new food

Ingredients

SUPPLEMENT

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Supercritical fluid extraction and application of bio active ingredients from plant materials

Supercritical fluid extraction (SFE) technology is a green technology that can be a replacement to the conventional solvent extraction technology, having less impact on the environment[1]. It surely meets the consumer demand for safe, natural and high quality ingredients. The industries using SFE technology are increasing nowadays and are also reported to have market advantage.

SFE is a process in which the gas beyond its critical temperature and pressure is fed to the extraction vessel in order to extract the desired compound/mixture of compounds from the product matrix (**Figure 1**, page 3). Under supercritical state the fluid has both the properties of gas and liquid, as it has density like liquid and viscosity like gas. This property enhances the diffusivity of the fluid in the product containing matrix and increases the rate of extraction of the targeted compound/s. SFE is a selective process in which the process variables, such as pretreatment to plant material, temperature, pressure, particle size, moisture content, solvent flow rate, extraction time and co-solvents (usually 1 to 10%) are varied according to the target compound to be extracted[2].

Important process variables

Pretreatment of the plant materials is an important step in preparation for SFE extraction. Pretreatment of the plant usually involves drying to reduce the moisture content and grinding for size reduction[3]. As water is polar in nature it may hinder the non-polar solvent like CO_2 and reduce the efficiency. Pressure and temperature are the interrelated parameters as they directly affect the solubility of the target compound as well as the solvent. Solvent flow rate and extraction time are also important parameters as they directly affect the throughput of the process and economics, hence they should be carefully controlled to improve the economics of the SFE process. Grinding reduces the particle size and impermeable plant tissues, thereby increasing the surface area for its interaction with the solvent. Hence, the smaller the particle size, the greater the extraction of target compound is expected; however, too small a particle size may create a compact bed and affect the diffusion of solvent into the solid matrix. The heat generated during the fine grinding of the plant material has to be controlled in order to avoid loss of volatile compounds. The application of low temperature grinding, such as cryogenic grinding, may further improve the grinding process, which results in high quality powder. In one of the studies conducted at Anand Agricultural University (AAU), on cryogenic grinding of coriander (*Coriandrum sativum* L.), it was observed that during the ambient grinding the amount of volatile oil obtained was 0.133% whereas in the case of cryogenic grinding the amount of volatile oil content was 0.607%. This indicated that in the case of cryogenic grinding 22% more volatile oil was retained as compared to ambient grinding. Similar studies have been conducted at AAU on cumin and cardamom and process parameters have been optimised.

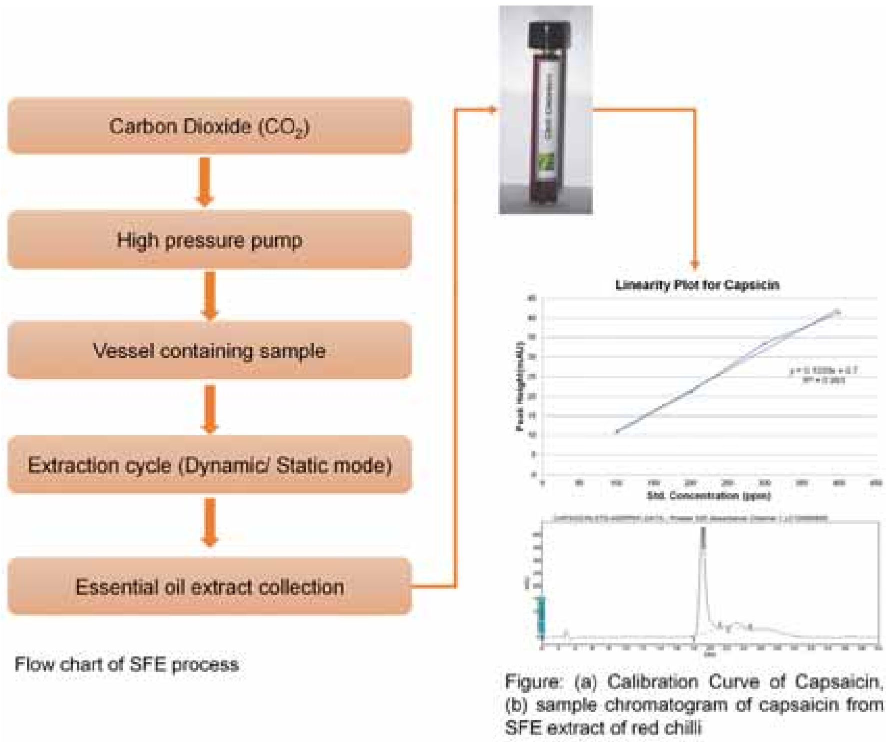

Figure 1: Typical flow chart for SFE extraction and characterisation of essential oil

