Oil Extraction from Enriched *Spirulina* Microalgae

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ABSTRACT

The aim of this work is to produce an edible oil from enriched *Spirulina platensis* using supercritical CO_2 extraction. Extractions have been carried out on dried and grounded biomass. Particular attention has been paid to the influence of operating conditions on oil yields, extraction kinetics and oil composition. Studied operating parameters are: pressure, temperature, and fill rate in the autoclave of the treated biomass. Experiments were performed at laboratory scale (9 to 13 g per batch) and at larger scale (950 g per batch).

INTRODUCTION

There is a growing interest in the use of microalgae for pharmaceutical and food applications over the last twenty years. Microalgae are able to accumulate oil up to 60% of their dry weight [1] and particularly when submitted to nitrogen defaults. The oil quality, due to the presence of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or γ-linolenic acid (GLA) [2-13], and antioxidants (i.e. β-caroten) [4,14-16], allows its use in the food and pharmaceutical industries. Many microalgae are even able to synthesize antimicrobial and antiviral molecules which increase the interest of pharmaceutical industries [13, 17-19]. *Spirulina platensis* is one of the most potential microalgae because of its high protein and nutritional content [20]. For example, some works have shown its benefit impacts on cancers [21, 22].

Whatever the application fields, the extraction of molecules of interest from dry seaweeds or microalgae is usually performed using organic solvents such as *n*-hexane but such widely-used solvents have major drawbacks: they are toxic, generally flammable and low selective solvents. This toxicity has a great impact as it is problematic for the people who handle them during processing and some residual traces remain in the extracted end-product and in the residue.

One alternative to avoid the use of toxic solvents to extract bio-oil or other molecules of interest is to carry out extraction using supercritical carbon dioxide (SC-CO_2) as solvent. This safe and non flammable solvent can be selective. This selectivity is obtained by varying pressure and temperature. Moreover the separation step to recover the targeted product may be avoided since CO_2 is gaseous at ambient pressure. In addition, the depressurization can be done step by step allowing a fractioning of the extracted compounds based on their solubility variation with pressure and temperature. The extract yields, which depend on experimental conditions, can be the same ones as for extraction processes using organic solvents and high for quite short extraction duration. Moreover, the quite low critical pressure and particularly the temperature of CO_2 allow its use for thermolabile compound extractions. Lastly, the environmental benefit in using microalgae is also more significant if extraction is done using a non pollutant solvent such as SC-CO_2.

$SC\text{-}CO_2$ is a non polar molecule; when the molecule of interest is not soluble, the solvent power can be increased using a safe and polar modifier like ethanol.

This technology is well-known today and is considered as a green process. Indeed, the use of organic solvent can be totally avoided depending on the chemical nature of the extracted molecules. The major part of CO_2 is recycled therefore decreasing the consumption per extracted mass. For the last three decades, a large number of industrial extraction plants using supercritical fluids have been constructed attesting that this technology is economically viable for a large number of applications.

The aim of this work is to extract a bio-oil from *Spirulina platensis* using supercritical carbon dioxide. A study of the influence of operating parameters: pressure, temperature, and fill rate of the autoclave on extraction yields and kinetics and on oil composition is proposed.

MATERIALS AND METHODS

Chemicals and microalgae

Instrument grade carbon dioxide (purity of 99.7%) was supplied by Air Liquide Méditerranée (France).

The studied microalgae was *Spirulina platensis* produced by Alpha Biotech (Asserac, France). After harvesting and centrifugation, microalgae have been subjected to drying under low temperature (35 °C). Depending on the sample of microalgae studied, oil content is understood between 10 and 16 %. Before extractions, microalgae were grounded and sieved.

Experimental set-up.

The set-up was supplied by Separex (Champigneulles, France). Figure 1 and 2 show a scheme and a photograph of the laboratory set-up, respectively. Experiments were performed in an extraction cell of 20 cm^3 corresponding to approximately 10 grams of dried microalgae.

One extraction experiment is performed as follows:

Liquid CO_2 (1) is cooled in a cryogenic bath (2), filtered and pumped (3) towards the extraction cell (6). Before the extraction autoclave, CO_2 is heated (4) until the chosen temperature. The extraction autoclave which contains a fixed bed of the dried powder is also heated. Pressure is controlled by a pressure gauge. After the extraction cell, CO_2 is released to gas state through an expansion valve (7). The extracted compounds are recovered in a collector (8).

