J. of Supercritical Fluids 92 (2014) 311–318

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Exploiting microalgae as a source of essential fatty acids by supercritical fluid extraction of lipids: Comparison between *Scenedesmus obliquus*, *Chlorella protothecoides* and *Nannochloropsis salina*

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ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form 6 June 2014
Accepted 7 June 2014
Available online 23 June 2014

Keywords:
Supercritical fluid extraction
Co-solvent
Microalgae
Essential fatty acids
α-Linolenic acid
Omega-3

ABSTRACT

Supercritical CO_2 extraction from microalgae is applied with the aim of obtaining an oil rich in α-linolenic (ALA) essential fatty acid and with a low ω6:ω3 ratio. The maximum extraction yield is obtained at 60 °C and 30 MPa with 0.4 kg/h of CO_2 and 5% of co-solvent (ethanol). When the effect of pressure, temperature and density on the supercritical extraction yield and solubility are studied, the thermodynamic cross-over is found at a pressure close to 30 MPa, while the extraction cross-over occurs at around 25 MPa. The experimental solubility data are correlated by literature empirical models. Mathematical models developed by Sovová are applied to describe the experimental extraction curves. Soxhlet extraction of lipids is also carried out, obtaining a similar fatty acids profile but proving to be less selective than $SCCO_2$ method. Among the three species of microalgae examined, results show that *Scenedesmus obliquus* oil is richer in ω-3 fatty acids and ALA than *Chlorella protothecoides* and *Nannochloropsis salina* lipids. The effect of the extraction parameters on ALA content and the fatty acid profile is also analysed, concluding that the ω-3 percentage is favoured by lower temperatures, lower pressures and shorter extraction times.

1. Introduction

Western diets are deficient in omega-3 fatty acids, and have excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA), and a very high ω6:ω3 ratio, promote the pathogenesis of many diseases, including cardiovascular ones, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA exert suppressive effects [1]. Therefore, also due to the difficulty of changing the nutritional habits of a whole society, in the last years many products enriched with omega-3 as nutritional supplements or functional foods have been developed to integrate the diet and reach a good ω6:ω3 ratio in blood without changing the diet too much [2].

The major current food source of omega-3 is fish. However, global fish stocks are in danger and consequently its production may decrease in the future if large amounts of omega-3 are needed. In addition to this, some fishes, especially marine ones like salmon, sardine, tuna, anchovy, mackerel or hake, are sometimes contaminated with heavy metals, as copper or mercury, and organic pollutants as PCBs or dioxins, which have a toxic effect for human health [3]. Furthermore, they have a limited capacity for synthesis of PUFA, so most of them are simply accumulated from their microalgae diet [4]. The idea of using microalgae to obtain essential fatty acids is increasingly being taken into account.

Besides producing lipids rich in PUFAs, microalgae offer important advantages over conventional oil crops. On the one hand, they can be cultivated under mild conditions, including short growth cycle with very simple nutrients supply, no field and season restrictions, and less extensive care [5,6]. Additionally, there is no need of the use of chemicals such as herbicides or pesticides thus reducing costs and environmental impacts [7]. On the other hand, the growth of microalgae can effectively remove phosphates and nitrates from wastewater and have greater photosynthetic

Abbreviations: ALA, α-linolenic acid C18:3ω3; LA, linoleic acid C18:2ω6; AA, arachidonic acid C20:4ω6; EPA, eicosapentaenoic acid C20:5ω3; DPA, docosapentanoic acid C22:5ω3; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; FFA, free fatty acids.

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http://dx.doi.org/10.1016/j.supflu.2014.06.013
0896-8446/

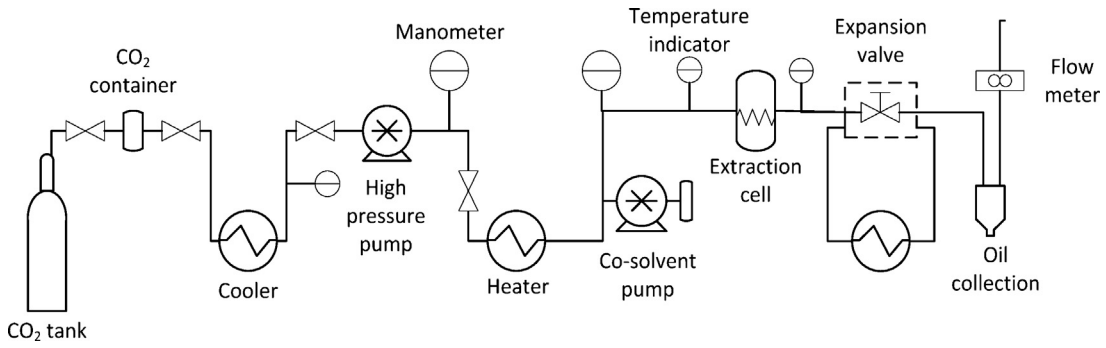

Fig. 1. Schematic diagram of the supercritical extraction equipment.

efficiency than terrestrial plants [6]. Specifically, microalgae growth actively utilizes and captures about 1.9 kg of CO_2 for every kg of dry biomass produced [8].