Due to the many process variables that affect the efficiency of the SFE extraction, systematic studies at lab scale are required for each product, which may have a different set of optimum process variables for the extraction of the target compound. There are different solvents, such as pentane, ethanol etc., that can be used in the SFE process, however, CO_2 is the most widely used in industry due to its many advantages. These include a low critical pressure (72.8 bar) and temperature (30.9°C); the fact that it's relatively non-toxic, non-flammable, low cost and can be easily removed from the extract leaving no residue. With the recent advances in SFE technology, it is now possible to recycle the CO_2 after decompression so that operational cost and emission in the atmosphere can be reduced.

"Pretreatment of the plant materials is an important step in preparation for SFE extraction"

Application of SFE

SFE can play a critical role in the nutraceutical and functional food industries because of its ability to extract product without solvent residue, high purity and no degradation of active compounds[4]. There have been extensive scientific studies reported on the extraction of different bioactive compounds, such as essential oils, phenolic compounds, vitamins, alkaloids, natural colours, etc., which were extracted from different parts of the plant. Some plant sources from which the bioactive compounds were extracted and studied are mentioned in Table 1. SFE can also be used to remove undesirable compounds such as organic pollutants, toxins, and pesticides. The bioactive compounds play a major role in improving human health and diet by acting as antimicrobial, antifungal, antioxidant, anti-inflammatory and other health promoting actions.

Essential oils are the volatile oils obtained from the plant sources and they impart characteristic flavour and fragrance to food products. SFE has been extensively studied for extraction of essential oils from

Table 1: The bioactivity of extracts obtained from different plant sources

Bioactivity	SCFE extract from Plant Source
Antioxidant	*Lavandula viridis, Mentha spicata, Vitis labrusca, Rubus sp., Bactris gasipaes,* grape marc, *Hymenaea courbaril* L., *Piper nigrum* L., *Vaccinium myrtillus* L., *Eugenia uniflora* L., *Pleurotus ostreatus, Campomanesia xanthocarpa, Copaifera langsdorffii*
Antitumor	*Bidens pilosa, Ceratonia siliqua, Prunus avium,* Rosemary, *Ganoderma lucidum, Cordia verbenacea*
Antimicrobial and Antibacterial	*Apium graveolens, Citrus sinensis, Eupatorium intermedium, Cyperus articulatus* L., *Eupatorium intermedium, Campomanesia xanthocarpa, Alpinia oxyphylla*
Anti-obesity and hypolipidemic	*Penaeus brasiliensis* and *Penaeus paulensis, Hippophae, Citrullus lanatus*

Table 2: SFE conditions for essential oil extraction from different plant materials

Plant material	Target compound	Optimum SFE conditions
Basil leaves (*Ocimum sanctum*)	Eugenol	Temperature 54°C Pressure 150 bar Particle size 0.8 mm Dynamic time 37.5 min
Mint (*Mentha spicata*)	Cavone	Temperature 48°C Pressure 150 bar Particle size 0.4 mm Dynamic time 38 min
Red chilli (*Capsicum annuum* L.)	Capsaicin	Temperature 65°C Pressure 200 bar Particle size 0.5 mm Dynamic time 90 min
Curry leaves (*Murraya koenigi* L.)	β-caryophyllene	Temperature 65°C Pressure 200 bar Particle size 0.5 mm Dynamic time 90 min

different plant sources. Some studies conducted at AAU, Anand are presented in Table 2 (page 3). For example, in the case of essential oil extraction from mint, carvon was the target compound. Based on the systematic study using experimental design, the optimum condition having maximum carvon extracted from mint leaves was identified. All the samples were analysed using GC-MS for their carvon content. The bioactivity of the essential oil from mint was also evaluated in terms of antimicrobial activity and highest antimicrobial activity against *E. coli* and a moderate activity against *S. typhi* and *S. aureus* was observed. In many reports the essential oils are reported as having bioactive properties, such as antioxidant activity, antimicrobial and anti-inflammatory. Essential oils are conventionally extracted from hydrodistillation and solvent extraction. The high temperature used in hydrodistillation may degrade the essential oil and alter their chemical structure, thus affecting their natural quality attributes. However, SFE technologies have been reported to be of superior quality than the conventional extraction technologies.

