Scientific Papers. Series B, Horticulture. Vol. LXVI, No. 2, 2022
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

LIPIDS EXTRACTION METHODS APPLIED ON *Nannochloropsis* sp. BIOMASS - A REVIEW

Carmen Gabriela CONSTANTIN, Ioana-Cătălina NICOLAE, Aurora DOBRIN, Andrei MOȚ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: carmen.constantin@qlab.usamv.ro

Abstract

Nannochloropsis sp. is a microalga of particular interest for the production of lipids containing omega 3 - fatty acids, specifically eicosapentaenoic acid (EPA), a fatty acid mostly found in the flesh of cold-water fish and crustaceans with high importance in human health. Because of the rigid cell wall structure of Nannochloropsis sp., the extract of EPA requires specific methods. There are certain critical points regarding lipidic extraction: the application in food and feed, and the fractionation methods that provide a high recovery rate of EPA. Therefore, green extraction methods have recently gained more and more interest, having minimal environmental and health impacts, as they use less or no organic solvents, being sustainable productive, and efficient. The methods used for lipid extraction should ensure that during the process, the lipid extraction is obtained without influencing the fatty acid composition. The purpose of this review is to summarize the existing research parameters regarding different green extraction methods applied for obtaining lipid fractions, emphasis on supercritical fluid extraction, ultrasound-assisted extraction, and accelerated solvent extraction methods.

Key words*: eicosapentaenoic acid, fractionation methods, microalgae.*

INTRODUCTION

As it is presented in many studies, in the global context the world population is increasing. Along with this, there will be an increase in the need for food and implicitly resources of any kind. Also, these resources in turn are renewable and non-renewable, both being exhaustible at a certain point.

In this context, we are forced to find or explore alternative sustainable edible sources that can satisfy human and animal nutritional needs and reduce competition in the use of traditional ones.

One such source rich in micronutrients are microalgae (Montoya-Arroyo et al., 2022).

Microalgae represent a group of autotrophic microorganisms living in aquatic and terrestrial ecosystems and producing organic substances by photosynthesis.

Today the main areas of use of the microalgae are biomass production (as a biological additive) and the cultivation for isolation of their biologically active substances (Vyacheslav Dolganyuk et al., 2020).

Microalgae are considered an alternative to unconventional sources of biologically active compounds and food supplements for animal and human nutrition (Lorenzo Zanella, Fabio Vianello, 2020). As a source of proteins, polysaccharides, lipids, polyunsaturated fatty acids, vitamins, pigments, phycobiliproteins, enzymes, etc., plays an antioxidant, antibacterial, antiviral, antitumor, regenerative, antihypertensive, neuroprotective, and immune-stimulant role. Considered one of the greatest primary producers of any aquatic habitat, they have high growth rates requiring only water, nutrients, and carbon dioxide (Salbitani et al., 2021).

Therefore, there is a demand for these compounds in domains such as medicine, the chemical industry, fish farming, the energy industry, and agriculture in the production of feed and functional foods (Vyacheslav Dolganyuk et al., 2020).

The consumption of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), like eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) is

associated with the health benefits, healthy, balanced diet and well-being (Douglas R. Tocher et al., 2019).

At present, the only commercial source of EPA is marine fish oil, a rather unsatisfactory source because of problems of contamination, taste, odour and stability. In addition, the presence of considerable amounts of other PUFAs in the fish oil complicates the EPA purification process, resulting in high retail prices of the pure product These factors have led to investigation of alternative EPA sources.

One such industrially promising species is *Nannochloropsis* sp. with elevated photosynthetic efficiency and lipid productivity.

Due to its high lipid content (37-60%), high yields of the omega-3 (ω-3), in particular, eicosapentaenoic acid (EPA) makes them become a candidate for commercial applications for human consumption (Salbitani et al., 2021).

An important issue in lipid extraction to consider is the selection of the right extraction method. In fact, extracting oil from biomass is necessary to choose a fully compatible solvent or a mixture of solvents (ratio) that will not alter its bioactivity.

The robust and complex cell wall structure of microalgae leads to the fact that the lipids are trapped in the cytoplasm by cell walls and cell membranes, so the lipids from the cells cannot be completely extracted, this aspect affecting the lipids' yield.

