Green technologies for isolation of marine bioactive compounds

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1.0 Introduction

Marine fauna and flora are reported to be richest repository of wider range of bioactive compounds. Polyunsaturated fatty acids, essential amino acids, unique proteins, vitamins, minerals, sulfated polysaccharides, pigments, etc., are present in marine organisms. It has been reported that marine bioactive compounds exhibit significant and biological properties contributing to their nutraceutical and pharmaceutical potential. By virtue of these properties, they are often reported to be safer alternatives to some of the synthetic drugs. Often marine bioactive compounds are reported to be extracted from various sources such as these compounds fish, shellfish, molluscs, crustaceans, echinoderms, seaweeds, microalgae and many other marine microbes. Further, there has been mounting evidence that the consumption of marine foods and biomolecules can reduce the incidence of many ailments. This coupled with the increased consumer awareness regarding the benefits of consumption of marine bioactive compounds has fostered its research. Accordingly, extraction of bioactive compounds is being attempted on a larger scale globally. Conventional solvent-based methods are often employed for extraction of bioactive compounds from marine sources. However, such methods are time consuming, require a longer duration and need further purification/concentration step. In addition to this, there is an increasing global concern on the use of organic solvents for extraction as they are not eco-friendly methods. Hence, innovative, sustainable and greener extraction technologies such as supercritical fluid extraction (SFE) were introduced as an alternative to conventional separation methods. SFE helps in the efficient extraction of thermolabile compounds as the process is often carried out at lower temperature. Apart from SFE, other greener technologies that are currently employed for extraction of marine bioactives include ultrasound-assisted extraction (UAE), microwave assisted extraction (MAE), eutectic solvents, deep eutectic solvents (DES), switchable solvents (SS), etc. Among all, SFE has garnered significant attention globally owing to its selectivity of extraction process.

2.0 Supercritical fluid extraction

Supercritical fluid extraction has emerged as one of the most feasible, economic, and environmentally friendly methods for extraction of commercially important bioactive compounds from natural resources. Recently, a substantial amount of research has been carried out to establish the effectiveness of SFE in extracting biomolecules from marine sources/fish-processing discards. In general, supercritical fluid extraction can be defined as a green eco-friendly technology wherein a supercritical fluid (SCF) is used as solvent for extraction of natural biomolecules without affecting their bioactivity. Supercritical fluid can be any substance whose temperature and pressure should be above its critical point (CP) to form a homogenous phase possessing properties of both liquid and gas, commonly referred to as the mesophase. In simpler terms, supercritical fluid can be defined as that form of matter in which the liquid and gaseous phases are indistinguishable (Lekshmi et al., 2023). The properties of SCFs are listed below:

- Highly compressed gas having properties of both gas and liquids;
- Have low viscosity, high diffusivity and hence enhanced transport properties;
- Can fine tune the solvating properties, density, and solubility by changing the pressure/temperature accordingly.

The most commonly used solvent in SFE is carbon dioxide (CO_2) as its critical conditions (critical pressure, Pc – 74 bar, critical temperature, Tc – 31.1°C) are easily achievable at ambient conditions. Non-toxicity, safety, non-flammable, inexpensive, availability, inertness, high diffusion coefficient, lower density, increased mass transfer makes it as an excellent candidate for SFE. Furthermore, such lower temperatures make it favorable for extraction of many thermolabile compounds. Being non-polar in nature, supercritical CO_2 (SC-CO_2) can extract only non-polar compounds or substances of lower polarity. For this reason, certain solubility enhancers known as co-solvents/modifiers can be added to increase the polarity of SC-CO_2. Methanol, ethanol, chloroform, ethane, etc., are some of the co-solvents being used in SFE among others. Among the available co-solvents, ethanol is the most recommended as it is non-toxic, miscible in CO_2 and food-grade (Liza et al., 2010)

2.1 Supercritical Fluid Extraction- Instrumentation

The instrumentation required to perform a successful SFE is commercially available. The supercritical fluid extractor unit consists of fluid source (CO_2 cylinder), pumps, extraction chamber, heat exchangers, collection chamber/separator, restrictors. 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.

2.2 Advantages of SFE

SFE have several advantages over the conventional methods and some are listed below:

• **Lower viscosity and high diffusion coefficient attributes**, SCFs enhanced transport properties, facilitating efficient extraction;

• **Solvent strength of SCF** can be modified by changing the extraction pressure and temperature; • SFE leaves no chemical residue making the process sustainable;

• Lower energy requirements;

• **Highly efficient** process in terms of increasing yield and lower extraction times;

• **No degradative changes** to the bioactive compounds extracted using SFE

•**Environmental safety**: As carbon dioxide is easy to remove simply by reducing the pressure, the chances of having solvent residues in the final product is negligible only when ethanol is used as co-solvent making the process more environment friendly

•**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

•**Speed**: Being a diffusion-based process and with the enhanced transport properties associated with supercritical CO2, the whole process can be finished in 45 min – 6 h which is very less compared with the conventional extraction techniques.

•**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**.

2.3 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.

