



**HAL**  
open science

## New procedure for extraction of algal lipids from wet biomass: A green clean and scalable process.

Celine Dejoye Tanzi, Maryline Abert, Farid Chemat

### ► To cite this version:

Celine Dejoye Tanzi, Maryline Abert, Farid Chemat. New procedure for extraction of algal lipids from wet biomass: A green clean and scalable process.. *Bioresource Technology*, 2013, 134, pp.271-275. 10.1016/j.biortech.2013.01.168 . hal-01328660

**HAL Id: hal-01328660**

**<https://hal.science/hal-01328660v1>**

Submitted on 29 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

New procedure for extraction of algal lipids from wet biomass: A green clean and scalable process

Celine Dejoye Tanzi, Maryline Abert Vian, Farid Chemat

PII: S0960-8524(13)00200-9  
DOI: <http://dx.doi.org/10.1016/j.biortech.2013.01.168>  
Reference: BITE 11332

To appear in: *Bioresource Technology*

Received Date: 13 December 2012  
Revised Date: 25 January 2013  
Accepted Date: 30 January 2013

Please cite this article as: Tanzi, C.D., Vian, M.A., Chemat, F., New procedure for extraction of algal lipids from wet biomass: A green clean and scalable process, *Bioresource Technology* (2013), doi: <http://dx.doi.org/10.1016/j.biortech.2013.01.168>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



# New procedure for extraction of algal lipids from wet biomass: A green clean and scalable process

Celine Dejoye Tanzi, Maryline Abert Vian\*, Farid Chemat

University of Avignon, INRA, UMR408, 84000 Avignon, France

## Corresponding author:

maryline.vian@univ-avignon.fr

## Abstract

A new procedure, called Simultaneous Extraction and Distillation Process (SDEP), for lipid extraction from wet microalgae (*Nannochloropsis oculata* and *Dunaliella salina*) was reported. This method does not require a pre-drying of the biomass and employs alternative solvents such as d-limonene,  $\alpha$ -pinene and p-cymene. This procedure has been compared with Soxhlet extraction (Sox) and Bligh & Dyer method (B&D). For *N. oculata*, results showed that SDEP-cymene provided similar lipid yields to B&D (21.45% and 23.78%), while SDEP-limonene and pinene provided lower yields (18.73% and 18.75% respectively). For *D. salina*, SDEP-Pinene provided the maximum lipid yield (3.29%) compared to the other solvents, which is quite close to B&D result (4.03%). No significant differences in terms of distribution of lipid classes and fatty acid composition have been obtained for different techniques. Evaluation of energy consumption indicates a substantial saving in the extraction cost by SDEP compared to the conventional extraction technique, Soxhlet.

**Keywords:** Wet lipid Extraction, Microalgae, *Nannochloropsis oculata*, *Dunaliella salina*, Terpenes

## 1. Introduction

Petroleum reserves depletion and global climate change have strongly encouraged the development of fuel production from various feedstocks such as

1 vegetable oils, waste cooking oils, animal fat and microalgae. Among these options,  
2  
3 microalgae have been recognized as potential good sources for biofuel production  
4  
5 because they synthesize and accumulate large quantities of neutral lipids (20–50 % dry  
6  
7 weight of biomass) and grow at high rates (Demirbas, 2008). In addition, microalgae  
8  
9 can grow on non-arable, nutrient-poor land that cannot support conventional agriculture  
10  
11 (Singh and Gu, 2010). A recent life-cycle assessment (LCA) of biofuel production from  
12  
13 microalgae feedstocks mentioned that drying and n-hexane extraction accounted for up  
14  
15 to 90% of the total process energy (Lardon et al., 2009). The extraction of crude oil is  
16  
17 usually performed with petroleum solvents such as conventional n-hexane, chloroform  
18  
19 and methanol, with techniques are highly energy-consumption and environmentally  
20  
21 damaging (Halim et al., n.d.). Other extraction processes such as supercritical CO<sub>2</sub>,  
22  
23 expelling, microwave-ultrasonic assisted extraction have also been reported (Cheng et  
24  
25 al., 2011). Many processes have been investigated and reported for converting directly  
26  
27 wet algae to crude biodiesel or biocrude (Anastasakis and Ross, 2011; Biller and Ross,  
28  
29 2011; Biller et al., 2011; Patil et al., 2011). But this biocrude differs from biodiesel that  
30  
31 we want to extract as it is composed primarily of hydrocarbons in contrast to biodiesel  
32  
33 which is composed of lipids in particular FAME after transesterification.  
34  
35  
36  
37  
38  
39  
40  
41

