Supercritical extraction: A green method for oil extraction from fish waste

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By definition, extraction is the removal of soluble material from an insoluble residue, either liquid or solid after treating with a solvent. Rate of diffusion of solute through the liquid boundary layer at the interface is the controlling factor in the process. The extract obtained by conventional procedures will be usually impure liquids often intended for external use. Hence, high quality extraction procedures are of considerable interest to obtain improved yields of drug derived from plant as well as animal sources. However, currently available conventional extraction methods are time consuming, requiring different solvents which are costly, often needing concentration step to improve yield. Limited selectivity and degradation of thermally labile compounds are also associated disadvantages with such methods. The solvents used and waste generated as a result of such extraction is also creating environmental hazards. An ideal extraction method should be swift, environmentally safe, yield quantitative recovery without degradation, and the extracts should be easily separated from the solvent. Henceforth, it is the need of the hour to replace conventional extraction methods with alternative green technology with improved extraction efficiency and low environmental impact. Supercritical fluid extraction (SFE) technology offers many features that can overcome many limitations of conventional extraction methods. Hence laboratories engaged in innovative research are currently developing SFE methods to replace conventional methodologies for routine analyses utilizing the high solvent power of supercritical fluids (SFs).

Supercritical Fluid

Matter exists in three most common phases which are solid, liquid, and gas. The phase of a pure simple substance depends on the temperature and pressure. Phase diagram shows a substance's phase at a given temperature and pressure as well as show the temperatures and pressures at which any two phases can coexist in equilibrium (Fig.1). The critical point refers to the temperature and pressure at which above which the substance can no longer be condensed into a liquid. Beyond the critical point, there is no longer an equilibrium curve to divide the liquid and gaseous regions; thus, the liquid and gas phases are no longer distinguishable. This region of the phase diagram is called the supercritical fluid region.

A supercritical fluid can be defined as a form of matter in which the liquid and gaseous phases are indistinguishable. Supercritical fluids are having more densities comparable to liquids. As a result, these fluids have solvating power. Supercritical fluid exhibits physicochemical properties intermediate between those of liquids and gases. Both liquid-like and gas-like characteristics of supercritical fluids make them unique for chemical separation. In particular, supercritical fluid densities, diffusivities, and viscosities fall into ranges between those of liquids and gases. Properties of supercritical fluid are given below:

(i) Supercritical fluids behave like gases and liquids in an interesting manner.

(ii) Supercritical fluids can lead to reactions, which are difficult to achieve in conventional solvents.

(iii) For most of the solutes, supercritical fluids have solvent power similar to light hydrocarbons.

(iv) Solubility of SFs increases with increasing density.

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

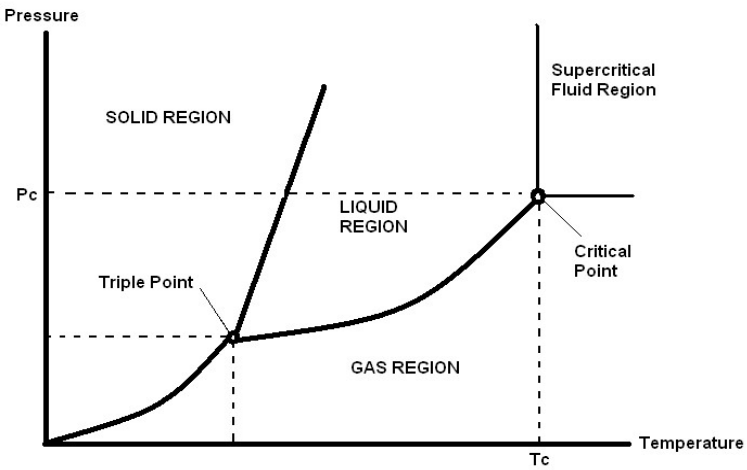

Figure 1: CO_2 Pressure - Temperature Phase diagram

Available Supercritical Fluids

The most popular SFE solvent is carbon dioxide. It is inexpensive, non-flammable, relatively nontoxic, low critical temperature and commercially available even at high purity. The SFE solvent supercriticalCO_2have extraction conditions above the critical temperature of 31°C and critical pressure of 74 bar.Supercritical$CO_2$is having density of around 200 bar pressure is close to that of hexane. The solvation characteristics are also similar to hexane since it acts as a non-polar solvent. Around the supercritical region, CO_2 can dissolve triglycerides at concentrations up to 1% mass. Other SFE solvents used are nitrous oxide (laughing gas), nitrogen, propane, ammonia, fluoroform, freons, and water. But in all the cases the number of disadvantages outweighs the advantages. Carbon dioxide does have a few disadvantages even though it is practically the only solvent for SFE. CO_2

has limited solvating power and expensive instrumentation is required to maintain high critical pressure. Critical properties of various solvents used in SFE are given below:

Critical properties of various solvents (Reid et al., 1987)

Solvent	Molecular weight (g/mol)	Critical temperature (K)	Critical pressure MPa (atm)	Critical density (g/cm^3)
Carbon dioxide (CO_2)	44.01	304.1	7.38 (72.8)	0.469
Water (H_2O) (acc. IAPWS)	18.015	647.096	22.064 (217.755)	0.322
Methane (CH_4)	16.04	190.4	4.60 (45.4)	0.162
Ethane (C_2H_6)	30.07	305.3	4.87 (48.1)	0.203
Propane (C_3H_8)	44.09	369.8	4.25 (41.9)	0.217
Ethylene (C_2H_4)	28.05	282.4	5.04 (49.7)	0.215
Propylene (C_3H_6)	42.08	364.9	4.60 (45.4)	0.232
Methanol (CH_3OH)	32.04	512.6	8.09 (79.8)	0.272
Ethanol (C_2H_5OH)	46.07	513.9	6.14 (60.6)	0.276
Acetone (C_3H_6O)	58.08	508.1	4.70 (46.4)	0.278

Supercritical Fluid Extraction (SFE)

SFE can be defined as the process of segregating one component from the matrix by using supercritical fluids as the solvent. Extraction is usually done from a solid matrix, but also possible from liquids. SFE is useful as a sample preparation step (for analytical purposes) or to strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). In SFE, the mobile phase is subjected to pressures and temperatures near or above the critical point for the purpose of enhancing the mobile phase solvating power. The process begins with CO_2 in vapour form. It is then compressed into a liquid before becoming supercritical.

Supercritical Fluid Extraction (SFE) System extracts chemical compounds using supercritical carbon dioxide instead of an organic solvent. The supercritical fluid state occurs when a fluid is above its critical temperature (Tc) and critical pressure (Pc), when it is between the typical gas and liquid state (Raventós *et al*., 2002). Manipulating the temperature and pressure of the fluid can solubilize the material of interest and selectively extract it. The sample is placed in an extraction vessel and pressurized with CO_2 to dissolve the sample. Transferred to a fraction collector, the contents are depressurized and the CO_2 loses its solvating power causing the desired material to precipitate. The condensed CO_2 can be recycled.

In SFE, the applications of supercritical carbon dioxide was having biggest interest, because it has a near ambient critical temperature (31^0C), thus biological materials can be processed at temperatures around 35^0C. The advantage here is that with a slight reduction in temperature or a slightly larger reduction in pressure can lead to precipitation of the entire solute. In addition, supercritical fluids can extract a product with minimal solvent residues. Utilization of SFE technology in decaffeinated coffee, cholesterol-free butter, low-fat meat, evening primrose oil, squalene from shark liver oil and many more. The solvation characteristics of supercritical CO_2 can be modified by the addition of an entrainer like ethanol (Doane-Weideman and Liescheski., 2004).

Instrumentation

The instrumentation required to perform a successful SFE is commercially available. The process begins with a clean source of fluid, which in most cases is a high-pressure cylinder of CO_2. A pump is used to increase the pressure of the fluid above its critical pressure. The working extraction pressure is determined by the density required to dissolve the target analytes from the sample. The sample is contained in the extraction chamber, which is heated to the desired extraction temperature above the critical point. The pressurized fluid is brought to temperature by the chamber and allowed to flow through the sample matrix to extract the analytes. After the sample, the analyte fluid flows to a restrictor, this controls the flow rate of the fluid. The restrictor maintains the high pressure of the fluid in the chamber. At the restrictor, the supercritical fluid loses its solvating strength as its pressure drops to atmosphere. After the restrictor, the analytes can be collected for analysis (Sapkale *et al*., 2010). Figure 2 shows a block diagram of a complete SFE system.

