Supercritical carbon dioxide extraction and fractionation of lipids from freeze-dried microalgae *Nannochloropsis oculata* and *Chlorella vulgaris*

Sara Obeid[a,b,c,*], Nicholas Beaufils[a], Séverine Camy[d], Hosni Takache[b,c], Ali Ismail[b,c], Pierre-Yves Pontalier[a]

[a] *Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, 4 allée Emile Monso, 31030 Toulouse cedex 4, France*
[b] *Département des Sciences et Technologies Alimentaires, Faculté des Sciences Agronomiques, Université Libanaise, Dekwaneh, Lebanon*
[c] *Platforme de Recherches et d'Analyses en Sciences de l'Environnement (PRASE), Ecole Doctorale des Sciences et Technologies, Université Libanaise, Hadath, Lebanon*
[d] *Laboratoire de Génie Chimique (LGC), INP-ENSIACET, Université de Toulouse, 31030 Toulouse, France*

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A B S T R A C T

This study deals with the selective extraction of neutral lipids from microalgae. We investigated the consequences of bypassing cell-wall disintegration before supercritical carbon dioxide extraction. Different operating parameters (use of co-solvent, pressure, and time) were tested on freeze-dried *Chlorella vulgaris* and *Nannochloropsis oculata*. The solid phase extraction technique (SPE) was used throughout the extraction process to assess variations in the yield of liberated neutral lipids, glycolipids, and phospholipids. Under operating conditions, 97% of neutral lipids were extracted from *C. vulgaris* using ethanol (10% v/v) as co-solvent. Neutral lipids from *N. oculata* represented most of the extracts (83%), whereas the proportion of glycolipids and phospholipids did not exceed 12.1% and 5.3%, respectively. Microscopic observation showed that cell wall integrity was maintained during the extraction process.

1. Introduction

Microalgal Lipids can generally be divided into two large classes based on the polarity of the molecular head group: polar and neutral lipids. Polar lipids can be categorized into phospholipids (PPL) and glycolipids (GL). They are essential structural components of cell membranes and organelle membranes that contribute to their flexibility, fluidity and selective permeability. Neutral lipids (NL) consist of acylglycerols and free fatty acids and are used for energy storage [1]. They are present as droplets in the chloroplast matrix and in the cytoplasm. Their extraction from the cellular matrix into the surrounding medium can be diminished by the rigidity of cell wall. Indeed, the microalgal lipids recovery involves several steps including cultivation, harvesting, drying and lipid extraction [2] [3]. Based on life cycle assessment studies, the oil extraction process consumes 90% of the energy, which signifies a need to improve the economic viability of the process [4].

Lipid extraction from microalgae is generally carried out using organic solvent. This is because chemical solvent has high selectivity and solubility towards lipids. However, such processes, which give rise to high lipids yields, cannot be used at industrial scale due to the high toxicity of organic solvents on human and environment [5].

In extraction using chemical solvent, diffusion is always the limiting factor in the overall mechanism [6]. However, this factor becomes more critical in microalgae as the cell wall may prohibits solvent from diffusing into the inner cell for lipid extraction. Therefore, mechanical pretreatments are generally introduced to enhance solvent diffusion efficiency and consequently, to improve microalgae lipid recovery rate. However, the methods used are nonselective and generally accompanied by cell wall destruction, leading to complex extracts of hydrophilic and hydrophobic components. Such treatments require additional costly stages in downstream processing for phase separation and compound purification. For example the presence of phospholipids in lipid extracts implies an additional degumming step to remove them either for food, health and energy applications [3].

Supercritical carbon dioxide ($ScCO_2$) extraction of microalgal lipids has received attention as an alternative to organic solvent extraction and has been shown to be an ideal method for extracting certain lipids [7]. Several studies reported high extraction yield using $ScCO_2$ [8] [9] [10]. However, from these works, it appears that the microalgae

Abbreviations: SPE, solid phase extraction; PPL, phospholipids; GL, glycolipids; NL, neutral lipids; $ScCO_2$, supercritical carbon dioxide

* Corresponding author at: Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, 4 allée Emile Monso, 31030 Toulouse cedex 4, France.
E-mail addresses: saraobeid.university.leb@live.com (S. Obeid), Pontalier@ensiacet.fr (P.-Y. Pontalier).

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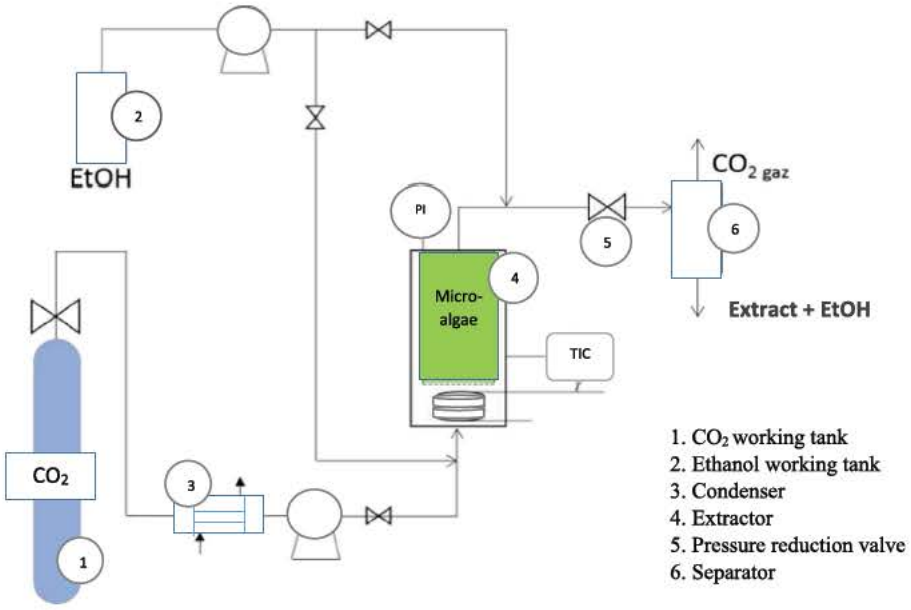

