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Oil extraction from enriched *Spirulina platensis* microalgae using supercritical carbon dioxide

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A B S T R A C T

This article deals with the extraction of neutral lipids and antioxidants from enriched *Spirulina platensis* microalgae using supercritical carbon dioxide (CO_2), and more particularly with the influence of experimental conditions on extraction yields and kinetics at laboratory and pilot scales. Preliminary studies were carried out at laboratory scale before the establishment of an experimental design: extraction curves were plotted for different autoclave fill rates, and under different conditions of pressure and temperature. Using a Response Surface Methodology, the significant influence of pressure on extraction efficiency was highlighted. Surface responses showed that, in the studied experimental field, mass loss increased when pressure, temperature, and CO_2/microalgae mass ratio increased. Extract analyses showed that oil extracts contained chlorophylls *a* and *b*, as well as β-carotene. Finally, larger-scale experiments were carried out with batches of 1 and 50 kg (scale-factors of 100 and 5000, respectively) and the results were consistent with those obtained at laboratory scale.

Keywords:
Supercritical CO_2
Extraction
Microalga
Neutral lipid
Antioxidant
RSM
Scale-up

1. Introduction

For several years now, microalgae have been the subject of a growing interest in research and industry for health-related and energy applications. Indeed, microalgae are photosynthetic organisms that have the ability to adapt to many environments (even the most extreme). Moreover, microalgae can synthesize high-added value compounds in significant quantity when subjected to particular growing conditions. For example, some species are able to accumulate high content of oil [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) [2–7] and antioxidants (i.e. β-carotene) [8], allows their use in food and pharmaceutical industries. Many microalgae are even able to synthesize antimicrobial and antiviral molecules which increases the interest of pharmaceutical industries [9–11]. Thanks to its content in phycocyanin, a water-soluble active compound, and thanks to its high protein content, *Spirulina platensis* is one of the microalgae with the greatest potential [12,13]. *Spirulina platensis* ranks first in the current global market with about 3000 tons of dry matter produced per year and is mainly cultivated in China, France, India, Japan, Myanmar, and USA. This microalga is used as a food supplement for human and animal nutrition, or as raw materials for cosmetic products. Furthermore, some works have been focussing on its beneficial impacts on cancer prevention [14].

The extraction of compounds 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: toxicity, flammability and low selectivity.

One alternative to avoid the use of such toxic and unsecured solvents to extract bio-oil or other compounds of interest is to carry out extraction using supercritical carbon dioxide (CO_2) as solvent. This solvent is Generally Recognized As Safe (GRAS) – non toxic, non-flammable – and may be rather selective. This selectivity is obtained by varying pressure and temperature. The separation step to recover the target product is done using simple depressurization since CO_2 is gaseous at ambient pressure. The depressurization can

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be multi-staged allowing a fractioning of the extracted compounds based on their solubility variation with density. The extract yield, which depends on experimental conditions, can be similar to those obtained through extraction processes using organic solvents and can be high for quite a short extraction time [15]. Moreover, the low critical temperature of CO_2 (304.21 K/31.06 °C) permits its use for thermolabile compound extractions. Lastly, its critical pressure (7.38 MPa) is quite low.

Supercritical CO_2 solubilizes non polar compounds as neutral lipids (triglycerides, free fatty acids...); when the compounds of interest are not soluble, the solvent power can be increased using safe and polar modifiers like ethanol or water.

This technology is well-known today and is considered as a green process. On an industrial scale, the major part of the CO_2 is recycled therefore decreasing the consumption per extracted mass. In summary, the use of supercritical CO_2 instead of organic solvents would provide a cleaner and more compact extraction process. For the last four decades, a significant number of industrial extraction plants using supercritical fluids have been built, attesting that this technology is economically viable for a large number of applications.

Some works in literature from 1995 to nowadays have dealt with the extraction of compounds of interest from microalgae using supercritical CO_2. In 2011 Crampon et al. [15] published a review reporting 80 articles about this subject: the supercritical CO_2 extraction of neutral lipids and other high-added value compounds from seaweeds and microalgae was described, as well as the operating conditions and pre-treatments associated. As also mentioned in literature, supercritical CO_2 extracts specifically the neutral lipids contained in microalgae; the extracted oil is free of phospholipids. This point is of great interest since the presence of phospholipids in lipid extracts implies an additional degumming step to remove them either for food and health, or energy applications. Another important point to highlight is the possibility to apply this technique on wet biomass containing water content up to 23 wt% [16].

Many articles particularly have focused on the extraction of compounds of interest from *Spirulina* (*Arthrospira*) *platensis or maxima*. Supercritical CO_2 extraction allows the extraction of neutral lipids [17,18] and particularly γ-linolenic acid (GLA) which has an important role in human metabolism and is used as a dietary supplement [19,3,4,20]. Indeed, thanks to the GLA, the human body is capable of producing dihomo-γ-linolenic acid which is a very important constituent of the phospholipids of the cell membrane. Some articles also mentioned the supercritical CO_2 extraction of carotenoids [18] and mainly β-carotene [21,22] as well as tocopherols [22,23].

