MICROWAVE AS A PRE-TREATMENT TO FACILITATE THE SUPERCRITICAL EXTRACTION OF LIPIDS FROM MICROALGAE

M. Solana[a]*, D. Hernández[b], M.C. García-González[b], A. Bertucco[a]

[a]Department of Industrial Engineering. University of Padova. Via Marzolo 9, 35131 Padova (Italy);
[b]Agricultural Technological Institute of Castilla y León. Ctra. Burgos, km. 119. 47071 Valladolid (Spain)
tel. +39-0498275462, e-mail: miriam.solanacipres@studenti.unipd.it;

ABSTRACT

This work addresses the $SCCO_2$ extraction from microalgae with the aim of studying the effect of a microwave pre-treatment on the lipid extraction process. Experimental extraction curves obtained from the species *Nannochloropsis gaditana*, *Tetraselmis* sp., *Isochrysis* T-ISO and *Scenedesmus almeriensis,* both with and without microwave pre-treatment, were fitted by the Sovová model of broken and intact cells. The total yields of the extraction are compared with the ones obtained by two traditional solvent methods: Soxhlet and Kochert. The fatty acid profiles of the four microalgae species extracted by Soxhlet and $SCCO_2$ are analysed by gas chromatography. This study shows that a microwave pre-treatment is essential to increase the yield of the extraction for the microalgae *S. almeriensis*. When the effect of the microwave pre-treatment is analysed on saturated, monounsaturated and polyunsaturated fatty acids, the conclusion is that a microwave pre-treatment for 1 minute increases the fatty acid content of the extract. However, when the microwave pre-treatment is applied for 5 minutes, the fatty acids are degraded.

INTRODUCTION

Microalgae are considered to be one of the most promising alternative sources for biodiesel [1] due to the potential high oil yield, which is about 16-70 times the oil (in terms of L/ha/year) that can be obtained from coconut, sunflower and palm [2]. Microalgae are also promising due to their high growth and photosynthetic rates enabling them to capture carbon faster than terrestrial crops, and to accumulate high lipid concentration in their biomass (up to 60%). In addition, they can be cultivated on non-arable lands, saline water medium and agro-industrial wastewaters, avoiding the use of herbicides or pesticides [3].

Nowadays, the search for the most viable microalgae oil extraction method in order to produce biodiesel as valuable bioactive molecules is subject to strong research. The feasibility of applying $SCCO_2$ process to extract microalgae lipids has already been demonstrated. For instance, Santana et al. [4] studied the supercritical carbon dioxide extraction from *Botryococcus braunii* for biodiesel production at pressures between 200 and 250 bar and temperatures of 50 and 80 °C. Other extraction methods have also widely been reported. In this sense, Balasubramanian et al. [5] designed a resonant continuous microwave processing system in conjunction with hexane for oil extraction from *Scenedesmus obliquus,* obtaining higher extraction yields than by $SCCO_2$. These results may be attributed to the effect of

microwaving, which causes a rapid alignment and realignment of dipoles in a polar solvent, resulting in a heat generation, which can alter and break down the cell structures, and facilitating the access and diffusion of CO_2 through the microalgae cell [5]. However, each microalgae species has different biological characteristics and in consequence, the effect of the microwave may be different. Therefore, in this study the results of the extraction from four different microalgae species are analysed: *Nannochloropsis gaditana*, *Tetraselmis* sp, *Isochrysis* T-ISO and *Scenedesmus almeriensis*. In this case, the microwave is studied as a pre-treatment previous to supercritical carbon dioxide extraction and compared with the results obtained with other extraction methods. The aim of this research is to find out the microalgae species whose supercritical extraction is positively affected by the microwave pre-treatment and to determine its effect on lipid content. These results could be useful to facilitate the development of an effective and energetically efficient process to carry out the oil extraction from microalgae.

MATERIALS AND METHODS

Materials and microalgae

Microalgae biomass was obtained in lyophilized form from the Food Innovation and Sustainability Center (Almería, Spain). *Isochrysis* T-ISO and *Tetraselmis* sp. were cultured according to Fabregas et al. [6]. *Nannochloropsis gaditana* B-3 and *Scenedesmus almeriensis* were cultured according González-López et al. [7] and Sánchez et al. [8], respectively. Microalgae powder was dried at 80 ºC until constant weight, ground and sieved before the experimental runs, obtaining a particle size distribution lower than 500μm.