Therefore, there are several gaps in choosing the extraction method for a specific compound from different complex matrices. The robust structure of *Nannochloropsis* sp., requires an integrated approach to lipid fraction extraction methods. Among other things, this will take into account several aspects related to the cell structure, the nature of the solvents used, the extraction yield and last but not least the extraction costs, all of which are raised to a scalable level.

In this context, the paper presents an analysis of the literature regarding the impact of the extraction methods associated to extraction solvents, preceded by cell disruption operations on lipid extraction from *Nannochloropsis* species.

MATERIALS AND METHODS

There are several types of extraction procedures both time and solvents consuming, such as conventional (Soxhlet, Bligh-Dyer and Folch). Also, conventional extraction techniques involve the use of organic solvents considered as not safe for humans and the environment (methanol, chloroform, acetone, etc.).

Therefore, the solvent extraction methods can be combined with the ultrasonic crushing method, microwave, autoclave, bead milling methods and other crushing methods to improve the rate of lipid extraction (Corrêa et al., 2021; Ren et al., 2021; Alhattab et al., 2019; D'Hondt et al., 2017; Naghdi et al., 2016). The solvent extraction methods have the advantages of high lipid yield, high lipid quality, and easy realization of large-scale production. However, usually in the extraction methods are used organic solvents which are toxic and volatile with a negative impact on all ecosystems (Naghdi et al., 2016). In recent years there is a demand to reduce the amount of solvent used in microalgae lipids extraction and also for greener, safer, and more natural products that do not require the involvement of toxic solvents thus minimizing the environmental impact (Ren et al., 2021; Imbimbo et al., 2020; Naghdi et al., 2016).

The solvents must be chosen depending on the compound polarity. The combination of polar and non-polar solvents leads to an increase in lipid extraction/recovery. The polar solvents have the ability to release the lipids from their protein-lipid complexes which facilitate their dissolving in the non-polar solvent (Abimbola et al., 2021; Naghdi et al., 2016). The lipid extraction/recovery is higher when these methods are applied on wet biomass, so the polar solvent can penetrate the water layer and make the lipids available for non-polar solvent solvation. The best solvent-free techniques are the ones which can be performed on a diverse variety of algae with low energy consumption and minimum initial set-up costs for infrastructure (Eikani et al., 2018; Naghdi et al., 2016).

Despite being well known and often used, conventional methods use toxic solvents and are not in accordance with environmental and

human health concerns (Corrêa et al., 2021). Bligh-Dyer and Folch methods are classical methods widely used which can be performed directly on microalgae because they extract lipid from the microalgal cell without the additional requirement of cell rupturing (Matos et al., 2019; Nagappan et al., 2019, Ranjith et al., 2015). The chloroform used in classic methods has been shown that is carcinogenic and ozone-depleting (Byrne et al., 2016; Chua et al., 2017).

Generally recognized as safety solvents like hexane, buthanol, ethanol, ethyl acetate (EtOAc), 2-methyl tetrahydrofuran (MeTHF), can be used alone or in combination for lipid extraction from microalgae by dissolving hydrophobic cell membrane components. Solvent mixtures like hexane-ethanol (4:1), and hexane-isopropanol have been demonstrated to be more efficient then using solvents alone, for lipid extraction. The alcohols from the mixture break the electrostatic forces and a hydrogen bond between membrane lipid and associated protein, so non-polar component of the solvent mixture can enter into the cell, followed by neutral lipid extraction (Nagappan et al., 2019; Ahmad et al., 2018).

According to Abimbola et al., 2021, ethanol efficiently extracts lipids from algae without the need for the cell disruption step. In their studies found that hexane extraction recovers oil from: *N. salina* and *N. oculata*, with about 60% Fatty acid methyl esters (FAME) content. Ethanol extraction method recovers more lipids at higher Total solids content (TS). The solvent biomass ratio was of 25. Ethanol extraction giving higher yields with 23% more recovery at 20 and 10 wt % TS while about 14% more recovery was obtained at 15 wt % TS.