42 However, various conventional methods are available for oil extraction, but they  
43  
44 generally require long extraction times, petroleum-based solvents, dried biomass with  
45  
46 water content no more than 10% and high energy inputs. Upon harvesting, typical  
47  
48 microalgal concentrations in cultures range from about 0.1-1.0% (w/v) (Cooney et al.,  
49  
50 2009). This means that as much as 1000 times the amount of water per unit weight of  
51  
52 microalgae must be removed before attempting oil extraction. The microalgae paste  
53  
54 obtained from centrifugation (dewatering step) contains as much as *ca.* 80% water  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 content. Therefore energy consumption for drying microalgae is dramatically high. A  
2  
3 lipid extraction step that eliminates biomass drying and petroleum solvent use could  
4  
5 lead to significant energy and cost savings.  
6  
7

8 Here we propose a new procedure of lipid extraction from microalgae, such as  
9  
10 *Nannochloropsis oculata* (*N. oculata*) and *Dunaliella salina* (*D. salina*), that does not  
11  
12 require drying of the harvested microalgal biomass and employs bio-solvents  
13  
14 recognized as environmentally safer (Virost et al., 2008). Terpenes are natural solvents  
15  
16 existing both in the citrus fruits and in many other plants, with extraordinary technical  
17  
18 and chemical properties. Mamidipally and Liu recently demonstrated that the industrial  
19  
20 extraction of oil from rice bran was possible by using terpene such as d-limonene  
21  
22 instead of the regular *n*-hexane (Mamidipally and Liu, 2004; Liu and Mamidipally,  
23  
24 2005).  
25  
26  
27  
28

29  
30 Extracted lipids obtained using this new procedure, conventional Soxhlet with *n*-  
31  
32 hexane and Bligh & Dyer method have been compared in term of total lipid content,  
33  
34 lipid classes distribution and fatty acid composition.  
35  
36

## 37 **2. Materials and methods**

### 38 2.1. Strain, culture and harvesting conditions

39  
40  
41 *N. oculata* and *D. salina* were obtained by Greensea Company (Meze, France). *N.*  
42  
43 *oculata* was incubated in tubular reactor at ambient temperature under deficiency  
44  
45 conditions to obtain a high rate of lipids in the biomass. *D. salina* was grown in photo-  
46  
47 bioreactor at ambient temperature with good sunniness under favourable conditions. For  
48  
49 both, harvesting is being performed by centrifugation, resulting in a 20% dry weight  
50  
51 paste that is directly frozen (-25°C).  
52  
53  
54

### 55 2.2. Extraction methods for total lipids

### 2.2.1 Bligh and Dyer method (B&D)

Total lipids content of both microalgae was determined using a modified B&D method (Bligh and Dyer, 1959) which is already described in Adam et al., 2012.

### 2.2.2 Soxhlet method (Sox)

Lipids were isolated from microalgae by means of Soxhlet extraction (F. Soxhlet, 1879). Lipids were extracted from 10 grams of dry microalgae for 8h using 300 mL of n-hexane. After the extraction, solvent 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<sub>2</sub> and re-suspended in solvent for HP-TLC or GC-FID analysis.

### 2.2.3 SDEP method

For SDEP extraction, 12 g ± 0.5 g of 20% dry weight microalgae paste were placed in a 500mL round-bottomed flask. 100mL of terpene solvent (p-cymene, d-limonene or α-pinene) was added in order to immerse the wet microalgae sample. The round-bottomed flask was surmounted by a modified Dean stark receiver with a 3-way valve and fitted with a condenser (Fig. 1). At the beginning of the experiment (step 1), the electrical heating was maximized until collection of the first droplets of microalgae water in the modified Dean stark receiver with a 3-way valve. Then the heating was adapted until most of the water had been distilled and it was continued to allow lipid extraction step (step 2) with terpene solvent. The extraction was performed for 30 min. Then, the terpene elimination took place (step 3 and 4) and for that water was re-introduced by adjusting the 3-way valve to form a binary water-terpene mixture. To eliminate d-limonene from the distillation flask, we used the property that terpenes are traditionally extracted from their matrix by using a technique called hydrodistillation