In mint, carvon was the target compound

Apart from extraction of the bioactive compounds, SFE technology has been reportedly used for supercritical drying, coating/impregnation and particle formation which have opened up new research areas for their applications in the pharmaceutical and nutraceutical industry. Supercritical drying has been used for the drying of wet gels, while reducing the shrinkage and resulting in a superior gel matrix. Particle design is also one of the emerging research areas where supercritical fluids are used in design and generation of particles, microspheres and microcapsules, which may find applications in pharmaceuticals and nutraceutical products. With the latest developments in SFE technology and extensive research carried out in the past, it may be concluded that this technology has enormous scope in high quality bioactives extraction from plant and other sources, to meet the current global demand of SFE extracts.

SFE has been extensively studied for extraction of essential oils from different plant sources

References and Further Reading

1. da Silva, R. P., Rocha-Santos, T. A., & Duarte, A. C. (2016). Supercritical fluid extraction of bioactive compounds. *TrAC Trends in Analytical Chemistry*, 76, 40-51
2. Martínez, J. L. (Ed.). (2007). Supercritical fluid extraction of nutraceuticals and bioactive compounds. CRC Press
3. Temelli, F., Saldaña, M. D. A., & Comin, L. (2012). Ch. 4.18 – Application of Supercritical Fluid Extraction in Food Processing A2 – Pawliszyn, Janusz *Comprehensive Sampling and Sample Preparation* (pp. 415-440). Oxford: Academic Press
4. Pereira, C. G., & Meireles, M. A. A. (2010). Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food and Bioprocess Technology, 3(3), 340-372.

About the Authors

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Nutritional properties of seaweeds

The Webster's Dictionary defines food as "a material consisting of protein, carbohydrate, and fat used in the body of an organism to sustain growth, repair, and vital processes, and to furnish energy". As such, the foods we eat affect our overall health, development, and well-being. Therefore, there is some irony in the fact that neurological diseases, particularly in developed countries, are on the rise[1], with the economic costs of these estimated to be in the €798 billion range[2]. Such neuro-degenerative disorders include Alzheimer's, dementia, Parkinson's Disease, Multiple Sclerosis, and Huntington's, among many others. Another sobering statistic around human health relates to cardiovascular diseases that remain the number one cause of death globally[3]. Indeed, the American Heart and Stroke Association reported in 2015 that approximately 2,150 Americans die each day from these diseases; that equates to one every 40 seconds[4]. While the reasons for these troubling statistics are both complicated and varied, food remains a fundamental component contributing to our overall health and wellness.

From the provision of all the brain essential nutrients, to high fibre contents and robust antioxidant capacities, macroalgae (seaweeds) can play important roles in human health and nutrition. The only nutritional element seaweeds collectively lack – in terms of a food to 'sustain growth, repair, and vital processes, and to furnish energy' – is calories. In these troubling 'obesogenic' times, that is not a bad attribute; in fact it is an important factor regarding the contention that seaweeds are ideal candidates for improving the nutritional quality of manufactured foods[5]. In their role as an ancient and natural food resource, seaweeds remained important to only a relatively small number of global consumers; mostly in Asia and a scattering of coastal populations around the world. However, today science is enlightening the general public in regard to the nutritional and wellness benefits of this fascinating precursor to land plants. It is estimated, for example, that around nine million tonnes of farmed seaweeds were used for direct human consumption in 2012[6]. Soon the expectation is that, in order to meet the demands of an ever increasingly educated public, food manufacturers will be formulating and producing a plethora of goods containing whole seaweeds and seaweed ingredients or extracts.