Dimethyl ether (DME), a green solvent, features a high affinity for both water and organic compounds with an ability to penetrate the cell walls and successfully extract lipids from microalgae without the requirement of drying the biomass (Wang et al., 2021; Nagappan et al., 2019).

With Soxhlet extraction using n-hexane as solvent was obtained a 45% (dw) yield of lipid from dried *Schizochytrium limacinum* and with ethanol, extract 48% (dw) lipid from dried *Synechocystis* PCC 6803. With supercritical extraction using CO_2 and ethanol was obtained a 34% (dw) yield of lipid from dried *S. limacinum* powder. 18.1% (dw) lipid yield from dried *Chlorella* spp. powder was obtained using a mixed extraction solvent of methanol: ethyl acetate at a volume ratio of 2:1.

Liquid dimethyl ether (DME) can be used also, for the extraction of lipids from microalgae. Liquid DME is partially miscible with water and has a high affinity for organic compounds. Thus, DME is suitable for extraction of lipids from wet biomass samples with simultaneous dewatering with a 25 mL DME for 8 mL of microalgae (Wang et al., 2021)

Extraction of lipid from microalgae can be performed also with water which it is food grade chemical, and resistant to auto-oxidation. The property of a low boiling point allows easy solvent recovery after initial extraction, thereby reducing the microalgal lipid extraction cost (Nagappan et al., 2019).

Bernaerts et al., 2020, used as a method of vacuum filtration and rotary evaporation using hexane: isopropanol (3:2 v/v) for lipid extraction from *Nannochloropsis* sp.

Herrero et al., 2004, obtained the higher oil yield from dried extract of *Spirulina* sp. using water and ethanol as solvents, carried out by Accelerated Solvent Extraction system (ASE 200), the extracted amount increases using higher extraction times and/or higher extraction temperatures (15 minutes at 170ºC).

Angles et al, (2017) found that the best solvents for lipid extraction were Methyl-tert-butyl ether (MTBE) and cyclopentylmethyl ether (CPME). They represent alternatives to chlorinated solvents or alkanes and they are followed by MeTHF and EtOAc, which are green solvents.

Iovine et al. (2019), used as solvents for accelerated solvent extraction by Dionex ASE 200, hexane and a mixture of chloroform, methanol/water (C/M/W) for accelerated solvent extraction at 50°C, two cycles of 10 minutes. Using hexane, the fatty acids obtained were 2.83 times higher than fatty acids obtained without pre-treatment (mechanical pre-treatment using the Planetary ball).

Sánchez-Camargo et al., 2018 used high-pressure homogenization (HPH) to break down the strong cell wall and supercritical fluid extraction (SFE) with pure CO_2 was applied as a first step to extract valuable compounds (such as non-polar lipids and pigments). Extraction of

the remaining residue for the recovery of bioactive compounds employing pressurized liquid extraction (PLE) with green solvents such as water and ethanol. Optimum extract was achieved with pure ethanol at 170°C for 20 min.

Blanco-Llamero et al. (2021) used Pressurized Liquid Extraction (PLE), carried out with ASE 350 DIONEX extractor using 20-25 mL from the following solvents: ethanol, 2-MeTHF, and different mixtures of hexane: ethanol (3:4), 2-MeTHF: ethanol (1:3), MTBE: ethanol (1:3), 2-MeTHF: isopropyl alcohol (1:3), and 2-MeTHF: isobutanol (1:3), heated to 90, 120, and 150°C and static extraction time was 15 min for each experiment. The mixtures of isopropanol, isobutanol, and MTBE produced the highest SFA content, ethanol, mixtures of 2-MeTHF: ethanol, 2-MeTHF: isobutanol, and mixtures of hexane: ethanol were the ones with higher PUFA content. The authors, also optimized the methods using a 1:3 ratio of 2-MeTHF and ethanol, with good results for the extraction of polar lipids with omega-3 PUFAs from algal biomass. 2-methyl-THF can be used successfully use as hexane substitute in solvent mixtures with alcohols when extracting by Pressurized Liquid Extraction polar compounds from microalgae (*N. gaditana*, *I. galbana*, *T. chuii*) being in agreement with green chemistry.

