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ABSTRACT

In the search for less environmental impacting extraction methods for obtaining high quality lipids for biofuels production, microwave-assisted extraction (MAE) technique was employed using Simultaneous Distillation and Extraction Process (SDEP) combined with *p*-cymene as bio-based solvent to extract lipids from fresh microalgae. In this work, the effects of microwave power and heating time on the yield and quality of extracted lipids from fresh microalgae were investigated. The optimized parameters were 10 min of extraction at 400 W, accounting for the highest yield of total lipids from *Dunaliella salina* (2.66 % w/w). In order to fully evaluate the effects of microwave and bio-based solvent on different cells and structures, the optimal conditions were applied to lipid extraction from other strains of microalgae. The comparison between the new extraction method and Soxhlet extraction, Bligh and Dyer and conventional SDEP methods resulted in a higher or similar extraction rate. The technique shows great potential in reducing extraction time (10 min for 93% extraction rate) when compared to Soxhlet (8 hours for 14.63% extraction rate) and conventional SDEP extraction (2 hours for 94% extraction rate).

1. Introduction

Biofuels have gained importance on the search for sustainable production of fossil-free energy. Among studied feedstocks, microalgae have shown great potential of obtaining high quality biofuel in large scale production, representing the highest yield biofuel crop [1,2]. However, from a global approach of environmental impact analysis of biofuel production systems, the lipid extraction from the biomass represents the most energy consuming stage from all production chain [3]. Therefore, the current challenge on biofuel production has become the search for less environmental impacting extraction methods in order to obtain a genuine green renewable source of energy.

A large number of lipid extraction procedures are available among conventional methods such as the Soxhlet (first developed for oleaginous seeds extraction), and the Bligh and Dyer method, which is specifically used for marine lipids extraction and still represents the standard method in this domain. However, some other innovative methods have been applied to recover target molecules for biofuel production, such as supercritical CO_2, expelling and microwave-ultrasonic assisted extraction have also been reported in order to shorten the extraction time [4–10].

However, most of the novel extraction methods require dried microalgal biomass with less than 10% water content, which implies the use of a prior drying step that can easily become expensive and time consuming. That is the main reason why the SDEP method for lipid extraction from microalgae was developed. Nevertheless, the SDEP method requires a long time of extraction as a result of the heating system. In the domain of natural molecules extraction, microwave use has shown great potential, especially when applied to replace conventional heating in standard procedures. A number of process intensification studies have been published using microwave energy such as the microwave accelerated dean stark, the ultrasound/microwave assisted extraction and others [11,12]. Also, the modification of a conventional method requires fewer adaptation and investments on an already implemented production chain.

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Therefore, a new method using microwave to assist the Simultaneous Distillation Extraction Process was developed and applied to four different microalgae strains and optimized. The quality of the obtained extracts was analyzed and compared to three extraction methods.

2. Experimental section

2.1. Chemicals and reagents

Extraction solvent *p*-cymene was of analytical grade and was supplied by Sigma Co. (USA). All other chemicals and organic solvents (methanol, sulfuric acid, NaCl, n-hexane, chloroform, methyl acetate, acetic acid, isopropanol, diethyl ether and KCl) used for the preparation of fatty acid derivatives and HP-TLC analysis were of analytical grade and were purchased from VWR International. Various glassware and SDEP apparatus used in extraction procedures and fatty acid methyl ester preparations were supplied by Legallais (Montferrier-sur-Lez, France).

2.2. Microalgae cultivation and harvesting

All microalgae strains (*Dunaliella salina* and *Nannochloropsis oculata*) were purchased from the Greensea Company (Meze, France). The *Dunaliella salina* strain used in this work is a "sauvage" strain that was grown in natural conditions, in a salt marsh, at ambient temperature with good sunniness under favourable conditions. *Nannochloropsis oculata* was incubated in tubular reactor at ambient temperature under deficiency conditions to obtain a high rate of lipids in the biomass. Each strain was harvested then centrifuged to have a microalgae paste at approximately 20% of dry weight. The biomass was frozen overnight at −20°C and slowly defrosted before each experiment.

2.3. Extraction of lipids

2.3.1. Determination of the total lipid content

The total lipid content in the all microalgae species was determined by using chloroform:methanol (1:2 v/v) according to Bligh and Dyer method (1957) [13]. The mixture was agitated during 20 min in an orbital shaker at room temperature. The lipid fraction was then separated and the solvent evaporated under a nitrogen stream. After gravimetry, the total lipid content was calculated as standard for further comparisons and expressed as percentage of dry weight.

