

# Evidence Of The Drying Technique's Impact On The Biomass Quality Of *Tetraselmis Subcordiformis* (Chlorophyceae)

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## Research Article

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# Abstract

Rapid drying, cost-effective and safe, will increase the viability of using microalgae for several bio-industrial applications. In this study, five different drying techniques of microalgal biomass were investigated. These include freeze-drying, oven drying, air drying, sun drying, and microwave drying. Morphology, metabolite content, FAME profiling, chlorophyll content, total organic carbon, and total nitrogen were analyzed. Results showed that the freeze-drying technique preserves the highest amounts of chlorophyll, proteins, and lipids. Oven drying underperformed as it retained the lowest amount of chlorophyll, protein, and lipid content. More importantly, FAME profiling results showed that air drying was the best technique in maintaining the highest amount of Polyunsaturated fatty acids and more specifically docosahexaenoic acid. Furthermore, this process requires the least capital and energy needs. The findings from this study confirmed that the drying technique affects the microalga biomass quality.

## 1. Introduction

Microalgae have drawn the attention of many researchers in recent times as a reliable and renewable source of energy (Ma et al., 2022). They have also been introduced as the third-generation biofuel and have been known to yield 30 times more energy per area unit compared to first- and second-generation biofuels (Chamola et al., 2019). Furthermore, they have a higher growth rate, carbon fixing ability and elevated lipid production compared to terrestrial plants (Show and Lee., 2014).

The major components of interest harvested from microalgae include proteins, lipids, carbohydrates and minor concentrations of vitamins, pigments, and sterols (Shaikh et al., 2021). These valuable products can be used to produce feedstocks for food and feed supplements, nutraceuticals, pharmaceuticals, and as chemicals (Lammens et al., 2012).

Microalgae can be cultivated at a large scale in photobioreactors or raceway ponds (Shi et al., 2018). This involves various stages, starting with the cultivation of the specific strain followed by harvesting through a series of steps including biomass separation, screening, thickening, dewatering, and drying and lastly biorefinery for extracting the products of interest (Show and Lee., 2014). It is crucial to optimize these steps for the efficient production of high-quality algal biomass. As dewatering and drying are vital elements in downstream extraction, it is important to consider the different techniques and their impacts on the overall production energy and cost and more importantly on the quality of biomass, products, and metabolites.

Dewatering the algae harvest efficiently ensures effective processing in downstream drying processes. Reduction of water through dewatering ensures lower cost and energy needs in overall drying steps as it aims to remove most of the water from the algae harvest (Show et al., 2015b). Dewatering requires approximately 20–40% of the energy requirements of the whole microalgal harvesting process (Yazdanabad et al., 2021). Although it is achieved through various mechanical processes such as filtration and centrifugation, a major setback has remained to be poor economic feasibility (Sharma et al.,

2013). Additionally, contamination risks need to be eliminated in case of production for human or animal consumption (Yazdanabad et al., 2021).

The drying process is also important as the dewatering of microalgae. It is also considered a crucial step as the algae slurry achieved from the upstream harvesting processes can be fragile. According to (Patil et al., 2008) drying process requires the most energy accounting for over 80% of the total cost of algal products such as biodiesel production. As algae can be susceptible to microbial contamination, mechanical damage and adversary storing conditions, which may lower the quality of products, it is important to dry algae efficiently for optimal storage (De Farias Neves et al., 2020). There are many ways of drying algae including conventional sun drying, hot air drying, freeze-drying, microwave drying, oven drying and spray drying (Brennan Owende, 2010).

The most traditional method of drying algae is sun-drying. The dewatered microalgae biomass is kept outside until the water content decreases to an acceptable limit. Oven drying utilizes high-temperature exposure for long periods of time to remove moisture from the algae biomass (Badmus et al., 2019). While freeze-drying is conducted by introducing the feed to lower temperatures to dry out and separate moisture from the biomass. Spray drying involves droplet/gas mixing, liquid atomization, and drying liquid droplets. Water droplets are atomized and sprayed down into a vertical tower that contains hot gases. The dried algae biomass is collected from the bottom of the tower and is available within a few seconds of the drying process (Hosseinizand et al., 2017).

Conventional methods such as sun drying, and oven drying are usually pursued as these methods do not require high energy and capital input. But these methods may not be preferred for reasons such as susceptibility to contamination from outside sources such as birds, insects, and microorganisms in the case of sun-drying (Show et al., 2015a). Furthermore, the method heavily relies on the weather, as it may not be feasible for areas with high rains and low sunlight zones. Another reason is the degradation of pigments such as chlorophyll due to direct solar radiation (Wang et al., 2019). Additionally, oven drying can negatively impact the heat-labile metabolites and bioactive compounds (Kadam et al., 2015). On the other hand, processes such as freeze-drying, and spray drying have become more common for drying the algae biomass. Freeze drying can be one of the safest forms of drying in terms of retaining important byproducts which may be lost otherwise. While spray drying process can be time efficient and produce high-value products (Molina Grima et al., 2003). The disadvantages of these methods are high operational and maintenance costs. Additionally, the spray drying method includes high-pressure mechanisms, which may rupture cells and degrade high value-added components such as pigments (Hosseinizand et al., 2018).

In a study conducted by (Ryckebosch et al., 2011) spray and freeze-drying of microalgae were conducted to investigate the effect on lipid and carotenoid stability. It was found that algae paste that was freeze-dried showed signs of lipolysis when stored, leading to lower lipid content. On the other hand, microalgae that were spray-dried were more susceptible to oxidation as there was the breakdown of carotenoids. While Morist et al., (2001) investigated spray drying influence on the fatty acid profile of *Arthrospira*

plants, no significant changes were found in the content of common fatty acids such as linoleic acid, palmitic acid and palmitoleic acid so on. In another study, freeze and air drying were compared for future commercialization prospects. Two microalgae strain *Chaetoceros sp.* and *Phaeodactylum tricornutum* were used and lipids were profiled. It was found that air drying led to the loss of almost 70% of the lipids compared to freeze-drying (Esquivel et al., 1993). Another effect that was noted by Oliveira et al., (2010) of air drying was oxidation of the biomass.

Finally, the choice of drying depends on the capital and energy sources available and on the importance of byproducts that need to be successfully attained from the algae harvest (Chen et al., 2015). Even though downstream processes such as dewatering and drying can be vital in the extraction of valuable high-quality algal biofuels and feedstocks for food supplements or animal feed, there is a lack of research outlining the importance of these processes (Ryckebosch et al., 2011).

