In some cases, the slope of the straight line corresponding to CER can be used as an indication of solubility, depending on the flow rate considered (for a more in-depth discussion, readers are referred to Rodrigues *et al*. [54]).

For liquid samples, the steps are similar although further complexity is introduced by including the dimensions of the column and the size and structure of the packing material in the counter-current column. Moreover, theoretical calculations of the efficiency of the separation, based on experimental measurements, are sometimes necessary to adjust the experimental conditions for challenging separations.

In the following section, an explanation of the main factors influencing the Supercritical Fluid Extraction process is presented

1. Raw Material (Particle Size, Porosity, Location of the Solute, Moisture Content)
2. Solubility (Pressure and Temperature)
3. Solvent Flow Rate (Solvent-to-Feed Ratio)
4. Use of Modifiers

9.1 Raw Material (Particle Size, Porosity, Location of the Solute, Moisture Content)

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. 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. [49]

As an illustration, the degree of crushing was a very significant factor in the extraction of carotenoids from Haematococcus pluvialis microalgae. [19] It was demonstrated how an increase in the crushing procedure produced an enhancement in the carotenoid extraction yield. This effect can be attributed to an increase of the mass transfer rate as a consequence of the lower particle size as well as to the increase of carotenoids in the medium as a result of the disruption of cells in the heavier crushing procedure. [19] Although supercritical solvents have higher diffusivity in the raw material matrix than liquids, a decrease in the sample particle size generally produces an increase in the extraction yield obtained due to the increase in the contact surface between sample and solvent, mainly when diffusion is limited by internal mass transfer resistance. Nevertheless, in some applications, for example, when dealing with samples of high-water content, the use of dispersing agents (e.g., diatomaceous earth) to avoid sample clogging together with hydro matrix to absorb the liquid portion from the sample can be useful.

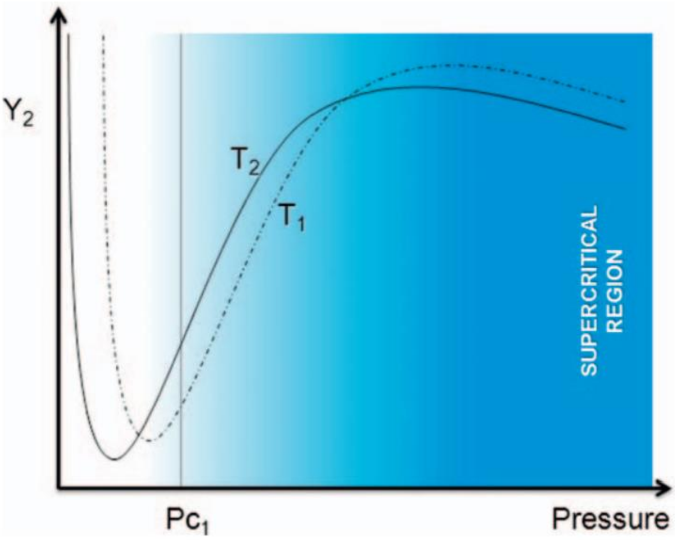
In general, drying the raw material is recommended; however, in some cases the presence of water is necessary to favour the interaction of the solvent with the solute, as in the extraction of caffeine from green coffee beans, or due to its role in the swelling of the cell, which facilitates the flow of the solvent into the cell. [49] In the case of liquid samples two main strategies are used: (a) to trap the liquid on a solid support (e.g. sepiolite) and to treat it like a solid or (b) to perform column counter-current extractions (see Figure 1.6 B). The first strategy is mainly used at a small scale since the employment of solid supports can increase the extraction costs. As mentioned, during counter-current 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. Hurtado-Benavides et al. studied the effect of the type, size, and structure of the column packing on the efficiency and performance of the counter-current system for the SFE of olive oil; results demonstrated the influence of these factors on the mass transfer ratio. [31] For instance, authors showed that the use of a column packing with high surface area provides similar results to decreasing the mean particle size of a solid raw material.