2.3.2. Conventional SDEP apparatus and procedure

For SDEP extraction, 12 g ± 0.5 g with 20% dry weight microalgae paste were placed into a 500 mL round-bottomed flask and dispersed using 100 mL of terpene solvent (*p*-cymene). A modified Dean stark receiver with a 3-way valve was added to the round-bottomed flask and fitted with a condenser. The SDEP procedure allowed elimination of microalgae water, extraction of lipids and elimination of terpene solvent in a single "in situ" step [14]. In the first step, most of the microalgae water was collected, giving place to the lipid extraction with terpene solvent in the second step and its subsequent elimination (by azeotropic distillation) in steps 3 and 4 by re-introducing water with the 3-way valve to form a binary water-terpene mixture. Terpene solvent was recovered from the water layer by phase separation in the modified Dean stark receiver and the extracted lipids were recovered from the water layer by phase separation in the distillation round-bottomed flask. Extractions were performed in triplicate and the mean values were reported. Lipid extracts were dried under a stream of N_2 and re-dispersed in solvent for HP-TLC or GC–FID analysis.

2.3.3. Simultaneous Distillation and Extraction Process assisted by microwave (SDEP/MW)

The principle of the new process of SDEP assisted by microwaves is illustrated in Fig. **1**. Microwave-integrated SDEP was performed in a Milestone ETHOS (Milestone, Italy) microwave oven. The multi-mode microwave reactor has a twin magnetron (2 x 800 W, 2.45 GHz) with a maximum delivered power of 1000 W variable in 10 W increments. Time, temperature, pressure and power were controlled with the "easy WAVE" software package during experiments and the temperature was monitored by an infrared sensor outside the reactor. The flask, the amounts of microalgae and solvent volumes used were the same as the conventional SDEP technique. The solvent used for this study is the *p*-cymene, since it has been recognized as the terpene solvent which allows the best extraction of marine lipid amount other terpene solvents such as limonene and pinene. In order to determine the range of power to be used, a screening process was performed by applying several ranges of power over 10 min. Each experiment was performed in triplicate and mean values were reported.

2.3.4. Soxhlet extraction apparatus and procedure

Since Soxhlet extraction is extensively employed in lipids extraction and is the reference methods for certain matrices, this technique was used on microalgae biomass in order to compare the yield and oil quality to the other used techniques [15]. The cellulose thimble (30 mm × 80 mm) was weighed and filled with 10 g of dry microalgae (Macherey-Nagel) and then placed in a Soxhlet apparatus. Lipids extraction was carried out over 8h using 300 mL of n-hexane. After the extraction, the major solvent contained in the distillation flask was eliminated with a vacuum rotary evaporator. Extractions were performed in triplicate and the mean values were reported. Lipid extracts were dried under a stream of N_2 and re-suspended in solvent for HP-TLC or GC-FID analysis.

2.3.5. Generalization

To assess the efficiency of the SDEP/MW, another microalgae strain was studied using optimal conditions and results were compared to conventional SDEP, Soxhlet and Bligh and Dyer methods. After extraction, *Nannochloropsis oculata* was analyzed in order to assess the effects of microwave on the cell membrane. All experiments were performed in triplicate.

2.4. *Lipid composition analysis*

2.4.1. Qualitative and quantitative analysis of lipids classes (HP-TLC)

Lipid classes in samples were determined by High Performance Thin-Layer Chromatography (HP-TLC), using a double development chromatography to separate polar and neutral classes. Lipids were detected by charring and quantified using a CAMAG 3TLC scanning densitometer (CAMAG, Muttenz, Switzerland) with identification of the classes against known polar and neutral lipid standards. Lipid extracts were loaded as a spot onto 20 × 10 cm Silica gel 60 F254 HP-TLC plates (Merck KGaA, Darmstadt, Germany) using an ATS 5 automatic TLC sampler (CAMAG, Switerland). The HP-TLC silica gel plates were developed with two mixed solvents in an ADC2 automatic developing chamber (CAMAG, Switerland). The first eluent was a mixture of methyl acetate/isopropanol/chloroform/methanol/KCl (0.25% solution) in a ratio of 25:25:25:10:9 v/v/v/v/v running to a height of 5.5 cm from the origin. And the second eluent was a mixture of n-hexane/diethyl ether/glacial acetic acid in a ratio of 80:20:2 v/v/v to a height of 8.5 cm from the origin. After dried, the plate was dipped for 6 seconds in a modified $CuSO_4$ reagent (20 g $CuSO_4$, 200 mL methanol, 8 mL H_2SO_4, and 8 mL H_3PO_4), then heated at 141 °C for 30 min on a TLC plate heater and finally scanned using a TLC Scanner 3 with WinCATs software (CAMAG). Lipid classes were identified and quantified against those of corresponding lipid standards.

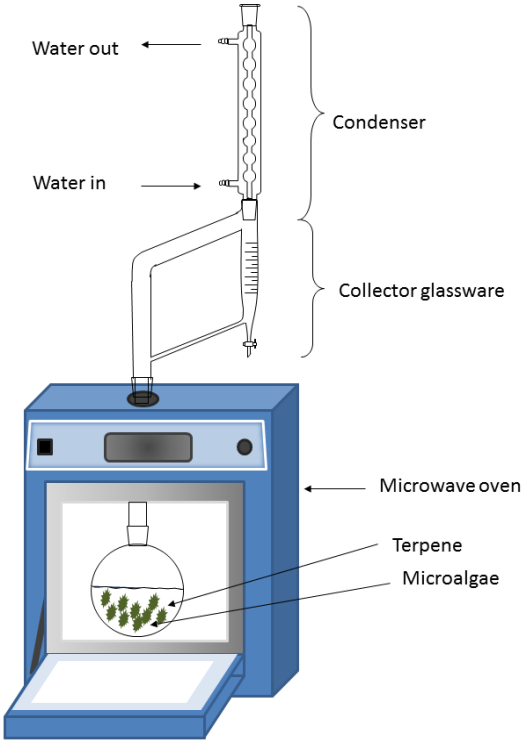

Fig. 1 - Simultaneous Distillation Extraction Process assisted by Microwave (SDEP/MW).