Concerning GLA extraction, pure supercritical CO_2 extraction yields are of the same order of magnitude than those obtained with *n*-hexane. Nevertheless, the extraction of GLA is enhanced by an addition of cosolvent (ethanol) [4].

The aim of this work was to extract a bio-oil predominantly containing neutral lipids and antioxidants from enriched *Spirulina platensis* using pure supercritical CO_2. In a first part, a laboratory scale study (10 g per batch) of the influence of operating parameters: pressure, temperature, CO_2 flow rate and fill rate of the autoclave on extraction yields and kinetics and on oil composition, is proposed. An experimental design focusing on the influence of pressure, temperature and CO_2/microalgae mass ratio is then presented and discussed. Finally, larger scale experiments with 1 kg and 50 kg of dry matter per batch were carried out corresponding to scale factors of 100 and 5000, respectively. To our knowledge, such a change of scale has never been discussed in the literature on supercritical CO_2 extraction from microalgae.

2. Materials and methods

2.1. Chemicals and microalgae

Carbon dioxide was supplied by Air Liquide Méditerranée (France) with a purity of 99.7%.

All reagents were AR grade (Carlo Erba, France).

Spirulina platensis microalgae were grown, harvested and supplied by AlgoSource SAS (Saint-Nazaire, France). Microalgae were enriched in neutral lipids by a production under nitrogen limitation. The total lipid content was comprised between 10 and 16 wt% of dry matter (supplier data) depending on the season and the cultivation method. After the harvest, the microalga suspension was centrifuged (Westfalia separator, France). The raw material thus recovered looked like a paste that needed to be dried since the moisture content remained at about 40%. This paste was then submitted to an extrusion in a 5 mm cylinder under a horizontal air flow at 308 K. Indeed, the supercritical CO_2 extraction kinetics of compounds of interest from microalgae are known to be enhanced when the initial biomass is dried under air flow in comparison with other drying methods like freeze-drying or spray-drying [16]. The resulting water content of dry biomass was about 5 wt%. Microalgae were supplied in the form of flakes and then frozen to limit lipid degradation.

At laboratory scale, the received microalga biomass was stored in a freezer at 255 K under an inert atmosphere. Before laboratory scale extractions, in order to increase extraction kinetics, microalgae were crushed using a cutting mill and then they were sieved. A fraction of the particles with an average size of less than 0.160 mm was isolated. After the extraction, the extracts (mainly composed of triglycerides) were recovered in *n*-hexane under an inert atmosphere in order to avoid their degradation into free fatty acids.

However, the storage conditions used at laboratory scale could not be applied at pilot scale because of practical difficulties. The microalgae were kept instead at room temperature and extracts were stored in closed recipients in a freezer.

2.2. Supercritical CO_2 extraction

Experiments were performed at laboratory and at pilot scales with different set-ups. Reproducibility of results was evaluated, extraction curves were plotted, and the influence of operating conditions were studied.

2.2.1. Experimental set-ups

The set-up used for the extractions at laboratory scale (Separex-4219) was supplied by Separex (Champigneulles, France). This apparatus allowed work to be done with three autoclaves (5, 10 and 20 cm^3) corresponding to batches comprised between 2 and 13 g of dry biomass. The maximum working pressure and fluid flow rate were 45 MPa and 1 kg h^{-1}.

A classical extraction was conducted as follows (Fig. 1): the autoclave was filled with the dry microalgae powder and heated until the desired temperature was reached. CO_2 was cooled by a cryogenic bath at 277 K, filtered and pumped into the extractor until the working pressure was reached. The pressure was controlled by a pressure gauge. Then the expansion valve was opened. A flow of supercritical CO_2 went through the microalga powder at constant pressure, temperature and flow rate during a predefined duration corresponding to the extraction duration. After passing through the extractor, the CO_2 was expanded through the expansion valve. The CO_2 became gaseous and extracted compounds were collected in a collecting container. The CO_2 flow rate was measured by a gas flow meter placed at the end of the extraction line. In general, the whole extraction curve was plotted and used to identify if there was a mass transfer limitation by diffusion.

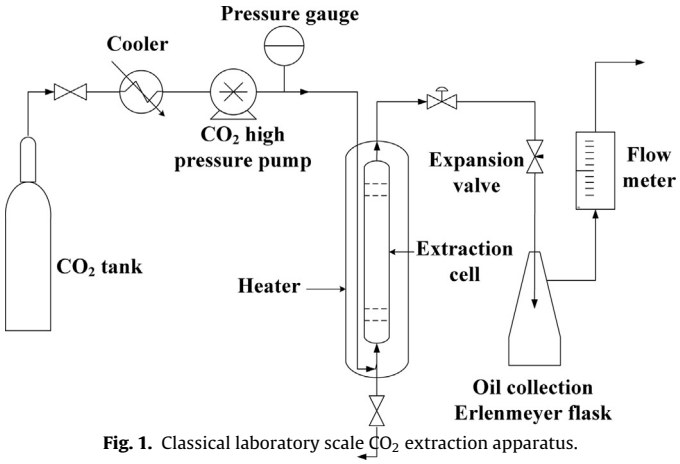

Fig. 1. Classical laboratory scale CO_2 extraction apparatus.