This study aims to investigate the effect of five different drying techniques on the nutritional quality of the microalgae biomass. For that purpose, an assessment of the Chlorophyll, proteins, lipids, and FAME content was performed to select the most suitable drying technique leading to maintaining high-quality biomass that can be used ultimately for animal-feed production therefore it will enhance the viability of the large-scale biomass production under arid climate.

## 2. Material And Methods

### 2.1. Microalgae Cultivation

One single colony of the local marine strain *Tetraselmis subcordiformis* QUCCCM50 was used to inoculate 5ml f/2 growth medium. The culture was incubated for 7 days in an illuminated shaker (Innova 44R, New Brunswick Scientific, USA) under the agitation of 150 rpm, an illumination of  $100 \mu\text{mol s}^{-1} \text{m}^{-2}$  with a light-dark cycle of 12:12 h and a temperature of 30°C, corresponding to the annual average temperature in Qatar. Subsequently, the culture was scaled up to 50ml then 200ml then 1L. All these cultures were incubated under the same previously described conditions. The 1L culture was used to inoculate 5L f/2 growth medium prior to being incubated under air bubbling, the light of  $400 \mu\text{mol s}^{-1} \text{m}^{-2}$  and room temperature. Ultimately, the 5L was scaled up to 20L and incubated under the latter conditions of light, temperature, and air bubbling. The cultures were performed in triplicate.

### 2.2. Drying techniques of the microalgae biomass

Five different techniques were tested such as (i) Freeze-drying (FD); (ii) Air Drying (AD); (iii) Sun drying (SD); (iv) Oven drying (OD) and (v) microwave drying (MD). For that purpose, 1L sample of *T. subcordiformis* culture was collected from each tank on the same day for each technique to be tested. The drying was performed in triplicate using 1L from each tank of 20L (1L sample per tank, and three replicates per drying technique). After harvesting using a cold centrifuge (SL16R, thermo-fisher) the wet biomass was spread into a glass plate prior to being dried via the following techniques:

- Freeze-drying (FD): After an ON incubation at – 80°C, the biomass will be transferred into a freeze dryer (Labconco, 7754067) for a duration of 48H,
- Sun drying (SD): wet biomass was incubated directly under the sun for 48H in a location that allows the maximum of illumination hours,
- Air drying (AD): biomass will be covered with 4 layers of aluminium and incubated at room temperature for 48H,
- Microwaves drying (MD): wet biomass was incubated in an oven (Panasonic MS3047) for a duration of 2H with a successive incubation of 15min until completely dry.
- Oven drying (OD): the biomass was incubated for 48H in an oven (Binder EP-53) under 90°C.

Treatments were performed in parallel and stopped after confirming a stable weight of the dry biomass. Morphological characterization of *T. subcordiformis* under the different drying techniques.

The morphology of the dried biomass of *T. subcordiformis* was investigated via light microscopy using light-microscopy (40×, Primo Star HAL Microscope, full Köhler, stage drive R, FOV 20, Carl Zeiss, Germany) and Scanning electron microscopy (SEM). For the SEM characterization, the dried algal cells issued from the different drying techniques were dehydrated in gradually increasing ethanol concentrations (up to 96% ethanol) then, transferred in formaldehyde dimethyl acetal for 24 and 2 hours, critical point dried with CO<sub>2</sub>, sputter coated with palladium/gold and examined with a (Nova™ Nano SEM 50 Series).

## 2.3. Metabolite's characterization

The total lipids were extracted from the dried algae biomass issued from the six previously described drying methods using a modified method of Folch et al., 1957). After extraction and drying, the total amount of lipid was determined gravimetrically and the lipid content (%) was determined using the following equations according to Arora et al., 2016):

$$\text{Lipid content (\%)} = \text{Total lipids (g)} / \text{Dry biomass (g)}$$

The total proteins content (% per g dry biomass) was assessed as per Saadaoui et al., (2016). 100 mg of dry microalgae biomass was subjected for total proteins extraction using a Sigma kit (Plant Total Protein Extraction PE0230, USA). Then, the total proteins were quantified using the Bradford assay (Bradford 1976). Chlorophyll extraction and quantification

50 mg of the dried *T. subcordiformis* issued from the 6 different methods tested were suspended in 1 mL of methanol 90% and kept at 60°C water bath until the biomass was colorless for chlorophyll extraction. Then, the mixture was centrifuged at 1500×g for 5 min after reaching room temperature. The chlorophyll concentration was calculated using the equations described by Porra et al., 1989) after reading the

extract absorption at different wavelengths (650 and 665 nm) using a spectrophotometer (Jenway, 6305, UK).

## 2.4. FAME profile determination

Fatty acid methyl esters (FAMES) were extracted using a one-step transesterification method (Saadaoui et al., 2016). For that purpose, 50 mg of dried biomass issued from the different drying techniques was added to an adequate volume of sulfuric acid (95%) and methanol (H<sub>2</sub>SO<sub>4</sub>:CH<sub>3</sub>OH, 1:10). Then, after 10 min of sonication (Branscon 1510, Mexico), the mixture was incubated for 2h at 80°C then transferred to a centrifuge tube containing distilled water and a mixture of hexane: chloroform (4:1). Finally, the FAME fraction was stabilized by the addition of BHT, followed by filtration, prior to GC-FID (Shimadzu, Japan) analysis.

## 2.5. Total Organic Carbon and Total Nitrogen of *T. subcordiformis*

Sample preparation included grinding the dried sample using a mortar and pestle. For determining the total organic carbon (TOC), 0.5mg of finely ground sample was placed into silver capsules. Acid (HCl) was added to the sample to eliminate all inorganic carbon in the form of carbon dioxide. The capsules were closed and fed into the autosampler of the FLASH 2000 NC Soil Analyzer (Thermo Scientific™, United States). For determination of Total Nitrogen (TN), 0.5 mg of dried finely ground samples were placed into tin capsules. The capsule was directly fed into the autosampler of the FLASH 2000 CHNS/O Organic Elemental Analyzer (Thermo Scientific™, United States).

## 2.6. Statistical analysis

All the drying techniques as well as the metabolic characterization were performed in triplicate. The reported values are the means of three independent samples while the error bars represent standard error. One-way ANOVA was used to determine significance difference ( $\alpha = 0.05\%$ ) between means.

# 3. Results

### 3.1. Characterization of the biomass and the cell morphology of *T. subcordiformis* after applying the different drying techniques.