Derwenskus et al. (2019), also use pressurized liquid extraction (PLE), performed using accelerated solvent extractor (ASE 350). In the study they used 5 g of dry (30% w/w) and wet algae biomass (*Phaeodactylum tricornutum UTEX 640* and *Chlorella vulgaris*), with biomass/solvent/water ratio (g/ml/ml) of 1/14/0 for dry biomass and 1/12/3 for wet biomass. Extraction temperatures were chosen between 50 and 150°C. Static extraction time was set to 20 min with a rinse volume of 60% and a nitrogen purge time of 300 s. The solvents used, where the following: ethanol, ethyl acetate and hexane. The more suitable extraction for triacyl glycerides (TAG) from wet biomass of *C. vulgaris*, was achieved with medium-polar solvents like ethyl acetate. Fatty acid yields of above 75% w/w were achieved for wet biomass of both microalgae in a single extraction step at temperatures of up to 150 °C.

Park et. Al., 2020, use for dry extraction 1 g of *N. oceanica* and hexane (96%), mixture of hexane and methanol (99.6%) (7:3, v/v), and mixture of chloroform (99.0%) and methanol (7:3, v/v). The total volume of each solvent was 40 mL. For wet extraction, 1 g of dry microalgae was at first mixed with 4 g of distilled water having a concentration of 200 g/L (80% water content). Amount of 5 g of wet microalgae was mixed with the solvents mention for dry extraction and also the total volume of each solvent was 40 mL. The samples were stirred at 1,000 rpm for 6 h at room temperature, and then distilled water was added for separation of the organic solvent layer, and after that 4.000 rpm centrifugation for 5 min. The best results of microalgae oil were obtained with hexane-methanol extraction.

CELL DISRUPTION PROCEDURES AND ASSOCIETED RESULTS

According to Laura Soto-Sierra et al. (2018), for releasing an intracellular compound, a disruption treatment is necessary. Preferably, one that selectively releases the compound using the least possible energy (Figure 1).

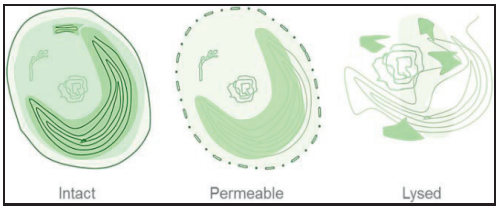

Figure 1. Levels of cell disruption. From non-disrupted (left) to complete cell disruption-lysis (right) (LauraSoto-Sierra et al., 2018)

Choosing a cell disruption method (Figure 2) may depend on the cell wall structure of the sample, compound location, size, solubility, and applied energy. Based on the disruption force, the methods can be classified as physical (drying, sonication, and pulsed electric field), mechanical (bead milling, homogenization) and chemical/biological (pH shift, enzymes, microwave, etc.).

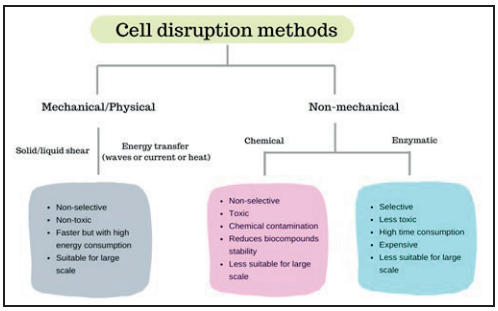

Figure 2. Cell disruption methods
(www. https://encyclopedia.pub/, 2022)

Bead beating includes mechanical stirring and grinding that cause disruption to the cell (ShunyuYao et al., 2018). Bead mill or bead beating are shaking vessels filled with quartz or metal, in which microalgae biomass are disrupted by agitation, friction, collision and grinding, mechanical stirring. Therefore, cells are damaged by direct impact with the beads (steel, zirconium, glass or ceramic) (LauraSoto-Sierra et al., 2018) at high speed. Also, the size of beads and the bead filling ratio are important. For microalgae the optimal beads diameter is 0.5 mm (Ashok Ganesan et al., 2022).
The disadvantage of this method is referring to the difficulty to scale-up (Fabiana Passos, 2015).

Sonication (Figure 3) is a physical treatment based on bubble cavitation by ultrasound waves that promote a non-specific cell-surface barrier disruption (Jose A.Gerde et al, 2012). The ultrasonication method for cell disruption is based on liquid-shear forces caused by emission of high frequency wave sounds.