Table 1
Reproducibility of extraction experiments.

Experiment	Mass loss/%	Average/%
$T = 328$ K; $P = 37$ MPa; $Q_{CO2} = 0.562$ kg h^{-1}		
1	8.8	8.9 ± 0.1
2	8.8	
3	8.9	
$T = 318$ K; $P = 28$ MPa; $Q_{CO2} = 0.791$ kg h^{-1}		
1	6.1	6.2 ± 0.1
2	6.3	
$T = 318$ K; $P = 46$ MPa; $Q_{CO2} = 0.867$ kg h^{-1}		
1	11.7	11.6 ± 0.3
2	11.3	
3	11.9	

At laboratory scale, the low mass of biomass introduced in the autoclave generates small amounts of extract difficult to recover. As a consequence, extracts were collected in pure *n*-hexane and stored in a CO_2 atmosphere at 255 K to avoid the hydrolysis of triglycerides.

Some other extractions were performed in autoclaves of 2L and 150L (HITEX, Vannes, France) corresponding to batches of 1 and 50 kg, respectively. The maximum pressure of this set-up was 29 MPa. At pilot scales, the amount of recovered extract was important and didn't require the dilution in *n*-hexane. It should be noted that at larger scales (batches of 1 kg and more), CO_2 was recycled.

2.2.2. Accuracy of measurements and reproducibility

The extraction performance was estimated relative to the mass losses of the extraction autoclave (Eq. (1)) or relative to the extraction yields (Eq. (2)).

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

$$\text{Neutral lipid yield}\,(\%) = \frac{\text{neutral lipid extracted(g)}}{\text{neutral lipid content(g)}} \times 100 \quad (2)$$

The accuracy on the gravimetric measurements for the estimation of the mass loss was in the order of 10^{-3} g. The uncertainties on mass losses were of 1.7 10^{-3}%. Pressure, temperature and CO_2 flow rate were read with an accuracy of 0.5 MPa, 1 K, and 7 10^{-3} kg h^{-1}.

The reproducibility of extraction experiments was tested at three different operating conditions. Each experiment was reproduced 2 or 3 times during an extraction time of 180 min. Reproducibility was estimated from the average deviation.

Table 1 gives the results of the reproducibility experiments. It shows that whatever the operating conditions, extractions were reproducible. The reproducibility on the weight loss of the vessel was less than 0.3%.

2.3. Analytical methods

2.3.1. Analysis of raw materials

2.3.1.1. Water content of dried microalgae. The water content of the raw material was determined as follows: 5 g of microalgae were placed in an oven Memmert (Schwabach, Germany) at 378 K until the sample mass remained constant. The water content was determined by the difference between the mass of microalgae before and after drying divided by the initial mass of microalgae.

2.3.1.2. Analysis of lipids. The qualitative composition of crude oils from microalgae was determined first by a Bligh and Dyer extraction [24] and then by gas chromatography coupled with mass spectrometry (GC/MS).

2.3.1.3. Fatty acid methyl ester (FAME) profiles. In order to identify and quantify the fatty acids present in the oil, lipids were methylated using the Morrison and Smith method [25]. The methylated fatty acids were purified by Thin Layer Chromatography (Adsorbil plus 1–250 mm – Hard Layer-20 × 20 cm – Alltech) and were analysed using Gas Chromatography (Autosystem XL, Perkin Elmer, France) with a polar Famewax Column (Restek, France).

2.3.2. Extract analysis

2.3.2.1. Phosphorus determination. The samples were first mineralized. The amount of phosphorus was then determined by ICP (Inductively Coupled Plasma) coupled with mass spectrometry. The results were given in ppm by weight, parts per million (mg phosphorus per kg of oil).

2.3.2.2. Dry fraction. The principle of this analysis is the same as the one used for determining the moisture content of microalgae. The result was in that case expressed relative to the solid and not to water.

2.3.2.3. Determination of lipid classes. The separation and quantification of lipid classes were performed by gas chromatography/flame ionization detector with an Iatroscan New MK5 (Iatron Laboratories Inc., Japan) with Chromarod S III quartz rods.

2.3.2.4. Antioxidant analysis. The identification and determination of the content of antioxidants (chlorophylls *a* and *b* and β-carotene) contained in the extracted oil was determined by UV–vis spectrophotometry using the absorbance value measured in a Biochrom Libra S4 spectrophotometer (Biochrom, Cambridge, UK). The wavelength used were 653 and 666 nm for chlorophyll, and 450 nm for β-carotene. A calibration curve using standards was plotted to determine the pigment concentration in extracts. The straight calibration curve of absorbance versus pigment concentration (mg mL^{-1}) was reliant on the Beer–Lambert law.