2.4.2. Preparation of fatty acids methyl ester (FAMEs)

FAMEs were prepared from the lipid extract using acid-catalyzed transmethylation as described by Li et al. [16]. 1 mL methanolic sulfuric acid (5%) solution was added to a specific amount of extracted oil. Triheptadecanoin (C17:0 TAG) was used as internal standard. The mixture was then heated during 90 min at 85°C. After, the flask was removed from heat and 1.5 mL of sodium chloride (0.9%) solution and 1 mL of *n*-hexane were added. The flask was closed and shook vigorously during 30 seconds then centrifuged at 4000 rpm during 2 min. A small amount of the organic layer was recovered and transferred to a vial before being injected directly in a gas chromatograph for analysis.

2.4.3. FAMEs analysis

Fatty acids methyl esters were separated, identified and quantified by gas chromatography coupled with flame ionization detector (GC-FID). Analyses were performed by using an Agilent 7820A (Kyoto, Japan) gas chromatograph. The instrument was equipped with a BD-EN14103 capillary column 30 m × 320 µm x 0.25 µm (Agilent) and the velocity of the carrier gas (He) was at 33 cm/s. Injection of 2 µL of the various samples were carried out with a split mode (split ratio 1:20) and the injector temperature was set at 250°C. Oven temperature was initially 50°C for 1 min and then progressed at a rate of 20°C/min from 50°C to 180°C and then increased from 180°C to 230°C at a rate of 2°C/min. The temperature was then held at 230°C over 10 min. FAMEs were identified by retention time and comparison with purified FAME standards (Sigma Co., USA).

3. Results and discussion

In this study, we focused on the potential of the new technique SDEP, which combines the advantages of the conventional SDEP (water distillation and lipid extraction on a single step) and microwave heating (reduction of experiment time). Fig **2**. shows a flowchart of the extractions that we were performed for this work.

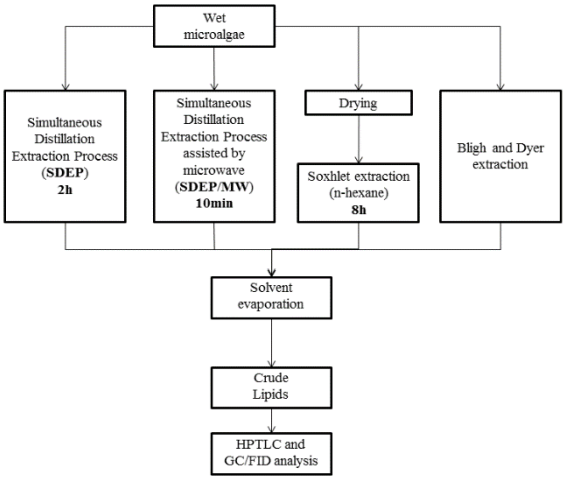

Fig. 2 – Diagram process.

3.1. Azeotropic distillation kinetics

Although *p*-cymene presents a low dielectric constant (2.34 at 20°C), this solvent is not fully transparent to microwaves. The azeotropic distillation using SDEP/MW procedure can be divided in four different phases (Fig. **3**). The first phase corresponds to the induction time. During this period, the temperature of the mixture teperne-fresh microalgae increased, even though water could not be recovered. Phase 2 corresponds to the azeotropic distillation of water and during this phase the water is easily removable from the biomass, which corresponds to the majority of microalgae water content (about 80%). During phase 3, the extraction rate is lower, since it corresponds to the extraction of intrinsic water, which is more difficult and takes longer to be removed from the microalgae paste. The lipid extraction starts when the majority of the water is extracted of the biomass. The last step (phase 4) corresponds to the end of water distillation and consequent lipid extraction, represented by a horizontal line on the graph. During this phase, there was no water left in the biomass and therefore *p*-cymene was the only remaining solvent.

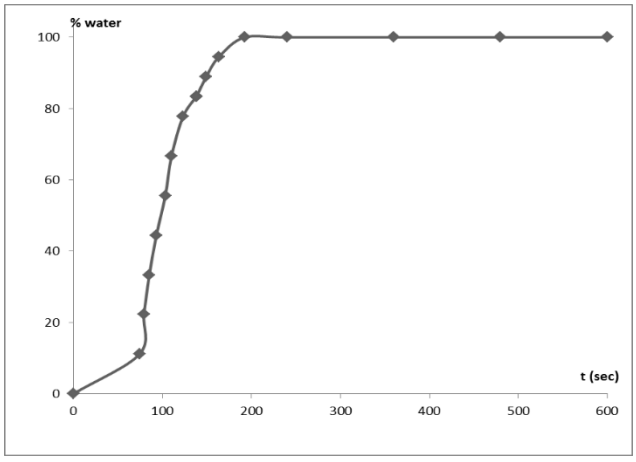

Fig. 3 – Distillation phases and water recovery as a function of time for SDEP/MW.