9.2 Solubility (Pressure and Temperature):

As previously mentioned, there are several physical parameters of the supercritical fluid that are highly dependent on the pair pressure–temperature. The design of processes using supercritical solvents is strongly dependent on the phase equilibrium scenario, which is highly sensitive to changes in operating conditions. Therefore, phase equilibrium engineering, that is, the systematic application of phase equilibrium knowledge to process development, plays a key role in the development and design of these processes. [72]

In general, both the yield of a solute and the separation selectivity, which are highly dependent on solubility properties, are determined by the operating pressure and temperature. At SFE conditions, the solvent solubilization capacity increases with pressure at constant temperature, therefore increasing the amount extracted from the raw material. In general terms, increasing the pressure leads to an exponential increase of the solubility close to the critical point (higher densities) (Figure 1.3 B). As a general rule, a component with high vapor pressure has higher solubility in a supercritical medium. Solubility of most components in supercritical fluids (SCFs) increases with the increase of the SCF density, which can be accomplished by increasing the extraction pressure. Other important aspects influencing solubility of components in the SCF are their polarity and molecular weight as well as extraction temperature. In SFE processes using CO_2, the component solubility is lowered as the polarity and/or the molecular weights of the solutes are increased.

Increasing the temperature, at constant pressure, promotes two opposite effects: it reduces the solvent power of CO_2 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. The balanced effect on solubility of the solute in the supercritical solvent will, in fact, depend on the operating pressure. Near the critical pressure, the effect of fluid density is predominant, thus, a moderate increase in temperature leads to a large decrease in the fluid density, and therefore, to a decrease in solute solubility. However, at high pressures, the increase in the vapor pressure prevails, thus the solubility increases with the temperature. This is called a retrograde behaviour of the solid solubility, as can be seen in Figure 1.9. At pressures above the P_c, the isotherms exhibit a maximum in solubility.



8Figure 1.9 Typical isotherms of solid solubility in SFE (T1>T2>T_{c1}).

When dealing with really complex matrices or extracting different components (like most natural product extraction processes), thermodynamic calculation can be very complicated. An alternative to performing highly complicated thermodynamic calculations consists on performing experimental designs to set up a robust extraction process.60 They offer a framework where researchers create an experiment, controlling experiment factors so as to predict or establish a result based on dependent and independent variables. Experimental designs give the possibility to evaluate interactions between factors, in this case pressure and temperature, while limiting the number of experiments

9.3 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.49 In general it is common to use S/F ratios around 25–100:1 for analytical and 5–15:1 for large-scale processes. In this sense it is important to consider the amount of CO_2 spent as a cost when dealing with industrial processes. In fact, when dealing with industrial scale only the first parts of the extraction curve (Figure 1.8) are considered; this is in contrast to analytical operations where quantitation is the main goal. Therefore, higher amounts of CO_2 can be consumed in analytical scale and higher S/F ratios are used.

S/F is also very important when dealing with counter-current column extractions. Generally, the efficiency of the column decreases as the CO_2 flow rate increases, since the HTU (height of a transfer unit) increases with increasing CO_2 loading, as demonstrated by Hurtado-Benavides *et al*.31 and Brunner et al.10

9.4 Use of Modifiers

CO_2 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, CO_2 has a limited ability to dissolve high-polarity compounds. To address this problem, small amounts of co-solvents (modifiers) are added to the CO_2 stream. The addition of modifiers to CO_2 can improve the extraction efficiency by raising the solubility of the solutes. Two mechanisms have been proposed by Pereira and Meireles 49 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. A key point when working with modifiers is to consider that the critical point of the mixture CO_2: modifier is different than the one of pure compounds and it also depends on the proportions of each.27 In fact, two or three different phases may coexist at the same conditions. It must be taken into account that modifiers are not gases at room conditions and, therefore, liquid residues are obtained in extracts and remaining matrix after SFE. This is the main reason for not recommending the use of methanol in the extraction of natural products since the presence of this toxic solvent can preclude the further use of the extracts, for instance, in food applications.

Ethanol is a GRAS solvent widely employed as a modifier for natural products extraction, although its final use will be determined by its ability to increase the solubility of the target compound in the CO_2: modifier mixture. Considering only toxicity and polarity, water can be suggested as an interesting modifier, but it presents several drawbacks such as the increase in the formation of ice blockages due to the Joule–Thompson effect in the separator vessel; the possible ionization and hydrolysis of compounds; and foam formation, attributed to the co-extraction of saponins 49 that thus leads to a loss of selectivity.