Regarding the small treated charges of raw material and the fact that the extracts contain both neutral lipids and pigments, the extraction efficiency has been evaluated through the mass loss (Eq. 1) of the vessel.

$$\text{Mass loss}\,(\%) = \frac{\text{initial mass (g) - mass after extraction (g)}}{\text{initial mass (g)}} \times 100 \qquad \text{Eq. 1}$$

The reproducibility of the experiment has been preliminary determined and estimated at less than 0.3 %, whatever the operating conditions.

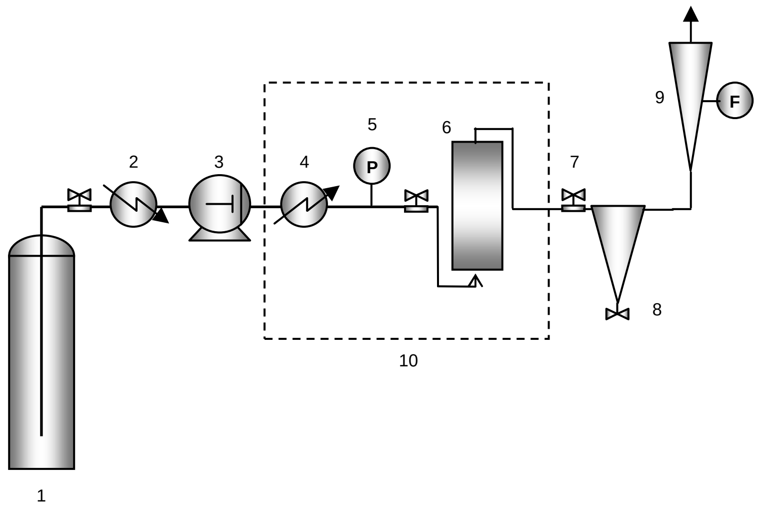

Figure 1: Experimental set-up. 1 – CO_2 cylinder; 2 – Cryogenic bath; 3 – High pressure volumetric pump ; 4 - Heat exchanger; 5 – Manometer ; 6 – Extraction cell; 7 – Expansion valve; 8 – Collector; 9 – Flow meter ; 10 – Thermoregulated area

Figure 2: Laboratory set-up.

Larger scale extraction was performed by HITEX (Vannes, France) in an autoclave of 2 L.

RESULTS

Influence of the fill rate

Fill rate may have an influence on mass transfer between the supercritical phase and the biomass, and then on extraction yields and/or extraction kinetics. In order to evaluate such influence, the extractions were conducted with 9.00, 10.70 and 13.25 g in an autoclave of 20 mL corresponding to a fill rate of 68, 80 and 100 %, respectively. The operating conditions were already determined thanks to a previous work [23]: pressure of 40 MPa, temperature of 333 K, CO_2 flow rate of 0.37 $kg.h^{-1}$. The particle diameter was less than 0.160 mm. Figure 3 shows the extraction curves obtained for each fill rate of the vessel.