(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

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

2.4 SFE for extraction of fish oil

The production of high-quality fish oil has assumed great significance in the recent past owing to its reported health benefits. Production of good quality fish oil depends to a greater extent on the type of raw material, quality of raw material, the type of extraction method and extraction conditions. Globally, there is an increasing trend to utilise the fish/seafood processing discards as raw material for production of high quality bioactive. It has been well documented that both fish and fish by-products can serve as excellent resources for extraction of omega-3 rich oil. SFE has evolved as one of the most feasible sustainable extraction methods for obtaining fish oil (Létisse *et al*., 2006). Rubio-Rodríguez *et al* (2012) have compared four different methods, such as cold extraction, wet reduction, enzymatic extraction and supercritical fluid extraction for obtaining oil from fish by-products. It was observed that among all methods, SFE can be a feasible method from the stability and safety point of view, as fish oil extracted by SFE had better oxidative stability and reduced arsenic content. Sahena *et al* (2010) has compared soxhlet extraction with SFE for obtaining oil from mackerel skin. It was observed that almost similar yield of fish oil was obtained using both the methods. Similarly, Sahena *et al* (2010) has analysed the fatty acid profile of oil extracted from various parts of Indian mackerel (*Rastrelliger kanagurta*) such as skin, flesh, viscera and head using various techniques of supercritical carbon dioxide (SC-CO_2), viz, continuous, cosolvent, soaking and pressure swing. All the extractions were carried out the same conditions (350 bar pressure, 60 °C temperature) and the efficiency of the process was compared with that of soxhlet. It was reported that among the different techniques employed, soaking and pressure swing techniques were efficient in extracting major polyunsaturated fatty acids, EPA and DHA.

Generally, the extraction efficiency of SFE are reported to be affected by change of major factors such as pressure, temperature, time, co-solvents etc. (Plaza and Rodríguez-Meizoso, 2013). Several researchers have investigated the effect of these variables on the yield, quality and quantity of bioactives obtained. Létisse *et al* (2006) have optimised the extraction conditions for obtaining EPA and DHA rich oil from sardine and efficiency was compared with that of conventional solvent based extraction. It was reported that about 11% of EPA and 13% of DHA were extracted from sardine at the optimised extraction conditions (300 bar pressure, 75 °C temperature, 45 min time). Though the extraction yields were better with solvent based extraction, it was suggested that SFE can be more advantageous from a time and quality point of view.

3.0 Other emerging methods for extraction of marine biomolecules

Apart from the most commonly employed methods like SFE, there are other environment friendly extraction techniques such as ultrasound assisted extraction (UAE), microwave assisted extraction (MAE) etc. UAE has found wider applications in seaweeds for isolation of carotenoids like fucoxanthin and phenolic compounds such as phlorotannin, pigments such as phycoerythrin, phycocyanin, and biopolymers such as carrageenan, laminarin, alginate, etc. Apart from this, UAE are finding excellent applications in isolation of fish proteins. By employing microwave assisted extraction, a range of biomolecules such as chitin, chitosan, hydroxyapatite, fucoidan, etc. can be isolated. Other than this, there are other extraction techniques which employs ionic liquids, DES, switchable solvents as extraction medium. DES are extraction mixtures comprising of a hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). Biodegradability, low toxicity, easiness in preparation, high thermal stability, low volatility, non-flammability, etc., makes DES one of the most efficient green extraction techniques (Xu et al., 2020). Das et al. (2016) have recently employed DES for extraction of kappa-carrageenan from red seaweed, *Kappaphycus alvarezii* and has reported that the hydrated DES were more effective in isolation.

4.0 Conclusions and future trends

Supercritical fluid extraction technology can offer attractive features for obtaining bioactive compounds 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. Accordingly, it is expected that the integration of single and combined technologies will lead to higher extraction yields and greater selectivity of such bioactive compounds with significant interest to the pharmaceutical industry. SFE can be regarded as a more sustainable, cleaner and environmental friendly extraction process in the research of bioactive compounds, 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:

- Das, A.K., Sharma, M., Mondal, D. and Prasad, K., 2016. Deep eutectic solvents as efficient solvent system for the extraction of κ-carrageenan from *Kappaphycus alvarezii*. Carbohydrate Polymers, 136, pp.930-935.
- Lekshmi R.G.K., Chatterjee, N.S. and Mathew, S., 2023. Green Technologies for Developing High-Value Novel Marine Nutraceuticals: Scope and Innovations. Advances in Fish Processing Technologies, pp.271-286.
- Létisse, M., Rozières, M., Hiol, A., Sergent, M and Comeau, L. 2006. Enrichment of EPA and DHA from sardine by supercritical fluid extraction without organic modifier: I. Optimization of extraction conditions. The Journal of supercritical fluids, 38 (1): 27- 36.
- Liza, M.S., Rahman, R.A., Mandana, B., Jinap, S., Rahmat, A., Zaidul, I.S.M. and Hamid, A., 2010. Supercritical carbon dioxide extraction of bioactive flavonoid from *Strobilanthes crispus* (Pecah Kaca). Food and Bioproducts Processing, 88(2-3), pp.319-326.
- Plaza, M and Rodríguez-Meizoso, I. 2013. Advanced extraction processes to obtain bioactives from marine foods. Bioactive compounds from marine foods: Plant and animal sources, 343-371.
- Rubio-Rodríguez, N., Sara, M., Beltrán, S., Jaime, I., Sanz, M.T and Rovira, J. 2012. Supercritical fluid extraction of fish oil from fish by-products: A comparison with other extraction methods. Journal of Food Engineering, 109 (2): 238-248.
- Sahena, F., Zaidul, I.S.M., Jinap, S., Jahurul, M.H.A., Khatib, A and Norulaini, N.A.N. 2010. Extraction of fish oil from the skin of Indian mackerel using supercritical fluids. Journal of Food Engineering, 99 (1): 63-69.