Two of the most abundant fatty acids in microalgae are linoleic acid (18:2ω6; LA) and α-linolenic acid (18:3ω3; ALA), which are considered the two parent essential fatty acids, since human body is able to synthesize the long chain PUFAs from them. In particular, omega-6 arachidonic acid (C20:4ω6; AA) can be synthesized by humans from LA, and omega-3 fatty acids, such as eicosapentaenoic acid (C20:5ω3; EPA), docosapentaenoic acid (C22:5ω3, DPA) and docosahexaenoic acid (C22:6ω3, DHA), from ALA [2]. These essential oils have been traditionally obtained by steam distillation and hydrodistillation. However, these separation techniques suffer a number of problems due to the thermal degradation of some compounds of the essential oil and partial hydrosolubilization and hydrolysis, which can affect its quality [9]. Supercritical fluid extraction has already been demonstrated as a good method in the production of omega-3 oil and omega-6 concentrates, avoiding the use of high temperatures and organic solvents. In addition, since CO_2 is gas at room temperature, it is easily removed when extraction is completed, thus it is safe for food applications and it can safely be recycled, which is an environmental benefit. Other advantage respect to other methods for lipid extraction is the lack of catalyst requirement [7].

The present study investigates the influence of supercritical fluid extraction conditions on the fatty acid profile of lipids from microalgae, with a focus on increasing the omega-3 ALA content. The results of three different species of microalgae are also compared. These data form the basis to analyse the feasibility of exploiting microalgae as a source of omega-3 rich oil, which could become an interesting option for pharmaceutical and food markets.

2. Materials and methods

2.1. Chemicals and microalgae

Carbon dioxide (4.0 type, purity greater than 99.99%) used as supercritical solvent was provided by Rivoira. Ethanol (≥99.8%), hexane (99.8%), methanol (99.8%) and chloroform (99%) were purchased from Sigma Aldrich.

Scenedesmus obliquus 276-7, *Chlorella protothecoides* 33.80 and *Nannochloropsis salina* 40.85 strains were obtained from SAG-Goettingen. The growth temperature was 24 ± 1 °C, with artificial light (fluorescent tubes) under a continuous photon flux density of 150 ± 10 μE m^{-2} s^{-1}, measured by a photoradiometer (LI-COR, Model LI-189). *C. protothecoides* and *S. obliquus*, freshwater species, were grown in BG11 medium, following SAG indications. *N. salina*, a marine species, was cultured in sterilized sea salts with 22 g L^{-1} solution enriched with f/2 Guillard solution modified by adding an excess of 1.5 g L^{-1} of $NaNO_3$. Medium was buffered with 40 mM Tris–HCl pH 8 to avoid alterations due to excess CO_2 supply. Maintenance and propagation of cultures were performed using the same medium added with 10 g L^{-1} of Plant Agar (Duchefa Biochemie). These conditions had been previously optimized in our laboratory [10,11].

After the harvest, the microalgae suspension was centrifuged 4425 rpm at 24 °C for 10 min. The centrifuged microalgae were kept refrigerated at a temperature of −20 °C until the oil extraction tests were performed.

Before extraction, microalgae were oven dried at 37 °C for two days, to reduce the water content to less than 20 wt%, since it is possible to extract oil from microalgae with water content in the biomass up to 20 wt% [12]. The water content of raw microalgae powder was evaluated in the following way: a mass of 0.1 g of microalgae powder was heated at 80 °C in an air flow oven until the mass no longer changed. By measuring the mass before and after the drying, we calculated the water loss. The water content is expressed as percentage with respect to the initial mass. After being dried for two days, a moisture content of 8% was left in *S. obliquus*, 20% in *C. protothecoides* and 16% in *N. salina*. The dried samples were ground and sieved before the tests, obtaining a particle size less than 0.5 mm.

2.2. Soxhlet extraction

Solvent extraction was carried out in a laboratory scale apparatus at ambient pressure. A mixture of methanol:chloroform 2:1 was used as solvent. The temperature of extraction was maintained at 105 °C during 18 h. Then the solvent was removed by a rotary evaporator at 40 °C and the extract was weighted.

2.3. Supercritical fluid extraction

A classical supercritical extraction process was specifically designed and constructed to carry out the microalgae oil extraction tests. Fig. 1 shows the schematic diagram of the equipment.

The operating procedure was as follows: the stainless steel vessel (extraction cell) was filled with 0.5 ± 0.1 g of dried microalgae powder and heated by a thermoresistance. The temperature of the internal flow was controlled before and after the extractor. The CO_2 was compressed through a high pressure pump and pre-heated before flowing through the vessel. The pressure was controlled by two gauges. The co-solvent (ethanol) was pumped by a HPLC pump (model PU-1580) and mixed with the CO_2 before the extractor.

The choice of ethanol as a co-solvent was based on literature data [13–15]. The ethanol concentration was 5%. A constant CO_2 flow rate of 0.4 ± 0.05 kg/h was kept in every run. The designed extraction pressures were 15, 20, 25 and 30 MPa. The minimum temperature was 45 °C, as the addition of 5% ethanol to CO_2 increases the critical temperature of the mixture to 42.5 °C [16].