1 thus inducing the use of an azeotropic distillation to below the boiling point of terpene  
2  
3 under the boiling point of the water (boiling point of the azeotrope : 97.4 °C). Terpene  
4  
5 solvent was recovered from the water layer by phase separation in the modified Dean  
6  
7 stark receiver and the extracted lipids were recovered from the water layer by phase  
8  
9 separation in the distillation round-bottomed flask. Thus, the SDEP procedure was  
10  
11 allowed elimination of microalgae water, extraction of lipids and elimination of terpene  
12  
13 solvent in a single “in situ” step. Terpene solvent was recuperated at 100% and purity  
14  
15 levels show that it can be recycled for other uses, including other SDEP processes.  
16  
17  
18 Lipid extracts were analysed by GC-FID (against an external calibration with the pure  
19  
20 solvent) and did not contain contamination by solvent (less than 0.01% of solvent in  
21  
22 lipid extract). Extractions were performed in triplicate and the mean values were  
23  
24 reported. Lipid extracts were dried under a stream of nitrogen and re-suspended in  
25  
26 solvent without purification for HP-TLC or GC-FID analysis.  
27  
28  
29  
30  
31

### 32 2.3. Analysis of total lipids

#### 33 2.3.1. Gravimetry

34  
35 Total lipid content was quantified by weight after total drying.  
36  
37

#### 38 2.3.2. High Performance Thin-Layer Chromatography (HP-TLC)

39  
40 Lipids were detected by charring and quantified using a CAMAG 3 TLC scanning  
41  
42 densitometer (CAMAG, Muttenz, Switzerland) with identification of the classes against  
43  
44 known polar and neutral lipid standards. Typically, Lipid extract was loaded as a spot  
45  
46 onto 20 × 10 cm silica gel 60 F254 HPTLC plates (Merck KGaA, Germany) using an  
47  
48 ATS 5 automatic TLC sampler (Camag, Switzerland). Plates were then developed in an  
49  
50 ADC2 automatic developing chamber (CAMAG, Switzerland) using first a methyl  
51  
52 acetate/isopropanol/chloroform/methanol/KCl (0.25% solution) (25:25:25:10:9) mixture  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62

1 running to a height of 5.5 cm from the origin and then a n-hexane/diethyl ether/glacial  
2 acetic acid mixture (80:20:2) to a height of 8.5 cm from the origin. After dried, the plate  
3 was dipped for 6s in a modified CuSO<sub>4</sub> reagent (20 g CuSO<sub>4</sub>, 200 ml methanol, 8 ml  
4 H<sub>2</sub>SO<sub>4</sub>, and 8 ml H<sub>3</sub>PO<sub>4</sub>) then heated at 141 °C for 30 min on a TLC plate heater and  
5 finally scanned using a TLC Scanner 3 with WinCATs software (CAMAG). The  
6 densitometry data are reported as values which are expressed as percent of lipid class in  
7 total microalgae lipids.  
8  
9

### 10 2.3.3 GC Analysis

11 Fatty acids methyl esters (FAMES) were separated, identified and quantified by gas  
12 chromatography coupled with flame ionization detector (GC-FID) using the method  
13 already described by Adam et al., 2012.  
14  
15

## 16 3. Results and discussion

### 17 3.1 Recovery of Crude lipids

18 The measurement of lipids recovery was based on the total amount of FAMES in each  
19 sample. So it is a real yield in total fatty acids, and it is certainly a much more accurate  
20 value than a yield obtained by gravimetric method. Table 1 shows the results of both  
21 microalgae for each extraction method. Three extraction methods, B&D, SDEP and Sox  
22 were tested with different solvents. Use of B&D extraction is the commonly accepted  
23 method for lipid extraction from biological tissues due to its high lipid yields (Certik et  
24 al., n.d.). Therefore, the B&D method was selected as standard to compare other  
25 extraction technique. As mentioned in Table 1, B&D method yielded 23.78% and  
26 4.03% lipids content for *N. oculata* and *D. salina* respectively. Crude lipids of *N.*  
27 *oculata* and *D. salina* obtained by SDEP procedure with three terpenes were higher than  
28 Sox and slightly lower than B&D reference method. First, these results can be explained  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1 by the difference of polarity between solvents; Liu and Mamidipally (Mamidipally and  
2  
3 Liu, 2004; Liu and Mamidipally, 2005) have already noted this effect that might be due  
4  
5 to the slightly more polar nature of terpenes and much more polar nature of  
6  
7 methanol/chloroforme mixture compared with *n*-hexane. In addition, in SDEP  
8  
9 procedure the matrix is in direct contact with the boiling solvent which is not the case  
10  
11 with the conventional Soxhlet. A higher dissolving ability of terpenes for lipids might  
12  
13 also be pointed out by the higher temperature used to boil this solvent which could  
14  
15 produce a lower viscosity of the analytes in the matrix and, accordingly, a better  
16  
17 diffusion rate of the solute from the solid phase to the solvent.  
18  
19  
20  
21

