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Recovery of microalgal biomass and metabolites from homogenised, swirl flash dried microalgae

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Abstract: Production of intracellular metabolites from microalgae involves various processing steps. Since algal drying and cell disruption are integral processes of the these operations, effects of novel swirl flash drying technique and cell cracking by high pressure liquid shear method were tested. Variations in biomass composition (focusing on the fatty acid and pigment composition) of two microalgal species, *Chlorella pyrenoidosa* and *Nannochloropsis salina*, were studied in order to investigate effects of novel drying technique, including whether recovery of bioactive compounds is more efficient on dried or wet biomass. Applying novel swirl flash drying technique showed no significant adverse effect on the fatty acid composition, including heat sensitive eicosapentaenoic acid (EPA), of tested microalgal species. Pigment and tocopherol composition of *C. pyrenoidosa* showed tendency to degrade after applying both cell cracking and drying treatment. Considering these data swirl flash drying technique has a potential as a new drying technique for microalgae biomass.

Key words: *Chlorella pyrenoidosa*, *Nannochloropsis salina*, algal drying, fatty acids, pigments

1. Introduction

Growing interest in natural and healthy foods and food ingredients is forcing development of novel products with functional ingredients. Microalgae have been recognized as a potential source for various valuable ingredients with positive health effects for commercial applications. These microorganisms are able to produce polyunsaturated fatty acids, pigments, vitamins, peptides and many other bioactive compounds. Production of intracellular metabolites from microalgae involves upstream and downstream processing steps. Upstream processing includes cultivation during which microalgal biomass is being produced, while downstream processing includes harvesting and recovery of the metabolites. After cultivation biomass needs to be harvested, up-concentrated and dried before further processing. Dewatered algae slurry is being dried in order to facilitate transportation, decrease the risk of unwanted deterioration and to extend the shelf life. However, drying of microalgae biomass is still very challenging and requires an innovative answer. Mostly because drying exerts a major economic constraint in the process and it may constitute up to 75% of the overall cost (Shelef et al. 1984; Show et al. 2013). Common drying methods include either natural traditional method as solar drying or using more advanced techniques such as freeze drying, spray drying, drum drying or fluidized bed drying (Show et al., 2015). Solar drying is cost effective compared to other techniques, but is also a relatively slow and unreliable method, which can result in altered nutritional value and safety of the final product. On the other hand, controlled freeze drying keeps the product temperature low enough during the process to avoid changes in the dried product appearance and characteristics. It is a gentle process in and commonly the best choice in order to preserve the quality of the biomass. However, the predominant deficiency of freeze drying is the high operating cost (Grima et al. 2003). Furthermore, if the biomass is not undergoing a heat treatment before freeze drying, the presence of lipolytic enzymes such as lipase will hydrolyse lipids and lead to formation of high levels of free fatty acids, which is undesirable. Spray drying is the most commonly used technique in commercial production, but despite the fact that it is a very efficient drying method, it may cause unwanted cell rupture and result in microalgae biomass degradation (Show et al. 2015). Shortcoming of spray drying may be its high-pressure atomization process, unless atomization is carried out without the use of nozzles which are susceptible to blockage, high capital and energy demands as well as thermal decomposition of bioactive compounds (Orset et al., 1991; Lin 1985)

The main consideration in the selection of the drying technology depends on the production scale and the final purpose for which the microalgae biomass is intended. Important factors to consider are also moisture content, quality requirements and maximum acceptable temperature for preventing thermal damage to the biomass. In this study, a prototype novel swirl flash dryer system, which was designed and constructed in one of our previous projects (Safafar 2017), was used for drying trials. In general, flash drying is achieved by rapid removal of moisture by injecting wet microalgae slurry into a hot air stream, which serves as a carrier for mass transfer of moisture from microalgae slurry to the gases (Debrand 1974). In the novel process design, liquid phase is being removed very fast, which requires less heat as a driving force so that the bioactive cell constituents are preserved. Swirl flash drying in theory is a combination of fluidized and flash drying and this technology has never been used for the drying of microalgae. In swirl flash dryer system, microalgae biomass is introduced to the drying chamber, which is distributed by the scraping paddles (which are moving close to the drying chamber walls) and fluidized in the stream of the spirally-flow stream of hot air. The energy consumption per unit of the drying by swirl flash dryer was estimated to be 28% less than spray drying for the same product (Safafar 2017). It uses less energy per kg of dry biomass, requires less investment and less area for the drying unit comparing to spray drying under the same condition (Katie 2000; Pertick et al. 2013). Furthermore, this system could be operated in small scale (10-100 kg of the slurry), which may be desired for the microalgae cultivation systems.