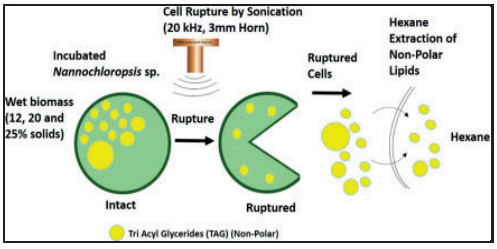

Figure 3. Cell disruption by sonication
(ShunyuYao, et al., 2018)

In liquid, these sound waves create gas bubbles or cavities that achieve a critical size and releasing large amounts of energy; during the extraction local temperature increases and forms hydroxyl radicals that damage the cell wall, and also may produce lipid hydroperoxides (Ying Liu, 2022). Sonication is able to disrupt (Figure 4) the cells at relatively low temperatures avoiding thermal protein denaturation. Using this method is low toxicity, and time saving and can be scaled-up and operated continuously.
Another possibility for cell disruption efficiency is cell weakening by incubation, preceding ultrasound extraction procedure (ShunyuYao et al., 2018). This consists on the dilution of microalgae biomass, obtaining a slurry, and incubated at 40°C on a plate, continuously stirring, during 24 hours.
As procedure, the extraction method is directly corelated to the microscopic evaluation of cell morphology.

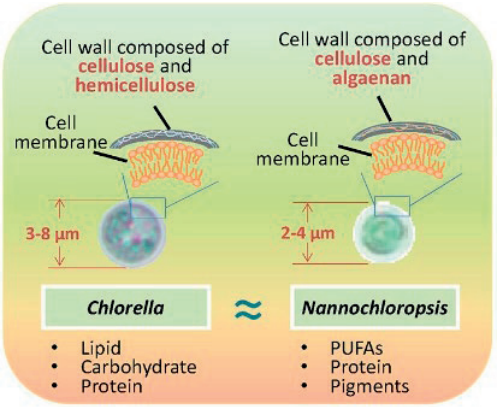

Figure 4. Cell rupture by ultrasonic treatment in *Nannochloropsis* and *Chlorella* species

It was observed that ultrasound could change the external structure of cell surface. The high local temperature and pressure caused by collapsing bubbles could lead to the breaking of *Nannochloropsis* cells into small fragments, and resulted in release of oil into the liquid (Figure 5).
Nevertheless, the extraction procedure involving the organic solvent penetrates the cell membrane and dissolves the lipids as well as the lipoproteins of chloroplast membranes. It has been found that cell disruption efficiency is strongly corelated to chlorophyll and carotenoids content (Aris Hosikian, 2010).

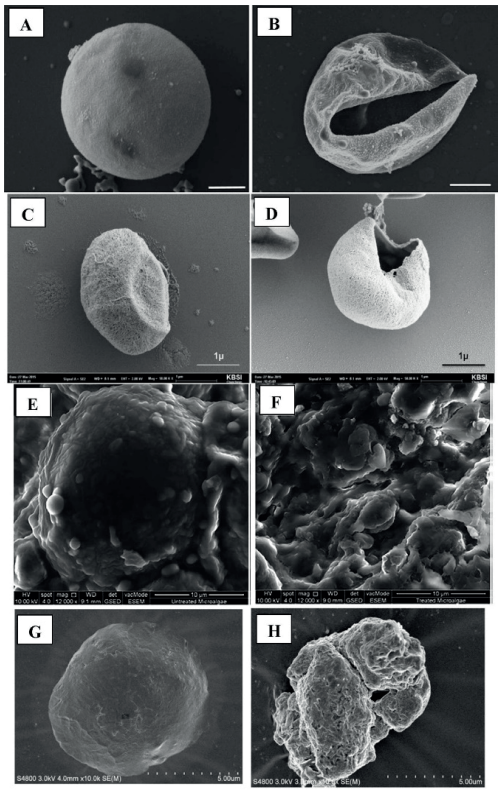

Figure 5. Deformation in cell surface induced by ultrasound (Ying Liu et al., 2022)

This aspect can be an important one in reducing the actual work time and costs, but also the selection of the type of extraction before GC-MS analysis

CONCLUSIONS

Taken together, our data showed that manipulation of photosynthetic pigments, can be an item related to the extraction procedure.
There are several generally recognized as safe solvents and green solvents that are used in the extraction methods for feed and food, but may be used in other components for reducing the environmental impact.
However, the extraction procedure is more efficient when is preceded by cell disruption technique or cell weakening procedure. is not very effective for some microalgae species and it is commonly combined with chemical treatments for efficiency improvement and to reduce energy demand.