### 22 3.2 Lipids class composition

23  
24 Similar to higher plants, microalgal lipids are composed of neutral lipids and polar  
25  
26 lipids. Under nutrient stress conditions, many microalgae tend to accumulate neutral  
27  
28 lipids (consist of triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols  
29  
30 (MAG) and free fatty acids (FFA)) and form lipid droplet localized in the cytoplasm as  
31  
32 a storage form of carbon and energy. Neutral lipids of the extracts were separated using  
33  
34 high-performance thin-layer chromatography (HP-TLC), quantified with densitometry  
35  
36 and presented as % of lipid class in total lipids extracted with B&D, SDEP and Sox for  
37  
38 both microalgae. Four standards in a mixture of monoacylglycerol (MAG),  
39  
40 diacylglycerol (DAG), triacylglycerol (TAG) and free fatty acids (C18) were deposited  
41  
42 on the plate. For *N. oculata*, HP-TLC results confirm that TAG, DAG and FFA were the  
43  
44 main components of the extracted lipids, but they contributed to the total lipid content in  
45  
46 different amounts. As shown in Fig. 2, TAG made up an important percentage of total  
47  
48 lipids (between 57 and 69% according to extraction method). Regarding *D.salina*, the  
49  
50 amount of TAG for B&D method is about 60%, 54% for Sox and ranging from 50 to  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 57% for SDEP procedures. The SDEP procedure with p-cymene solvent gives a higher  
2  
3 quantity of TAG than d-limonene and  $\alpha$ -pinene. Results obtained with the SDEP  
4  
5 method including different terpene were almost similar with those obtained by both  
6  
7 conventional Sox and B&D method.  
8  
9

### 10 3.3 Fatty acid compositions

11 Table 1 shows the classes' variability of FAMES in connection with the extraction  
12  
13 method used. The nature of FAMES composition in *N. oculata* and *D. salina* was  
14  
15 determined by GC analysis. According to Table 1, the main fatty acids for *N. oculata*  
16  
17 were palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids.  
18  
19 These four fatty acids represent more than 80% of the total fatty acid composition of the  
20  
21 extracted oil. Other fatty acids such as myristic (C14:0), palmitoleic (C16:1),  
22  
23 palmitolenic (C16:2), hirigonic (C16:3) or stearic (C18:0) acids were also noted with a  
24  
25 less predominant peak area. Arachidic (C20:0) and behenic (C22:0) acids were found in  
26  
27 trace levels. No significant differences were detected according different extraction  
28  
29 methods and solvents. The fatty acid composition of *N. oculata* sp. displayed in Table 1  
30  
31 is different to the profile presented by Adam et al. (Adam et al., 2012). Despite similar  
32  
33 results regarding the respective proportions of C16:0 and C14:0, the authors report *N.*  
34  
35 *oculata* to contain particularly high levels of C16:1 and C25:3, with corresponding  
36  
37 reduction in C18:1, C18:2 and C18:3. Fatty acid profile of microalgal species is known  
38  
39 to be function of its culturing conditions and environmental conditions (Olofsson et al.,  
40  
41 2012). For *D. salina* sp., palmitic (C16:0), oleic (C18:1n9), hexadecatetraenoic (C16:4),  
42  
43 linoleic (C18:2) and linolenic (C18:3) acids were identified as the principal fatty acids  
44  
45 in *D. salina* extracted lipids. The data in table reveals that total unsaturated fatty acids  
46  
47 were about 45-60%, while saturated fatty acids were about 40-50%. Results obtained  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 with SDEP method were almost similar with those obtained by both conventional Sox  
2 extraction and B&D method. The sum percentages of saturated, mono- and poly-  
3  
4 extraction and B&D method. The sum percentages of saturated, mono- and poly-  
5  
6 unsaturated fatty acids were in line with those of several tables dealing with the fatty  
7  
8 acid composition of *N. oculata* and *D. salina* oil found in literature. As a conclusion, it  
9  
10 can be said that the proportion of the different fatty acids as well as the proportion of  
11  
12 SFAs, PUFAs, or MUFAs has not been affected by the unusual conditions used in our  
13  
14 experiment, in other words, the use of terpenes as solvent do not involve extraneous  
15  
16 effects and/or artefacts on the composition of the lipids extracted.  
17  
18