Sometimes modifiers are not only used to increase the polarity of the solvent phase, but also to improve the extraction rate of non-polar solvents. The use of oil as modifier came from the observations of Bamberger et al.64 that the solubility of a less volatile lipid component was significantly enhanced by the presence of a more volatile triglyceride species in the system. Sun and Temelli demonstrated the ability of vegetable oils used as modifiers to enhance the yield of carotenoids (non-polar and with low volatility) from carrot; without a co-solvent, the extraction yield had a very small variation with changes in pressure and temperature, but when canola oil was employed, extraction yields increased by 3–4 times. The extraction yield of total carotenoids with SC-CO_2 using 5% canola oil addition (w/w) was substantially higher than that obtained by traditional solvent extraction.

10. ADVANTAGES AND DRAWBACKS OF SUPERCRITICAL CO_2 (SC-CO_2):

There are a large number of compounds that can be used as a fluid in supercritical techniques, but by far the most widely used is carbon dioxide. From the viewpoint of pharmaceutical, nutraceutical and food applications it is a good solvent, because it is non-toxic, non-flammable, inexpensive, easy to remove from the product and its critical temperature and pressure are relatively low (T_c=31.1°C, p_c=72 bar) make it important for food and natural products sample preparation, is the ability of SFE using CO_2 to be operated at low temperatures using a non-oxidant medium, which allows the extraction of thermally labile or easily oxidized compounds. It is environmental friendly and generally recognized as safe by FDA and EFSA. These properties make it suitable for extracting, for example, thermally labile and non-polar bioactive compounds but, because of its non-polar nature, it cannot be used for dissolving polar molecules.

The solubility of polar compounds and the selectivity of the process can be increased by adding small quantities of other solvents, such as ethanol, in the fluid that named as co-solvent or modifier. On one hand, it decreases the processing times, increases yields and makes it possible to use milder processing conditions, but on the other, it complicates system thermodynamics and increases capital costs [3]. The use of high purity SFE-grade CO_2 is not required but impurity and moisture in industrial grade CO_2 can accumulate and may interfere with further analytical operations (gas or liquid chromatography). Thus, an on-line fluid clean-up system may be used to remove trace contaminants. An important drawback of SC-CO_2 and 5most of the other supercritical fluids is that predominantly, a non-polar extraction fluid, such as CO_2, is used.

Therefore, a logical trend to widen the application range of this technique is the study of new methods to decrease analyte polarity to make them more soluble in non-polar supercritical fluids. In this sense, chemical in situ derivatization has been applied to improve the selectivity of the extraction towards a specific group of compounds [33,56]. So the solvent power of SC-CO_2 can be summarized by a few rules:

- It dissolves non-polar or slightly polar compounds.
- The solvent power for low molecular weight compounds is high and decreases with increasing molecular weight.
- SC-CO_2 has high affinity with oxygenated organic compounds of medium molecular weight.
- Free fatty acids and their glycerides exhibit low solubilities.
- Pigments are even less soluble.
- Water has a low solubility
- Proteins, polysaccharides, sugars and mineral salts are insoluble and;
- SC-CO_2 is capable of separating compounds that are less volatile, have a higher molecular weight and/or are more polar as pressure increases [49].

11. SPECIAL APPLICATIONS OF SUPERCRITICAL FLUIDS TO FOOD PROCESSING:

1. Removal of fat from foods
2. Enrichment of vitamin E from natural sources
3. Removal of alcohol from wine and beer, and related applications
4. Encapsulation of liquids for engineering solid products
5. Extraction and characterization of functional compounds:

12. RECENT ADVANCES AND APPLICATIONS OF SUPERCRITICAL FLUID EXTRACTION:

Current Applications of Supercritical Fluid Extraction Supercritical fluid-based technologies are involved in a broad spectrum of industrial applications that have experienced significant progress in recent years. Extraction with supercritical fluids has been applied extensively in the food, pharmaceuticals, and cosmetics industries.