Fig. 1. Description of the supercritical carbon dioxide pilot.

pretreatment is highly significant to perform an efficient extraction with $ScCO_2$. In major cases, prior to supercritical extraction, mechanical pretreatment was carried out and high amount of organic co solvent was used for increasing lipid extraction yield all in sharply decreasing selective NL extraction. Patil et al. [9] subjected *Nannochlorpsis* biomass to microwave pretreatment prior to supercritical extraction and added an azeotropic mixture (hexane/ethanol) as co solvent to enhance $ScCO_2$ affinity towards NL, giving a low selective extraction. Increasing the co extraction of polar lipids necessitates therefore further purification steps. Cheng et al. [10] studied NL extraction from freeze dried *Pavlova* sp. subjected to cell disruption prior to extraction. The highest extrac tion yield of 98.7% was observed using $ScCO_2$ with bead beating as pretreatment. However, low selectivity of lipid extraction was obtained due to cell disintegration and as result, further purification steps were necessary. Recently, Crampon et al. [11] reported the use of $ScCO_2$ at industrial scale for the extraction of NL from *Spirulina platensis* without cell disruption. However *S. platensis* is known to have a vulnerable cell wall and did not require a severe cell disruption contrary to lipid rich microalgae having a robust cell wall like *C. vulgaris* and *N. oculata* [12].

The aim of this study was to investigate the extraction of NL from *C. vulgaris* and *N. oculata* using $ScCO_2$ without cell disruption. On the one hand, we study the effect of $ScCO_2$ key parameters on the extraction yield: temperature, pressure and addition of polar modifier (ethanol 10% v/v). Such addition of polar modifier allows to understand the effect of polar lipid co extraction on NL extraction yield. On the second hand, the selectivity of NL extraction was evaluated by analyzing the composition of microalgal lipid extracts all long the process using solid phase extraction (SPE) technique. To our knowledge, similar study has never been discussed in the literature.

2. Materials and methods

2.1. Microalgae biomass

C. vulgaris and *N. oculata* used in this study were provided freeze dried by Alpha Biotech (Assérac, France). These two species were used because they possess an extra rigid membrane, due to their growth under stressful conditions [12]. *N. oculata* was cultivated in modified Conway media under conditions of N nutrient deficiency to favor the accumulation of lipids, whereas the microalga *C. vulgaris* was cultivated in Sueoka media under conditions of high N nutrient concentration to favor protein accumulation. Biomass have been harvested during the exponential growth phase and concentrated by centrifugation (20 24% dry weight). The frozen paste of crude microalgae was freeze dried in a Fisher Bioblock Scientific Alpha 2 4 LD Plus device (Illkirch, France). During freeze drying, the pressure was reduced to 0.01 bar, and the temperature decreased to −80 °C for 48 h to give a completely dry biomass. After freeze drying process, freeze dried aggregates were slightly spread with a laboratory spatula. The mean diameter of parti cles was measured using Mastersizer 2000 granulometer (Malvern In struments Ltd.) showing a value around 200 µm.

2.2. Total lipids content

The term "total lipids" represents the amount of all extractable li pids initially present in the algal biomass. This amount served as a re ference throughout the study to evaluate the effectiveness of lipid ex traction using $ScCO_2$. Total extractable lipids were measured after extraction in a Soxhlet apparatus using a slightly modified method based on the two methods developed by Blight and Dyer (1959) and Folch (1957), using a mixture of polar and non polar solvents: chloro form/methanol (0.35/0.65, V/V) [13] [14] [15]. The microalgal bio mass (3 g) was placed in a cartridge filter paper in 200 mL solvent. The Soxhlet extractor was operated for multiple cycles for 18 h to ensure the extraction of all extractable lipids.

2.3. $ScCO_2$ extraction

Extraction was carried out using a pilot unit developed by the company Separex (Nancy, France). The pilot was composed of a 25 cm^3 autoclave (Fig. 1). This unit could be operated at pressures up to 1000 bar and a temperature up to 250 °C. At the beginning of the ex periment, the extractor was filled with determined mass of freeze dried microalgae powder (Table 1) and the module was supplied with liquid CO_2 through a pump which was maintained at a temperature below 7 °C to keep the CO_2 in the liquid state. Ethanol was introduced into the extractor either as a co solvent or a cleaning fluid.