In terms of protein content, seaweeds can be an excellent source, although total amounts depend upon species and culture conditions. Dulse (*Palmaria palmata*), for example, can range between 8-35%

protein and some nori species (*Pyropia*) have been shown to contain as much as 47% protein. The carbohydrate content of seaweeds is most significant in relation to the high proportion of soluble and insoluble fibres associated with them. These function both as important pre-biotic agents, enhancing gut health and improving bacterial populations, and by providing bulking effects, facilitating smooth passage through the gastrointestinal tract. The fat content of seaweeds is relatively low and mainly consists of lipids, or fatty acids. However, it is noteworthy that algae – both microalgae and macroalgae – are the rudimentary sources of the essential n-3 polyunsaturated fatty acids, docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA). It is actually at this lower level of the food chain where those vital fatty acids are synthesised de novo, and they are then consumed and concentrated by fish and shellfish[7,8]. Significantly, the ratio of n-6 to n-3 in seaweeds (and fish) is approximately 1:1, very similar to the proportions of the fatty acids in the human brain. Disturbingly, the wide global availability of n-6 in our foods has skewed that important ratio such that, in the United States, for example, the ratio of n-6 to n-3 is actually in the vicinity of 20; in Denmark it is around eight; and in Mexico it is 25. The ideal is considered to be 1-5[9].

Historically Asian and costal populations ate more seaweed, but now this is expanding

In addition to the macronutrients necessary for sustaining human growth and development, seaweeds also contain all the brain essential nutrients necessary for neural growth and protection, cognition, learning, and memory. This point is especially important with respect to maternal diets as brain development begins with the foetus and continues until approximately age two. Deficiencies in the neonate of vitamin B_{12}, zinc, iodine, iron, or DHA, for example, can lead to impaired learning and poor cognitive development even later in life. Some seaweeds are viable sources of B_{12} and *Pyropia* is one of them, however only a small proportion of the over 10,000 seaweed species in the world have been adequately analysed for this essential vitamin, which can only be obtained from our diet. Also critical for human cognitive function and development, linking neuronal pathways and playing a pivotal role in learning and memory, is the transition metal, zinc. After iron, this is the most abundant metal nutrient in the body, and it is present in all tissues. Zinc is normally present in a minor plasma pool within the human body and it has a rapid turnover rate, such that a daily intake is required to achieve steady state and to maintain and support all the functions it is involved in. With their less diverse diets, many elderly people are also susceptible to zinc deficiencies, as this mineral is present in relatively small amounts in most foods. Good sources of zinc are certain cuts of meat, such as liver, oysters and crustaceans and, of course, seaweeds.

A report published by WHO[10] stated that iodine deficiency was the world's greatest, single cause of preventable mental impairment, and its deficiency negatively impacts the intellectual ability of an individual by between 10 and 15 IQ points. It is widely recognised that seaweeds are a robust source of iodine and they are often referred to in precautionary terms with respect to potential toxicity that could result from excessive consumption of certain species, particularly brown seaweeds. However, iodine concentrations vary considerably among species and Nitschke and Stengel[11] analysed 19 species of seaweeds for iodine content and reported 359-1920$\mu g \cdot g^{-1}$ (fresh weight) in the browns; 9-174$\mu g \cdot g^{-1}$ in the reds; and 3-14$\mu g \cdot g^{-1}$ in the green seaweeds measured. Studies on iodine bioavailability from an edible seaweed administered daily to a group of iodine-insufficient women for two weeks significantly increased urinary iodine excretion and the concentrations of thyroid stimulating hormone[12]. The researchers concluded that, with adequate analytical testing, the inclusion of seaweed in staple foods would serve as an effective alternative to fortification with salt or KI (potassium iodide) as a means to combat iodine deficiency.

Seaweeds are a substantial source of prebiotic fibres, amongst other proven health benefits

Considering the environments in which seaweeds grow and survive – at the interface of land and sea – it is no wonder they are robust sources of antioxidants and antioxidant compounds. The stresses they are exposed to include tidal extremes, high light conditions, often vigorous wave action, rain and wind, herbivory, and, in colder climates, ice and snow, and yet they survive and reproduce. The free radical scavenging activities of seaweeds have demonstrated anti-inflammatory and neuro-protective effects in humans and animals, making seaweeds potent food sources for not only balancing nutrition, but by providing avenues for cellular homeostasis[13]. Reactive oxygen species (ROS) underlie the pathologies associated with all forms of systemic duress, and the adult human brain is particularly prone to oxidative stress, in part, as a result of its high oxygen consumption. This effect of oxygen amount per unit of tissue mass is even more pronounced in young children with smaller bodies but not proportionately smaller brains.