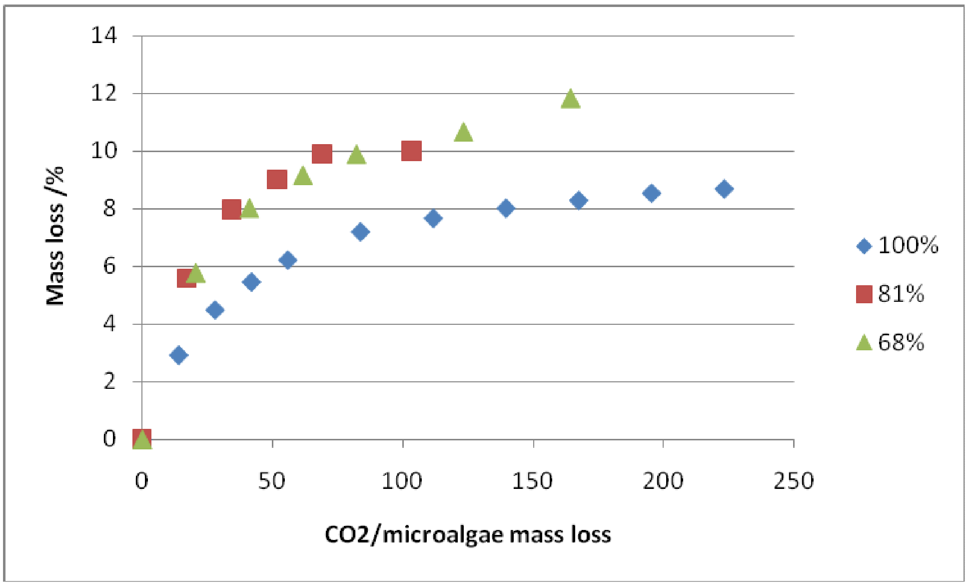

Figure 3: Extraction curves obtained at several fill rates

The extraction curves obtained show that when the powder is packed, the mass transfer is obviously more limited. To obtain high mass losses, it is advised to work with a vessel filled up to 80 %.

Influence of pressure.

In a previous work [23], an experimental design has been presented in order to evaluate the influence of operating parameters on mass losses. Three parameters were studied through the experimental design: pressure, temperature and CO_2/microalgae mass ratio. The extraction experiments were carried out by modifying the following parameters: temperature from 318 to 338 K, pressure from 28 to 46 MPa and CO_2/microalgae mass ratio from 80 to 200. Each experiment was performed during 90 minutes.
Figure 3 remembers the surface responses that illustrate the influence of the three studied parameters, pressure (Figure 3.a), temperature (Figure 3.a-b) and CO_2/microalgae mass ratio (Figure 3.b), on mass loss.

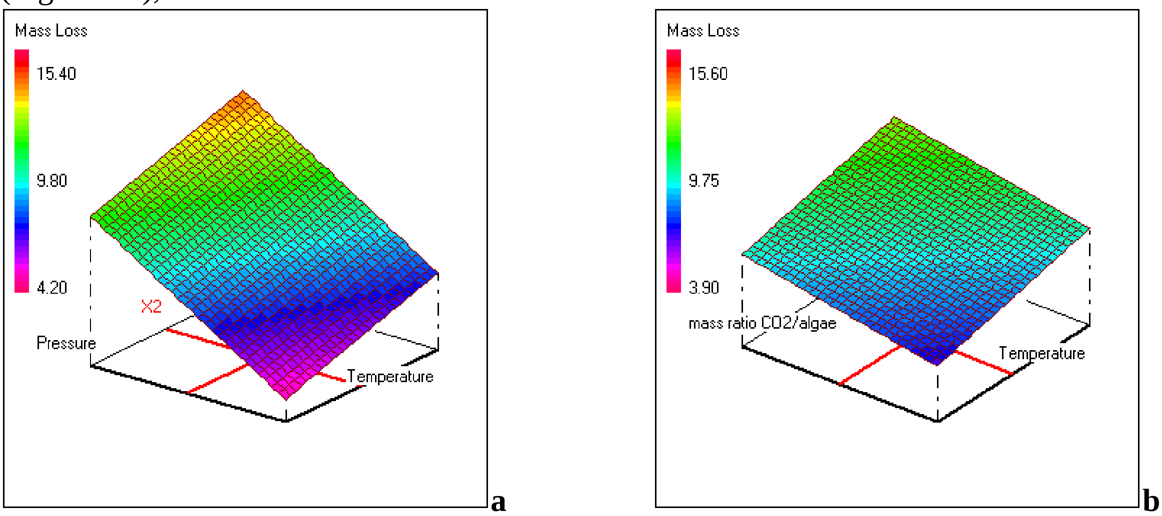

Figure 3: Response surfaces for the influence of pressure (a), temperature (a and b) and the mass ratio CO_2/algae (b)

It appears that pressure is the most influent parameter. Mass losses increase significantly with pressure. The temperature also plays an important role. In the pressure range studied, the higher the temperature, the higher the mass losses.

In order to evaluate the influence of pressure on extraction kinetics, extraction curves were plotted (figure 4) at several pressures: 10, 20, and 30 MPa. Each curve was obtained at 333 K, with a fill rate of 68 % and a CO_2 flow rate of 0.45 $kg.h^{-1}$. The particle diameter was less than 0.160 mm.