Several extractions were performed by varying the working pressure and solvent polarity (addition of ethanol 10% v/v). All supercritical extractions were carried out under constant temperature of 50 °C. A simple extraction consisted of one step of $ScCO_2$ extraction with or without ethanol as co solvent (10% v/v), whereas a double extraction

Table 1
Operating conditions for $ScCO_2$ extraction applied to freeze-dried *C. vulgaris* and *N. oculata*, with and without ethanol (10%) as co-solvent.

Microalgae	T (°C)	P (bar)	CO_2 flow rate CO_2 (g/min)	Biomass (g)	Solvent rate (g/g·min ^{1})	Ethanol as co-solvent	Ethanol flow rate (g/min)	Operation time (min)
Simple extraction								
C. vulgaris	50	250	25	12.1	0.45	Yes	1.9	230
N. oculata	50	250	25	25.4	1.02	No	–	210
N. oculata	50	450	25	8.8	0.35	No	–	240
N. oculata	50	750	25	8.8	0.35	No	–	240
Double extraction								
C. vulgaris	50	250	25	25.4	1.02	No	–	210
Residue	50	250	25	23.4	0.87	Yes	1.9	210

Note: Simple extraction: $ScCO_2$ extraction with 10% ethanol as co-solvent. Double extraction: $ScCO_2$ extraction with addition of ethanol at t = 210 min. Solvent rate is the ratio of incorporated biomass (g) with respect to the CO_2 flow rate (g/min), as the extraction is semi-continuous.
C. vulgaris: *Chlorella vulgaris*, *N. oculata*: *Nannochloropsis oculata*, T: temperature, P: pressure, CO_2: carbon dioxide, $ScCO_2$: supercritical carbon dioxide.

consisted of first performing $ScCO_2$ extraction followed by a second $ScCO_2$ extraction with co solvent (Table 1).

2.4. Fractionation of neutral and polar lipids

All lipids extracted from *N. oculata* and *C. vulgaris*, either by $ScCO_2$ or Soxhlet, were fractionated into neutral and polar lipids using the solid phase extraction method (SPE) described by Juaneda and Rocquelin [16]. The cartridges were conditioned by slowly adding 5 mL chloroform followed by 5 mL methanol, reaching the top of the gel to ensure good rinsing. A sample of 25 mg lipids was applied to the top of the silica cartridges Supelco (500 mg, Sigma Aldrich). The NL fraction was eluted with 10 mL chloroform, whereas the GL and PPL fractions were eluted with 10 mL acetone and methanol, respectively. Once eluted and freeze dried under liquid nitrogen, every fraction was measured gravimetrically. The contents of neutral and polar lipids are expressed in g/g dry biomass.

2.5. Microscopic observation

Microscopically qualitative approach was done to characterize any cell disintegration of the biomass. Observation at high magnifications (×1000) was performed before and directly after $ScCO_2$ treatments. A small quantity of cell suspension was placed on a specific plate (Nikon SMZ 1500). The images were captured under a constant exposure and illumination by Nikon eclipse E600 camera.

2.6. Statistical analysis

Results for microalgae characterization in terms of lipid content were based on 3 replicates for 3 experiments (n = 9). All supercritical extraction data were presented as mean values ± standard deviations of three experiments. Statistically significant differences ($p < 0.05$) between the means were evaluated using one way analysis of variance (ANOVA) and the Tukey's test on XLTAT software version 2018.1.

3. Results and discussion

3.1. Microalgae characterization

The lipids composition of the studied microalgae was defined ac cording to the values following Soxhlet extraction [17]. The maximal lipids extraction yield was obtained after 18 h: 0.399 ± 0.016 and 0.244 ± 0.010 g/g dry weigh for *N. oculata* and *C. vulgaris*, respec tively (Table 2).

The proportions of various lipids classes were evaluated by SPE fractionation. *N. oculata* was rich in NL, comprising 44.0 ± 1.6% of its total lipids content. The high lipids content of *N. oculata* may be ex plained by the growth conditions. In fact, the studied strain was grown under conditions of normal CO_2 concentration and nitrogen depletion. Cultivation under such conditions can induce lipids accumulation which surpasses that during normal growth conditions, as well as fa voring the production of mostly NL [18].

In contrast to *N. oculata*, *C. vulgaris* was grown under conditions of high N nutrient levels. Microalgae grown under such conditions have polar lipids as predominant lipids, which are located in chloroplasts and cell membranes [19].

C. vulgaris had a lower NL content (26.0 ± 0.4% of its total lipids) than *N. oculata*. This suggests that *C. vulgaris* did not accumulate large amounts of reserve lipids due to the growth conditions. Hence, most of the available lipids were polar, especially GL, which represented 59.0 ± 0.8% of the total lipid content. Our results concerning the li pids composition of *N. oculata* and *C. vulgaris* are in accordance with several studies in the literature [20] [21] [22]. These results were used as reference values throughout this study.

3.2. Supercritical extraction yield and kinetics

3.2.1. Effect of pressure on lipids extraction from N. oculata

Fig. 2 shows the effect of pressure on the extraction yield of mi croalgal lipids from freeze dried *N. oculata* by $ScCO_2$. Extraction at 250 bar resulted in a very low yield (15%). Increasing the pressure to 450 and 750 bar led to an increase of the extraction yield (20%). Sev eral studies reported that the extraction efficiency of $ScCO_2$ increases with pressure [23] [24]. In fact, increasing the pressure results in an increase in the solubility of NL in $ScCO_2$. Moreover, the adsorption capacity of solute decreases at high pressure [25] [26]. At least, part of the lipids droplets bounded to the cell matrix may desorb during ex traction at high pressure.