2.4. Experimental design

An experimental design combined with Response Surface Methodology (RSM) using Nemrodw software (LPRAI, Marseille, France) was established in order to study the influence of three operating parameters upon extraction performance: pressure, temperature and CO_2/microalgae mass ratio. The operating domain was chosen as follows: pressure varied from 28 to 46 MPa, temperature from 318 to 338 K and CO_2/microalgae mass ratio from 80 to 200.

The three factors were chosen for entry values of the experimental design and for each, two levels at the extremity were considered. A factorial design composed of 2^3 experiments was performed. X_1,

Table 2
Factors and levels studied for the experimental design.

Parameter	Temperature T/K	Pressure P/MPa	CO_2/microalgae mass ratio
Factor	X_1	X_2	X_3
Maximum parameter value	338	46	200
High level	(+1)	(+1)	(+1)
Minimum parameter value	318	28	80
Low level	(−1)	(−1)	(−1)

Table 3
Operating extraction conditions carried out.

Experiment number	X_1 T/K	X_2 P/MPa	X_3 CO_2-over-feed ratio
1	318	28	80
2	338	28	80
3	318	46	80
4	338	46	80
5	318	28	200
6	318	28	200
7	338	28	200
8	318	46	200
9	318	46	200
10	318	46	200
11	338	46	200
12	328	37	140

X_2 and X_3 were the temperature, pressure, and CO_2/microalgae mass ratio, respectively. Table 2 shows the factors and levels chosen for the experimental design. The response or the output of the experimental design, noted as Y, was the mass loss in the autoclave. The different operating conditions of the experiments carried out in this study are presented in Table 3. The factorial design was composed of 8 possible combinations between the two levels of the factors. It was deduced from an Hadamard matrix to which an experiment at the medium level was added. 2 experiments were repeated (experiments 5 and 6, and experiments 8, 9, and 10) in order to quantify the error.

Second-order response surface models were used to express the variation of oil recovery (Y) as a function of the independent variables X_1, X_2 and X_3 (Eq. (3)):

$$Y(\%) = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (3)$$

where Y represented the response variable, b_0 was a constant. b_i, b_{ij} and b_{ijk} were the linear and interactive factors. It is a degree 3 synergistic model classical for this type of matrix. The quadratic coefficients were imposed to be null. The other coefficients of the model were determined by using Nemrodw software (LPRAI, Marseille, France). The agreement of model fit was evaluated using the determination coefficient R^2.

3. Results and discussions

Preliminary experiments were conducted before the experimental design in order to delimit the temperature and pressure domain of study.

3.1. Qualitative analysis of lipids contained in dry microalgal biomass before supercritical CO_2 extraction

After the air flow drying the microalgal biomass was characterized. The water content was estimated at about 5 wt%.

The lipids contained in the microalgal biomass before supercritical CO_2 extraction were also analysed. The lipids were extracted by the Bligh and Dyer method [24] and separated by Gas Chromatography.

Table 4
Lipid profile of microalgae before supercritical CO_2 extraction.

Fatty acids	Concentration of fatty acids/μg g^{-1} dry microalgae)	Relative proportions/%
Saturated (SFA)		
C8	78	0.07
C10	27	0.02
C12	27	0.02
C14	515	0.46
C16	70860	63.48
C18	1468	1.32
Mono-unsaturated (MUFA)		
C16:1	8955	8.02
C18:1n-9	3274	2.93
C18:1 n-7	588	0.53
Poly-unsaturated (PUFA)		
C18:2	15321	13.73
C18:3	10505	9.42
Σ SFAs	72975	65.38
Σ MUFAs	12817	11.48
Σ PUFAs	25826	23.14

Table 4 shows the lipid profile of oil contained in a sample of microalgal biomass before supercritical extraction. For all analysed samples, the mean lipidic content including polar and non-polar lipids varied between 10 and 16% which corresponds to the supplier data.

The original microalgal oil was rich in saturated fatty acids from C16 up to C18. We could note a non-negligible amount of γ-linolenic acid (GLA) (C18:3).

3.2. Extraction curves at different operating conditions

Extraction curves were plotted at several operating conditions in order to i) estimate the influence of operating parameters on extraction yields and kinetics, ii) accumulate data to feed a mathematical model [26]. Instead of plotting extraction curves versus time, a most commonly used parameter was chosen: the CO_2 mass flow rate-over-the mass of biomass.

3.2.1. Influence of fill rate

Fill rate should have an influence on mass transfer between biomass and supercritical CO_2, and then on 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 fill rates of 68, 81, and 100%, respectively. The operating conditions were fixed at a pressure of 40 MPa, temperature of 333 K, CO_2 flow rate of 0.37 kg h^{-1}.

Fig. 2 shows the extraction curves obtained for each filling of the vessel. Filling the autoclave with 68 and 81% of biomass led to similar results. However, the extraction curves showed that when the powder is packed, the mass transfer is disadvantaged probably due to preferential paths. To obtain a high quantity of extract, it is advised to work with a vessel filled up to 80%.