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

Solvent extraction

Two solvent extraction methods were performed. First, lipids were extracted with methanol-chloroform 1:2 (v/v) used as a mixture of solvents, following the method proposed by Kochert [9]. Once the extraction was done, the lipids were quantified by gravimetric analysis. Then, another solvent extraction was carried out by traditional Soxhlet apparatus using methanol: chloroform 2:1 (v/v) as solvents. The temperature of extraction was kept at 105 °C. After 18 hours of extraction, the extract was separated from the solvent by a rotatory evaporator at 41 ± 0.1 °C.

Supercritical fluid extraction

Supercritical extraction tests were performed by a laboratory scale equipment. The method was conducted as follows: First, the stainless steel vessel was filled with 0.5 g of dried microalgae powder. Then, the CO_2 was pumped at the desired pressure, which was measured by two gauges. A pre-heater before the vessel and a thermo-resistance around the extractor maintained the desired temperature, which was measured in the internal flow before and after the vessel. The co-solvent, in a concentration of 5% respect to the solvent, was pumped by an intelligent HPLC pump (Jasco PU-1580) and mixed with the CO_2 before the extraction vessel.

After extraction, the mixture of the solvent, co-solvent and extract was expanded by a valve inserted in a water bath at 40 °C, to avoid CO_2 freezing caused by sudden expansion. Extract samples, collected every 15 minutes in ethanol, were separated from ethanol by a rotary evaporator. All extractions were carried out during 90 minutes. A constant CO_2 flow rate of 0.4±0.05 kg/h, measured by a flow meter after the depressurization, was kept in every test. The extraction pressure was 30 MPa and the temperature was kept at 45 ± 2.2 °C, as the addition of 5% ethanol to CO_2 increases the critical temperature of the mixture to 42.5°C [10].

The microwave pre-treatment was carried out in a KOR-612R microwave (Daewoo, Seul, Sout Korea) for 1 and 5 minutes, at 1.2 kW and 2.45 GHz. The microalgae biomass had been previously suspended in 50 mL of distilled water to set a concentration of 200 g VSS (volatile suspended solids) L-1. This procedure was made once. Then, a $SCCO_2$ extraction was performed on pre-treated biomass.

Fatty acids analysis

The fatty acids composition of the extracted oil was measured by gas chromatography. The method was performed according to reported procedures [11,12]. A GC Agilent Technologies (Model 7890) with a FID detector was used. The columns were Supelco (75 m × 180 µm × 0.14 µm film thickness) Model 23348-U and J&W (3.8 m × 250 × 0.25 µm film thickness) Model 190915-431.The carrier gas was H_2.

RESULTS

Comparison between Kochert, Soxhlet and $SCCO_2$ extraction methods

Preliminary extraction tests were carried out with the aim of comparing the amount of extract obtained by different methods in terms of mass of extract respect to the initial mass of dried microalgae powder loaded into the extractor. As shown in Table 1, *N. gaditana* was the only microalgae strain in which Kochert method yielded higher extract percentage (19.1%) than Soxhlet method (17.8%). For *Isochrysis* T-ISO, *S. almeriensis* and *Tetraselmis* sp. the Soxhlet extraction lipid yield increased considerably with respect to the yields obtained by Kochert method. Therefore, the microalgae grinding with alumina was probably efficient enough to break down *N. gaditana* cell walls, but it was unable to break down cell walls of different nature.

Table 1: Extraction percentage (wt.%) from three different extraction methods.