### 20 3.4 Energy consumption

22 We conducted a comparison on energy consumption of SDEP process and the  
23 current method to extract lipids, Soxhlet. Alternatively, the input power consumption  
24  
25 was monitored using a separate Wattmeter at the entrance of electrical heater power  
26  
27 supply (i.e. at the entrance of electrical heater, rotary evaporator and cooler power  
28  
29 supply). For Soxhlet extraction, drying biomass is required. So, in our calculation we  
30  
31 have taken into account the freeze-drying of microalgae for 48h. We have added the  
32  
33 energy consumption of all apparatus involved in each extraction technique. The energy  
34  
35 requirement to perform the extraction, based on the power consumptions for 1g of  
36  
37 extracted lipids, was 8.84 kWh for Soxhlet while this value was 2.15 kWh for SDEP.  
38  
39 This indicates a substantial saving in the extraction cost by SDEP compared to the  
40  
41 conventional extraction technique, Soxhlet.  
42  
43  
44  
45  
46  
47  
48

## 49 4. Conclusion

51 The new procedure has undergone reproducible results in a substantially shorter  
52  
53 time. The efficiency of SDEP is considerably higher than the conventional procedure in  
54  
55 terms of lipid yield and if we take into account short distillation and extraction times  
56  
57  
58  
59  
60  
61  
62

1 required, cost and energy used of the process. This study has clearly demonstrated that  
2  
3 this procedure can be used to extract lipid directly from wet microalgae with an  
4  
5 extraction yield similar to that of the B&D method, with only minor variation in the  
6  
7 fatty acids distribution.  
8  
9

## 10 11 **Acknowledgements**

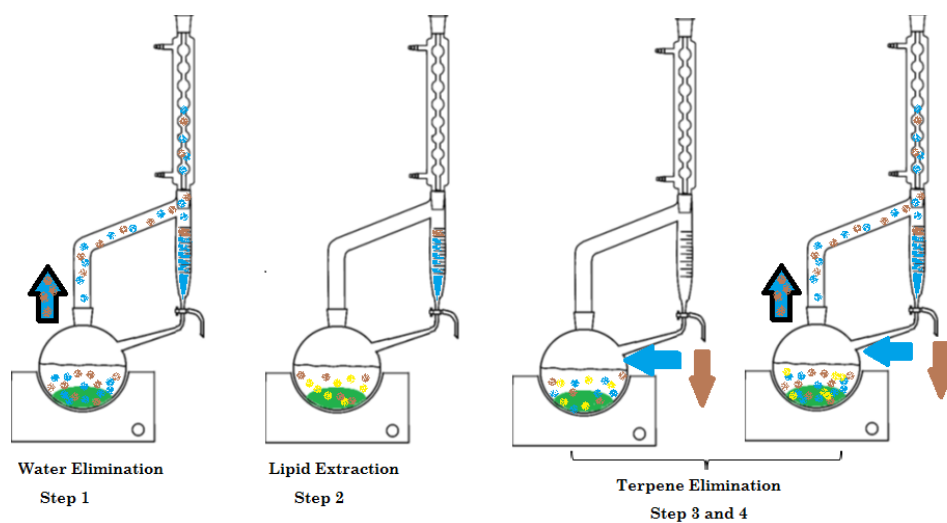
12  
13  
14  
15 The authors are grateful for the financial support of the Salinalgue program (FUI-  
16  
17 France) coordinated by La Compagnie du Vent (Montpellier, France).  
18  
19  
20  
21