Understanding the possible effects of drying methods on microalgae biomass quality is very important to improve the efficiency of algal high value ingredients production. Therefore, in this study effects of two different drying methods (novel swirl flash drying and freeze drying) on the biomass composition of two microalgal specie, *Chlorella pyrenoidosa* (also known as *Auxenochlorella pyrenoidosa*) and *Nannocloropsis salina* (also known as *Microchloropsis salina*), were compared (focusing on the fatty acid and pigment composition), including whether recovery of bioactive compounds is more efficient on dried or wet biomass. In order to enhance extraction of bioactive compounds, microalgae cell walls often needs to be cracked. Since high pressure liquid shear methods such as homogenization have proven to be successful on microalgae cell disruption (Spiden et al. 2013), this technology has been used in order to evaluate whether recovery of metabolites from dry microalgae biomass was more efficient with or without this pre-treatment. During this mechanical process, liquid dispersion of a cell

biosuspension is forced by high pressure through a micrometric disruption chamber. As a result, cell suspension is exposed to intense fluid-mechanical stresses, including shear, turbulence, elongation, and cavitation that may cause the physical disruption of the cell wall and other cell constituents (Chisti and Moo-Young, 1986).

2. Materials and methods

2.1. Microalgae biomass

Two microalgae, *Chlorella pyrenoidosa* (SAG 211-8k) and *Nannochloropsis salina* (SAG 40.85) were selected for drying trials and to evaluate its effect on the biochemical composition. *C. pyrenoidosa* was cultivated in industrial scale in Ecoduna (Bruck an der Leitha, Austria) and *N. salina* was cultivated in microalgal pilot plant facilities in Kalundborg (Denmark) (Safafar et al., 2016). Harvesting was done at early stationary phase by cross-flow microfiltration from LiqTech (SiC ceramic membrane, 0.1 µm) and the biomass was up concentrated by centrifugation (6500 *x g* for *N. salina* and 3000 *x g* for *C. pyrenoidosa*) prior to shipment to Technical University of Denmark (DTU). Final dry matter (DM) of *C. pyrenoidosa* and *N. salina* after centrifugation was 12.5 ± 0.1 % and 17.0±2.0 % (w/w), respectively.

2.2. Biomass pretreatment – cell rupture

High-pressure homogenization (Rannie 110, SPX Flow Technology, Søborg, Denmark) was applied to the microalgae samples of *C. pyrenoidosa* in order to investigate the effect of cell rupture on the recovery of microalgal biomass and metabolites. Positive displacement piston pump was used to accelerate the slurry flow through an orifice within an assembly of specially designed valves creating high shear forces, which result in cell wall destruction. Around 1.5 L of cooled (≈ +5 C°) microalgal slurry of *C. pyrenoidosa* (dry solid content 12.5% w/w) passed the nozzles at a pressure of 150 M Pa and flow rate of 250 ml/min. The same sample was running two times through the homogenizer since our preliminary experiments showed that there was almost no cell rupture detected after the first run. After homogenization the degree of cell rupture was evaluated visually by optical microscopy and then the samples were stored at -20 °C.

2.3. Drying experiments

Microalgal biomass samples of *N. salina* and *C. pyrenoidosa* were dried by freeze drying in a pilot scale freeze dryer (Beta 1-8, Martin Christ GmbH, Osterode, Germany) and by swirl flash drying prototype (**Fig. 1**). Freeze drying was carried out by placing the frozen samples (at -20 °C) under a vacuum where heat energy is then added to the product causing the ice to sublime. The drying conditions for the swirl flash drying are shown in **Table 1**. Dry biomass was stored at -20 °C prior to chemical analysis.