Extract percentage[a]	Kochert	Soxhlet	$SCCO_2$
Isochrysis T-ISO	12.7	23.1	14.7
N. gaditana	19.1	17.7	12.9
S. almeriensis	15.7	22.4	13.2
Tetraselmis sp.	14.5	18.1	14.1

[a] Extract percentage= (mass of microalgae extract/mass of dried microalgae powder)×100

Comparing Soxhlet and $SCCO_2$ methods, a higher lipid yield was obtained by the first one from all microalgae strains tested, as reported in Table 1. However, the FFA content of the oil extracted by Soxhlet was lower for all the microalgae species (Table 2). This method extracts

both polar and non-polar lipids, and a considerable concentration of other components as pigments and waxes [13]. It is also noted in Table 2 that the content of polyunsaturated fatty acids on the oil extracted by $SCCO_2$ is higher respect to the lipids extracted by Soxhlet. Therefore, increasing temperature when the lipids are extracted by Soxhlet (from 45°C to 105 °C), may degrade the double bonds of the polyunsaturated fatty acids. This was especially significant in *N. gaditana*, and particularly on the long chain unsaturated fatty acids. As shown in Table 2, the amount of C20:5 ω3 (EPA) varied from 3.6 to 7.1 on Soxhlet and $SCCO_2$ extraction methods respectively; and the fatty acid C22:6 ω3 DHA, that was found on an amount of 6.3 mg/g by $SCCO_2$, was not detected on the oil extracted by Soxhlet.

Table 2: Comparison between the fatty acids profile, in terms of mg FFA/g dried microalgae powder, when Soxhlet and $SCCO_2$ are used to extract lipids from different microalgae species.

	Isochrysis T-ISO		*N. gaditana*		*S. almeriensis*		*Tetraselmis* sp.	
	Soxhlet	$SCCO_2$	Soxhlet	$SCCO_2$	Soxhlet	$SCCO_2$	Soxhlet	$SCCO_2$
C 14:0	14.1	13.6	4.8	4.9	6.3	7.9	2.0	2.7
C 16:0	10.2	14.4	19.8	20.3	22.6	20.9	16.8	18.8
C 18:0	n.d.	2.6	n.d.	0.9	n.d.	1.7	n.d.	0.6
Others[a]	1.9	0.5	2.7	7.8	3.4	4.3	2.0	2.8
Total SFA	26.2	31.1	27.3	34.0	32.3	34.7	20.8	25.0
C 16:1 ω9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.5	2.9
C 16:1cis ω7	5.0	4.6	21.2	22.7	26.6	28.9	5.6	6.6
C 18:1cis ω9	11.0	8.0	6.2	3.7	3.7	3.9	3.2	6.0
Others[a]	2.5	0.7	2.9	2.4	2.3	3.8	2.6	3.1
Total MFA	18.5	13.3	30.2	28.8	32.6	36.7	11.3	15.7
C 16:2 ω6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.4	2.2
C 16:3 ω3	0.8	n.d.	n.d.	n.d.	n.d.	n.d.	3.3	3.1
C 16:4 ω3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.4	8.7
C 18:2 ω6 (LA)	3.7	3.8	2.1	3.0	2.6	2.4	4.4	5.1
C 18:3 ω3 (ALA)	5.0	5.4	n.d.	4.2	n.d.	n.d.	11.0	13.0
C 18:4 ω3	11.2	11.4	8.6	8.9	n.d.	n.d.	1.7	2.3
C 18:5 ω3	1.4	1.5	1.0	1.1	n.d.	n.d.	n.d.	n.d.
C 20:4 ω6	n.d.	n.d.	1.3	1.9	n.d.	n.d.	n.d.	n.d.
C 20:5 ω3 (EPA)	n.d.	n.d.	3.6	7.1	5.9	6.6	1.1	1.6
C 22:6 ω3 (DHA)	2.5	2.9	n.d.	6.3	n.d.	n.d.	n.d.	n.d.
Others[a]	4.3	6.0	2.7	0.6	3.5	4.1	2.8	3.2
Total PFA	28.9	31.0	19.4	33.1	11.9	13.1	34.1	39.1
Total FFA	73.6	75.4	76.9	95.9	76.8	84.5	66.2	79.8

n.d.: not detected. SFA: saturated fatty acids; MFA: monounsaturated fatty acids; PFA: polyunsaturated fatty acids ALA, α-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid ; DHA, docosahexaenoic acid
[a]: amount of fatty acids lower than 1% by mass.