The assessment of the *T. subcordiformis* biomass quality generated from different techniques evidenced different colours and textures. The biomass issued from freeze drying was light green like the wet biomass. While the other four techniques led to biomass with a dark green colour. We also noticed that the biomass issued from the air drying, sun drying and oven drying led to very dry and hard biomass. Also, sun drying led to the appearance of a specific orange colour that might correspond to carotenoids (Fig. 1A).

Furthermore, we noticed that the effect on the *T. subcordiformis* cell morphology was specifically related to the drying technique applied. Results proved that freeze-dried cells presented similar morphology to the

wet cells with a length of 10mm. However, cells subjected to microwave and oven drying showed smaller sizes with almost no internal vesicles. An irregular shape with a small size was observed in the case of sun-drying with the appearance of orange vesicles. However, air-dried cells presented regular size and shape but with fewer vesicles (Fig. 1B). The Scanning electron microscopy also evidenced differences (Fig. 1C). Both freeze and sun-dried cells were separated from each other's. The freeze-dried cells were intact; however, the sun-dried cells were broken. The other three techniques led to cells piled together.

## 3.2. Chlorophyll content of the biomass after different drying techniques

Results proved the presence of significant difference between the five different techniques in terms of Chlorophyll content ( $a < 0.05$ ). The highest Chlorophyll amount was observed in the case of the freeze-dried biomass with  $95.27 \pm 3.01 \text{ mg L}^{-1}$  followed by the microwave drying ( $91.63 \pm 0.47 \text{ mg L}^{-1}$ ). The sun and air drying led to a similar chlorophyll amount of  $85 \text{ mg L}^{-1}$ . The lowest amount of chlorophyll was detected in the case of oven drying ( $62.6 \pm 7.9 \text{ mg L}^{-1}$ ) with a decrease of 34% compared to the freeze-dried biomass (Fig. 2).

## 3.3. Assessment of the metabolites content of the biomass after different drying techniques

A significant difference was observed between protein contents of the microalgal biomass obtained after applying the five different drying techniques ( $a < 0.05$ ). The highest protein content was observed in the case of freeze-drying with  $24.17 \pm 0.7 \text{ \% dry wt}^{-1}$ , followed by sun drying ( $22.24 \pm 1.6 \text{ \% dry wt}^{-1}$ ) then oven drying ( $20.8 \pm 1.86 \text{ \% dry wt}^{-1}$ ). These two techniques showed similar results without any significant difference. The least amount was observed in the case of air and microwaves drying showing a similar amount of  $\sim 19 \text{ \% dry wt}^{-1}$  (Fig. 3A).

Similarly to the protein content, a significant difference was observed between the different tested techniques in terms of lipid content ( $a < 0.05$ ) and the highest amount was observed in the case of freeze-dried biomass  $26.51 \pm 2.2 \text{ \% dry wt}^{-1}$  followed by biomass issued from sun and oven drying with a similar amount of  $\sim 2 \text{ \% dry wt}^{-1}$ . The least amount was also observed in the case of air and microwaves drying showing a lipid content of  $\sim 1 \text{ \% dry wt}^{-1}$  (Fig. 3B).

## 3.4. Assessment of the FAME profiling of *T. subcordiformis* after the different drying techniques

Results proved that the FAME composition was heavily affected by the drying technique (Table 1). The highest total amount of FAME was observed in the case of freeze drying (522.65 ppm) followed by air drying (159.44 ppm), then microwaves drying (134.51 ppm). Oven drying and sun drying led to a similar amount of FAME  $\sim 80$  ppm. Regarding PUFAs amount, we noticed that the air drying showed the highest amount of 80.92 ppm, followed by the freeze-drying (68.1 ppm) then microwave drying (50.72 ppm) then

oven drying (39.33 ppm). However, the sun-drying led to the lowest PUFAS amount of 9.23ppm. Moreover, results evidenced that ALA (C18:3) was observed under the five different techniques however significantly different amounts were observed. Freeze-dried biomass showed the highest ALA amount with  $44.09 \pm 4.7$  ppm followed by air-dried biomass ( $25.25 \pm 1.25$  ppm) then microwave drying ( $16.02 \pm 2.9$  ppm). Sun drying and oven drying showed similar and very low ALA amounts of 5 ppm.

More importantly, we noticed that the DHA (C22:6) was present only in the case of air drying, microwave drying, and the highest amount was observed in the case of air drying ( $9.89 \pm 3.45$  ppm). Finally, we noticed that sun drying and oven drying resulted in the total absence of the DHA.

Table 1  
FAME profiling of the *T. subcordiformis* biomass obtained after the 6 different drying techniques

FAME (ppm)	Freeze drying	Sun drying	Oven drying	Air drying	Microwaves drying
C16:0	481 ± 46.56	32.68±1.6	30.32±5.16	52.92±1.34	58.81±7.7
C16:1	17.56±1.41	-	-	-	3.09±0.9
C18:0	30.71 ± 3.00	2.86±0.1	5.08±0.82	4.80±0.47	5.74±0.7
C18:1 (Oleate)	14.58±1.14	3.48±0.9	5.85±3.51	-	-
C18:1 Vaccenate	15.62±3.21	1.36±0	1.31±0.27	2.46±0.16	2.36±0.3
C18:2	9.99±2.21	0.54±0.1	0.63±0.38	4.12±0.3	4.50±0.5
C18:3 (ALA)	44.09±4.7	4.61±0.2	6.41±1.31	25.25±1.25	16.02±2.9
C20:0 Arachidate	4.32±0.82	7.09±0.4	7.71±1.31	7.93±0.24	6.65±1.2
C20:1	8.13±1.48	-	-	4.24±0.15	4.19±0.5
C20:2	14.02±4.74	4.08±0.2	5.15±0.87	18.61±0.68	12.49±1.9
C20:4	-	-	27.15±4.59	23.05±1.79	12.99±2.5
C22:6 (DHA)	-	-	-	9.89±3.45	4.72±1
C24:0	6.62±0.92	23.26±1.6	-	6.17±1.47	2.94±0.2
<b>Total FAME</b>	522.65	79.96	89.59	159.44	134.51
<b>SFA</b>	522.65	65.89	43.11	71.82	74.14
<b>MUFA</b>	55.89	84.80	96.75	166.14	144.17
<b>PUFA</b>	68.1	9.23	39.33	80.92	50.72

### 3.5. Total Carbon and Nitrogen of the microalgae biomass after the different drying techniques

Results proved the absence of significant difference in terms of nitrogen and hydrogen amounts of the microalgae biomass obtained after the 6 different drying techniques (Table 2). However, slight differences were observed in the case of total Organic carbon. Freeze drying led to the highest TOC of (35.18% weight basis) and microwave drying led to the lowest (30.70 ± 0.03 % weight basis). The four other techniques led a very similar TOC of ~ 33% weight basis.