Microscopic observation of *N. oculata* subjected to $ScCO_2$ treatment showed maintained cell wall integrity (Fig. 3). The presence of such structure could limit the mass transfer of the solvent and then the ex traction of NL which may explain the low extraction yield obtained for all pressures.

3.2.2. Effect of medium polarity on lipid extraction from C. vulgaris

The solubility of lipids in $ScCO_2$ or ethanol depends on their che mical composition. Studied *C. vulgaris strain* was grown under high N nutrient growth conditions. It was thus expected to have a low lipids content, mainly composed of polar lipids (GL and PPL) as shown in Table 2.

Given the relatively high polarity of the initial lipids fraction, we tested the addition of ethanol as co solvent to enhance its solubiliza tion/extraction (Fig. 4). The addition of ethanol (10% v/v) at the be ginning of the process markedly enhanced the extraction kinetics during the first 100 min relative to the first stage (absence of ethanol) of the double extraction procedure. In the presence of co solvent, the final yield reached almost 40.3 ± 1.1% of the total extract (Fig. 4A).

The extraction of lipids was likely controlled initially by their so lubility in $ScCO_2$/ethanol and then, after the break of the curve, it was

Table 2
Lipids fractions (NL, GL, PPL) relative to total lipids and dry matter in *C. vulgaris* and *N. oculata*. Results are based on 3 replicates for 3 experiments ± SD (n = 9).

	C. vulgaris			*N. oculata*		
Total lipids (g/g DM)	0.244 ± 0.010			0.399 ± 0.016		
	NL	GL	PPL	NL	GL	PPL
Fraction in total lipids (%)	26.0 ± 0.4	59.0 ± 0.8	15.0 ± 0.6	44.0 ± 1.6	30.1 ± 0.8	25.9 ± 0.9

Note: *C. vulgaris*: *Chlorella vulgaris*, *N. oculata*: *Nannochloropsis oculata*, NL: neutral lipids, GL: glycolipids, PPL: phospholipids.

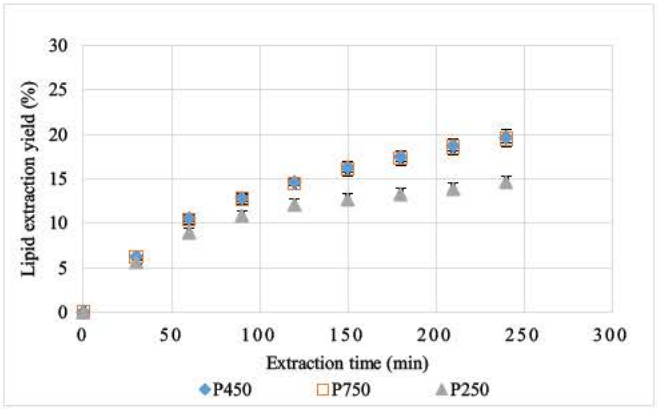

Fig. 2. Lipid extraction yield (extracted/total extractable) from *Nannochloropsis oculata* over time using supercritical CO_2 ($ScCO_2$) extraction under different applied pressures (P): 250, 450, or 750 bar. Data shown as mean ± SD (n = 3).

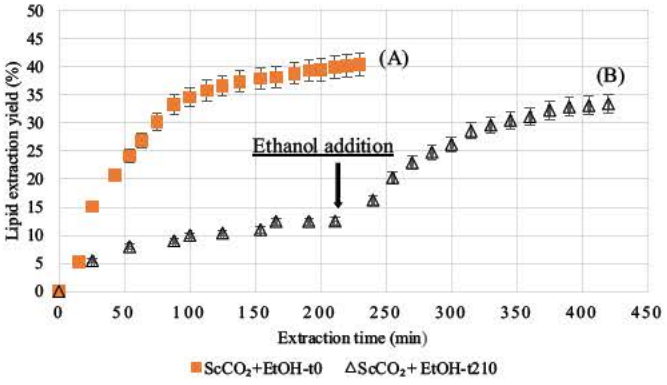

Fig. 4. Lipid extraction yield (extracted lipids/total extractable lipids) from *Chlorella vulgaris* aver time under P = 250 bar. (A) Simple supercritical CO_2 ($ScCO_2$) extraction with ethanol (10% v/v) as co-solvent, (B) double extraction: $ScCO_2$ extraction with addition of ethanol at t = 210 min. Data shown as mean ± SD (n = 3).

probably related to lipids desorption from the cell matrix [27]. The non extracted lipids remain adsorbed on the microalgal matrix and the equilibrium phase are described by the adsorption isotherm. The ad sorption capacity can be estimated from the point at which the slope of the extraction curve suddenly decreases.