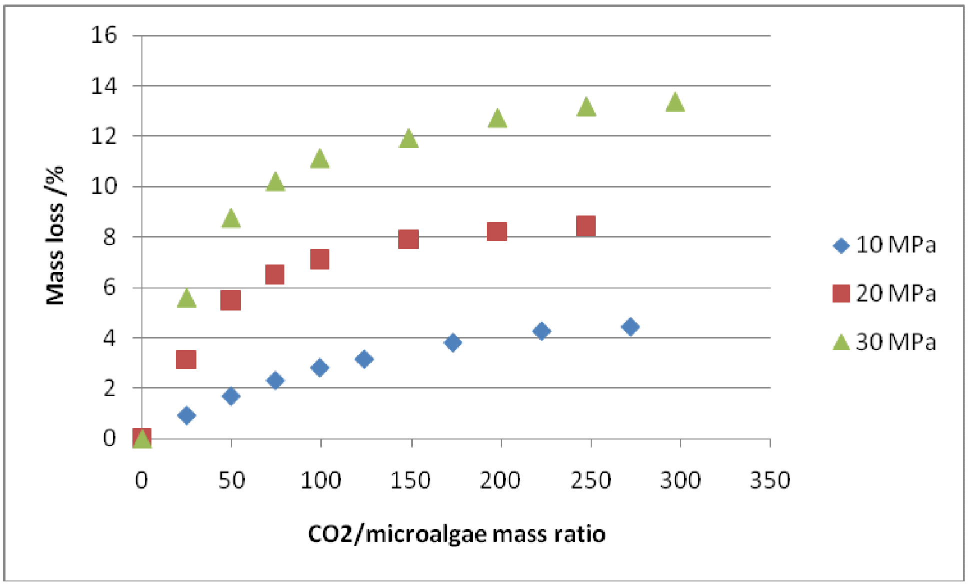

Figure 4: Influence of pressure on extraction kinetics

The extraction is limited by the diffusion phenomenon. Pressure plays a significant role on yields and kinetics. The pressure increase has a positive influence on the results since the solubility of molecules in supercritical CO_2 generally increases when the pressure increases.

Influence of temperature.

In this work, the temperature effect has been studied under 10 and 20 MPa. Figures 5 and 6 shows the extraction curves obtained.

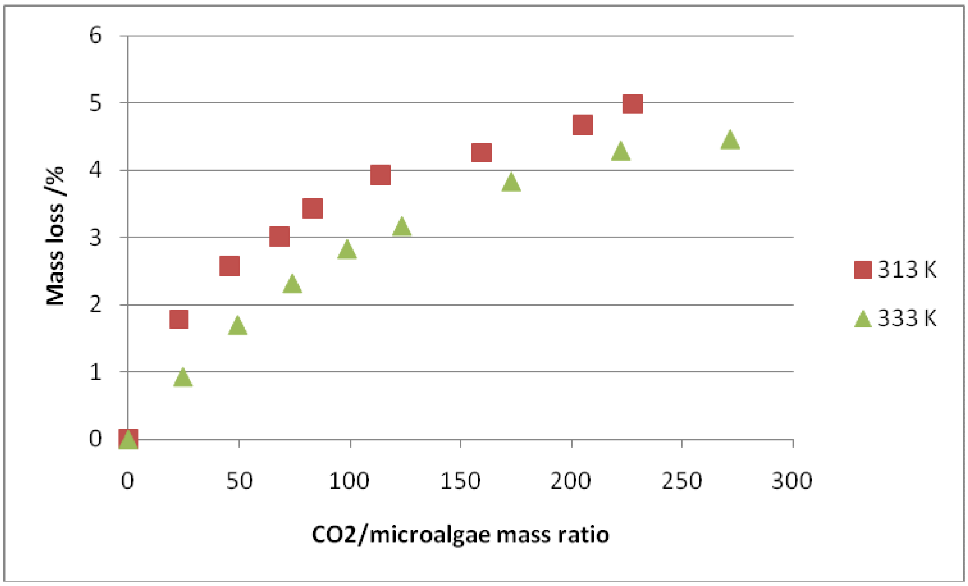

Figure 5: Influence of temperature under 10 MPa.

