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Supercritical CO_2 extraction of γ-linolenic acid (GLA) from the cyanobacterium *Arthrospira* (*Spirulina*)*maxima*: experiments and modeling

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Abstract

The freeze-dried biomass of *Arthrospira* was submitted to supercritical CO_2 extraction, in a flow apparatus, at temperatures of 50 and 60 °C, pressures of 250 and 300 bar and flow-rates of 12.8, 19.6 and 29.5 g CO_2/min. The achieved extraction yields, using pure CO_2, were low and increased slightly with pressure and temperature and decreased with the flow rate.

In order to increase the extraction yield of the lipids, namely GLA, which is mostly contained in glycolipid fractions, a polar compound (ethanol) was mixed with the freeze-dried biomass. The presence of ethanol increased both lipid and GLA yields relatively to the extraction with pure CO_2.

A comparison between supercritical extraction and organic solvent extraction was also carried out, in what concerns lipid yields and fatty acid composition of total lipids.

Furthermore, a plug flow model, in which the resistance to internal mass transfer is considered to be the controlling step, was applied to the supercritical CO_2 extraction of lipids from the *Arthrospira maxima*.

Keywords: *Arthrospira maxima*; γ-Linolenic acid; Plug flow model

1. Introduction

Evening primrose, borage, blackcurrant, fungi (*Mortierella* and *Aspergillus*) and cyanobacteria *Arthrospira* (*Spirulina*) are important sources of γ-linolenic acid [1].

Low endogenous concentrations of this compound are associated to several dysfunctions in human metabolism, such as dermatitis, diabetes, schizophrenia, rheumatoid arthritis and pre-menstrual syndrome. This behavior is due to a deficient function of the Δ6-desaturase enzyme, which is responsible for the conversion of the linoleic acid (C18:2 ω6) into γ-linolenic acid (C18:3 ω6) [2,3].

The use of GLA in medical and dietary applications led to the need of better methods of extraction and purification of this fatty acid from the natural source [4]. Supercritical fluid extraction is a new separation technique very promising to obtain the GLA and other valuable lipids. Carbon dioxide is the most used supercritical solvent, because the obtained extracts are toxic solvent free and the degradation of thermal labile components is avoided due to the moderate temperature used in the process [5]. Supercritical fluid extraction of GLA using this and other solvents has been carried out: from fungi (*Mortierella ramanniana*) using CO_2, N_2O, CHF_3 and

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SF_6 [6], evening primrose seeds [7] and *Spirulina* [8,9,10] using CO_2, borage seeds with CO_2 and a mixture of CO_2 and propane [11].

The objectives of this work are to carry out the supercritical CO_2 extraction of lipids, namely GLA, from *Arthrospira maxima*, and to assess the influence of several parameters, namely flow rate, in order to identify the mass transfer mechanism.

2. Materials and methods

For the supercritical fluid extraction experiments, a flow apparatus was used (Fig. 1). The apparatus allows to carry out studies at the temperature range from 25 to 80 °C and pressures up to 300 bar [12].

The liquid CO_2 flowing from a cylinder is compressed, with a pump (CP), into the extraction vessel of 1 L (V), after passing through the heat exchanger (S2). The preset extraction temperature is reached with the aid of a water jacket enveloping the extraction vessel. The pressure, measured by a Bourdon type manometer (M2), is controlled by the back-pressure regulator (BP). After flowing upward through the vegetable bed into the extraction vessel, the supercritical fluid is expanded in the separators of 0.27 L (SP1 and SP2). The gas flow rate is monitored with a rotameter (FL) and the total volume of CO_2 is determined with the dry test meter (DTM).

In this work, the supercritical fluid extraction was carried out using 40 g of *Arthrospira* (containing 28% ash). On the other hand, conditions of extraction were: CO_2 flow rates of 12.8, 19.6 and 29.5 g min^{-1}, pressures of 250 and 300 bar and temperatures of 50 and 60 °C. The extracts were collected in the first separator (SP1), at a pressure of 20 bar, and the temperature was controlled with an ice bath. The amount of extract obtained was assessed gravimetrically.