Probably, the most extended use of SFE is in the food field. A high variety of samples, type of materials, target compounds and procedures have been published in the last years.

A relatively new group of applications that have been recently developed includes the extraction and fractionation of carbohydrates by SFE. This technique, using supercritical CO2, as it is massively employed, is not at first sight the best option for this group of compounds, given the low polarity of carbon dioxide; however, using CO_2 with a relatively low amount of polar entrainer, authors demonstrated its usefulness. A full factorial design was used to evaluate the influence of extraction pressure, temperature and modifier flow rate (and consequently proportion of modifier) to effectively fractionate carbohydrate mixtures formed by lactose and lactulose. Results obtained were analyzed by multiple linear regression determining as optimum values 100 bar, 100 ^{o}C and 0.2 ml/min of co-solvent (4% modifier) for the selective recovery of lactulose [43]. This strategy was also applied to other carbohydrates [42,46]. All these works demonstrated the great importance of the modifier nature to obtain selective fractionations, being ethanol/water the most successfully used co-solvent. In fact, the solubilities of different carbohydrates in supercritical carbon dioxide with ethanol/water as co-solvent were experimentally obtained and thermodynamically modelled [45]

Crude vegetable oils are widely used in the food industry for a number of applications. Usually, oils have to be refined to remove undesirable compounds before consumption; during this refining process, valuable compounds contained in the oils can be also lost. SFE has been suggested as an alternative to refining procedures, to obtain extracts enriched with the particular compounds of interest; this is the case for instance of wheat germ oil [19], green coffee oil obtained by pressing [18], rice bran oil [13] or crude palm oil [17].

SFE has been also used to remove unwanted compounds from other matrices; for example, supercritical CO_2 with water as cosolvent has been employed to selectively extract caffeine from green tea while avoiding the extraction of antioxidants from the matrix [36]. The selectivity of the different extraction conditions studied was confirmed by HPLC.

Essential oils [2,41,65], fatty acids [2,41,65] and/or bioactive compounds [41,26 ,9 ,67] have been extracted from fruits and vegetables using supercritical CO_2. Soy isoflavones have been also widely studied; aqueous methanol seems to be the most appropriate modifier to carry out isoflavones supercritical CO_2 extractions [37], although the use of acetonitrile has been also tested [37]. Daidzein and genistein were successfully extracted at high pressures, from 350 to 500 bar [71,70]. In all of these studies, HPLC was the analytical tool selected to determine these flavonoids. These compounds as well as other bioactives have been extracted by SFE prior analysis from a number of matrices: stilbenes such as cajaninstilbene from pigeonpie [37], cinnamic derivatives from propolis [12] or carotenoids and flavonoids from black rice [47]

One of the few works dealing with SFE using a solvent different from CO_2 is related to fatty acid extraction from a fish oil sample [21]. In this case, the ability of ethane as an alternative to the more common use of CO_2 to extract fatty acids was evaluated. Besides, a thermodynamic modelling was used to obtain the most appropriate conditions to extract the highest possible amounts of eicosapentanoic (EPA) and docohexaenoic (DHA) acids. Ethane provided better selectivity and higher solubility for EPA and DHA esters as compared to other esters containing the same number of carbons. Applying 60 ˚C and 84 bar, a concentration of 60% of both fatty-acids ethyl esters could be obtained using ethane as supercritical solvent [21].

SFE has been widely used to value food industry by-products; these products are generated during food manufacturing and normally do not have any commercial value. By-products extraction allows the removal of valuable/interesting compounds that otherwise cannot be utilized. Rice wine lees were investigated for the extraction of polyphenols with antioxidant activity [69]. The amount of modifier (ethanol) used, combined with supercritical carbon dioxide, was shown to be the most critical factor to maximize polyphenols extraction. Soxhlet ethanol extraction was compared with SC-CO_2 using ethanol as co-solvent. Polyphenols content was only 43% of that obtained by Soxhlet ethanol extraction, although in contrast, SFE needed significantly shorter extraction times [69]