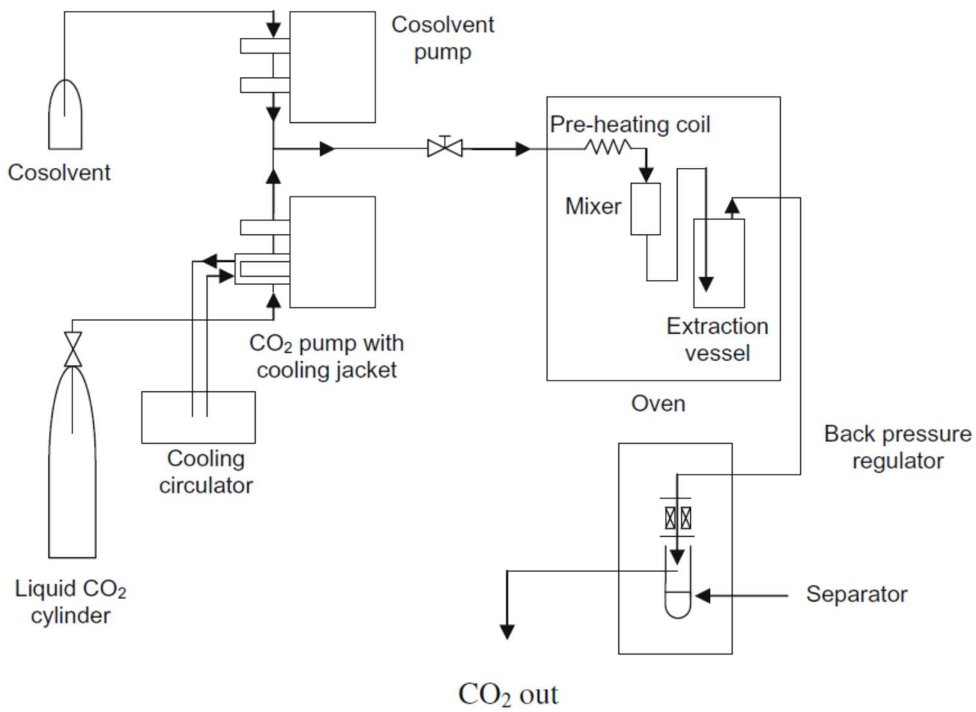

Fig.2: Schematic diagram of Supercritical fluid extraction apparatus

Advantages of SFE

- Environmental safety: SFE is a substitute to liquid extraction which uses organic solvents such as hexane or dichloromethane. There is always chance of solvent residue in the extract and matrix and there is always some level of environmental contamination from their use. Whereas carbon dioxide is easy to remove simply by reducing the pressure, leaving almost no trace and it is also environmentally benign. The use of SFE with CO_2 is also approved by the Soil Association for organic products. The CO_2 used is largely a by-product of industrial processes or brewing, and its use in SFE does not cause any extra emissions.
- Selectivity: By changing the pressure and the temperature, the solvent strength of a supercritical fluid can be altered. For example, volatile oils can be extracted from a plant with low pressures (100 bar), whereas liquid extraction would also remove lipids. By SFE, lipids can be removed using pure CO_2 at higher pressures, and then phospholipids can be removed by adding ethanol to the solvent.
- Speed: It is a fast process and completed in 10 to 60 minutes. It is a diffusion-based process, with the solvent required to diffuse into the matrix, and the extracted material to diffuse out of the matrix into the solvent.
- Purity: A supercritical fluid can be separated from an analyte by releasing pressure so that the product will be almost pure.
- Recovery: Recovery of analytes is simpler as compared to conventional techniques.
- Supercritical fluids are cheap, inert and nontoxic. Thus, they are readily disposed off after an extraction is completed by allowing them to evaporate into the atmosphere.

Efficiency in sample preparation

Because SFE has several distinct physical properties, it is regarded as a promising alternative technique to conventional solvent extraction. Some of its major advantages are summarized as follows:

(1) Super critical Fluids have higher diffusion coefficients and lower viscosities than a liquid solvent. So, solubility and diffusivity in such fluids tends to be much higher than in liquids, resulting in comparatively fast reactions (Mira *et al*., 1999).