Table 2

Microalgae Biomass properties after the different drying techniques. N: Nitrogen, H: Hydrogen, TOC: Total organic Carbon

	<b>N % dry wight basis</b>	<b>TOC % dry weight basis</b>	<b>H % dry weight basis</b>
<b>Freeze drying</b>	4.62 ± 0.04	35.18 ± 0.06	6.21 ±0.06
<b>Sun drying</b>	5.06 ± 0.03	32.93 ± 0.5	5.97 ± 0.06
<b>Air drying</b>	5.06 ± 0.12	32.84 ± 0.15	6.12 ± 0.06
<b>Microwaves drying</b>	4.48 ± 0.01	30.70 ± 0.033	5.14 ± 0.08
<b>Oven drying</b>	5.36 ± 0.1	34.04 ±0.15	5.85 ± 0.14

## 4. Discussion

This study investigates the various drying techniques to determine the best possible method for the preservation of strain and biomass quality. Extensive analyses were conducted to view all the different possible changes the various drying techniques can produce. Produced biomass and morphology assessment showed that the most suitable method for drying is freeze-drying as it preserves the size of the microalgae, which was similar to cells from the wet biomass (Fig. 1B). Our study aligns with Min et al., (2022) study, which states that freeze-drying does not harm the strains cell wall, hence producing a high-value product. On the other hand, air drying, sun drying and oven drying technique which are the easiest to perform produced extremely dry and stiff biomass. Scanning electron microscopy confirmed this constatation as it revealed a surface with cells attached to each other in the form of a membrane. The morphology of air-dried cells showed less vesicle formation, and sun-dried cells showed irregular cell shape with orange vesicle formation. These changes may be due to internal changes in the cell, for example, vesicle loss from air dried samples may be the reason for low lipid content in the biomass obtained and sun drying may have led to the deterioration of green pigments and production of carotenoids, as response to the light stress, leading to change in colour, also noted in Hosseinizand, et al., (2017) study. The presence of carotenoids was confirmed by spectral scanning showing high peak of absorbance at 420nm. These pigments were released outside the cells and led to the orange colour of the

sun-dried biomass. This was confirmed by scanning electron microscopy proving the sun-dried cells presented a broken cell membrane (Fig. 1C).

The highest chlorophyll content was visible in freeze dried samples, this was possible as this method is safe and preserves the quality of cells, ensuring the viability of pigments in the biomass and ensuring no degradation takes place. The second-best method for chlorophyll pigment preservation in cells was microwave drying. This may be because it is the least time-consuming method with only 2 hours of drying, which may have ensured that the pigment is not lost. De Farias Neves, et al., (2019) suggest that chlorophyll concentration can decrease significantly after 40°C and that temperature for drying should be optimized beforehand. While the lowest chlorophyll content was found to be, in biomass obtained from oven drying, with 34% less chlorophyll compared to the freeze-drying method. All drying methods other than freeze and air drying, utilize a heat source, which explains the decrease in chlorophyll content for most heat using methods, except for microwave, and this may be due to thermal degradation of the pigments (Silva, et al., 2019; Hosseinizand, et al., 2017). Additionally, a study on *Spirulina* strain showed adverse effects on the cell by products and on pigments at temperatures exceeding 45°C (Sarada, et al., 1999).

The same trend was observed for both lipid and protein content, the maximum value was retrieved from biomass that was freeze dried. The second most effective method for metabolite preservation was noted to be sun drying, followed by oven drying followed by oven and sun drying techniques that presents similar data and the lowest amounts were observed in the case of microwaves and air drying that also present similar results. Our result aligned well with the findings of Desmorieux and Hernandez (2004) who analyzed five different drying techniques on spirulina cyanobacterium and proved that freeze drying retained the highest amount of sugars and proteins.

Our results of higher protein production in high temperature oven drying aligns with the research of Nelson (2015), but it was suggested that this may be an anomaly and may be due to increased signal through higher cell digestibility in high heat.

FAME profiling analysis showed that the provision and absence of different fatty acids depended on the drying method used. For example, even though the total FAME was highest for freeze dried biomass, it retained the lowest amount of MUFAs. Most of the total FAME from freeze dried sample can be attributed to SFAs. Furthermore, highest concentration of ALA was noted but DHAs were completely absent in the freeze-dried biomass. In a study by Nelson, (2015), it was noted that drying using heat above 60°C prevents the conversion of triglycerides to free fatty acids, due to lipase deactivation. This may be the reason for the lowest concentration of FAME for all drying methods which use heat, especially sun drying and oven drying methods. On the other hand, an unexpected finding was the highest concentration of MUFAs and PUFAs found in air dried biomass, also with the highest amount of DHAs. This may be as the sample is dried naturally under room temperature conditions, these fatty acids are preserved and may be heat sensitive, hence the other drying techniques may have reduced them. Following air drying, microwave showed higher concentration of FAME, as suggested by Nelson, (2015) that shorter heating

time at high heat is better than heating for longer time which is the case in oven and sun drying. Accordingly, air drying is the most suitable drying technique to produce algae biomass enriched with omega 3 fatty acids

Overall, through all the analyses conducted, the best suitor for preservation of biomass quality, freeze drying takes the lead, although it does have some disadvantages, which can be significant when deciding which drying method to use. For example, freeze drying requires 45.75 kWh/kg of energy, while comparatively solar and microwave drying require only 0.01–0.1 and 26.2–34.9 kWh/kg respectively (Guldhe et al., 2014). Another disadvantage may be the large capital investment needed for large scale algal product recovery (Ochoa-Martínez et al., 2012). Solar drying is an easy and energy efficient method but requires a large area and can depend on the weather of site, making it unreliable. Furthermore, in our study, solar drying did not provide significant results. Furthermore, it was reported that cell destruction and transmutation can be avoided below a certain critical temperature. Figuring out this temperature can be vital for the strain being used as it will avoid loss of important byproducts produced by the cells (Guldhe et al., 2014).

This study provides a list of analyses which focus on the different byproducts of interest extracted from microalgal biomass such as pigments like chlorophyll, proteins, lipids, and fatty acids more specifically PUFAs such as DHAs and ALAs. As noted in this study, depending on the product of interest, production scale and capital available, drying techniques should be considered accordingly (de Carvalho, et al., 2020). However very few studies are reported on considering the different techniques and their effect on the byproduct yields.