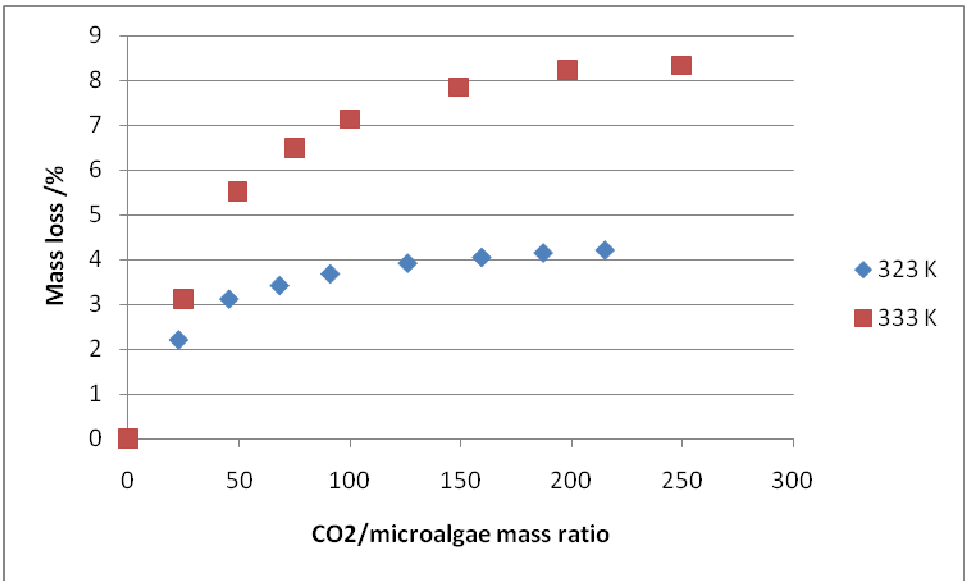

Figure 6: Influence of temperature under 20 MPa.

The retrograde behavior is well illustrated by figures 5 and 6. Under 10 MPa, mass losses obtained at 313 K are higher than those obtained at 323 K whereas under 20 MPa, the opposite effect is observed.

Influence of pressure and temperature on the oil pigment content.

The β-caroten and chlorophyll a and b contents have been determined for samples of oil obtained at different operating conditions. Table 1 summarises the operating conditions corresponding to the analysed samples and table 2 gives the pigment contents. All extractions were performed with a CO_2 flow rate of 0.4 $kg.h^{-1}$ and a particle size less than 0.160 mm.

Table 1: Operating conditions for extracted oils.

Sample	Temperature /K	Pressure /MPa
1	333	40
2	313	40
3	313	10

Table 2: Pigment contents (mg/g of oil).

Sample	β-caroten	Chlorophyll a	Chlorophyll b
1	0.22	0.40	1.50
2	0.09	0.20	0.65
3	0.11	0.26	0.64

The extraction of pigments seems to be strongly influenced by temperature. Indeed, under 313 K, working under 10 or 40 MPa leads to the same composition.

Larger scale extraction.

This experiment has been conducted by HITEX (Vannes, France) in an autoclave of 2 L (i.e. a batch of 950 g with a neutral lipid content of 12%). The average particle size is 0.5 mm. Extraction was performed under 29 MPa, 333 K and a CO_2 flow rate of 8.5 $kg.h^{-1}$. At a solvent/biomass mass ratio of 50, the mass loss was of 6.3 %. Such results may be improved with a lower particle size.

CONCLUSION

The aim of this work was to perform supercritical CO_2 extraction from lipid enriched *Spirulina platensis*. The influence of pressure and temperature on extraction kinetics and yields, and on pigment content in the extract have been studied. As expected, the pressure is the most influent parameter on extraction yields and kinetics. The retrograde behavior has been well illustrated. The inversion pressure is understood between 10 and 20 MPa. Concerning the influence of operating parameters on pigment content, it appears that temperature is the most significant parameter. The pigment content in the extract is higher when the temperature increases. A test at larger scale has been conducted and led to a lower extraction efficiency probably due to a bigger average particle size. All the data collected will be used to develop a mathematical model in order to predict extraction curves.

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