2.4. Analytical analysis

Protein content

The protein content in the samples was estimated based on total nitrogen content, which was determined using the Dumas method (Elementar, Mt. Laurel, NJ, USA) for the quantitative measurements of protein in different substances. Approximately 1 g of the dried microalgal biomass was used for analyses. The following steps were automated including sample combustion in a chamber at a high temperature (900 °C) in the presence of oxygen. Estimation of the total protein content was done using total nitrogen determination and conversion factor 4.44 (Lopez at al. 2010). Conversion factor value of 4.44 is recommended to be used to estimate the protein content if total nitrogen is measured. It was determined based on testing several microalgae and cyanobacteria undergoing rapid growth. Protein content is reported as % of dry biomass. Analyses were carried out in duplicate.

Total lipid content

Lipid extraction was done as described by Bligh & Dyer (1959), but with a reduced amount of solvent (30 mL of each). Approximately 1 g of the dried microalgal biomass was weighted and placed into extraction glass. Before extraction samples were sonicated in sonication bath (Buch & Holm A/S, Herlev, Denmark) for 10 min at 10-15 °C in order to enhance cell rupture. Extraction was carried out by subsequent addition of methanol, chloroform and water while stirring. In order to separate methanol/water phase from chloroform/oil phase, samples were centrifuged at 1400 x *g* for 10 min. Bligh & Dyer extracts were used for analyses of oil content, fatty acids and tocopherols. Determination of total lipid content was done by weighing 15 g of extract in beakers and keeping it overnight in a fume hood in order to evaporate chloroform. Lipid content was calculated using the equation below (1).

$$\% \text{ lipid } = \frac{g\,(lipid) \times specified\ mass\ on\ chloroform\ phase\ (41\ g) \times 100}{(g\,(extract) - g\,(lipid) \times g\,(sample)} \quad [1]$$

Fatty acids

Fatty acid profile was determined based on the American Oil Chemist´s Society (AOCS) official method Ce 1i-07 (Firestone 2009) with some modifications. Approximately 5 g of Bligh & Dyer extract was weighed in methylation glass tube and evaporated under the stream of nitrogen until dryness. A mixture containing 100 µL of internal standard solution (C23:0), 200 µL of heptane with butylated hydroxytoluene (BHT) and 100 µL of toluene was added to the dry extract. Samples were methylated in a microwave oven (Microwave 3000 SOLV, Anton Paar, Ashland, VA, USA) for 10 min at 100 °C and power of 500 watts. After methylation, heptane with BHT (0.7 mL) and saturated salt water (1 mL) were added. The upper phase (heptane) was transferred into GC vials and analyzed by gas chromatography equipped with flame ionization detector (GC-FID). (HP-5890 A, Agilent Technologies, Santa Clara, CA, USA). Fatty acid methyl esters were separated by the GC column Agilent DB wax 127-7012 (10 µm x 100 µm x 0.1 µm) (Agilent technologies, Santa Clara, CA, USA). Standard mix of fatty acids methyl esters (Sigma, St. Louis, MO, USA) was used for fatty acid identification. Fatty acids were quantified as area % of total fatty acids. Analyses were carried out in duplicate.

Tocopherols

Approximately 1 g of Bligh & Dyer extract was weighed in glass tube and evaporated to dryness in darkness and under a stream of nitrogen. Dry extract was re-dissolved in a mixture of isopropanol: heptane (0.5:99.5, v/v). The solution was filtered by 0.22 µm PTFE syringe filter and 20 µL of filtrate was injected to HPLC. Analysis was done based on the AOCS official method as (Firestone, 2009) using an Agilent 1100 Liquid Chromatograph equipped with a fluorescence detector, with the excitation wavelength set at 290 nm and emission wavelength at 330. The separation was carried out in isocratic mode by Spherisorb column (150 mm x 46 mm with 3 µm particle size) at room temperature using a mixture of isopropanol: n- heptane (0.5:99.5, v/v) as mobile phase. Elution flow rate was 1 ml min^{-1}. Analyses were carried out in duplicate.

Pigments

Pigment analysis was done according to the method described by Safafar et al. (2015). Approximately 0.05 g of the dried algal biomass was weighted in centrifugation tubes. Extraction was carried out by methanol containing BHT in a sonication bath (Buch & Holm A/S, Herlev, Denmark) for 15 min at 5±2 °C. Pigment analysis was performed by HPLC using Agilent 1100 Liquid Chromatograph with diode array detector (DAD). Separation was carried out on a Zorbax Eclipse C8 column 150 mm x 46 mm x 3.5 µm (Phenomenex Inc. CA, USA) at 60°C. The mobile phase was a mixture of 70% methanol + 30% of 0.028 M tertiary butyl ammonium acetate in water and methanol at a flow rate of 1.1 mL min^{-1} with total acquisition time of 40 min. DHI pigment standard mix (DHI LAB Products, Horsholm, Denmark) was used for identification of peaks. Detection of chlorophylls and carotenoids was done at 660 nm and 440 nm, respectively, and for internal standard (BHT) at 280 nm. Pigments are reported as µg g^{-1} dry biomass. Analyses were carried out in duplicate.