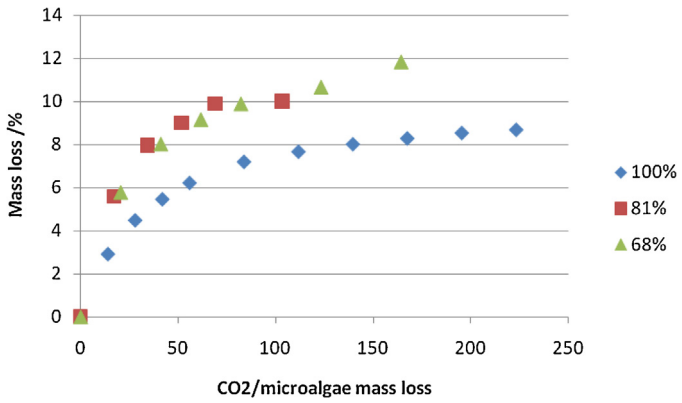

Fig. 2. Extraction curves obtained for several fill rates.

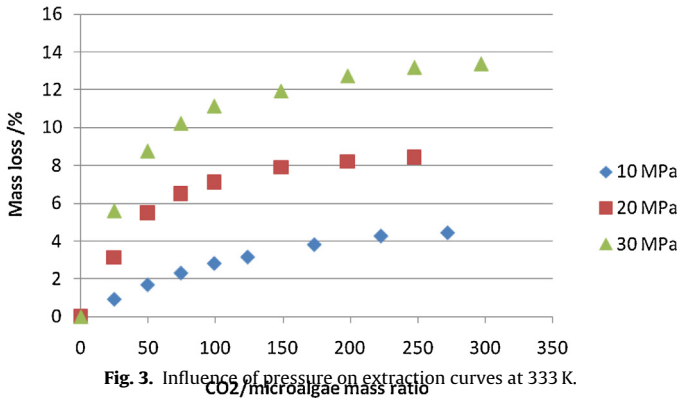

Fig. 3. Influence of pressure on extraction curves at 333 K.

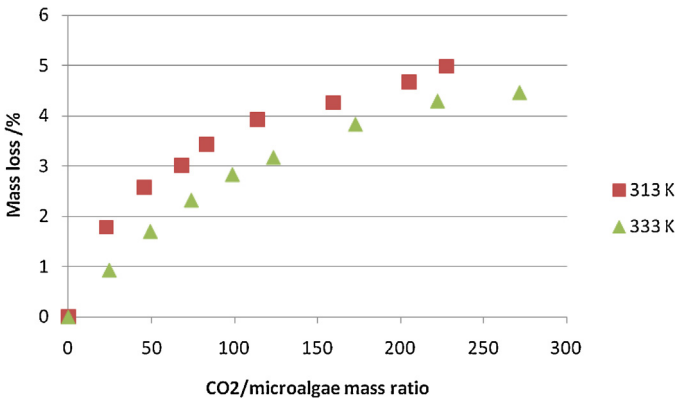

Fig. 4. Influence of temperature under 10 MPa.

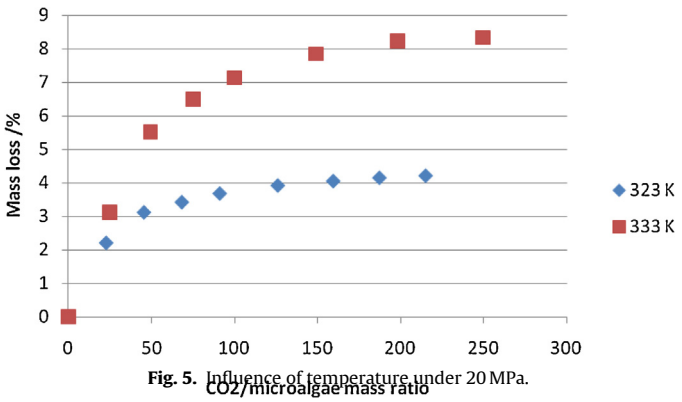

Fig. 5. Influence of temperature under 20 MPa.

3.2.2. *Influence of pressure*

In order to evaluate the influence of pressure on extraction kinetics and yields, extraction curves were plotted (Fig. 3) under several pressures: 10, 20, and 30 MPa. Each curve was obtained at a fill rate of 68%, 333 K, with a CO_2 flow rate of 0.45 kg h^{-1}.

Fig. 3 shows that pressure had a positive influence on yields as the solubility of compounds in supercritical CO_2 generally increases when the pressure increases. Working under too low pressures may drastically decrease the solubility of compounds in the solvent and finally decrease yields for a given extraction duration. The extraction curves seemed to be limited by the diffusion phenomenon but pressure had a positive impact on kinetics. Working under high pressure led to more rapid extractions, and then with a lower consumption of CO_2 for a given extraction duration.