3.2. Optimization of Microwave Power

3.2.1. Yield Optimization

The high affinity between lipids and terpenes solvents allowed the extraction of lipids from wet matrix using the SDEP and SDEP/MW methods. The mean value found for lipid content was 2.82 ± 0.03% for the conventional SDEP, and the results from SDEP/MW were based on percentage of the total yield expected. Table 1 shows the ranges of power used on *Dunaliella salina* in order to determine the optimal microwave power for the SDEP/MW procedure (which was based on the lipid yield). In this table, the results from methods comparison between SDEP/MW, SDEP, Bligh and Dyer, and Soxhlet showed that the highest lipid extraction rates were obtained for SDEP and SDEP/MW (when compared to the Bligh and Dyer reference method) and the Soxhlet method provided the lowest lipid yield. These results for the Soxhlet extraction can be explained by the reduced diffusivity of *n*-hexane through the cell when compared to terpene solvent. The boiling point of terpenes are substantially higher than *n*-hexane, requiring a higher temperature during the Soxhlet extraction, which can result in a lower viscosity of the aim compounds (lipids) in the matrix and, in consequence, a better diffusion rate of the solutes from the solid phase to the solvent, resulting in the observed lower yield for the Soxhlet procedure.

Regarding conventional SDEP, this method is a time-consuming process (around 2 h) that relies on heat to increase the mass transfer rate from the outside to the inside of the sample matrix. On the other hand, microwave-assisted extraction is a fast extraction process (10 min) that relies on heat from the inside to the outside of the sample matrix, due to the fact that microwave energy can penetrate materials through molecular interaction. Concerning efficiency, the comparison of SDEP/MW with conventional SDEP shows in general that the lipid yields obtained by SDEP/MW were slightly lower than those provided by conventional SDEP and Bligh and Dyer. The lowest power used (100 W) did not allow lipid extraction neither water recovery, since the energy provide was not enough to create the azeotropic mixture required for lipid extraction. From 200 to 400 W, the power applied was sufficient to extract lipids from the biomass and the lipid yield increased with the power. From 500 to 700 W, only 56% of the lipids contained in the microalgae paste were recovered, therefore this range of power showed less effectiveness for lipid extraction. In fact, these powers represent a strong irradiation for the solution volume (paste and solvent). The mixture heats quickly without allow efficient extraction of lipids in the terpene solvent. Thus, 400 W was chosen as the optimal power to be applied in the SDEP/MW, since it resulted in the highest yield.

3.2.2. Extracts Composition

One of the main aspects of the study here developed was to check if the proposed approach produced degradation of the lipids by the actions of microwaves. With this purpose, the extracts obtained by both SDEP/MW and conventional methods (SDEP, Soxhlet and Bligh and Dyer) were subjected to several chromatographic analysis and few differences due to the extraction system were found.

From the High Performance Thin Layer Chromatography (HP-TLC) analysis, the lipids separation is shown in the plate (Fig. **4**) and monoglycerides, diglycerides, free fatty acids, triglycerides and polar lipids were detected and quantified. The positions of the different glycerides after separation were determined from the relative distance of standards migration. The separation was based on the partition coefficients of the components of the reaction mixture. For all extracts, the distribution of the different lipid classes was obtained as a percentage of total detected areas in the densitometer. The monoglycerides and diglycerides were not found in the extracts; the results indicate that the majority of lipids are neutral, triacylglycerol and free fatty acids. The results of polar lipids quantification showed this class of compounds represents less than 0.1% of extracted lipids of *Dunaliella salina*. Although the TAG