The CO_2 (99.995% purity) was purchased from Air Liquide (Portugal). The *Arthrospira* (*Spirulina*) *maxima* (Geitler LB 2342) used in the experiments was grown from a strain of the University of Texas culture collection (Austin, USA). The growth was carried out with a *Spirulina* medium [13]. The *Arthrospira* was harvested using a nylon plankton mesh (33 μm) and then was freeze-dried at −20 °C under N_2.

Prior to the supercritical studies the biomass was ground and sieved and only particles with a diameter <0.2 mm were used in the experiments.

Several lipid extractions with organic solvents were carried out at 25 °C, for 2 h, with magnetic stirring (100 rpm), in light protected vessels under nitrogen using 500 mg of dry *Arthrospira*. The solvents used were ethanol, acetone, hexane and a mixture of chloroform, methanol and water (Bligh and Dyer method [14]).

To determine the content in fatty acids of the freeze-dried biomass and of the several supercritical extracts, they were transesterified according to the modified [15] Lepage and Roy method [16].

The supercritical extracts were analyzed in a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a fused-silica DB-5 column (J&W; 30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was programmed to 170 °C during 5 min, then was raised from 170 to 250 °C at 4 °C min^{-1} and held at this temperature for 5 min. The injector and detector temperatures were 195 and 290 °C, respectively. Helium was used as a carrier gas, at a flow rate of 1.4 ml min^{-1} and the split ratio was 1:50. Heptadecanoic acid (Sigma) was used as internal standard. Peak identification and response factor calculation was carried out using standards (Sigma).

3. Results and discussion

The cyanobacterium *Arthrospira maxima* was submitted to supercritical fluid CO_2 extraction at the following conditions: flow rates from 12.8 to 29.5 g min^{-1} of CO_2, at a pressure of 250 bar and temperature of 50 °C. Furthermore, to assess the effect of pressure and temperature, at a flow rate of 19.6 g min^{-1}, experiments were carried out at 300 bar and temperatures of 50 and 60 °C. Yield in lipids, collected at regular time intervals, for the several conditions, are shown in Figs. 2 and 3 as a function of the CO_2 mass.

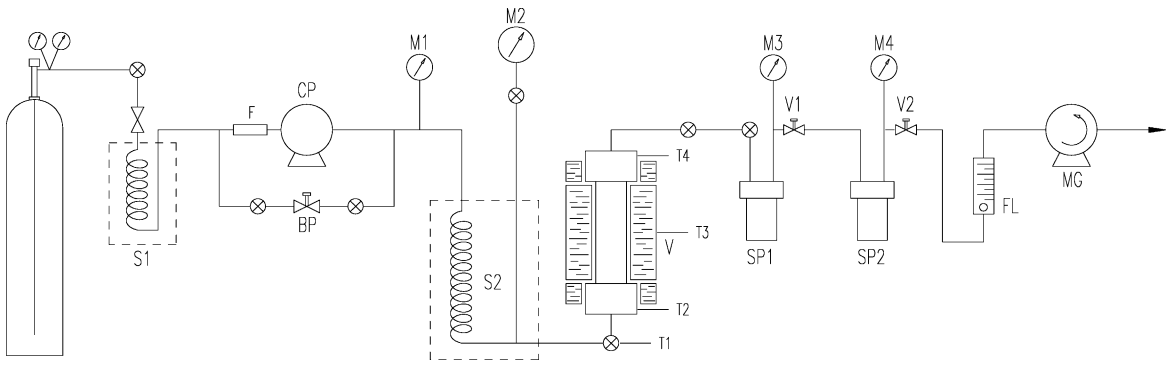

Fig. 1. Diagram of the supercritical fluid extraction apparatus. S1, Ice cooler; F, filter; CP, circulating pump; BP, back-pressure regulator; M1–M4, manometers; S2, heat exchanger; V, extraction vessel; SP1 and SP2, separators; V1 and V2, valves; FL, rotameter; DTM, dry test meter.