## 5. Conclusion

The drying technique heavily affected the microalgal metabolite composition and more specifically the pigment, metabolites content and the FAME profiling as well. The selection of the best technique depends on the application targeted. Air drying is the most suitable technique to be applied since it is safe to the PUFAs (ALA and DHA), low cost, environment friendly. Accordingly, this would enhance the viability of the large-scale biomass production for high-quality food supplement production.

## Declarations

### Author Contributions:

H.M.J writing—original draft, Conceptualisation, writing—review and editing; M.C data Acquisition, writing—review and editing, SAS writing—original draft preparation; writing—review and editing; TB: data acquisition. I.S: Conceptualisation, Methodology, writing—original draft preparation, writing—review and editing and funding acquisition.

### Competing interests

The authors declare that they have no competing interests

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## References

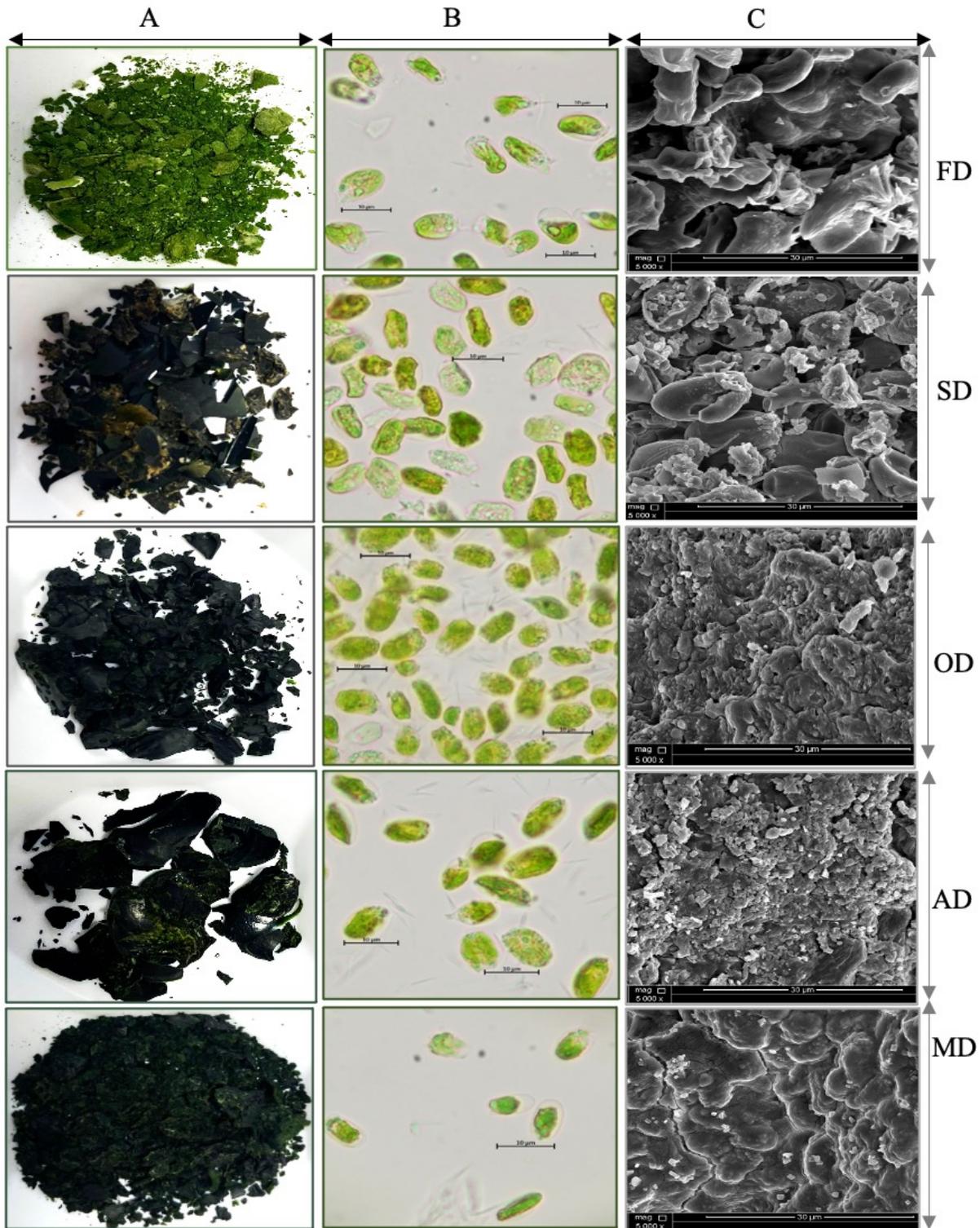
1. Arora, N., Patel, A., Pruthi, P. A., Pruthi, V., 2016. Synergistic dynamics of nitrogen and phosphorous influences lipid productivity in *Chlorella minutissima* for biodiesel production. *Bioresour. Technol.*, 213, 79–87. <https://doi.org/10.1016/j.biortech.2016.02.112>
2. Badmus, U.O., Taggart, M.A.; Boyd, K.G., 2019. The effect of different drying methods on certain nutritionally important chemical constituents in edible brown seaweeds. *J. Appl. Phycol.* 31, 3883–3897. <https://doi.org/10.1007/s10811-019-01846-1>
3. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
4. Brennan, L., Owende, P., 2010. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sust. Energ Rev.* 14, 557–577. <https://doi.org/10.1016/j.rser.2009.10.009>
5. Chamola, R., Khan, Mohd. F., Raj, A., Verma, M., Jain, S., 2019. Response surface methodology-based optimization of in situ transesterification of dry algae with methanol, H<sub>2</sub>SO<sub>4</sub> and NaOH. *Fuel.* 239, 511–520. <https://doi.org/10.1016/j.fuel.2018.11.038>
6. Chen, C.L., Chang, J.S., Lee, D.J., 2015. Dewatering and Drying Methods for Microalgae. *Dry. Technol.* 33, 443–454. <https://doi.org/10.1080/07373937.2014.997881>
7. De Carvalho, J. C.; Magalhães, A. I., de Melo Pereira, G.V., Medeiros, A.B.P., Sydney, E. B., Rodrigues, C., Aulestia, D.T.M., de Souza Vandenberghe, L.P., Soccol, V.T., Soccol, C.R., 2020. Microalgal biomass pretreatment for integrated processing into biofuels, food, and feed. *Bioresour. Technol.* 300, 122719. <https://doi.org/10.1016/j.biortech.2019.122719>
8. De Farias Neves, F., Demarco, M., Tribuzi, G., 2020. Drying and Quality of Microalgal Powders for Human Alimentation. In M. Vítová (Ed.), *Microalgae—From Physiology to Application.*, IntechOpen.