## 22 **References**

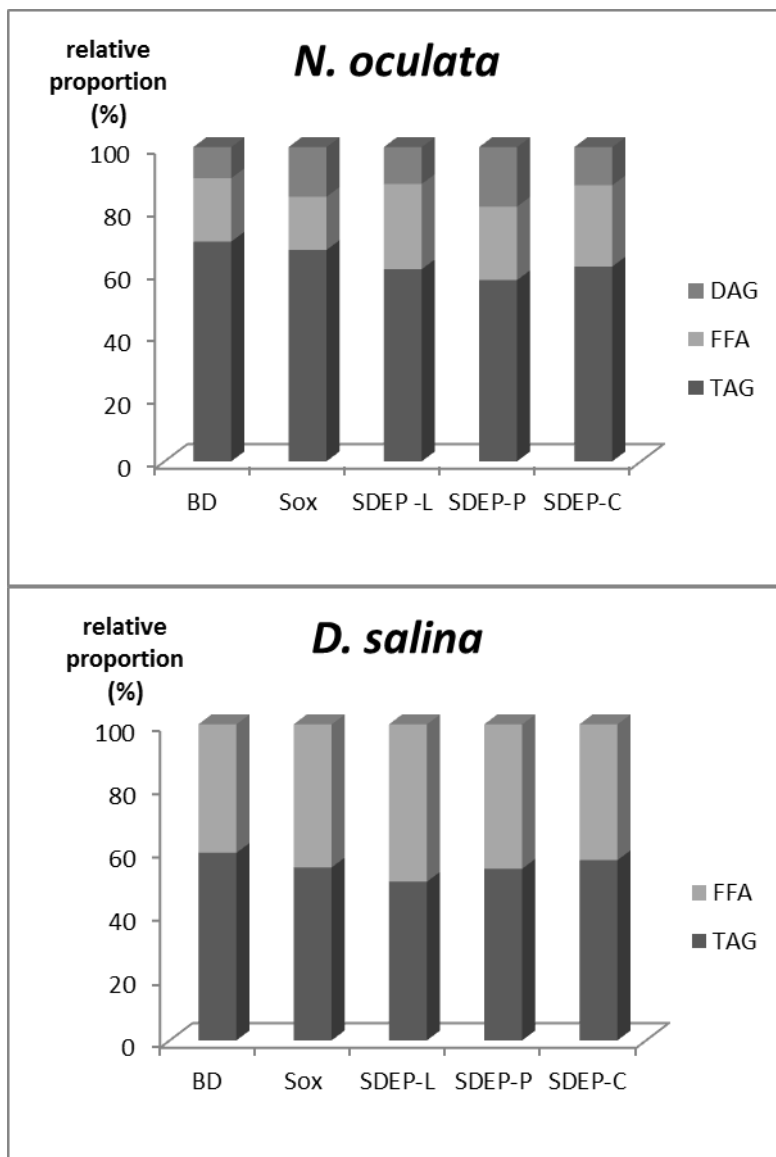
- 23  
24  
25 1. Adam, F., Abert-Vian, M., Peltier, G., Chemat, F., 2012. “Solvent-free”  
26  
27 ultrasound-assisted extraction of lipids from fresh microalgae cells: A green,  
28  
29 clean and scalable process. *Bioresource Technology* 114, 457–465.  
30  
31
- 32 2. Anastasakis, K., Ross, A.B., 2011. Hydrothermal liquefaction of the brown  
33  
34 macro-alga *Laminaria Saccharina*: Effect of reaction conditions on product  
35  
36 distribution and composition. *Bioresource Technology* 102, 4876–4883.  
37  
38
- 39 3. Biller, P., Riley, R., Ross, A.B., 2011. Catalytic hydrothermal processing of  
40  
41 microalgae: Decomposition and upgrading of lipids. *Bioresource Technology*  
42  
43 102, 4841–4848.  
44  
45
- 46 4. Biller, P., Ross, A.B., 2011. Potential yields and properties of oil from the  
47  
48 hydrothermal liquefaction of microalgae with different biochemical content.  
49  
50 *Bioresource Technology* 102, 215–225.  
51  
52
- 53 5. Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and  
54  
55 purification. *Biochemistry and Cell Biology* 37, 911–917.  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1 6. Certik, M., Andrasi, P., Sajbidor, J., n.d. Effect of extraction methods on lipid  
2  
3 yield and fatty acid composition of lipid classes containing  $\gamma$ -linolenic acid  
4  
5 extracted from fungi. *Journal of the American Oil Chemists' Society* 73, 357–  
6  
7 365.  
8  
9
- 10 7. Cheng, C.-H., Du, T.-B., Pi, H.-C., Jang, S.-M., Lin, Y.-H., Lee, H.-T., 2011.  
11  
12 Comparative study of lipid extraction from microalgae by organic solvent and  
13  
14 supercritical CO<sub>2</sub>. *Bioresource Technology* 102, 10151–10153.  
15  
16  
17
- 18 8. Cooney, M., Young, G., Nagle, N., 2009. Extraction of Bio-oils from  
19  
20 Microalgae. *Separation & Purification Revs.* 38, 291–325.  
21  
22