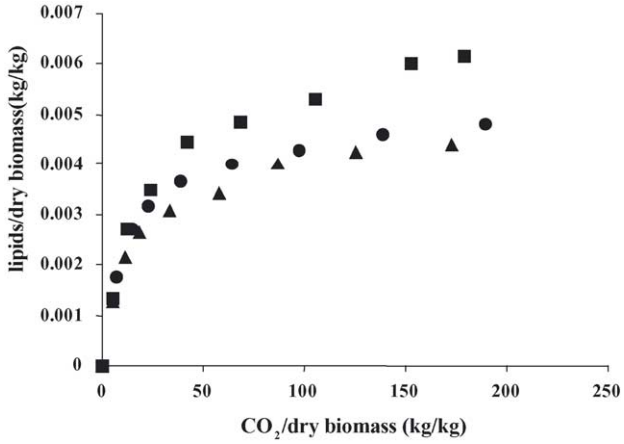

Fig. 2. Extraction of lipids from *Arthrospira* as a function of carbon dioxide mass at a flow rate of 19.6 g min^{-1}. (▲) 250 bar/50 °C; (●) 300 bar/50 °C; (■) 300 bar/60 °C.

At constant flow rate, the yield increased with the pressure at constant temperature and with temperature at constant pressure (Fig. 2). The pressure improved the solvent power of the supercritical fluid, due to the increase of its density. On the other hand, at constant pressure and flow rate, the yield increased with the temperature. In fact, the temperature increases the vapor pressure of the lipids, leading also to its higher solubility. Moreover, the yield decreases with the flow rate (Fig. 3), at constant pressure and temperature, being this behavior possibly due to mass transfer resistances inside the *Spirulina* particles.

With the aim of increasing the extraction yield in both GLA and other lipids the dried biomass was mixed with ethanol, a biocompatible solvent, previously to its introduction in the extractor. Two amounts of ethanol (100% and 20% of the total dried matter) were tested.

In Fig. 4 are presented the cumulative curves of the extracted lipids. The lower amount of ethanol (20%) did not

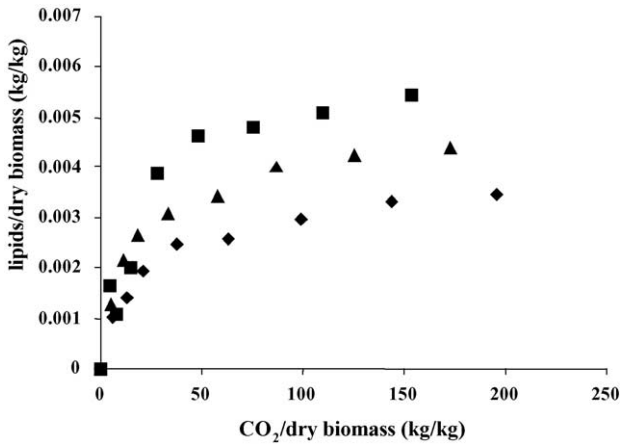

Fig. 3. Extraction of lipids from *Arthrospira* as a function of carbon dioxide mass at 250 bar and 60 °C, at several flow rates: (■) 12.8 g min^{-1}; (▲) 19.6 g min^{-1}; (♦) 29.5 g min^{-1}.