2020. <https://doi.org/10.5772/intechopen.89324>
9. Desmorieux, H., Hernandez, F., 2004. Biochemical and Physical Criteria of Spirulina after Different Drying Processes. Proceedings of the 14th International Drying Symposium (IDS 2004), São Paulo, 22-25 August 2004, 900-907.
  10. Esquivel, B.C., Lobina, D. V., Sandoval, F. C., 1993. The biochemical composition of two diatoms after different preservation techniques. *Comp. Biochem. Physiol. B, Biochem.* 105, 369–373. [https://doi.org/10.1016/0305-0491\(93\)90243-X](https://doi.org/10.1016/0305-0491(93)90243-X)
  11. Folch, J., Lees, M., Sloane Stanley, G.H.A., 1957. Simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226, 497–509.
  12. Guldhe, A., Singh, B., Rawat, I., Ramluckan, K., Bux, F., 2014. Efficacy of drying and cell disruption techniques on lipid recovery from microalgae for biodiesel production. *Fuel.* 128, 46–52. <https://doi.org/10.1016/j.fuel.2014.02.059>
  13. Hosseinizand, H., Lim, C. J., Webb, E., Sokhansanj, S., 2017. Economic analysis of drying microalgae *Chlorella* in a conveyor belt dryer with recycled heat from a power plant. *Appl. Therm. Eng.* 124, 525–532. <https://doi.org/10.1016/j.applthermaleng.2017.06.047>
  14. Hosseinizand, H., Sokhansanj, S, Lim, C.J., 2018. Studying the drying mechanism of microalgae *Chlorella vulgaris* and the optimum drying temperature to preserve quality characteristics. *Dry. Technol.* 36, 1049–1060. <https://doi.org/10.1080/07373937.2017.1369986>
  15. Kadam, S.U., Álvarez, C., Tiwari, B.K., O'Donnell, C.P., 2015. Processing of seaweeds. In *Seaweed Sustainability* (pp. 61–78.) Elsevier. <https://doi.org/10.1016/B978-0-12-418697-2.00004-0>
  16. Lammens, T. M., Franssen, M. C. R., Scott, E. L., Sanders, J. P.M., 2012. Availability of protein-derived amino acids as feedstock for the production of bio-based chemicals. *Biomass Bioenerg.* 44, 168–181. <https://doi.org/10.1016/j.biombioe.2012.04.021>
  17. Ma, X.; Mi, Y.; Zhao, C.; Wei, Q., 2022. A comprehensive review on carbon source effect of microalgae lipid accumulation for biofuel production. *Sc. Total Environ.* 806, 151387. <https://doi.org/10.1016/j.scitotenv.2021.151387>
  18. Min, K.H., Kim, D.H., Ki, M.R. Pack, S.P., 2022. Recent progress in flocculation, dewatering, and drying technologies for microalgae utilization: Scalable and low-cost harvesting process development. *Bioresour. Technol.* 344, 126404. <https://doi.org/10.1016/j.biortech.2021.126404>
  19. Molina, G.E., Belarbi, E.H., Acién Fernández, F.G., Robles Medina, A., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: Process options and economics. *Biotechnol. Adv.* 20, 491–515. [https://doi.org/10.1016/S0734-9750\(02\)00050-2](https://doi.org/10.1016/S0734-9750(02)00050-2)
  20. Morist, A. Montesinos, J. L. Cusidó, J. A. Gòdia, F., 2001. Recovery and treatment of *Spirulina platensis* cells cultured in a continuous photobioreactor to be used as food. *Process Biochem.* 37, 535–547. [https://doi.org/10.1016/S0032-9592\(01\)00230-8](https://doi.org/10.1016/S0032-9592(01)00230-8)
  21. Nelson, J. A., 2015. "Postharvest Degradation of Microalgae: Effect of Temperature and Water Activity". All Graduate Theses and Dissertations. 4458.
  22. <https://digitalcommons.usu.edu/etd/4458>

23. Ochoa-Martínez, C.I., Quintero, P.T., Ayala, A.A., Ortiz, M.J., 2011. Drying characteristics of mango slices using the Refractance Window™ technique. *J. Food Eng.* 109, 69–75.  
<https://doi.org/10.1016/j.jfoodeng.2011.09.032>
24. Oliveira, E.G.; Duarte, J.H.; Moraes, K.; Crexi, V.T.; Pinto, L.A.A., 2010. Optimisation of *Spirulina platensis* convective drying: Evaluation of phycocyanin loss and lipid oxidation: Optimisation of *Spirulina* convective drying. *Int. J. Food Sci. Technol.* 45, 1572–1578.  
<https://doi.org/10.1111/j.1365-2621.2010.02299.x>
25. Patil, V.; Tran, K.-Q.; Giselrød, H. R., 2008. Towards Sustainable Production of Biofuels from Microalgae. *Int. J. Mol. Sci.* 9, 1188–1195. <https://doi.org/10.3390/ijms9071188>
26. Porra, R. J.; Thompson, W. A.; Kriedemann, P. E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. et Biophys. Acta (BBA) - Bioenerg.* 975, 384–394.  
[https://doi.org/10.1016/S0005-2728\(89\)80347-0](https://doi.org/10.1016/S0005-2728(89)80347-0)
27. Ryckebosch, E., Muylaert, K., Eeckhout, M., Ruysen, T., Foubert, I. 2011. Influence of Drying and Storage on Lipid and Carotenoid Stability of the Microalga *Phaeodactylum tricornutum*. *J. Agric. Food Chem.* 59, 11063–11069. <https://doi.org/10.1021/jf2025456>
28. Saadaoui, I., Al Ghazal, G., Bounnit, T., Al Khulaifi, F., Al Jabri, H., Potts, M., 2016. Evidence of thermo and halotolerant *Nannochloris* isolate suitable for biodiesel production in Qatar Culture Collection of Cyanobacteria and Microalgae. *Algal Res.* 2016, 14, 39–47.  
<https://doi.org/10.1016/j.algal.2015.12.019>
29. Sarada, R., Pillai, M. G., Ravishankar, G. A., 1999. Phycocyanin from *Spirulina* sp: Influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. *Process Biochem.* 34, 795–801. [https://doi.org/10.1016/S0032-9592\(98\)00153-8](https://doi.org/10.1016/S0032-9592(98)00153-8)
30. Shaikh, S. M. R., Hassan, M. K., Mustafa N.S., S., Sayadi, S., Ayesha, A. I., Vasagar, V., 2021. A comprehensive review on harvesting of microalgae using Polyacrylamide-Based Flocculants: Potentials and challenges. *Sep. Purif. Technol.* 277, 119508.  
<https://doi.org/10.1016/j.seppur.2021.119508>
31. Sharma, K. K., Garg, S., Li, Y., Malekizadeh, A., Schenk, P. M., 2013. Critical analysis of current Microalgae dewatering techniques. *Biofuels.* 4, 397–407. <https://doi.org/10.4155/bfs.13.25>
32. Shi, Z., Zhao, B., Tang, S., Yang, X. 2013. Hydrotreating lipids for aviation biofuels derived from extraction of wet and dry algae. *J. Clean. Prod.* 204, 906–915.  
<https://doi.org/10.1016/j.jclepro.2018.08.351>
33. Show, K.Y., Lee, D.J., 2014. Algal Biomass Harvesting. In *Biofuels from Algae* (pp. 85–110. Elsevier. <https://doi.org/10.1016/B978-0-444-59558-4.00005-X>
34. Show, K.Y., Lee, D.J., Mujumdar, A. S. 2015. Advances and Challenges on Algae Harvesting and Drying. *Dry. Technol.* 33, 386–394. <https://doi.org/10.1080/07373937.2014.948554>