Seaweeds provide many essential nutrients

A considerable volume of new research is centred around the microbes reported to be in us and on us, and what they do to contribute to human health and wellness. It is startlingly clear that the several trillion human gut microbes and the metabolites they produce influence both physical and psychological health. Even emotional and cognitive function may be impacted by our gut microbes, as has repeatedly been shown to be the case in rodents[14]. Obviously it is important to cultivate beneficial gut microbes, and products in the marketplace to this end include probiotics actually containing some of the 'good bugs' and prebiotics that are selectively fermented to provide food for the beneficial microbes. Prebiotics mostly consist of specialised polysaccharides/oligosaccharides from certain dietary fibres that are not absorbed or digested in the small intestine, but are ultimately utilised in the large bowel, producing short chain fatty acids (SCFAs) which are known to have health promoting effects. Seaweeds are a substantial source of prebiotic fibres and several research studies on chickens, rats, pigs, nematodes and cell cultures have shown improved intestinal histo-morphology, increased abundance of beneficial microbes and increased SCFA production with the administration of dietary seaweeds[15]. Classified according to their chemical diversity, seaweed-derived marine oligosaccharides include chitosans, laminarins, alginates, fucoidans, carrageenans and ulvans – all of which constitute significant fermentable fibre components. Without a doubt, seaweeds can provide versatile, nutrient dense, health promoting foods and food ingredients. The integration of physical health, psychological wellness and human consciousness points to the treatment of our bodies as whole systems, rather than as isolated parts. Food quality and nutritional balance are fundamental to the growth, repair, and vital processes, as well as the energy required to sustain the entire unit. Dietary seaweeds can play an important, nay critical, role.

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Lynn Cornish has an undergraduate degree from Dalhousie University, Faculty of Agriculture (BSc. Soil Science) obtained in 1988, followed by a MSc. in Biology from St. Francis Xavier University (2013). Since then she has worked in agriculture, both as a consultant, as well as in the management of her own farming operation. However, the study and cultivation of seaweeds is now her career. She has worked for Acadian Seaplants Limited for 21 years. Her official role as Seed Stock Manager means she is responsible for the annual production of the macroalgal inoculum for our commercial seaweed food-production facility. In addition, she researches and writes about the many health benefits associated with dietary seaweeds, and has published review papers on this very important aspect of marine macroalgae.

References

1. Science Daily 2013 https://www.sciencedaily.com/releases/2013/05/130510075502.htm
2. A.Gustavsson *et al.* (2011) Cost of disorders of the brain in Europe 2010. Eur. Neuropsychopharmacol. 21(10):718-779. doi: 10.1016/j.euroneuro.2011.08.008
3. http://www.who.int/mediacentre/factsheets/fs317/en/
4. https://www.heart.org/idc/groups/ahamah-public/@wcm/@sop/@smd/documents/downloadable/ucm_470707.pdf
5. M.L. Cornish *et al.* (2015) A role for dietary macroalgae in the amelioration of certain risk factors associated with cardiovascular disease. Phycologia 54: 649-666
6. http://www.fao.org/3/a-i2727e.pdf
7. L. Burri et al. (2012) Marine omega-3 phospholipids: metabolism and biological activities. Int J Mol Sci 13:15401-15419
8. L. Paiva *et al.* (2014) Edible Azorean macroalgae as source of rich nutrients with impact on human health. Food Chem 164:128-135
9. O. G. Mouritsen (2016) Deliciousness of food and a proper balance in fatty acid composition as means to improve human health and regulate food intake. Flavour 5:1
10. http://www.who.int/nutrition/publications/VMNIS_Iodine_deficiency_in_Europe.pdf
11. U. Nitschke and D.B. Stengel (2015) A new HPLC method for the detection of iodine applied to natural samples of edible seaweeds and commercial food products. Food Chem. 172:326-334.
12. E. Combet *et al.* (2014) Low-level seaweed supplementation improves iodine status in iodine-insufficient women. Br J Nutr 112:753-761
13. M.L. Cornish and D.J. Garbary (2010) Antioxidants from macroalgae: potential applications in human health and nutrition. Algae 25(4):155-171
14. J.F. Cryan and T.G. Dinan (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Neurosci 13:701-712
15. M.L. Cornish *et al.* (2016) Consumption of seaweeds and the human brain. J Appl Phycol (in press).