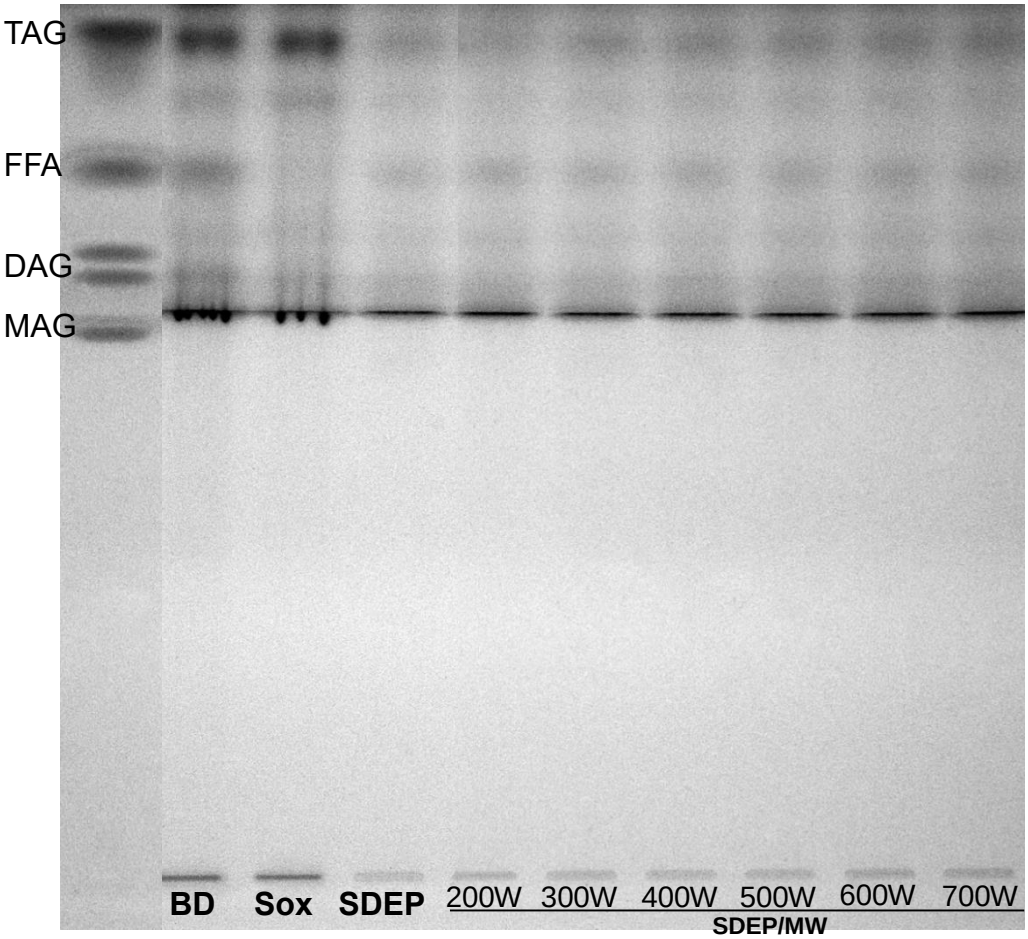

Fig. 4 – HPTLC plate where the different lipid classes detected can be visualized in extracts from Bligh and Dyer, Soxhlet, conventional SDEP and different ranges of power of SDEP/MW methods.

proportion is higher for Soxhlet extracts (due to the high affinity between TAG and *n*-hexane solvent), the lipid yield is strongly inferior when compared to the other methods (Bligh and Dyer, SDEP and SDEP/MW) as shown in the Table 1. The comparison of quantified TAG measured by HP-TLC/densitometry for the three remaining extraction methods (Bligh and Dyer, conventional SDEP and SDEP/MW) showed similar amounts for all three methods, although SDEP and SDEP/MW extracts presented a higher concentration in TAG than Bligh and Dyer extracts. In the power optimization for SDEP/MW, 400 W allowed the recovery of the highest TAG proportion.

The fatty acids composition of lipids extracted from *D. salina* using the four methods (Bligh and Dyer, Soxhlet *n*-hexane, SDEP, SDEP/MW) was determined using GC analysis (Table 1). In the lipids extracted from *D. salina*, palmitic (C16:0), oleic (C18:1n9), hexadecatetraenoic (C16:4), linoleic (C18:2n6) and alpha linolenic (C18:3n3) acids were commonly dominant. Fatty acids extracted by Bligh and Dyer, Soxhlet, conventional SDEP and SDEP/MW showed very similar profiles, although Bligh and Dyer method accounted for the highest amounts of PUFAs among the four methods. The lower PUFAs amount in the Soxhlet, conventional SDEP and SDEP/MW methods could be caused by slight degradation due to heating unlike Bligh and Dyer, which is a cold extraction method. The amounts of remaining fatty acids (oleic, linoleic and alpha linolenic acids) extracted by SDEP/MW were very similar to those extracted by SDEP method. The power optimization the lipid yield and profile of fatty acids, 400 W was the most efficient power to extract lipids from *D. salina*.

3.3. Generalization

Another microalgae strain with different cell wall compositions was used in order to assess the efficiency of new SDEP/MW process. Results are summarized in Table 2. *N. oculata* was chosen to represent microalgae, with a cell wall more resistant than *D. salina*.

The comparison of lipid yields obtained from the three methods (Bligh and Dyer, SDEP and SDEP/MW) showed similar results for *N. oculata* (23.78%, 21.45% and 18.20%, respectively), and for *D. salina* (2.86%, 2.82% and 2.66%, respectively). Therefore, the cell wall composition will have a strong impact on the lipid yield, which can depend on the extraction method.