- 23 9. Demirbas, A., 2008. Comparison of transesterification methods for production of  
24  
25 biodiesel from vegetable oils and fats. *Energy Conversion and Management* 49,  
26  
27 125–130.  
28  
29
- 30 10. F. Soxhlet, 1879. . *Dinglers' Polyt. J.* 232, 461.  
31  
32
- 33 11. Halim, R., Danquah, M.K., Webley, P.A., n.d. Extraction of Oil from  
34  
35 Microalgae for Biodiesel Production: A Review. *Biotechnology Advances.*  
36  
37
- 38 12. , A., Sialve, B., Steyer, J.-P., Bernard, O., 2009. Life-Cycle  
39  
40 Assessment of Biodiesel Production from Microalgae. *Environ. Sci. Technol.*  
41  
42 43, 6475–6481.  
43  
44
- 45 13. Liu, S.X., Mamidipally, P.K., 2005. Quality Comparison of Rice Bran Oil  
46  
47 Extracted with d-Limonene and Hexane. *Cereal Chemistry* 82, 209–215.  
48  
49
- 50 14. Mamidipally, P.K., Liu, S.X., 2004. First approach on rice bran oil extraction  
51  
52 using limonene. *European Journal of Lipid Science and Technology* 106, 122–  
53  
54 125.  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
15. Olofsson, M., Lamela, T., Nilsson, E., Bergé, J.P., Del Pino, V., Uronen, P., Legrand, C., 2012. Seasonal Variation of Lipids and Fatty Acids of the Microalgae *Nannochloropsis oculata* Grown in Outdoor Large-Scale Photobioreactors. *Energies* 5, 1577–1592.
16. Patil, P.D., Gude, V.G., Mannarswamy, A., Deng, S., Cooke, P., Munson-McGee, S., Rhodes, I., Lammers, P., Nirmalakhandan, N., 2011. Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions. *Bioresource Technology* 102, 118–122.
17. Singh, J., Gu, S., 2010. Commercialization potential of microalgae for biofuels production. *Renewable and Sustainable Energy Reviews* 14, 2596–2610.
18. Virot, M., Tomao, V., Ginies, C., Visinoni, F., Chemat, F., 2008. Green procedure with a green solvent for fats and oils' determination: Microwave-integrated Soxhlet using limonene followed by microwave Clevenger distillation. *Journal of Chromatography A* 1196-1197, 147–152.