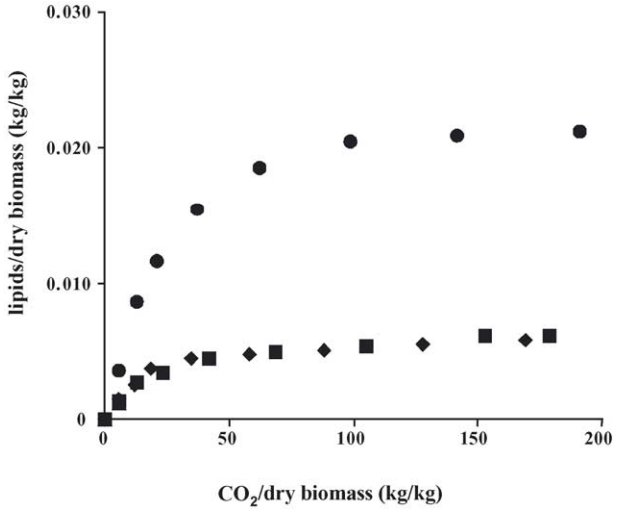

Fig. 4. Yield of extracted lipids from *Arthrospira maxima* as a function of carbon dioxide mass (flow rate = 19.6 g min^{-1}). (♦) 300 bar/60 °C (no entrainers); (■) 300 bar/60 °C (with 20% ethanol); (●) 300 bar/60 °C (with 100% ethanol).

lead to any increase in lipid yield when compared with pure CO_2. However, the higher amount of ethanol led to a significant increase of both the extraction rate and yield. Santos et al. [8], also showed that when the samples of *Spirulina platensis* contained moisture, the yield of the extraction with CO_2 was higher than when completely dried samples were used.

The ethanol can have an entrainment effect on the extraction of the lipids from *Arthrospira*, which are for the larger part polar [3]. On the other hand, the ethanol can also have, probably, an effect of destruction of the cellular walls [17], improving the amount of available lipids for extraction.

In Fig. 5 are represented the cumulative yields in GLA as a function of CO_2 mass for several conditions. This figure shows that the use of ethanol mixed with the dry biomass led

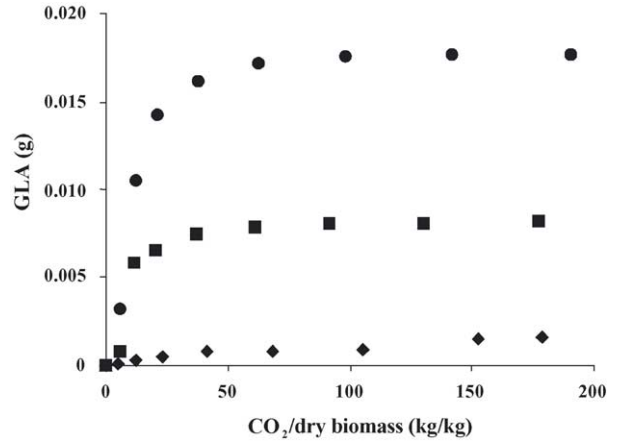

Fig. 5. Yield of extracted GLA from *Arthrospira maxima* as a function of carbon dioxide mass. Conditions of extraction: 300 bar, 60 °C, flow rate of 19.6 g min^{-1}. (♦) no entrainers; (■) with 20% ethanol; (●) with 100% ethanol.

Table 1
Lipid and GLA yield for several conditions

Conditions	Lipid content (% dry biomass)	GLA extracted (%)
250 bar/50 °C	0.4	1.1
300 bar/50 °C	0.5	1.2
300 bar/60 °C	0.6	2.8
300 bar/60 °C/ethanol (100%)	2.1	31.3
Hexane	1.8	1.0
Acetone	3.3	48.0
Ethanol	4.0	72.0
Solvent mixture (Bligh and Dyer)	5.5	73.0

to a higher yield of GLA, when compared with the use of pure CO_2.

The extraction yield of the GLA increases with the amount of ethanol mixed with the biomass. The first part of the curves in Fig. 5 corresponds to the ethanol effect. These results are expected since GLA is primarily concentrated in polar lipids and especially in glycolipids [4].

The comparison of the supercritical extraction (gas flow rate of 19.6 g min^{-1}) with the extraction by organic solvents was also carried out. Lipid and GLA contents are summarised in Table 1 for several conditions and solvents.