One of the most interesting recent applications of SFE in food analysis sample treatment dealt with the determination of amino acids profiles in different genetically modified varieties of maize and soybean [8]. Supercritical CO_2 modified with 35% methanol at 80 ºC and 120 bar was employed to extract the amino acids, that were afterwards analyzed by GC–MS. These optimal extraction conditions were provided by a central composite design and the statistical analysis of the obtained data. The sum of the areas of the aromatic amino acids was selected as the experimental design response variable. This way, several genetically modified organisms (GMO) could be directly compared to their respective isogenic non-transgenic varieties. Some statistical differences among the amino acids contents in the samples studied were observed [8]. The extraction with modified CO_2 allowed a faster and more efficient recovery of amino acids from maize and soybean grains compared with previous techniques.

12.1 Application of SC-CO2 in Food Industry

One of the primary trends in the food industry is the demand for all-natural food ingredients free of chemical additives. Natural food antioxidants are derivatives of plant by-products. Food waste valorization is under intense worldwide investigation topic and supercritical fluid extraction has been reported as one of the best ways to valorize agro-industry by-products. Those extracts showed sustainable use in food industries as a good source of phenolic compounds with antioxidant activities. For example, the extract of mango peel has been used as a natural antioxidant in sunflower oil to control lipid oxidation; the optimal conditions were 25 MPa, 60 ºC and 15% ethanol in water by using a Box–Behnken design. Supercritical fluid extraction is also applied in the extraction of cholesterol and other lipids from egg yolk, extraction of lipids and cholesterol from meat and meat products, decaffeination of coffee and tea [39], extraction of hops [55,59], extraction of bioactive compounds [9], extraction of free amino acids [67], extraction of lipids and cholesterol from fish [22], extraction of natural glycosides [20], fractionation of natural colourings, natural flavourings, and fragrances from several foodstuffs [7], and the separation of spices and essential oils [14,61,24,8].

12.2. Application of SC-CO2 in the Pharmaceuticals Industry

In the pharmaceuticals industry, development in the properties of active pharmaceutical ingredients is generally preferred. A significant challenge in this respect is particle size reduction, which increases bioavailability [35]. The primary use of supercritical fluids in the pharmaceuticals industry involves processes such as particle formation for drug delivery systems and crystal engineering; complexing cyclodextrins; coating, foaming and tissue engineering; impregnation [3] and purification of pharmaceutical excipients; and sterilization and solvent removal.

The best application of supercritical fluids in pharmaceuticals deals with the isolation of bioactive compounds from a mixture (purification from reactions, quantification of an active enantiomer, extraction from a natural matrix) [39]. Different plants have been used for the extraction of compounds to be used in pharmacy, such as Catharanthus roseus, a rich source of alkaloids, from which two dimeric alkaloids were extracted that are extensively used as antineoplastic drugs vinblastine and vincristine [22,35]. Artemisia annual. exhibits a vigorous antimalarial activity due to the existence of artemisinin and its derivatives (like dehydro-artemisinin). These compounds are particularly active against drug-resistant strains of Plasmodium falciparum [6]. Melocactus zehntneri is a medicinal cactus that is unusual pharmacologically because it contains iso-quinoline and indole alkaloids, which are used in several drugs.

12.3. Application of SC-CO_2 in the Cosmetics Industry

Cosmetics are daily use products that are currently available in a different market. Nowadays, people are interested in natural products, and they have realized that one of the most active ingredients of cosmetics is the antioxidant, which can interrupt radical-chain processes, help skin repair systems, improve cell rejuvenation and prevent skin-cancer. Different research has shown that SC-CO_2 extraction is a great technique that provides natural extracts with attractive fragrances or active ingredients that add value to the cosmetics by enhancing their practical action and attributes. SC-CO_2 has been used for the isolation of antioxidants and parabens from cosmetic products. Vogt *et al.,* (The effect of selected supercritical CO2 plant extract addition on user properties of shower gels). investigated the extract of blackcurrant seeds, strawberry seeds, hop cones, and mint leaves obtained by SC-CO_2 for the production of shower gels and shampoo, and the result showed that those natural extracts could be used as ingredients in shower gels.

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KEYWORDS

Supercritical fluid extraction

Super critical fluid

Solvent extraction

Modifiers

Food industry

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