2.5. Statistical analysis

All compositional analyses were performed in two replicates. The results are given as the mean (± standard deviation). Analysis of variance (two-way ANOVA) was used to evaluate the effect of homogenization and drying technique on chemical composition of biomass. Data have met the assumption of normality and homogeneity of variance. Tukey's post hoc test was used to detect significant differences between groups where p values < 0.01 were considered significant. The Statistica v. 13.2 software (USA) was used for all statistical analyses.

3. Results

Effect of drying and high-pressure homogenization

The effect of homogenization on the cell rupture of *C. pyrenoidosa* is shown in **Picture 1**. Detectable cell rupture can be seen within the *C. pyrenoidosa* cells after homogenization. Optical micrographs are indicating that there is a mixture of material associated with cellular fragments and extracellular polymeric substances (EPS) (**Picture 1b**), extracted by the cell under stress conditions (Rossi et al. 2008). Results showed significantly higher lipid yield in dried and homogenized samples compared to non-dried and non-homogenized biomass (**Table 2**). On the other hand, protein content did not show any significant deviations between tested samples. The protein determination method that we used is

based on the complete combustion at a high temperature (900 °C) (section 2.4) so there is no extraction step included that may be affected by differently treated biomass. Effect of drying by swirl flash dryer on the fatty acid composition of *N. salina* is shown in **Table 3**. Results are showing that there was no significant adverse effect on the heat-sensitive polyunsaturated fatty acids, including EPA. The fatty acid profile of *C. pyrenoidosa* also showed no significant difference among applied treatments (**Table 4**). Evaluation of the pigment composition of *N. salina* confirmed that there was no significant adverse effect due to the drying process (**Fig. 2**). There was no significant degradation in concentration of heat-sensitive carotenoids in the swirl flash dried biomass compared to the intact, non-dried biomass. Slightly lower concentration of violaxanthin was detected in the dried samples (3217 ± 248 compared to 3882 ± 21 μg g^{-1} DW), which may be the result of the heat-induced degradation. Ryckebosch et al. (2011) found that freeze dried microalgae were more susceptible to lipolysis than spray-dried microalgae, thus spray dried microalgae were more sensitive to pigment oxidation than freeze dried microalgae, possibly due to breakdown of protecting carotenoids upon spray drying. Leach et al. (1998) reported that spray drying affected β-carotene recoveries from *D. salina*, varying between 57% and 91%, depending on the drying temperature. Chlorophylls can convert at high temperature to the undesirable degraded products (pheophytins) which function as a photoperoxidant resulting in reduction of the storage time of the dried microalgae (Hosikian et al. 2010). Concentrations of pheophytin and other chlorophyll degradation products were negligible in the present study, which demonstrate that the drying treatment did not alter the chlorophylls structure. Compared to *N. salina*, both freeze and flash drying had an adverse effect on pigment composition of *C. pyrenoidosa* (**Fig. 3**). The highest concentration of total pigments was in non-dried, non-homogenized biomass (**Table 5**). Chlorophylls showed significantly higher recovery in the intact biomass (non-dried, non-homogenized) compared to dried and homogenized samples, which may indicate that both drying and high pressure liquid shear methods reduce chlorophyll content *in C. pyrenoidosa*, specifically when both treatments are subsequently applied. On the other hand, recovery of total carotenoids showed no significant difference between dried and non-dried biomass. Carotenoids are thermo-sensitive and may decompose during the drying process, or any other heating process (Takaichi 2011). However, the concentration of carotenoids was found to be significantly lower in the non-dried biomass, which suggests that drying (both freeze drying and swirl flash drying) may enhance carotenoids extractability. Also, there was no significant reduction in carotenoid content after applying tested drying methods, for both homogenized

and non-homogenized samples. Since chlorophylls are not heat-sensitive as carotenoids, oxidation seems to be the reason of low chlorophyll recovery after drying, which is in agreement with Stramarkou et al. (2017) study.