Among organic solvents, hexane led to the lowest yield in lipids and GLA, while for the biocompatible organic solvents the highest yield in lipids and GLA was obtained with ethanol. These results can be explained in terms of a low extraction of lipids associated to the cell membranes, which need solvent mixtures containing alcohols to counterbalance the hydrogen bonding and ionic forces between the lipids and proteins [17].

Supercritical extraction of lipids from *Arthrospira* (*Spirulina*) *maxima* with pure carbon dioxide showed a low yield, when compared with the total lipids extracted with non-biocompatible mixture of organic solvents (Bligh and Dyer method). The highest yields in both lipids and GLA were obtained at 300 bar and 60 °C. These yields increased significantly when ethanol (100% in reference to dried biomass) was used mixed with the biomass. On the other hand, they are below those obtained with acetone and ethanol, although higher than that obtained with hexane. However, it is possible to improve them by increasing the amount of ethanol in the system.

In Table 2 is presented the content in fatty acids of the extracted lipids (after transesterification) for several conditions and it was verified that the mixture containing the higher amount of ethanol led to the highest yields and selectivity in both GLA and palmitic acid.

Table 2
Fatty acids content (mg) in the extracts for several conditions

Conditions	C16:0	C18:0	C18:1	C18:2	C18:3
250 bar/50 °C	0.94	0.17	0.018	0.074	0.60
300 bar/50 °C	1.06	0.16	0.21	0.75	0.68
300 bar/60 °C	2.66	0.43	0.54	1.95	1.56
300 bar/60 °C/ethanol (100%)	21.3	1.63	1.44	5.10	17.67

C16:0: palmitic acid; C18:0: stearic acid; C18:1: oleic acid; C18:2: linoleic acid; C18:3: γ-linolenic acid (GLA).

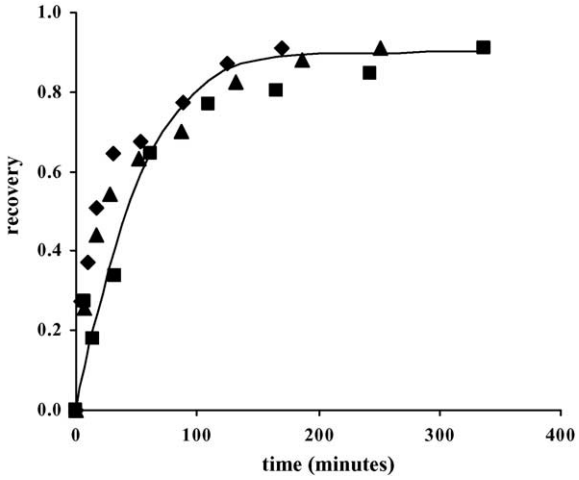

Fig. 6. Calculated and experimental recovery of lipids, at 250 bar and 50 °C, for several flow rates, as a function of time. (■) flow rate of 12.8 g min^{-1}; (▲) flow rate of 19.6 g min^{-1}; (♦) flow rate of 29.5 g min^{-1} (— model).

3.1. Modeling

When the extraction yields of lipids are plotted against the CO_2 mass used, the yield decreases with the flow rate (Fig. 3), as occurred with *Spirulina platensis* [8] and with the sage oil [18]. However, when the recovery (ratio of the extracted lipids/available lipids) is plotted against the time there is a fair coincidence of the points for the several flow-rates used (Fig. 6). The available lipids for each extraction were determined asymptotically from the extraction curves. This behavior is consistent with a mass transfer process inside the *Spirulina* particles, being the resistance of external film of solvent considered negligible.

Several mathematical models that assume plug flow of the fluid through the fixed bed of solid material (seeds, leaves, etc.) were already published when the extraction is controlled by the external mass transfer [20], by the internal one [18] and by a combination of both [21].