Effect of the microwave pre-treatment on the extraction yield

The extraction kinetics of *N. gaditana*, *Tetraselmis* sp., *Isochrysis* T-ISO and *S. almeriensis* by $SCCO_2$ are shown in Figure 1. The yield, *e*, was calculated as the mass of extract collected divided by the insoluble microalgae biomass. In kinetics evaluation, the curve modelling was performed using the model published by Sovová in 2005 [14], already applied to fit

supercritical extraction curves from microalgae [15]. The model equations are described by Mouahid et al. [15].

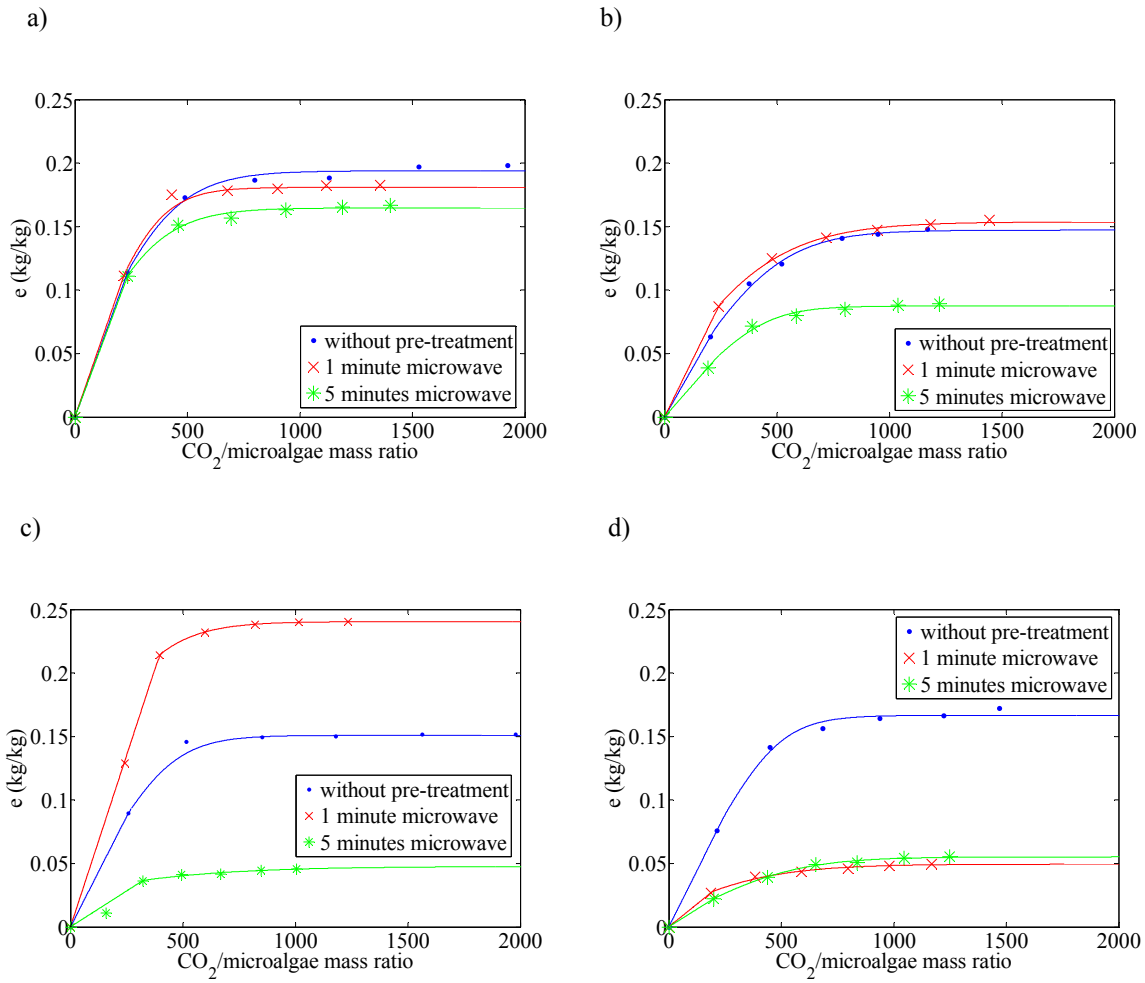

Figure 1: $SCCO_2$ extraction curves of lipids from the microalgae species *Isochrysis* T-ISO (a), *N.gaditana* (b), *S. almeriensis* (c) and *Tetraselmis* sp.(d), experimental and modelling. The curves represent e, the extraction yield ($kg_{extract}/kg_{insoluble\ solid}$) as a function of the CO_2/insoluble microalgae mass ratio.