3.2.3. *Influence of temperature*

The temperature effect was studied from 313 to 333 K under pressures of 10 and 20 MPa. Figs. 4 and 5 show the extraction curves obtained.

The retrograde behavior is well illustrated by Figs. 4 and 5. The retrograde solubility is characteristic to supercritical fluids and is explained by the complex influence of temperature on solubility because of the competitive effects of two phenomena: the vapor pressure of the solute which increases when temperature increases, and the supercritical solvent power which decreases when temperature increases while its density decreases. Depending on pressure conditions, one effect is predominant over the other. At high pressure, when the fluid is quite incompressible, solubility increases thereby following the increase in vapor pressure of the solute. At low pressure, solubility decreases when temperature increases because of the predominant effect of the decrease in density.

Figs. 4 and 5 thus show that under 10 MPa, mass losses obtained at 313 K were higher than those obtained at 333 K whereas under 20 MPa, the influence of temperature was the opposite. The inversion pressure is thus understood between 10 and 20 MPa. It could be concluded that above 20 MPa, an increase in temperature would have a positive effect on extraction yield.

3.3. *Experimental design for response surface methodology (RSM)*

In the present work, the extractions were performed at temperatures in the range of 318–338 K, pressures from 28 to 46 MPa and CO_2 flow rates between 0.32 and 0.81 kg h^{-1} corresponding to CO_2/microalgae mass ratios from 80 to 200. These chosen parameters have a great impact on extraction performance: the first two parameters influence the solubility of extracted compounds in the supercritical CO_2 as well as the mass transfer by diffusion whereas the last parameter is important to deduce the optimum amount of solvent to be used for a given amount of biomass. It is therefore connected to the cost of the operation. This experimental domain was determined based on the preliminary experiments and literature. The pressure was chosen above 20 MPa in order to avoid the retrograde solubility area and enhance mass losses as illustrated in Figs. 3–5.

For all experiments, an extraction duration of 90 min was fixed. The microalgae mass was on average 6 g in an extractor of 10 mL corresponding to an average fill rate of 80%.

Table 5 gives the mass loss obtained for each experiment.

The obtained mass loss covered a large range of values of 4.29–16.11%. As expected, the highest mass loss was reached for experiment 11 at the highest studied temperature, pressure, and CO_2/microalgae mass ratio, i.e. 338 K, 46 MPa, and 200, respectively.

Table 5
Mass losses obtained at different operating conditions.

Experiment number	T/K	P/MPa	CO_2/microalgae mass ratio	Mass loss/%
1	318	28	80	4.29
2	338	28	80	5.48
3	318	46	80	9.85
4	338	46	80	12.56
5 6	318 318	28 28	200 200	6.08 6.25
7	338	28	200	8.19
8 9 10	318318318	464646	200 200 200	11.9111.3411.66
11	338	46	200	16.11
12	328	37	140	9.00

Table 6
Coefficients of the response surface equation.

Coefficient	Value	Significance %
b_0	9.247	0.600
b_1	1.307	4.5
b_2	3.267	1.80
b_3	1.232	4.77
b_{12}	0.483	12.1
b_{13}	0.332	17.3
b_{23}	0.108	45.2
b_{123}	0.102	46.7

Table 7
Predicted oil mass losses and deviations relative to experimental data.

Experiment number	Y calc/%	Y exp/%	Deviation
1	4.259	4.29	0.031
2	5.449	5.48	0.031
3	9.819	9.85	0.031
4	12.529	12.56	0.031
5	6.049	6.08	0.031
6	8.159	8.19	0.031
7	11.629	11.66	0.031
8	16.079	16.11	0.031
9	9.247	9.00	−0.247

Experimental oil yields were used to determine the coefficients of the response surface equation (Eq. (3)). Estimated coefficients are given in Table 6. The most significant factor is X_2, i.e. the pressure.

The following polynomial equation (Eq. (4)) correctly represents the experimental data ($R^2 = 0.999$). The calculated mass loss and experimental data are compared in Table 7.

$$Y(\%) = 9.247 + 1.307X_1 + 3.267X_2 + 1.232X_3 + 0.483X_1X_2 + 0.332X_1X_3 + 0.108X_2X_3 + 0.102X_1X_2X_3 \quad (4)$$

Fig. 6(a) and (b) shows the response surfaces illustrating the influence of pressure and temperature, and temperature and CO_2/microalgae mass ratio on mass losses, respectively. In the experimental field of study, the most influent parameter on extraction mass loss was the pressure while the least influent was the CO_2 flow rate. The evolution of extracted yield with each parameter was as described in literature [15]: the solubility of neutral lipids in supercritical CO_2 increases when the pressure increases. From 28 up to 46 MPa the influence of temperature on mass losses was confirmed from the previous measurements: an increase in temperature increases the solubility of neutral lipids in supercritical CO_2.

The microalgal biomass lipidic content including polar and non-polar lipids is on average 16% (supplier data), and using supercritical CO_2 it was possible to extract up to 16% of the initial biomass. Nevertheless, it is known that polar lipids are not extracted by supercritical CO_2 [15,16], so these high mass losses can be explained mainly by the presence of water in the extracts and, to a much lesser extent, by the presence of antioxidants.