ACKNOWLEDGEMENTS

This work was realized within the project Unlocking the potential of microalgae for the valorisation of brewery waste products into omega-3 rich animal feed and fertilisers-AlgaeBrew submitted to Program 3 "European and International Cooperation" organized by UEFISCDI, Project Code ERANET-SUSFOOD-FOSC-AlgaeBrew, within the Research Contract no. 303/2022, stage no. I/2022 (01.04.2022 - 31.12.2022).

REFERENCES

Abimbola. T., Christodoulatos, C. and Lawal, A. (2021). Performance and optimization studies of oil extraction from *Nannochloropsis* spp. and *Scenedesmus obliquus*, *Journal of Cleaner Production*, *311*, 1-14.

Ahmad F. B., Zhang, Z., Doherty, W.O. S. & O'HARA, I.M. (2018). Optimising extraction of microalgal oil using accelerated solvent extraction by response surface methodology. *Journal of Engineering Science and Technology*, *13*(4), 964–976.

Alhattab, M., Kermanshahi-Pour, A. & Brooks, M. S. L. (2019). Microalgae disruption techniques for product recovery: influence of cell wall composition. *J Appl Phycol 31*, 61–88.

Angles E., Jaouen, P., Pruvost, J. & Marchal., L. (2017). Wet lipid extraction from the microalga *Nannochloropsis* sp.: Disruption, physiological effects and solvent screening *Algal Research - Biomass, Biofuels and Bioproducts*, Elsevier, *21*, 27–34.

Adil Mouahid, Kanitta Seengeon, Mathieu Martino, Christelle Crampon, Avery Kramer, et al. (2020). Selective extraction of neutral lipids and pigments from *Nannochloropsis salina* and *Nannochloropsis maritima* using supercritical CO_2 extraction: Effects of process parameters and pre-treatment. *Journal of Supercritical Fluids, Elsevier*, 165, pp. 104934.

Aisamuddin Ardi Zainal Abidin, Mohanrajh Suntarajh, Zetty Norhana Balia Yusof. (2020). Transformation of a Malaysian species of *Nannochloropsis*: gateway to construction of transgenic microalgae as vaccine delivery system to aquatic organisms, *Bioengineered***,** 11, p. 1071.

Ashok Ganesan, Prachi Nawkarkar, Shashi Kumar (2022). Chapter 17 - Algal biodiesel: technology, hurdles, and future directions, Editor(s): Sanjay Sahay, Handbook of Biofuels, Academic Press, Pages 331-351, ISBN 9780128228104.

Vítor, A. C. M., Francisco, A. E., Silva, J., Pinho, M., Huws, S. A., Santos-Silva, J., Bessa, R. J. B., & Alves, S. P. (2021). Freeze-dried *Nannochloropsis oceanica* biomass protects eicosapentaenoic acid (EPA) from metabolization in the rumen of lambs. Scientific Reports, *11*, 1-17.

Bernaerts T.M.M., Verstreken, H., Dejonghe, C., Gheysen, L., Foubert, I., Tara Grauwet, T. & Van Loey, A.M. (2020). Cell disruption of *Nannochloropsis* sp. improves *in vitro* bioaccessibility of carotenoids and ω3-LC-PUFA, *Journal of Functional Foods*, *65*, 1-10.

Blanco-Llamero, C. & Señoráns, F.J. (2021). Biobased Solvents for Pressurized Liquid Extraction of *Nannochloropsis gaditana* Omega-3 Lipids. *Mar. Drugs*, *19*, 107.