(2) In Super critical Fluid extraction, the solvation power of the fluid can be controlled by changing pressure (P) or temperature (T); so, it may achieve a remarkably high selectivity. This solvation power of SFs is useful for the extraction of complex samples.

(3) In Super critical Fluid extraction, fresh fluid is continuously passes through the sample; therefore it can provide complete extraction (Stashenko *et al*., 196).

In addition to these benefits, another advantage of SFE over conventional methods is that, it involves less duration and minimal usage of organic solvents. It was shown that SFE for 30–60 min provides higher recoveries than several hours of Soxhlet extraction (Reindl *et al*., 1994)

Applications:

SFE applications in the food, pharmaceutical, and fine chemical industries:

- Decaffeinating of coffee and tea
- Extraction of essential oils (vegetable and fish oils)
- Extraction of flavors from natural resources (nutraceuticals)
- Extraction of ingredients from spices and red peppers
- Extraction of fat from food products
- Fractionation of polymeric materials
- Extraction from natural products
- Photo–resist cleaning
- Precision part cleaning

Supercritical fluid extraction of fish oil

Polyunsaturated fatty acids, especially omega-3, are essential in human nutrition since they play an important role in the organism and prevent several diseases. Fish has been the traditional source to obtain omega-3 enriched oil and concentrates. Supercritical fluid extraction was demonstrated as green method in the production of omega-3 oil and omega-3 concentrates, avoiding the use of high temperatures and organic solvents. The most widely used SCF is carbon dioxide that is considered a green solvent; CO_2 is non toxic, cheap and non flammable. It has mild critical conditions (T_c=304.15 K and P_c=7.38 MPa) that allow to process thermolabile compounds, as omega-3 PUFA, and makes operation costs not too expensive, and is gaseous under ambient conditions and therefore easy to separate from the processed products after processing. Supercritical CO_2 is also a good solvent of fats and oils, and a lot of research has been made to determine the solubility of the different oil components, specifically fish oil components and related compounds, and to study the viability of oil extraction and fractionation. A comparison of the oils obtained by SFE over freeze-dried fish by-products and by other methods carried out (cold extraction, wet reduction and enzymatic extraction), shows that SFE is a useful method to prevent lipid oxidation, especially in fish oils rich in omega-3. In spite of involving higher inversion costs, SFE presents some advantages over other extraction processes. Furthermore, fractionation of the extract in two separators after SFE is an easy way to enhance fish oil quality by reducing the amount of free fatty acids as well as some oxidation products.

Supercritical fluid extraction of fish oil starts with liquid CO_2 contained in the high pressure cylinder. Pressure will be then increased above its critical pressure by the help of pumps. The pressurised fluid will be then bought to the extraction vessel where in it will be heated to a temperature above its critical point. The SCF is then allowed to interact with the fish sample loaded in the extraction chamber for the required time of experiment and at the desired pressure. SCF along with the target analyte (oil) then moves to the restrictor, which maintains the high pressure of fluid when inside the extraction vessel. At the restrictor, SCF pressure is brought down to the atmospheric pressure and as a result, loses its solvating power. The oil will be collected inside the collection vessel.

Conclusions and future trends

The valorisation of fish by-products by recovering their oil has a great interest in the fish industry, especially when the oil is rich in triglycerides and has a high content of omega-3 polyunsaturated fatty acids. The extraction process used to obtain omega-3 rich oils has been also shown to be important to obtain the best oil quality regarding lipid oxidation, pollutants content and sensory properties. Supercritical fluid extraction technology can offer attractive features for obtaining fish oil and overcome many limitations that exist in other extraction methodologies. SFE allows the control of fluid density by changing its pressure and/or temperature thus providing faster extraction rates. SFE can be regarded as a more sustainable, cleaner and environmental friendly extraction of fish oil, while providing tools and technology output for future laboratorial and industrial development. Smart, systematic development of SFE can be expected to consolidate it into an advantageous alternative to conventional solid-liquid extraction, so that its real, great potential can be fully realized. Supercritical fluid extraction cannot be considered as a fully mature technology. Knowledge of the chemical properties of both the analyte and the matrix is important for SFE. In addition, one must ensure that mechanics of SFE have been optimized.

Suggested Readings

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