Tocopherol content in freeze and swirl flash dried biomass is presented in **Fig. 4**. Tocopherol is a lipid-soluble antioxidant that is light and temperature sensitive (Carballo-Cardenas et al. 2003). Results showed that there was significantly higher tocopherol content in dried, non-homogenized biomass compared to dried-homogenized biomass (both swirl flash and freeze drying), which indicates that cracking the cells before drying may affect degradation of tocopherols. However, swirl flash dried biomass that was not exposed to the homogenization treatment prior to drying showed no significant difference in tocopherol content compared to the freeze dried biomass. Tocopherol content had similar trend as chlorophyll content in tested microalgal biomass, which confirms earlier given explanations.

4. Discussion

Homogenizers are using pumps to accelerate the liquid flow to a high velocity creating high shear forces, which can destroy cell walls. Cell disruption is desirable in order to enhance recovery of intracellular metabolites. Mendes-Pinto et al. (2001) reported that three times more astaxanthin from *Haematococcus* was obtained after applying high-pressure homogenization, to disrupt its thick and resistant cell wall, compared to the intact biomass. Intense fluid-mechanical stresses such as shear, elongation, turbulence, and cavitation may cause the physical disruption of the cell wall and membranes, as well as the macromolecules such as proteins. However, if high temperature is being used in dehydration process it can enhance deterioration of these released value-added bioactive compounds.

Fatty acids

It has been reported that drying process causes some structural changes in the cells and enables the cellular components such as neutral lipids to be easily extracted (Show et al. 2015, Taher et al. 2014, Ryckebosch 2013). Unchanged cell structure of non-dried biomass holds a resistance to solvent diffusion through the cell membrane, which may lead to low extraction efficiencies of lipids (**Table 2**). However, some previous studies reported identical lipid levels from a given sample both after drying and without any drying (Chatsungnoen and Chisti, 2016). One of the explanations for the contradicting

findings could be that existing studies have tested different microalgae species and applied slightly different extraction methods (repeated solvent extractions, different DM concentration in the wet sample, different centrifugal forces used for up concentrating...). Our results indicate that the drying step enhances lipid extractability in *Chlorella pyrenoidosa* but it doesn't necessarily exclude other factors that may interact during the extraction procedure and which could have affected the results.

Algal drying and cell disruption are high-cost integral processes when it comes to bioactive compounds recovery. As an example, for a product such as eicosapentaenoic acid (EPA, 20:5(n-3)), which is a nutraceutical having therapeutic benefits in disease management, 60% of the costs arise from the recovery processes (Grima et al. 2003). Results of this study showed no significant change in fatty acid composition of tested microalgae (**Table 3,4**). One of the justifications is a relatively rapid drying process that enables short retention of the biomass at high temperatures (3.3 seconds drying time for *N. salina* slurry with 17.0±2.0% of dry matter) that contributes to the bioactive content preservation. For comparison, non-continuous drying process of the thick slurry from *Scenedesmus* sp. took 72 h by sun drying, 12 h by oven drying at 60°C and 24 h by freeze drying (Guldhe et al. 2014). In microalgae, membrane lipids commonly include polyunsaturated fatty acids (PUFAs), such as EPA and DHA (docosahexaenoic acid). Since the results are showing that there was no significant difference in relative composition of the EPA and other fatty acids of *N. salina*, it can be concluded that there was no oxidation caused by the swirl flash drying treatment. Microalgal lipid components, especially PUFAs, have shown swift response to several parameters like temperature, pH, light and nutrient depletion (Borges et al. 2011; Gao et al. 2013). Guldhe et al. (2014) compared different drying methods and their effect on the fatty acid composition in microalgae. His study showed significant differences in percentage of saturated and unsaturated fatty acids among all applied methods. He reported that oven dried and freeze dried biomass had higher percentage of saturated fatty acid than sun dried biomass while, surprisingly, freeze drying showed lowest percentage of PUFAs compared to the other two methods. Spray drying, as an efficient drying method commonly used for microalgae drying, can rupture the algal cells due to high-pressure in the atomizer nozzles, which means that quality of pigments can be destroyed. Ryckebosch et al. (2011) found that freeze dried microalgae were more susceptible to lipolysis than spray-dried microalgae, thus spray dried microalgae were more sensitive to pigment oxidation than freeze dried microalgae, possibly due to breakdown of protecting carotenoids

upon spray drying. Leach et al. (1998) reported that spray drying affected β-carotenoid recoveries from *D. salina*, varying between 57% and 91%, depending on the drying temperature.