Lipids extraction from *C. vulgaris* using $ScCO_2$ without ethanol showed stabilization of the extraction yield at almost 12.5 ± 0.6% after 120 min (Fig. 4B). Ethanol (10% v/v) addition after 210 min in creased the yield. The addition of ethanol modified the solvent (CO_2) properties, shown by the increased slope for the linear portion of the curve. Changing solvent polarity allowed further extraction of mole cules that were not accessible to the $ScCO_2$. Indeed, combining ethanol (a polar solvent) with CO_2 has been shown to improve the extraction of various lipids classes (neutral, polar) as reported in the literature [28]. Microscopic observation showed that *C. vulgaris* microalgae preserve its cell wall integrity after $ScCO_2$ treatment (Fig. 5).

3.3. Extracts composition

3.3.1. N. oculata extracts composition

Lipids extraction from *N. oculata* by $ScCO_2$ under 450 and 750 bar, resulted in comparable quantities of extracted lipids of similar quality. Under 450 bar, NL represented 70.0 ± 1.3% of the extracts at the be ginning and 80 ± 2% by the end of the extraction (Fig. 6A). The fraction of PPL did not exceed 7.1 ± 0.3% throughout the analysis, while GL made up 26.0 ± 1.1% of the extracts at the beginning and

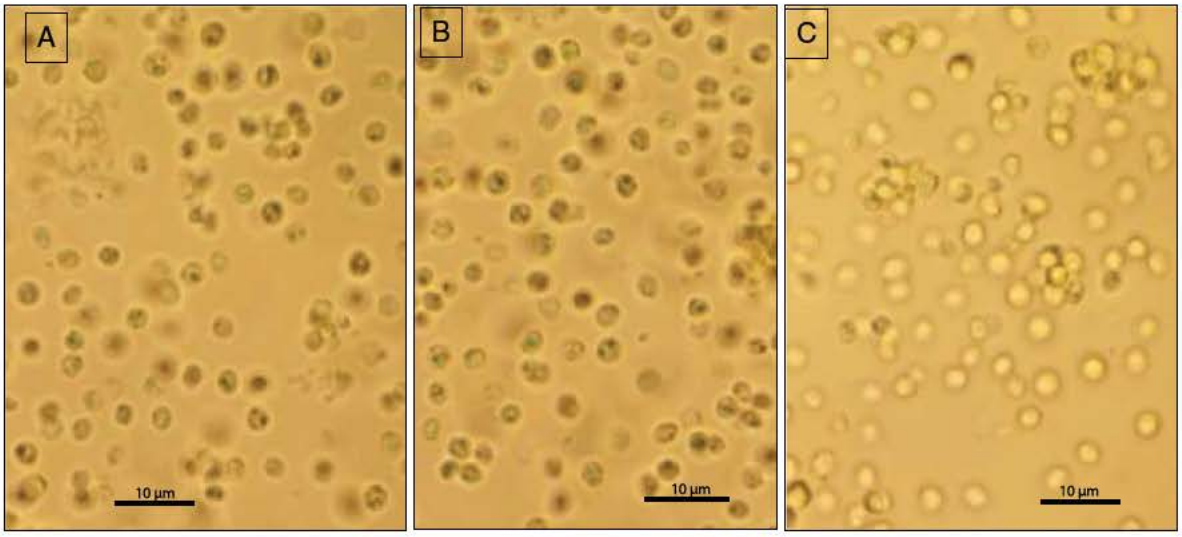

Fig. 3. Microscopic observation at high magnification (×1000) of *Nannochloropsis oculata* cells after supercritical CO_2 ($ScCO_2$) extraction. (A) Control before extraction, (B) After extraction under 450 bar, (C) After extraction under 750 bar.