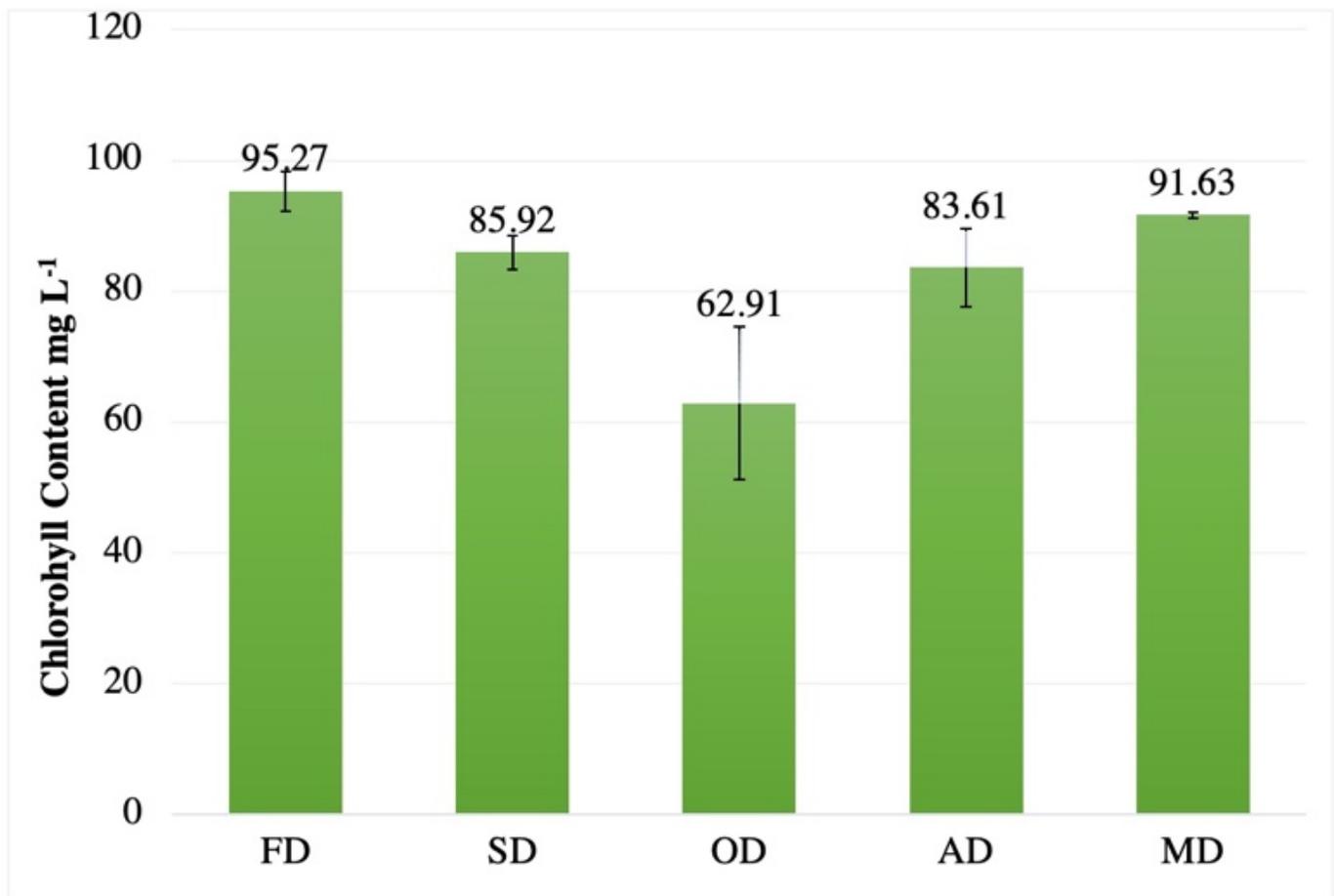
35. Show, K.Y., Lee, D.J., Tay, J.H., Lee, T.M., Chang, J.S., 2015 Microalgal drying and cell disruption – Recent advances. *Bioresour. Technol.* 2015; 184, 258–266.  
<https://doi.org/10.1016/j.biortech.2014.10.139>
36. Silva, A., Abreu, H, Silva, A., Cardoso, S., 2019. Effect of Oven-Drying on the Recovery of Valuable Compounds from *Ulva rigida*, *Gracilaria* sp. And *Fucus vesiculosus*. *Mar. Drugs.* 17, 90.  
<https://doi.org/10.3390/md17020090>
37. Wang, J., Zhang, M. Fang, Z. Recent development in efficient processing technology for edible algae: A review. *Trends Food Sc. Technol.* 2019, 88, 251–259. <https://doi.org/10.1016/j.tifs.2019.03.032>
38. Yazdanabad, S. K., Samimi, A., Shokrollahzadeh, S., Kalhori, D. M., Moazami, N., Ibáñez González, M. J., Mazzuca Sobczuk, T., Molina Grima, E., 2021. Microalgae biomass dewatering by forward osmosis: Review and critical challenges. *Algal Res.* 2021, 56, 102323.  
<https://doi.org/10.1016/j.algal.2021.102323>