Pigments

Both freeze and flash drying had an adverse effect on pigment composition of *C. pyrenoidosa* (**Fig. 3**). These deviations between species can be attributed to the morphological differences of the microalgal cell wall. *Chlorella* average cell size is 5-9 μm, compared to the *N. salina*, 2-4 μm so the degree of rupture may be also higher. It is important to point out that the *Nannochloropsis* sp. has thick cell wall that is relatively hard to break down (Beacham et al. 2014). Due to this morphological characteristic, bioactive compounds, such as PUFAs and pigments, are being additionally protected from unwanted degradation during stress condition such as harvesting and drying.

Interestingly, drying processes significantly affected pigment composition (mainly chlorophylls) of *C. pyrenoidosa* (**Fig. 3**), but the fatty acids profile showed no adverse effect. For the swirl flash drying technique these results could be justified by the fact that the heat treatment of microalgae biomass inactivates enzymes such as lipase and lipoxygenase, which can degrade the cellular lipids. For the freeze dried biomass, presence of various natural antioxidants in the microalgae, including tocopherols and carotenoids, which mostly remain intact during the freeze drying, might inhibit lipid oxidation, which results in the PUFAs preservation. Furthermore, it should be noted that *C. pyrenoidosa* in general contained very high concentrations of pigments and low concentrations of long chain PUFAs. Deviations in the pigment composition of *C. pyrenoidosa* under tested treatments may be affected by several factors. High temperatures during drying process may rupture the cell wall to some extent so the extractability might be enhanced or it may destroy the pigments. However, that would not give an explanation for freeze dried microalgae biomass. For freeze dried samples, ice crystallization, which occurs during the freezing stage of the freeze drying, may have a crucial role. Even though freeze drying is commonly used laboratory scale method and it is considered as a gentle drying technique for microalgae, it should be noted that slow freezing rates between -20 to -60°C will cause development of large ice crystals in the intercellular spaces and result in the displacement of the constituent parts. If the cells are rapidly frozen (e.g. in liquid nitrogen at -196°C), small intercellular ice crystals that would be formed would not affect the cell morphology (Lin 1985). On the other hand, creating large ice crystals may break the cell walls and in that way extractability of the pigments might be enhanced. That would

explain significant difference in carotenoid concentration between freeze dried and non-dried homogenized samples (3853 ± 162 compared to 2234 ± 65 μg g^{-1} DW).

Tocopherols

Tocopherols are natural antioxidant, known as free radical scavengers, which means they can inhibit or retard lipid oxidation. This suggests that tocopherols may be decomposed as a result of decreasing the instability of PUFA-s by scavenging oxidation induced radicals. Therefore, it is important to preserve these antioxidants also after the drying process for the stability of the microalgal biomass. As mentioned earlier, high temperature during drying may inactivate enzymes responsible for lipid oxidation. However, the enzymes remain intact in freeze-dried microalgae biomass, where antioxidants may have a crucial role in preventing unwanted lipid oxidation.

5. Conclusion

Drying technique for microalgae should be designed to eradicate possible deterioration of the sensitive bioactive compounds while being efficient and energy saving. A novel prototoype swirl flash dryer tested in this study was shown to be a promising drying technique for a microalgae biomass. Data showed no adverse effect on the fatty acid composition in microalgal biomass of drying by swirl flash dryer (for both *N. salina* and *C. pyrenoidosa*). That indicates that a high temperature and shear force applied during the drying process did not cause any degradation of fatty acids, which could be justified by the short drying time. High pressure homogenization and drying treatments caused degradation of chlorophylls and tocopherols in *C. pyrenoidosa* and, per contra, enhanced carotenoids extraction. The disruption of the microalgal cell wall seems to be the main factor that contributed to the degradation of these bioactive compounds by increasing their accessibility during the drying process.