As illustrated in Figure 1a, the microwave pre-treatment slightly affects the supercritical extraction from *Isochrysis* T-ISO, since the total yield obtained after 90 minutes of extraction only decreased from 0.20 to 0.17 when a previous microwave pre-treatment for 5 minutes was applied. In the case of *N. gaditana* (Figure 1b), practically no differences were found on the yield when a microwave pre-treatment for 1 minute was carried out. However, after 5 minutes of microwave pre-treatment, the yield decreased from 0.14 to 0.08. *S. almeriensis* lipid yield increased considerably (from 0.15 to 0.24) when biomass was microwaved for 1 minute compared to $SCCO_2$, as shown Figure 1c. This result may be attributed to the particular characteristics of this microalga cell wall, which is described as one of the most resistant [16, 17]. Thus, probably $SCCO_2$ alone is not able to break down the cell wall of *S. almeriensis*, in order to access and extract lipid content and only the microwave pre-treatment is able to enhance lipid release. However, when microwave pre-treatments were performed for 5 minutes, a considerable decrease of lipid yield was observed, obtaining only a yield of 0.05. The microalgae strain *Tetraselmis* sp. was negatively affected by the microwave pre-treatment

regardless of the time. The yield achieved without microwave pre-treatment was 0.17, whereas only 0.05 and 0.06 were obtained after 1 minute and 5 minutes of pre-treatment, respectively (Figure 1d).

Effect of the microwave pre-treatment on the fatty acids classes

Fatty acids obtained after $SCCO_2$ extraction (with and without 1 minute of microwave pre-treatment) are shown in Figure 2. Fatty acids are classified in saturated, monounsaturated and polyunsaturated. For *Isochrysis* T-ISO and *N. gaditana*, the total content of fatty acids extracted is higher when the microwave pre-treatment is applied (from 75.4 to 92.8 mg/g for *Isochrysis* T-ISO and from 95.9 to 107.8 mg/g for *N. gaditana* respectively). It is also noted worthy the increase of polyunsaturated fatty acids when a previous microwave pre-treatment is applied in the microalgae strain *Isochrysis* T-ISO. Hence, a microwave pre-treatment does not degrade the fatty acids and facilitates the access of the CO_2 into the microalgae cells.

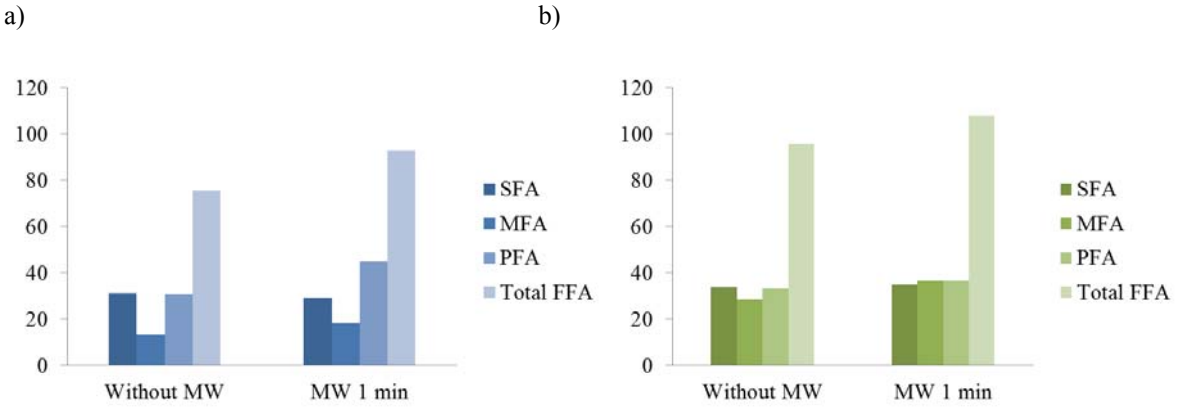

Figure 2: Comparison of the fatty acids classes (mg/g microalgae) when $SCCO_2$ is carried out without a previous microwave (MW) pre-treatment and when a microwave pre-treatment is applied for 1 minute from *Isochrysis* T-ISO (a) and *N. gaditana* (b). SFA: saturated fatty acids; MFA: monounsaturated fatty acids; PFA: polyunsaturated fatty acids; FFA: free fatty acids.