Byrne F. P., Jin,S., Paggiola,G., Petchey, T. H. M. & Clark, J. H. (2016). Thomas J. Farmer, Andrew J. Hunt, C. Robert McElroy & James Sherwood, Tools and techniques for solvent selection: green solvent selection guides, *Sustainable Chemical Processes*, *4*: 7, 1-24.

Chua E. T. & Schenk P. M. (2017). A biorefinery for *Nannochloropsis*: Induction, harvesting, and extraction of EPA-rich oil and high-value protein, *Bioresource Technology*, *244*, Part 2, 1416-1424.

Chua E. T., Shekh A.Y., Eltanahy E., Thomas-Hall S. R. and Schenk P. M. (2020). Effective harvesting of *Nannochloropsis* microalgae using mushroom chitosan: A Pilot-Scale Study. Front. Bioeng. Biotechnol. 8:771.

D'Hondt E., Martín-Juárez J., Bolado S., Kasperoviciene, J. Koreiviene, J. Sulcius, S. Elst, K. & Bastiaens L. (2017). 6 - Cell disruption technologies, Editor(s): Cristina Gonzalez-Fernandez, Raúl Muñoz, In Woodhead Publishing Series in Energy, Microalgae-Based Biofuels and Bioproducts, Woodhead Publishing, 133-154, ISBN 9780081010235.

Derwenskus F., Metz F., Gille A., Schmid-Staiger U., Briviba K., Schließmann U., & Hirth, T. (2019). Pressurized extraction of unsaturated fatty acids and carotenoids from wet Chlorella vulgaris and Phaeodactylum tricornutum biomass using subcritical liquids. *GCB Bioenergy*. *2019*;11: 335–344.

Eikani M.H., Khandan, N., Feyzi, E., & Ebrahimi I. M. (2018). A shrinking core model for Nannochloropsis salina oil extraction using subcritical water, *Renewable Energy*, *131*, 2019, 660-666.

Fabiana Passos, Enrica Uggetti, Hélène Carrère, Ivet Ferrer (2015). Chapter 11 - Algal Biomass: Physical Pretreatments, Editor(s): Ashok Pandey, Sangeeta Negi, Parameswaran Binod, Christian Larroche, Pretreatment of Biomass, Elsevier, Pages 195-226, ISBN 9780128000809.

Herrero, M., Ibáñez, E., Cifuentes, A., & Señoráns, J. (2004). Pressurized liquid extracts from Spirulina platensis microalga. Determination of their antioxidant activity and preliminary analysis by micellar electrokinetic chromatography. *Journal of chromatography. A*, *1047*(2), 195–203.

Hamed Safafar, Michael Z. Hass, Per Møller, Susan L. Holdt 1 and Charlotte Jacobsen (2016). High-EPA biomass from Nannochloropsis salina cultivated in a flat-panel photo-bioreactor on a process water-enriched growth medium. *Marine Drugs* 14, 144.

Hosikian A., Lim S., Halim R., Danquah Michael K. (2010). Chlorophyll extraction from microalgae: A review on the process engineering, *Bioprocess Development for Biofuels and Bioproducts,* 1-12.

Imbimbo, P., D'Elia, L., Liberti, D., Olivieri G.& Monti D.M. (2020). Towards green extraction methods from microalgae learning from the classics. Appl Microbiol Biotechnol 104: 9067–9077.

Iovine A., Cerbone A., Mehariya S., Musmarra D., Casella P. & Molino A. (2019). Effect of mechanical pretreatment on Nannochloropsis gaditana on the extraction of omega-3 by using accelerated solvent extraction technology, *Chemical engineering transactions*, *74*. 943-948.

Jose A. Gerde, Melissa Montalbo-Lomboy, Linxing Yao, David Grewell, Tong Wang. (2012). Evaluation of microalgae cell disruption by ultrasonic treatment, Bioresource Technology, 125:175-181, ISSN 0960-8524.

Laura Soto-Sierra, Petya Stoykova, Zivko L.Nikolov (2018). Extraction and fractionation of microalgae-based protein products, *Algal Research*, 36, 175-192.

Lorenzo Zanella, Fabio Vianello, Microalgae of the genus Nannochloropsis: Chemical composition and functional implications for human nutrition, *Journal of Functional Foods*, 68, 103919.