Effect of the swirl flash drying method on the recovery of microalgal biomass and metabolites may differ between different specie depending on the cell morphology and biochemical composition. Therefore, more studies are needed on several different microalgal species, including testing of different drying parameters by swirl flash drying method.

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References

Beachem TA, Bradley C, White DA, Bond P, Ali ST (2014) Lipid productivity and cell wall ultrastructure of six strains of *Nannochloropsis*: Implications for biofuel production and downstream processing. Algal Res 6:64-69.

Bligh EG, Dyer WJ, (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917.

Borges L, Morón-Villarreyes JA, D'Oca MGM, Abreu PC, (2011) Effects of flocculants on lipid extraction and fatty acid composition of the microalgae *Nannochloropsis oculata* and *Thalassiosira weissflogii*. Biomass Bioenergy 35:4449–54.

Carballo-Cardenas EC, Tuan PM, Janssen M, Wijffels RH (2003) Vitamin E (alpha tocopherol) production by the marine microalgae *Dunaliella tertiolecta* and *Tetraselmis suecica* in batch cultivation. Biomol Eng 20:139-47.

Chisti Y, Moo-Young M (1986) Disruption of microbial cells for intracellular products. Enzyme Microb Technol 8:194-204.

Debrand S (1974) Heat transfer during a flash drying process. Ind Eng Chem Process Des 13:396-404.

Firestone D, AOAC (2009) Official methods and recommended practices of the AOCS, 5th ed. American Oil Chemist's Society, Urbana, IL, USA.

Gao Y, Yang M, Wang C (2013) Nutrient deprivation enhances lipid content in marine microalgae. Bioresour Technol 147:484-91.

Grima EM, Belarbi EH, Acién Fernández FG, Medina AR, Chisti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20:491-515.

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.

376 Hosikian A, Lim Su, Halim R, Danquah MK (2010) Chlorophyll extraction from microalgae: A
377 review on the process engineering aspects. Int J Chem Eng 2010:1-11, article ID 391632.
378 Katie A (2000) APV Dryer Handbook. APV, Rosemount, Illinois.
379 Lin LP (1985) Microstructure of spray-dried and freeze-dried microalgal powders. Food Structure
380 4(2), article 17.
381 López CVG, García MCC, Fernández FGA, Bustos CS, Chisti Y, Sevilla JMF (2010) Protein
382 measurements of microalgal and cyanobacterial biomass. Bioresour Technol 101(19):7587-7591.
383 Mendes-Pinto M, Raposo M, Bowen J (2001) Evaluation of different cell disruption processes on
384 encysted cells of *Haematococcus pluvialis*: effects on astaxanthin recovery and implications for
385 bio-availability. J App Phycol 13:19-24.
386 Orset S, Leach GC, Morais R, Young J (1999) Spray-drying of the microalga *Dunaliella salina*:
387 effects on beta-carotene content and isomer composition. J Agric Food Chem 47:4782–4790.
388 Petrick I, Dombrowski L, Kröger M, Beckert T, Kuchling T, Kureti S (2013) DBFZ Report No. 16:
389 Algae Biorefinery - Material and energy use of algae, 1-165.
390 Rossi N, Derouiniot-Chaplain M.P, Legentilhomme P, Petit I (2008) *Arthrospira platensis*
391 harvesting with membranes: fouling phenomenon with limiting and critical flux. Bioresour Technol
392 99:6162–6167.
393 Ryckebosch E, Muylaert K, Eeckhout M, Ruyssen T, Foubert I (2011) Influence of drying and
394 storage on lipid and carotenoid stability of the microalga *Phaeodactylum tricornutum*. J Agric Food
395 Chem 59:11063-11069.
396 Ryckebosch E (2013) Microalgae as alternativesource of long chain omega-3 polyunsaturated fatty
397 acids. PhD thesis, KU Leuven, Faculty of Bioscience Engineering, Kortrijk, Belgium.
398 Safafar H, Hass MZ, Møller P, Holdt SL, Jacobsen C (2016) High-EPA biomass from
399 *Nannochloropsis salina* cultivated in a flat-panel photo-bioreactor on a process water enriched
400 growth medium. *Mar Drugs* 14:144.
401 Safafar H, Jacobsen C, Reesbøll CA, Møller P (2017) An algae drier and a system for drying algae
402 paste and/or liquid algae biomass, PCT/DK2017/050403.
403 Safafar H, van Wagenen J, Møller P, Jacobsen C (2015) Carotenoids, phenolic compounds and
404 tocopherols contribute to the antioxidative properties of some microalgae species grown on
405 industrial wastewater. Mar Drugs 13:7339–7356.