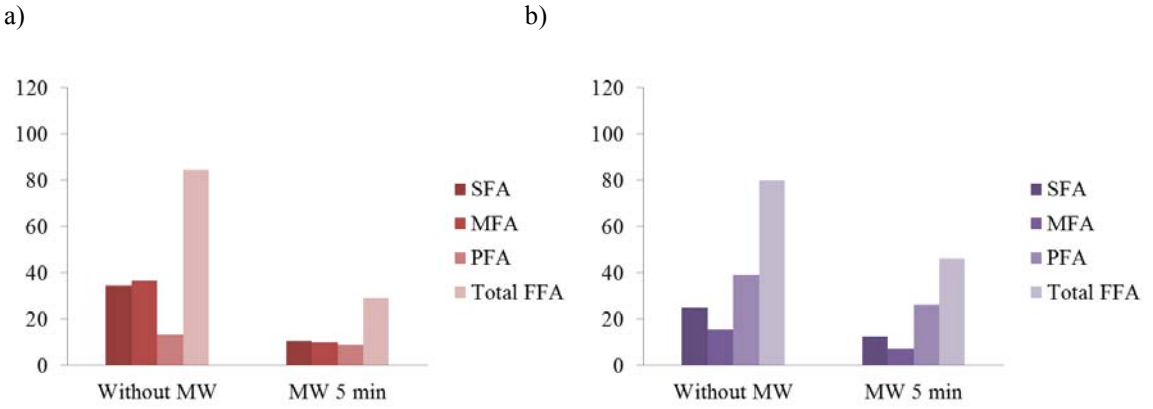

Figure 3: Comparison of the fatty acids classes (mg/g microalgae) when $SCCO_2$ is carried out without a previous microwave (MW) pre-treatment and when a microwave pre-treatment is applied for 5 minutes from *S. almeriensis* (a) and *Tetraselmis* sp. (b), SFA: saturated fatty acids; MFA: monounsaturated fatty acids; PFA: polyunsaturated fatty acids; FFA: free fatty acids.

The influence of the microwave pre-treatment for 5 minutes on *S. almeriensis* and *Tetraselmis* sp. fatty acids is illustrated in Figure 3. It shows that, when microwave pre-treatments are performed for 5 minutes, a considerable decrease in lipid yield is obtained. This remarkable decrease may be elicited by the high temperatures reached during the microwave process, which could propitiate FFA degradation and consequently lower lipids extraction by $SCCO_2$.

CONCLUSION

Comparing the extraction efficiency in terms of extract/dried microalgae (wt.%), Soxhlet method is better than Kochert and $SCCO_2$ methods in all the microalgae species studied, except for *N. gaditana,* for which the Kochert method increases slightly the amount of extract. Nevertheless, the total fatty acids extracted by Soxhlet in terms of mg FFA/g dried microalgae powder is lower than the one obtained by $SCCO_2$ and consequently, it is confirmed that $SCCO_2$ is a more selective method. The microwave pre-treatment affects the $SCCO_2$ extraction diversely depending on the microalgae strain and the time of microwave. It is remarkable that the supercritical extraction on *S. almeriensis,* whose wall is described as one of the most resistant, is strongly improved by the microwave pre-treatment. The total content of fatty acids extracted is higher when the microwave pre-treatment is applied for one minute on the two microalgae strains investigated, *Isochrysis* T-ISO and *N. gaditana.* However, exposing the microalgae to microwave during 5 minutes lowers lipids extraction by $SCCO_2$ regardless of the species. To sum up, microwave is a promising pre-treatment that could improve the results of the supercritical fluid extraction from microalgae obtained so far.

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