## Figures



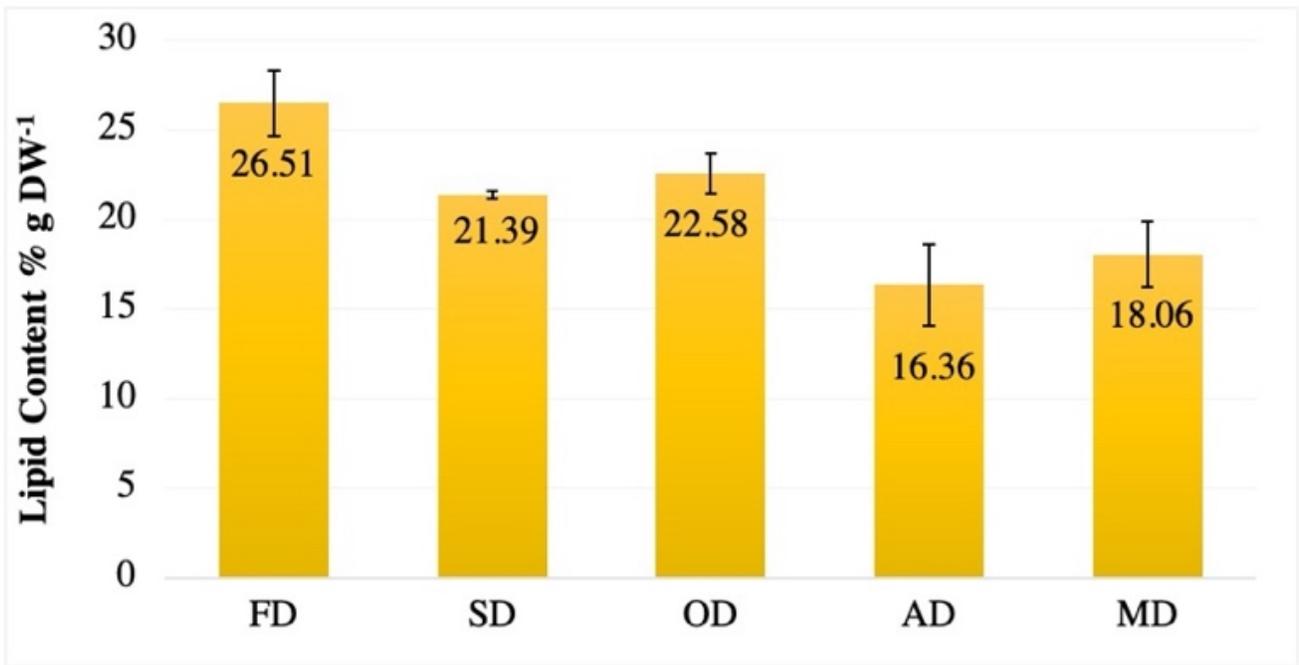
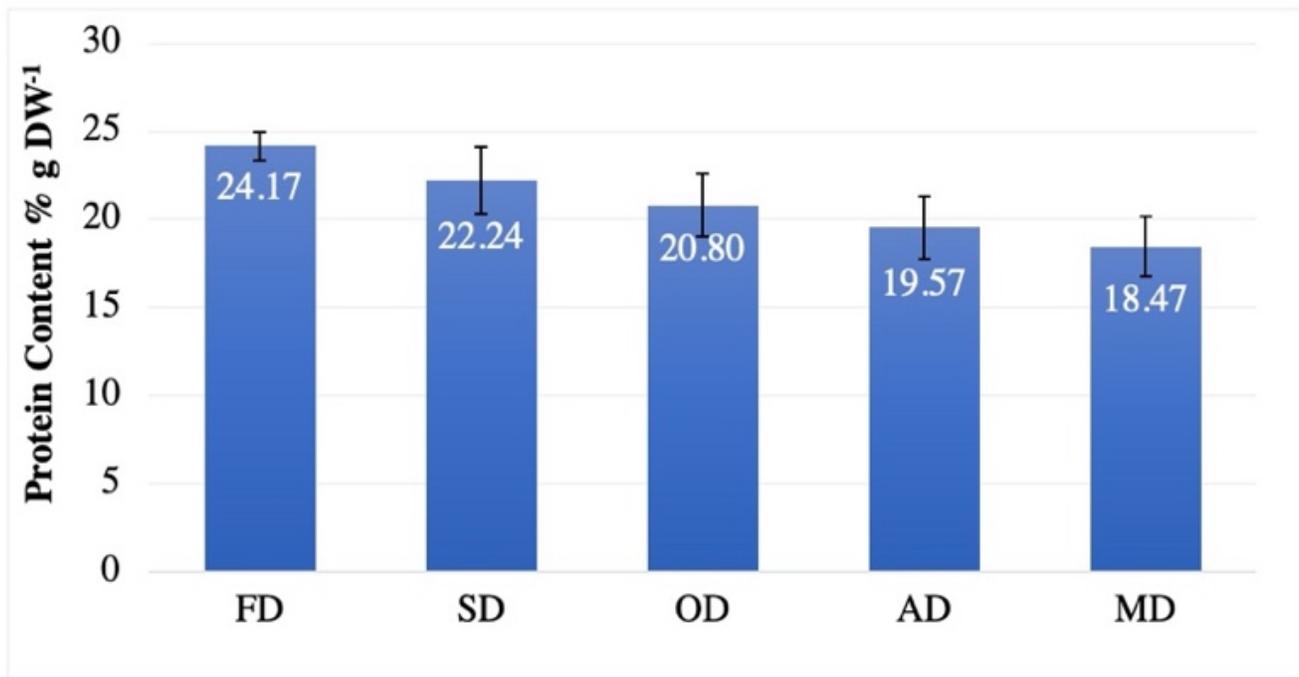
**Figure 1**

Biomass and cell morphology after different drying techniques. A: Biomass characterization and B: Cell morphology under light microscopy with a magnification of 100x. C: Scanning Electron Microscopy HV: 10.00 kV, WD: 5.0 mm and magnification: 5000X. FD: freeze drying; MD: Microwave drying; AD: Air drying; SD: Sun drying; OD: Oven drying.



**Figure 2**

Chlorophyll content of the microalgae biomass after different drying techniques. FD: freeze drying; SD: sun drying; OD: oven drying; AD: air drying; MD: microwave drying



**Figure 3**

Metabolite content of the *T. subcordiformis* biomass dried under the different drying techniques.

A: content and B: Lipid content. FD: freeze drying; MD: microwave drying; SD: sun drying; OD: oven drying; AD: air drying

## Supplementary Files

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