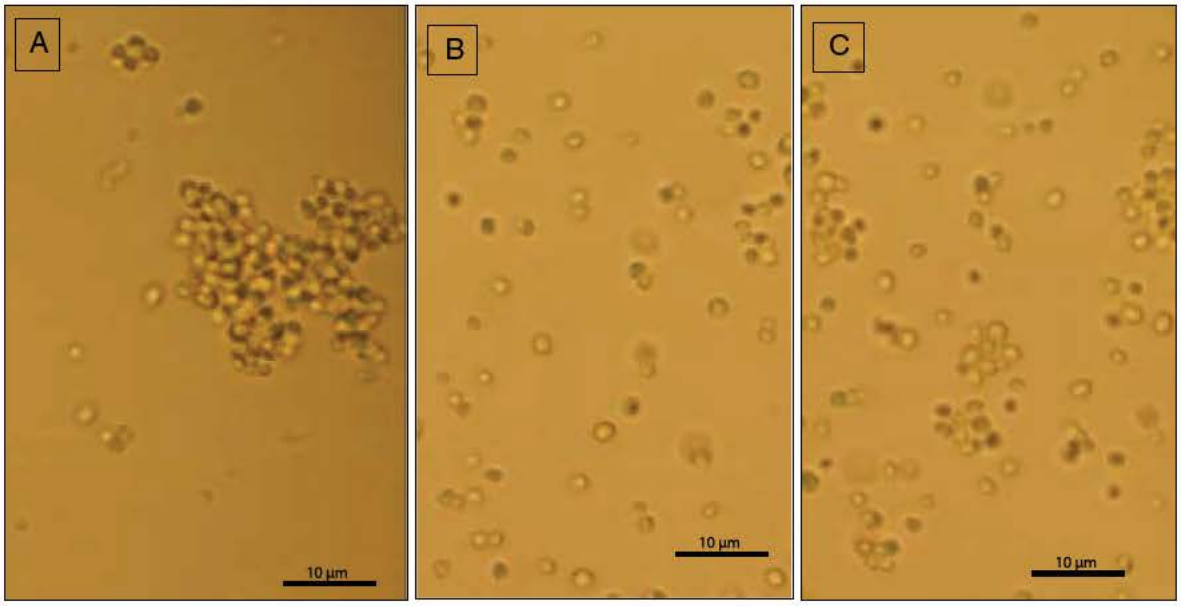

Fig. 5. Microscopic observation at high magnifications (×1000) of the *Chlorella vulgaris* cells after supercritical CO_2 ($ScCO_2$) extraction. (A) Control before extraction (B) After double extraction (C) After simple extraction.

decreased to 15.2 ± 0.7% by the end of the extraction. Similar lipids fraction profiles were obtained under higher pressure extraction con dition (750 bar), showing no significant difference ($p > 0.05$). NL re presented most of the extracts with 70.0 ± 1.7% at the beginning, rising to 83.0 ± 3.3% after 211 min, whereas the proportion of GL and PPL did not exceed 12.1 ± 0.65% and 5.3 ± 0.3%, respectively, by the end of the extraction process (Fig. 7A). These results are in ac cordance with those reported by Temelli [29].

In this experimental set up, the yield of extracted lipids under 450 or 750 bar was essentially identical. Processing under a pressure of 450 bar, allowed the extraction of 41.9 ± 1.2% of total NL, 16.0 ± 0.8% of total GL and 4.5 ± 0.2% of total PPL initially con tained in *N. oculata* (Fig. 6B). Similar results were obtained at 750 bar where 43.2 ± 1.05% of NL initially contained in *N. oculata* were ex tracted (Fig. 7B). The GL and PPL extraction curves rapidly reached steady state, yielding 14.7 ± 0.8% and 3.8 ± 0.2% of their total content, respectively. These results were better than those obtained by Patil et al. [9] in terms of experimental simplicity, selectivity, and purity of extract. They subjected *Nannochlorpsis* biomass to microwave pretreatment prior to supercritical extraction to improve the efficiency of NL extraction and added an azeotropic mixture (hexane/ethanol) as co solvent to enhance $ScCO_2$ fluid affinity towards NL. In terms of se lectivity, our results were also better than those of Tang et al. [30] who obtained a partial fraction of total lipid content using ethanol (95%, v/ v) as co solvent under high pressure, which increased the co extraction of polar lipids, necessitating then further purification steps.

Increasing the pressure from 450 to 750 bar did not provide any additional benefit in terms of NL extraction yield. Global yields at t = 210 min were essentially the same, in accordance with the results of Crampon et al. [31] who reported a negligeable difference in the yield of extracted lipids from *N. oculata* between 500 and 850 bar.

Contrary to GL and PPL, the NL extraction yield continued to line arly increase even after 4 h, under both applied pressures, and would have reached much higher values if the extraction time was prolonged. This may be due to high pressure causing the formation of holes in the microalgae cell wall, allowing $ScCO_2$ to reach the NL located in the cytoplasm as droplets, and extract them. The yield of GL and PPL reached a steady state after 2 h, suggesting that the external cell wall and membranes of the inner organelles, rich in polar lipids, were not substantially damaged and supporting the hypothesis of holes

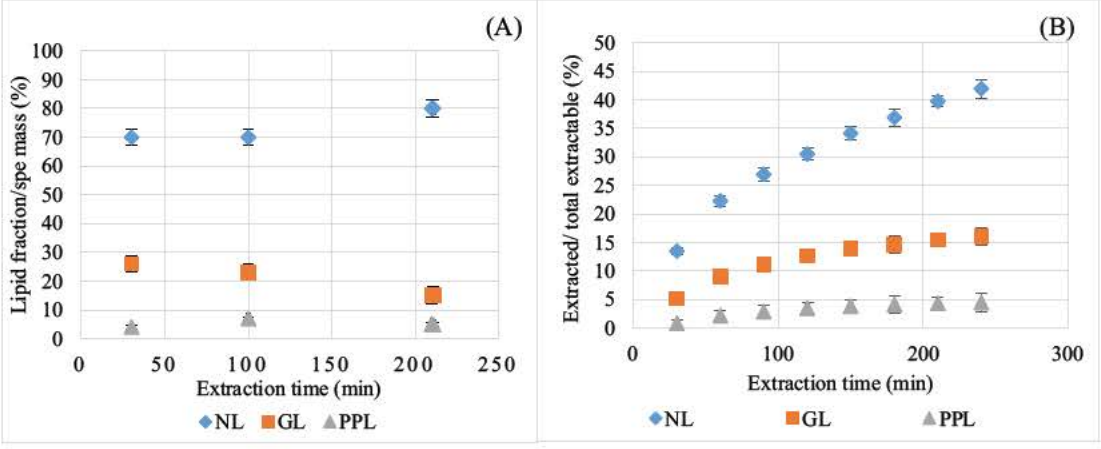

Fig. 6. Variation of proportions (A) and yield (B) of NL (neutral lipids), GL (glycolipids) and PPL (phospholipids) in the supercritical CO_2 ($ScCO_2$) extracts of *Nannochloropsis oculata* over time (450 bar and 50 °C). Data shown as mean ± SD (n = 3).