Table 8
Operating conditions of extraction and corresponding antioxidant contents (mg g^{-1} of extracted oil).

Temperature/K	Pressure/MPa	β-carotene	Chlorophyll *a*	Chlorophyll *b*
333	40	0.22	0.40	1.50
313	40	0.09	0.20	0.65
313	10	0.11	0.26	0.64

3.4. Influence of pressure and temperature on antioxidant content in the extracted oil

The brown pigmentation of extracts is mainly due to the presence of chlorophylls and carotenoids. Indeed, under certain operating conditions, supercritical CO_2 also dissolved these antioxidants with the oil and their solubility increased when the pressure increased, as for neutral lipids.

The β-carotene and chlorophyll *a* and *b* contents were determined using UV–vis spectrophotometry for samples of oil obtained at different operating conditions. Table 8 relates the operating conditions and gives the corresponding antioxidant 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.

As expected, antioxidant contents were higher when extraction was performed under the highest pressure and temperature. However, the extraction of antioxidants seemed to be strongly influenced by temperature. Indeed, the content of antioxidants was more than doubled when the temperature was increased from 313 K to 333 K. A variation of pressure did not have a significant effect. Indeed, working under 10 or 40 MPa led to the same composition.

3.5. Larger scale extractions

Larger scale experiments were conducted with microalgae containing a neutral lipid content of 12% with an average particle size of 0.5 mm.

A first extraction was performed in an autoclave of 2L for a batch of 1 kg of dry biomass corresponding to a fill rate on an average of 75%. Extraction was carried out under 29 MPa, 333 K and a CO_2 flow rate of 8.5 kg h^{-1}. One batch was carried out without any adjuvant and another one with cellulose in view to increase the mass transfer between supercritical CO_2 and biomass. Fig. 7 gives the extraction curves obtained and shows that the transfer was rapidly limited by diffusion. Moreover, the presence or not of adjuvant did not have a significant influence on the extraction performance.

Another experiment was performed in an autoclave of 150L for a batch of 50 kg (average fill rate of 50%). Extraction was performed under 29 MPa, 333 K and a CO_2 flow rate of 550 kg h^{-1}. Fig. 8 gives the extraction curve obtained.

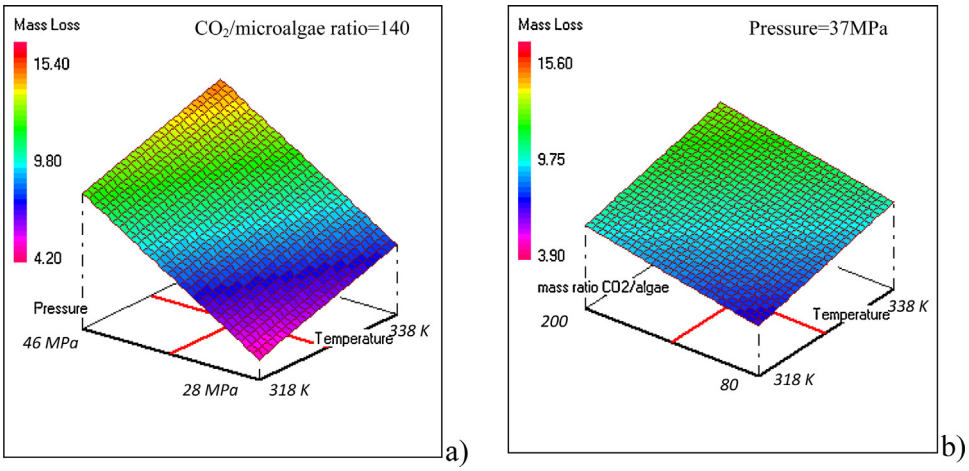

Fig. 6. Response surfaces illustrating the influence of pressure, temperature on mass loss (a) and temperature and CO_2/microalgae mass ratio on mass loss (b).

Table 9
Comparison between lab-scale and pilot plant results.

Scale	Autoclave volume/L (mass dry biomass)	P/MPa	T/K	CO_2 flow rate kg h^{-1}	Mass loss at CO_2-over-feed ratio = 50
Lab-scale	20 mL (10 g)	30	333	0.5	8.8[a]
×100	2 L (1 kg)	29	333	8.5	6.2[b]
×5000	150 L (50 kg)	29	333	550	6[b]

[a] d < 160 μm.
[b] d = 500 μm.