Therefore, considering that the intra-particle mass transfer resistance controls the process, a model, that considers a plug flow of the supercritical fluid through the bed of *Spirulina* with the following differential mass balance equations, can be used:

$$\rho u \frac{\partial y}{\partial h} + \rho \varepsilon \frac{\partial y}{\partial t} + (1-\varepsilon) \quad \rho_s \frac{\partial x}{\partial t} = 0 \tag{1}$$

$$(1-\varepsilon)\frac{\partial x}{\partial t} = -A_p K (x - x^*) \tag{2}$$

in which ρ_s (kg/m^3) is the solid density, ρ (kg/m^3) the solvent density, ε the porosity of the solid bed, u (m/s) the superficial velocity, t (s) the time, A_p (m^2/m^3) the specific area, K (m/s) the internal mass transfer coefficient, y (kg/kg) the concentration of lipids in the fluid phase, x (kg/kg) the lipid

Table 3
Parameters used in the plug flow model

Concentration of extractable lipids in *Spirulina* (g/dm^3)	1.7–2.6
Density of the solid matrix (g/dm^3)	617
Porosity	0.1
Internal diameter of the extractor (mm)	45.8
Density of the supercritical fluid (g/dm^3)	834
Particle diameter (mm)	0.2

weight concentration in the solid, x^* the weight concentration of lipids in the interface solid–fluid (kg/kg).

To solve Eqs. (1) and (2) it is necessary to know how y and x^* are related [18]. A linear dependence was considered, $y = kx^*$, where k is a partition coefficient, which was estimated as being 0.025, according to the concentration in supercritical CO_2 of lipids from other micro-algae with similar lipid content [19]. The boundary and initial conditions are $h = 0$, $y = 0$, for any t and for $t = 0$, $y = 0$ and $x = x_0$. This last value was obtained asymptotically from the extraction curve, for each flow rate.

In Table 3 are shown some of the parameters used in the application of the plug flow model.

The extractor was divided in n stages, each stage becoming a perfect mixture extractor, and the system of partial differential equations (PDEs) could be transformed in a system of $2n$ ordinary differential (ODEs), Eqs. (3) and (4):

$$\frac{\rho u n}{H}(y_n - y_{n-1}) + \rho\varepsilon\frac{\mathrm{d}y_n}{\mathrm{d}t} + (1-\varepsilon)\rho_s\frac{\mathrm{d}x_n}{\mathrm{d}t} = 0 \quad (3)$$

$$(1-\varepsilon)\frac{\mathrm{d}x_n}{\mathrm{d}t} = -A_p K(x_n - x_n^*) \quad (4)$$

with the initial conditions at $t = 0$, $x_n = x_0$ and $y_n = 0$, and where H is the bed height, x_n the concentration in solid phase at stage n, y_n the concentration in fluid phase at stage n and x_n^* the concentration at solid–fluid interface (which is in equilibrium with the fluid phase).

To solve these ODEs, the Runge–Kutta of 4th order method was used, considering the extractor divided in 10 stages.

A_pK was determined by fitting and it was related to the internal time of diffusion and to the diffusivity of the solute. The internal time of diffusion can be evaluated from the equation $t_i = (1-\varepsilon)/A_pK$ [18]. This time can be related with the internal diffusion coefficient D_i, $t_i = r^2/15D_i$ (spherical particles), where r is the mean particle radius [22]. The value determined to the diffusivity of the "solute" (lipids) was $D_i = 7.4 \times 10^{-13}$ m^2/s.

Fig. 6 shows that a fair representation of the extraction can be achieved with the plug flow model.

4. Conclusions

Supercritical extraction of lipids from *Arthrospira maxima* with pure supercritical carbon dioxide showed a low yield, when compared with the total lipids extracted with a mixture of chloroform, methanol and water, as well as with acetone and ethanol. The GLA content in the supercritical extracts was also low.

The increase in pressure and temperature led to an improvement in lipid and GLA yields. In contrast, the yield decreased with the increase in flow rate of the supercritical fluid.

The presence of ethanol increased both lipid and GLA yields relatively to the extraction with pure CO_2.

Furthermore, a plug flow model, in which it is considered that the internal resistance to mass transfer controls the supercritical extraction of lipids from *Arthrospira maxima*, gave a fair representation of the yield cumulative curves.

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