Matos Â. P., Teixeira M. S., Corrêa F. M. P. S., Machado M. M., Werner R. I. S., Aguiar A. C., Cubas A. L. V., Sant'Anna E. S.& Moecke E. H. S. (2019). Disruption of *Nannochloropsis gaditana* (Eustigmatophyceae) rigid cell wall by non-thermal plasma prior to lipid extraction and its effect on fatty acid composition, *Brazilian Journal of Chemical Engineering 36*(4). 1419–1428.

Montoya-Arroyo, A.; Lehnert, K.; Muñoz-González, A.; Schmid-Staiger, U.; Vetter, W.; Frank, J. (2022). Tocochromanol profiles in *Chlorella sorokiniana*, *Nannochloropsis limnetica* and *Tetraselmis suecica* confirm the presence of 110 -α-tocomonoenol in cultured microalgae independently of species and origin. *Foods*, 11, 396.

Nagappan S., Saravanan Devendran S., Tsai P-C., Dinakaran S., Dahms H-U. & Ponnusamy, V.K. (2019). Passive cell disruption lipid extraction methods of microalgae for biofuel production - A review, *Fuel*, *252*. 699-709.

Naghdi G., F., González González L. M., Chan W., & Schenk P. M. (2016). Progress on lipid extraction from wet algal biomass for biodiesel production. *Microbial biotechnology*, *9*(6), 718–726.

Nashwa A. H. Fetyan, Abo El-Khair B. El-Sayed, Fatma M. Ibrahim, Yasser A. Attia, Mahmoud W. Sadik. (2022). Bioethanol production from defatted biomass of Nannochloropsis oculata microalgae grown under mixotrophic conditions. *Environmental Science and Pollution Research*, 29: 2588–2597.

Park, J.-Y., Kim, M.-C., Cheng,J., Yang,W. & Kim, D-K. (2020). Extraction of microalgal oil from Nannochloropsis oceanica by potassium hydroxide-assisted solvent extraction for heterogeneous transesterification. *Renewable Energy*. *162*, 2056-2065.

Salbitani G., Del Prete F., Carfagna S., Sansone G., Barone C. M. A. (2021). Enhancement of pigments production by Nannochloropsis oculata cells in

response to bicarbonate supply. *Sustainability*, 13: 11904.

Sánchez-Camargo A., Pleite N., Mendiola J. A., Cifuentes A., Herrero M., Gilbert-López B., & Ibáñez, E. (2018). Development of green extraction processes for Nannochloropsis gaditana biomass valorization. *Electrophoresis*, 10.1002/elps.201800122.

Quesada-Salas, M.C., Delfau-Bonnet, G.; Willig, G.; Préat, N.; Allais, F.; Ioannou, I. (2021). Optimization and comparison of three cell disruption processes on lipid extraction from microalgae. *Processes*. 9: 369.

Ranjith Kumar R, Hanumantha Rao P and Arumugam M. (2015). Lipid extraction methods from microalgae: a comprehensive review. *Front. Energy Res*. 2:61.

Ren, X., Wei, C., Yan, Q., Shan, X., Wu, M., Zhao, X.& Song, Y. (2021). Optimization of a novel lipid extraction process from microalgae. *Sci Rep 11*, 20221.

Shunyu Yao, Srinivas Mettu, Sam Q.K. Law, Muthupandian Ashokkumar, Gregory J.O. Martin. (2018). The effect of high-intensity ultrasound on cell disruption and lipid extraction from high-solids viscous slurries of *Nannochloropsis* sp. biomass, *Algal Research*, 35: 341-348.

Tocher D. R., Betancor M.B., Sprague M., Olsen R.E., Napier J.A. (2019). Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: Bridging the gap between supply and demand. *Nutrients.* 11(1):89.

Wang, Q., Oshita, K. & Takaoka, M. (2021). Effective lipid extraction from undewatered microalgae liquid using subcritical dimethyl ether. *Biotechnol Biofuels*, *14*, 17.

Ying L., Xin Liu., Yan C., Wenqiao Y. (2022). Ultrasound for microalgal cell disruption and product extraction: A review, *Ultrasonics Sonochemistry*, 87, 106054, ISSN 1350-4177.