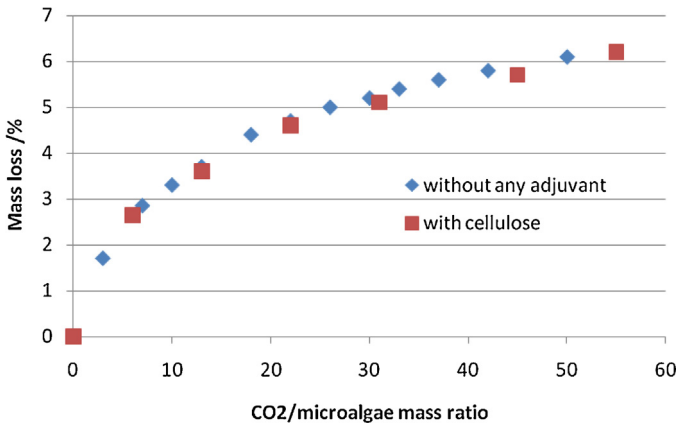

Fig. 7. Extraction curves obtained with a batch of 1 kg of dry biomass without any adjuvant and with cellulose at 29 MPa, 333 K and a CO_2 flow rate of 8.5 kg h^{-1}.

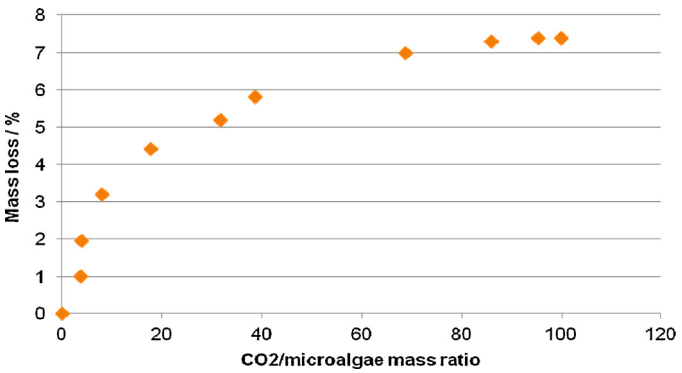

Fig. 8. Extraction curves obtained with a batch of 50 kg under 29 MPa, 333 K and a CO_2 flow rate of 550 kg h^{-1}.

These two larger scale experiments correspond to scale factors of 100 and 5000, respectively. Table 9 compares the results obtained at lab-scale and at pilot scales. For both experiments (batch of 1 kg and batch of 50 kg), at a solvent/biomass mass ratio of 50, the mass loss obtained was close to 6% (Figs. 7 and 8, respectively). At laboratory scale, the mass loss obtained under 30 MPa and 333 K for the same solvent-over feed ratio was 8.8% but for a particle size less than 160 μm (Fig. 3). The mass losses obtained for the three different scales (laboratory, ×100, and ×5000) are thus of the same order of magnitude at the same solvent/biomass mass ratio. This feature was already observed under 50 and 85 MPa in previous works carried out on microalgae [16].

Finally, the oils extracted at larger scales were analysed: phosphorus determination, dry extract and composition of oils were determined.

3.5.1. *Phosphorus determination*

The phosphorus content of oils extracted by supercritical CO_2 was less than 10 mg kg^{-1} (limit of detection of the analysis apparatus). This expected result is explained by the fact that supercritical CO_2 does not solubilize phospholipids.

3.5.2. *Dry extract*

The dry extract of extracted oils is about 96–99 wt%. One part of residual water (initial water content in the biomass was on an average of 5 wt%) is then extracted.

3.5.3. *Composition of oils*

Table 10 shows the lipid classes of the extract obtained with the batch of 50 kg under 29 MPa and at 333 K.

The analysed oil showed a high content in free fatty acids to the detriment of triglycerides. This feature has already been observed in previous works [16]. Indeed, at laboratory scale, the low quantity of microalgal biomass is stored in a freezer under inert atmosphere, but this way of storing cannot be applied at larger scale. The batch of 50 kg of microalgal biomass was stored at ambient temperature potentially leading to the degradation of triglycerides in free

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Table 10
Lipid classes of the extract obtained with the batch of 50 kg.

Lipid classes	Hydrocarbons	FFAs[a]	Triglycerides	Cholesterol	Antioxidants
Composition/wt%	1.55	86.10	2.41	2.42	7.51

[a] Free Fatty Acids.

fatty acids. Another explanation can be the substantial content of water in the extract, perhaps also leading to the hydrolysis of the extracted triglycerides in free fatty acids. However, this content in free fatty acids does not compromise the nutritional value of extracted oils.

4. Conclusions

The aim of this work was to perform supercritical CO_2 extraction from neutral lipid enriched *Spirulina platensis*. The influence of operating conditions: pressure, temperature, CO_2 flow rate, fill rate on extraction kinetics and/or yields, and on antioxidant contents was studied. As expected, the pressure is the most influent parameter on extraction yields and kinetics. The retrograde behavior was also well illustrated. The inversion pressure is understood between 10 and 20 MPa. Concerning the influence of operating parameters on antioxidant content, it appears that temperature is the most significant parameter. The higher the temperature, the higher the antioxidant content. Extractions at larger scale (scale factors of 100 and 5000) were conducted and led to encouraging results since the mass loss was of the same order of magnitude as at laboratory scale for an equivalent solvent-over-feed ratio. The collected data will be used to develop a mathematical model in order to predict extraction curves.

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