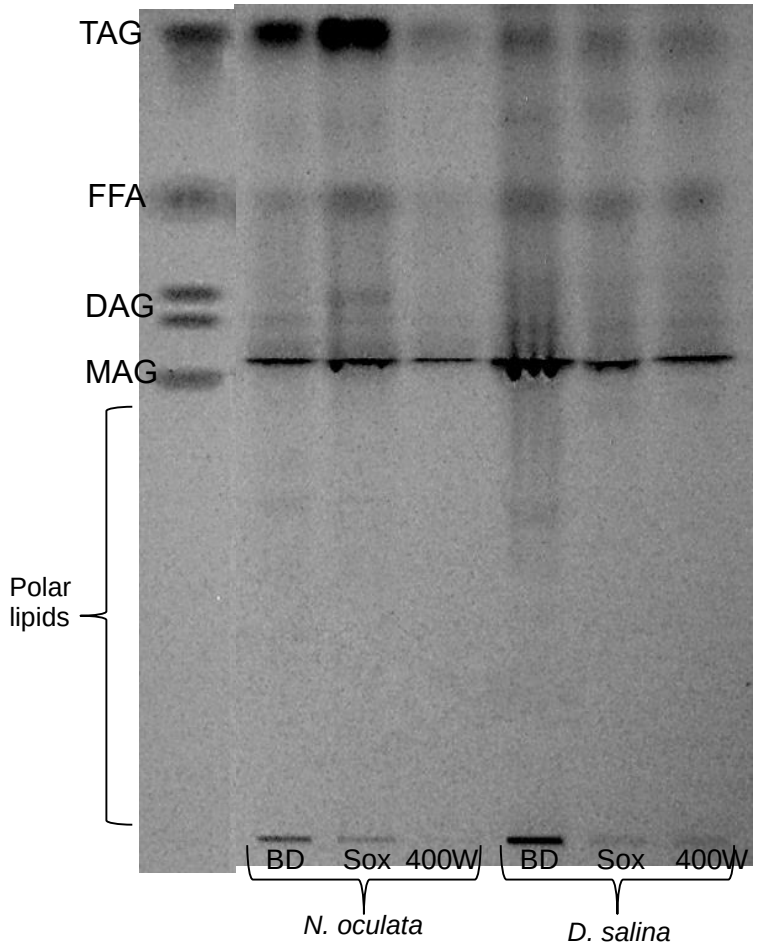

Fig. 5 – HP-TLC plate where the different lipid classes detected of all screening extracts.TAG Triacylglycerol, FFA Free fatty acids, DAG Diacylglycerol, MAG Monoacylglycerol, BD Bligh and Dyer, SoxSoxhlet.

The qualitative lipids analysis showed that the fraction of polar compounds is inexistent in *D. salina* and *N. oculata* extracts (Fig. **5**). For *N. oculata*, the Bligh and Dyer method resulted in the highest proportion of TAG among the three methods, while SDEP and SDEP/MW resulted in similar TAG proportions (67.98%, 57.94% and 57.15%, respectively). In this case, it is possible that TAG was degraded in DAG or FFA for SDEP and SDEP/MW. This heterogeneity of results concerning TAG proportion between methods and microalgae strains might be due to degradation of TAG in DAG or FFA and, because of the differences between the strains, the fatty acids might have more or less affinity with the solvents (*p*-cymene, methanol and chloroform).

For quantification and fatty acids profile of different lipid extracts from the different techniques, fatty acids were converted into FAMEs derivatives and analyzed by GC/FID. Identification was thus performed and the relative percentages in peak area of the compounds are presented. Table 2 reports the major identified fatty acids for the extraction of lipids from *N. oculata*. As observed in Table 2, in *N. oculata* strain, palmitic (C16), oleic (C18:1n9), linoleic (C18:2n6) and alpha linolenic (C18:3n3) acids relative percentage were the most predominant. The other compounds identified for this microalgae were myristic (C14:0), palmitoleic (C16:1n9 and n7), hexadecadienoic (C16:2), hexadecatrienoic (C16:3), stearic (C18:0) and oleic (C18:1n7) acids. All experimental results (Bligh and Dyer, SDEP and SDEP/MW) are in good agreement with the literature data for major fatty acids identification for *N. oculata* [17–21]. The classification of the extracted compounds was here represented in three fractions: SFAs, MUFAs and PUFAs. The main SFAs quantified in extracts were myristic (C14:0) and palmitic (C16:0) acids. Only palmitoleic (C16:1n7) and oleic (C18:1n9) acids were obtained in the MUFA's fraction. The major PUFAs extracted were hexadecadienoic (C16:2), hexadecatrienoic (C16:3), linoleic (C18:2n6), arachidonic (C20:4n6) and eicosapentenoic (C20:5n6) acids. The comparison between extracts obtained using SDEP/MW, conventional SDEP and Bligh and Dyer methods showed the same compounds were extracted with an equivalent relative proportion, which is in good agreement with the literature.

Even though some differences were observed in terms of crude oil due to the high-resistance of cell walls, the SDEP/MW method showed a wide applicability, since it was suitable for lipid extraction in several microalgae strains. Concerning the application of this

Table 2 – Lipid yield, lipid classes composition and fatty acids composition in function of the microwave power.