**Fig. 1.** Simultaneous Distillation Extraction Process (SDEP) (blue: water biomass, brown: terpene solvent, yellow: lipids, green: microalgae)



**Fig. 2.** Distribution of lipid classes by HP-TLC



**Table 1:** Crude lipids, distribution of lipid class and fatty acid composition of extracts obtained by different extraction methods. (PUFAs polyunsaturated fatty acids, MUFAs monounsaturated fatty acids, SFAs Saturated fatty acids)

	<i>N. oculata</i>									<i>D. salina</i>										
	B&D		SDEP		$\alpha$ -pinene		p-cymene		Sox	B&D		SDEP		$\alpha$ -pinene		p-cymene		Sox		
<b>Lipids Yield (%)</b>	23.78	$\pm 2.13$	18.73	$\pm 6.58$	18.75	$\pm 3.01$	21.45	$\pm 2.64$	8.31	$\pm 1.05$	4.03	$\pm 0.01$	2.94	$\pm 0.02$	3.29	$\pm 0.05$	2.99	$\pm 0.01$	1.90	$\pm 0.03$
<b>Lipid class</b>																				
<b>Lipid composition</b>																				
FFA : Free fatty acid	20.21	$\pm 0.03$	27.12	$\pm 0.12$	23.42	$\pm 0.27$	25.84	$\pm 0.32$	16.92	$\pm 0.13$	40.64	$\pm 0.36$	49.77	$\pm 0.08$	47.55	$\pm 0.32$	42.95	$\pm 0.01$	45.25	$\pm 0.09$
TAG : Triacylglycerol	69.94	$\pm 0.16$	61.18	$\pm 0.05$	57.67	$\pm 0.65$	62.02	$\pm 0.82$	67.30	$\pm 2.68$	59.36	$\pm 0.02$	50.23	$\pm 0.20$	52.45	$\pm 0.05$	57.05	$\pm 0.24$	54.75	$\pm 0.04$
DAG: Diacylglycerol	9.85	$\pm 0.06$	11.71	$\pm 0.06$	18.91	$\pm 0.09$	12.15	$\pm 0.15$	15.78	$\pm 0.12$	-		-		-		-		-	
<b>Fatty acids</b>																				
<b>Fatty acid composition</b>																				
<b>Saturated</b>																				
C14:0	1.20	$\pm 0.02$	1.52	$\pm 0.03$	1.43	$\pm 0.01$	0.72	$\pm 0.97$	1.37	$\pm 0.10$	3.37	$\pm 0.45$	6.74	$\pm 0.17$	3.69	$\pm 0.02$	3.61	$\pm 0.01$	1.01	$\pm 0.03$
C16:0	20.65	$\pm 0.09$	21.86	$\pm 0.41$	21.90	$\pm 0.01$	21.69	$\pm 0.03$	21.55	$\pm 0.28$	46.46	$\pm 0.31$	34.84	$\pm 1.05$	43.36	$\pm 0.15$	42.66	$\pm 0.06$	38.67	$\pm 1.65$
C18:0	1.62	$\pm 0.05$	1.84	$\pm 0.11$	1.75	$\pm 0.01$	1.88	$\pm 0.09$	1.72	$\pm 0.01$	1.26	$\pm 0.04$	3.13	$\pm 0.06$	1.01	$\pm 0.03$	1.17	$\pm 0.02$	0.81	$\pm 0.07$
<b>Mono-unsaturated</b>																				
C14:1	-		-		-		-		-		0.43	$\pm 0.01$	2.02	$\pm 0.02$	0.60	$\pm 0.01$	0.48	$\pm 0.02$	0.19	$\pm 0.02$
C16:1(n-9)	1.07	$\pm 0.01$	1.25	$\pm 0.05$	1.12	$\pm 0.01$	1.10	$\pm 0.01$	1.13	$\pm 0.02$	0.69	$\pm 0.09$	0.94	$\pm 0.09$	0.91	$\pm 0.01$	0.58	$\pm 0.01$	1.52	$\pm 0.58$
C16:1(n-7)	2.76	$\pm 0.02$	3.26	$\pm 0.01$	3.11	$\pm 0.01$	3.12	$\pm 0.05$	2.83	$\pm 0.11$	0.54	$\pm 0.01$	1.88	$\pm 0.34$	0.89	$\pm 0.01$	0.52	$\pm 0.01$	1.77	$\pm 0.24$
C18:1(n-9)	27.46	$\pm 0.04$	26.03	$\pm 0.02$	26.27	$\pm 0.11$	26.62	$\pm 0.73$	26.87	$\pm 0.36$	6.50	$\pm 0.14$	12.61	$\pm 0.19$	7.55	$\pm 0.03$	7.67	$\pm 0.01$	6.80	$\pm 0.27$
C18:1(n-7)	2.78	$\pm 0.02$	2.59	$\pm 0.24$	2.52	$\pm 0.11$	2.45	$\pm 0.15$	2.35	$\pm 0.04$	-		-		-		-		-	
<b>Poly-unsaturated</b>																				
C16:2	2.46	$\pm 0.01$	2.73	$\pm 0.13$	2.48	$\pm 0.01$	2.46	$\pm 0.03$	2.53	$\pm 0.03$	0.08	$\pm 0.01$	2.42	$\pm 0.08$	1.04	$\pm 0.01$	0.91	$\pm 0.01$	0.82	$\pm 0.06$
C16:3	4.76	$\pm 0.03$	4.84	$\pm 0.04$	4.75	$\pm 0.03$	4.74	$\pm 0.05$	4.90	$\pm 0.07$	1.18	$\pm 0.01$	1.64	$\pm 0.06$	0.37	$\pm 0.07$	0.27	$\pm 0.01$	0.91	$\pm 0.05$
C16:4	-		-		-		-		-		9.22	$\pm 0.17$	9.20	$\pm 0.03$	9.16	$\pm 0.04$	9.94	$\pm 0.01$	8.37	$\pm 0.22$
C18:2(n-6)	14.70	$\pm 0.01$	14.91	$\pm 0.09$	15.08	$\pm 0.03$	15.50	$\pm 0.64$	15.14	$\pm 0.20$	6.95	$\pm 0.09$	7.89	$\pm 0.99$	7.98	$\pm 0.15$	7.65	$\pm 0.17$	8.60	$\pm 0.50$
C18:3(n-3)	20.09	$\pm 0.07$	19.18	$\pm 0.36$	19.60	$\pm 0.01$	19.73	$\pm 0.24$	20.06	$\pm 0.32$	20.32	$\pm 0.10$	16.70	$\pm 0.10$	23.44	$\pm 0.09$	24.66	$\pm 0.05$	30.54	$\pm 0.14$
$\Sigma$ SFAs	23.47		25.22		25.08		24.28		24.64		51.09		44.71		48.06		47.44		40.49	
$\Sigma$ MUFAs	34.07		33.13		33.01		33.29		33.18		8.16		17.44		9.95		9.25		10.28	
$\Sigma$ PUFAs	42.01		41.66		41.90		42.43		42.62		37.75		37.85		41.99		43.43		49.24	

### Highlights

- A new extraction procedure of lipids from wet microalgae
- *Nannochloropsis oculata* and *dunaliella salina* were chosen as a microalgae
- Influence of operating parameters on the extraction yields will be studied
- Utilization of terpenes bio-solvents recognized as environmentally safer

ACCEPTED MANUSCRIPT