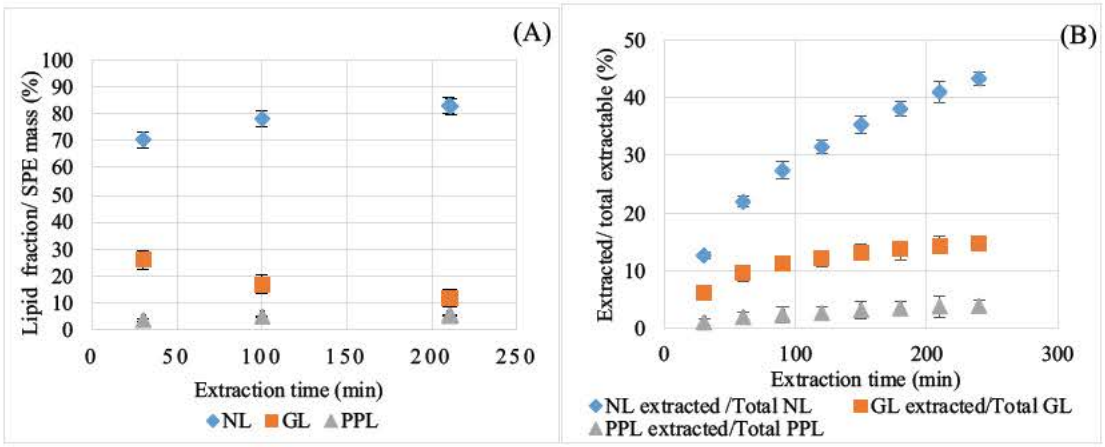

Fig. 7. Variation of proportions (A) and yield (B) of NL (neutral lipids), GL (glycolipids) and PPL (phospholipids) in the supercritical CO_2 ($ScCO_2$) extracts of *Nannochloropsis oculata* over time (750 bar and 50 °C). Data shown as mean ± SD (n = 3).

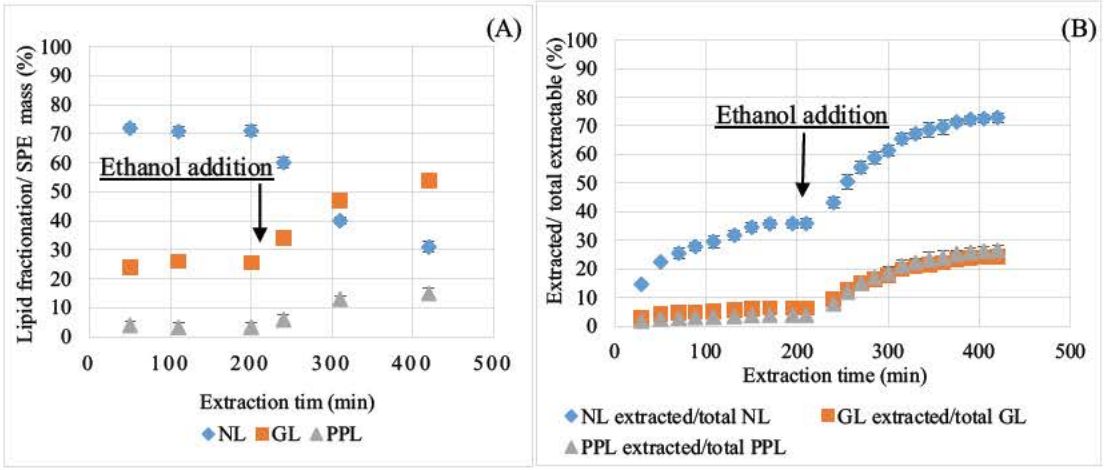

Fig. 8. Variation of proportions (A) and yield (B) of NL (neutral lipids), GL (glycolipids) and PPL (phospholipids) in the supercritical CO_2 ($ScCO_2$) of *Chlorella vulgaris* extracts after double extraction over time. Data shown as mean ± SD (n = 3).