	Bligh and Dyer	Soxhlet	Conventional SDEP	SDEP/MW					
				200	300	400	500	600	700
Oil Yield									
(g/100g of dry matter)	2.86 ± 0.26	0.41 ± 0.03	2.82 ± 0.03	1.81 ± 0.50	2.38 ± 0.01	2.68 ± 0.03	1.88 ± 0.01	1.9 ± 0.03	1.80 ± 0.01
Lipid Classes Composition (%)									
FFA	42.39 ± 0.03	26.29 ± 0.27	39.25 ± 0.12	47.01 ± 0.08	41.41 ± 0.19	36.87 ± 0.07	42.17 ± 0.03	42.27 ± 0.11	38.74 ± 0.40
TAG	57.61 ± 0.12	73.71 ± 0.09	60.75 ± 0.01	52.99 ± 0.20	58.59 ± 0.08	63.13 ± 0.36	57.83 ± 0.65	57.73 ± 0.04	61.26 ± 0.06
Fatty Acids Composition (%)									
Saturated									
C14	0.39 ± 0.04	1.42 ± 0.14	2.33 ± 0.33	2.63 ± 0.03	2.47 ± 0.03	2.40 ± 0.07	2.41 ± 0.05	2.43 ± 0.01	2.15 ± 0.01
C16	43.10 ± 2.99	40.75 ± 0.51	40.76 ± 0.19	46.28 ± 0.45	40.14 ± 0.01	39.40 ± 0.06	40.57 ± 0.31	41.66 ± 0.02	40.49 ± 0.17
C18	0.70 ± 0.06	0.93 ± 0.12	1.01 ± 0.02	1.17 ± 0.06	1.02 ± 0.03	0.98 ± 0.02	1.06 ± 0.01	1.19 ± 0.01	1.10 ± 0.03
Mono-unsaturated									
C16:1 n9	0.18 ± 0.03	0.82 ± 0.26	0.64 ± 0.12	0.92 ± 0.24	0.96 ± 0.01	0.86 ± 0.01	0.94 ± 0.01	0.91 ± 0.01	0.95 ± 0.01
C16:1 n7	0.22 ± 0.04	0.57 ± 0.50	0.62 ± 0.07	0.31 ± 0.17	1.05 ± 0.01	0.98 ± 0.01	1.05 ± 0.01	1.00 ± 0.01	1.00 ± 0.01
C18:1 n9	6.22 ± 0.30	6.66 ± 0.13	7.35 ± 0.05	8.13 ± 0.19	7.50 ± 0.05	7.38 ± 0.04	7.72 ± 0.05	7.91 ± 0.01	7.84 ± 0.01
C18:1 n7	0.82 ± 0.53	1.08 ± 0.01	1.05 ± 0.01	1.14 ± 0.06	1.10 ± 0.06	1.08 ± 0.01	1.15 ± 0.01	1.17 ± 0.01	1.13 ± 0.01
Poly-unsaturated									
C16:2	0.25 ± 0.02	0.58 ± 0.08	0.72 ± 0.01	0.77 ± 0.08	0.81 ± 0.05	0.84 ± 0.01	0.82 ± 0.01	0.88 ± 0.01	0.87 ± 0.01
C16:3	0.51 ± 0.01	0.95 ± 0.18	1.15 ± 0.01	1.15 ± 0.20	1.11 ± 0.01	1.11 ± 0.01	1.13 ± 0.01	1.09 ± 0.01	1.17 ± 0.01
C16:4	5.17 ± 0.57	8.58 ± 0.23	8.26 ± 0.05	7.79 ± 0.12	8.26 ± 0.02	8.79 ± 0.03	7.72 ± 0.60	6.67 ± 0.03	6.63 ± 0.04
C18:2 n6	8.32 ± 0.08	7.19 ± 0.52	8.48 ± 0.06	8.49 ± 0.64	7.98 ± 0.01	8.12 ± 0.02	8.06 ± 0.04	8.17 ± 0.01	8.52 ± 0.03
C18:3 n3	34.11 ± 3.60	30.47 ± 1.68	27.63 ± 0.10	21.21 ± 3.37	27.60 ± 0.02	28.05 ± 0.09	27.35 ± 0.13	26.91 ± 0.02	28.14 ± 0.28
ΣSFAs	44.19	43.10	44.10	50.08	43.63	42.78	44.04	45.28	43.74
ΣMUFAs	7.44	9.13	9.66	10.50	10.61	10.30	10.86	10.99	10.92
ΣPUFAs	49.36	47.77	46.24	39.41	45.76	46.91	45.08	43.72	45.33

FFA: Free Fatty Acids, TAG: Triacylglycerides, SFAs: Saturated Fatty Acids, MUFA: Mono-unsaturated Fatty Acids, PUFA: Poly-unsaturated Fatty Acid.
Tolerance expressed as standard deviation, n = 3

composition and cell wall resistance should be evaluated and an optimization of microwave power should be performed for each microalgae strain. Therefore, this work shows the potential of this innovative technology using SDEP/MW, a more environmentally safer extraction procedure to be applied in the lipid extraction for biofuel production purposes, resulting in a high quality.