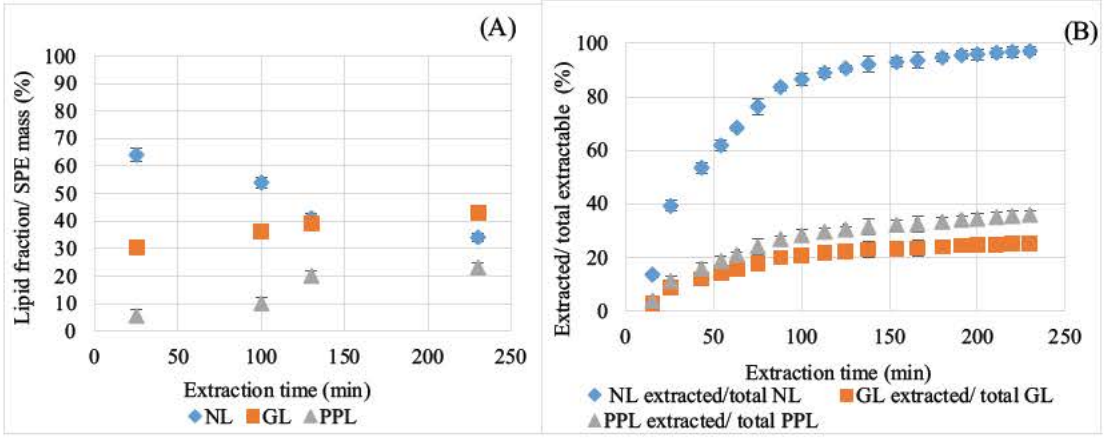

Fig. 9. Variation of proportions (A) and yield (B) of NL (neutral lipids), GL (glycolipids) and PPL (phospholipids) in the supercritical CO_2 ($ScCO_2$) of *Chlorella vulgaris* extracts after simple extraction over time. Data shown as mean ± SD (n = 3).

formation in the membranes rather than complete disintegration. In general, the amount of extractable solute depends on the percentage of microalgae cells opened by the disintegration step (not used in our study) and the fact that the solute from intact cells is not available for extraction by $ScCO_2$. The formation of holes through exposure to $ScCO_2$ was reported in the study of Solana et al. [32].

NL extraction may be also influenced by the contact time with the solvent. This suggests an effect of residence time in which more ad sorption sites of lipid molecules may be damaged over time, resulting in a longer time for the extraction to reach equilibrium. Our results sug gest that a longer extraction time for both extraction conditions (450 and 750 bar) would increase the yield of NL only. Consequently, the

final product becomes purer and consists mainly of NL. This may avoid extensive purification steps, which could compensate for the costs associated with the long extraction time.

3.3.2. *C. vulgaris* extracts composition

We fractionated samples of total lipids extracts obtained from *C. vulgaris* under double extraction ($ScCO_2$ followed by $ScCO_2$ + ethanol 10% as co solvent added at t = 210 min) into NL, GL, and PPL, by the SPE technique (Fig. 8). It can be seen that during the first stage of double extraction that NL represented 70.2 ± 2.5% of the obtained extracts when using only $ScCO_2$ as solvent. PPL were a minor fraction (5.0 ± 0.3%), whereas GL represented 25.3 ± 1.3% of the extracted material (Fig. 8A). Moreover, the yield of extracted GL and PPL was relatively low (6.7 and 3.5%, respectively), whereas almost 35.8 ± 0.9% of the total NL originally contained in *C. vulgaris* were extracted. The addition of ethanol to the stabilized extraction (at t = 210 min) significantly increased the NL, PPL, and GL extraction yield. Almost 72.9 ± 1.3% of total NL, 26.4 ± 1.5% of total PPL, and 26.1 ± 1.2% of total GL were obtained by the end of extraction (Fig. 8B). These results may be due to the increase in the polarity of the medium after the injection of ethanol which may boost the extraction of both polar and non polar lipids as reported by Moreau et al. [28] and Temelli [29].

Adding ethanol (10% v/v) at the beginning of the extraction did not give the same results as those of the double extraction. The NL fraction represented 64.1 ± 2.9% of the initial extracts but decreased to 34.0 ± 1.6% by the end of extraction (Fig. 9A). The GL and PPL fractions progressively increased over time to reach after 230 min 43.5 ± 1.7% and 23.0 ± 0.9% of the total extract, respectively.

Using simple extraction, the steady state was promptly reached for all lipid fractions (Fig. 9B). However, in this case, the extraction yield of NL was higher (97.1 ± 0.5%) than that for GL and PPL (25.3 ± 1.0% and 35.9 ± 1.5%, respectively). Our results competed with Moradi Kheibari and Ahmadzadeh [33] who studied NL extraction from *C. vulgaris* using $ScCO_2$ with a disruption step and a higher pressure.

Furthermore, the NL extraction yield was higher than that obtained by double extraction. This could be explained by the fact that the extraction of polar lipids may promote the extraction of NL due to existing interactions between different types of lipids (polar/non polar) [28] [29]. Using double extraction, the lipid molecules remaining in the matrix from the first stage become highly viscous and form agglomerates that hindered their diffusion during the second step [34]. This could limit the extraction of NL remaining in the biomass which could not pass through the non disintegrated cell wall as shown by microscopic observation.

4. Conclusion

During this study, $ScCO_2$ extraction was carried out for selective recovery of neutral lipids from *C. vulgaris* and *N. oculata* microalgae without preliminary cell disruption step. Extraction yield of total lipids from *N. oculata* at 450 and 750 bar (20%) was higher than that at 250 bar (15%). Neutral lipids extracts from *N. oculata* represented 83% of total lipids extracts, whereas the proportion of glycolipids and phospholipids did not exceed 12.1% and 5.3%, respectively. Adding ethanol (10% v/v) as co solvent led to the extraction of 97% of neutral lipids from *C. vulgaris*.

Residues from $ScCO_2$ extraction, composed mainly of hydrophilic fraction (proteins, polysaccharides), will undergo in future study an optimized aqueous extraction, in aim to recover total protein fraction without any cell disruption pretreatment. This deals with the fundamental algorefinery concept, by taking advantages from the whole biomass components, making the large scale application of microalgae feasible and economically viable.

Conflict of interest

The authors declare no conflict of interest.

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