4. Cost, cleanliness, and safety considerations

SDEP/MW is proposed as an "environmentally friendly" extraction method suitable for lipid extraction from microalgae. The reduced cost of extraction is clearly advantageous for the proposed SDEP/MW method in terms of energy, solvent used and time. Conventional procedure required an extraction time of 8h for Soxhlet and 2h for SDEP. The SDEP/MW method required heating for 10 min only. The energy required to perform the two extraction methods are, respectively, 8 kWh for Soxhlet (drying biomass, electrical energy for heating and evaporating), 1.05 kWh for conventional SDEP (for drying, heating and evaporating) and 0.6 kWh for SDEP/MW (electrical energy for microwave supply). The power consumption was determined with a Wattmeter at the microwave generator entrance and the electrical heater power supply.

Table 2 – Lipid extraction of two microalgae strains by conventional SDEP and SDEP/MW.

	D. salina			*N. oculata*		
	BD	**SDEP**	**SDEP/MW**	**BD**	**SDEP**	**SDEP/MW**
Oil Yield (%)	2.86 **±0.26**	2.82 **±0.03**	2.66 **±0.03**	23.78 **±2.13**	21.45 **±2.64**	18.20 **±0.28**
Lipid class composition						
FFA	42.39 **±0.09**	39.25 **±0.07**	36.87 **±0.32**	20.59 **±0.10**	23.75 **±0.07**	21.04 **±0.87**
TAG	57.61 **±0.11**	60.75 **±0.05**	63.13 **±0.41**	67.98 **±0.05**	57.94 **±0.53**	57.15 **±0.06**
DAG	-	-	-	11.43 **±0.18**	18.31 **±0.90**	21.81 **±0.28**
Other polar lipids	-	-	-	-	-	-
Fatty acids composition						
Saturated						
C14	0.39 **±0.04**	2.31 **±0.03**	2.40 **±0.07**	1.20 **±0.02**	0.72 **±0.97**	1.24 **±0.01**
C16	43.10 **±2.99**	40.36 **±0.19**	39.40 **±0.06**	20.65 **±0.09**	21.69 **±0.03**	20.32 **±0.04**
C18	0.70 **±0.06**	0.99 **±0.02**	0.98 **±0.02**	1.62 **±0.05**	1.88 **±0.09**	1.57 **±0.01**
Mono-unsaturated						
C15:1	-	-	-	-	-	-
C16:1 n9	0.18 **±0.03**	0.63 **±0.12**	0.86 **±0.01**	1.07 **±0.01**	1.10 **±0.01**	1.07 **±0.01**
C16:1 n7	0.22 **±0.04**	0.61 **±0.07**	0.98 **±0.01**	2.76 **±0.02**	3.12 **±0.05**	2.98 **±0.01**
C18:1 n9	6.22 **±0.30**	7.27 **±0.05**	7.38 **±0.04**	27.46 **±0.04**	26.62 **±0.73**	26.79 **±0.01**
C18:1 n7	0.82 **±0.53**	1.04 **±0.01**	1.08 **±0.01**	2.78 **±0.02**	2.45 **±0.15**	2.46 **±0.09**
Poly-unsaturated						
C16:2	0.25 **±0.02**	0.72 **±0.01**	0.84 **±0.01**	2.46 **±0.01**	2.46 **±0.03**	2.48 **±0.04**
C16:3	0.51 **±0.01**	1.14 **±0.01**	1.11 **±0.01**	4.76 **±0.03**	4.74 **±0.05**	5.03 **±0.01**
C16:4	5.17 **±0.57**	8.18 **±0.06**	8.78 **±0.03**	-	-	-
C18:2 n6	8.32 **±0.08**	8.40 **±0.06**	8.12 **±0.02**	14.70 **±0.01**	15.50 **±0.64**	14.82 **±0.07**
C18:3 n3	34.11 **±3.60**	27.36 **±0.10**	28.05 **±0.09**	20.09 **±0.07**	19.73 **±0.24**	21.14 **±0.01**
C20:2 n6	-	-	-	-	-	-
C20:3 n6	-	-	-	-	-	-
C20:4 n6	-	-	-	-	-	-
C20:5 n6	-	-	-	-	-	-
∑SFAs	44.19	43.66	42.78	23.47	24.29	23.13
∑MUFAs	7.44	9.55	10.30	34.07	33.29	33.30
∑PUFAs	48.36	45.80	46.90	42.01	42.43	43.47

5. Conclusion

For the first time, heating with microwaves was used as an energy source for a rapid heating of the mixture solvent/wet microalgae and in second time for lipid extraction with a SDEP apparatus. The validation of this new procedure has been checked by applying it to lipid extraction from microalgae. The new method has undergone reproducibility when optimal conditions were applied. The efficiency of this new technique SDEP/MW is considerably higher than conventional procedure Soxhlet or SDEP method if short extraction time required, cost and energy used and cleanliness of the process are taken into account. Therefore, SDEP/MW method could be appropriate for lipid extraction in a biodiesel purpose.

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