406 Shelef G, Sukenik A, Green M (1984) Microalgae harvesting and processing: a literature review.
407 doi:10.2172/6204677
408 Show KY, Lee DJ, Chang JS (2013) Algal biomass dehydration. Bioresour Technol 135:720-729.
409 Show KY, Lee DJ, Tay J, Lee T, Chang JS (2015) Microalgal drying and cell disruption - Recent
410 advances. Bioresour Technol 258-266.
411 Spiden EM, Yap BHJ, Hill DRA, Kentish SE, Scales PJ, Martin GJO (2013) Quantitative
412 evaluation of the ease of rupture of industrially promising microalgae by high pressure
413 homogenization. Bioresour Technol 140:165-171.
414 Stramarkou M, Papadaki S, Kyeiakopoulou K, Krokida M (2017) Effect of drying and extraction
415 conditions on the recovery of bioactive compunds from *Chlorella vulgaris*. J Appl Phycol 29:2947-
416 2960.
417 Taher H, Al-Zuhair S, Al-Marzouqi AH, Haik Y, Farid M (2014) Effective extraction of microalgae
418 lipids from wet biomass for biodiesel production. Biomass Bioenerg 66:159-167.
419 Takaichi S (2011) Carotenoids in algae: distributions, biosynthesis and functions. Mar Drugs
420 9:1101-1118.
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Tables and figures

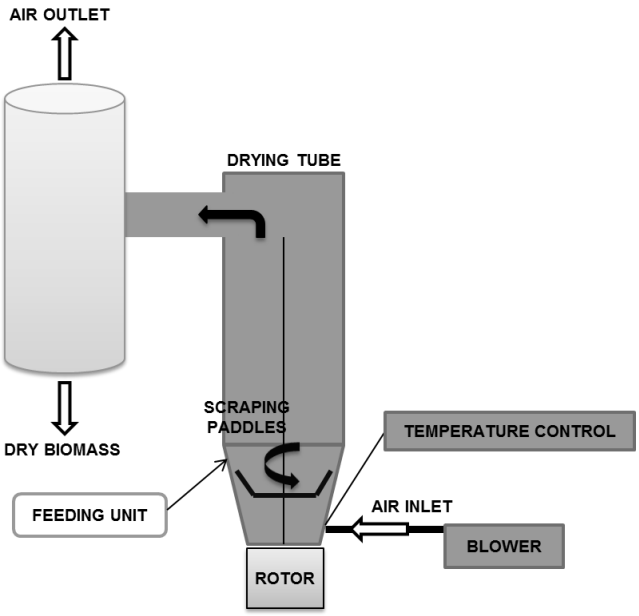
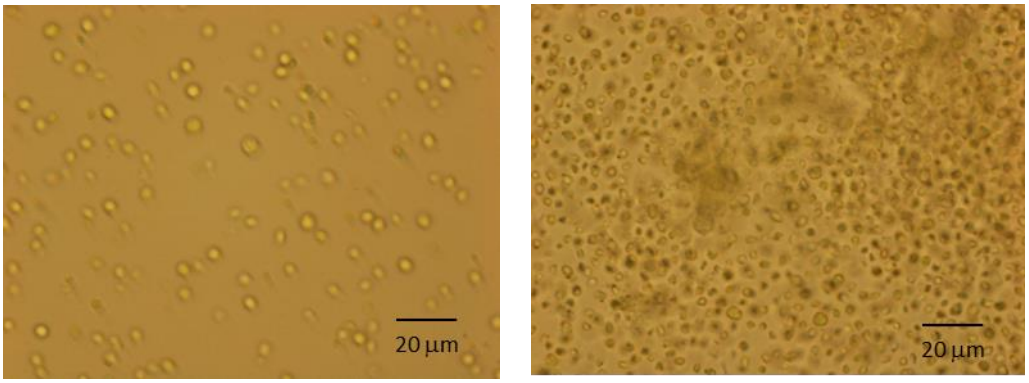


Figure 1. Schematic of prototype swirl flash dryer. Microalgae paste was introduced to the drying tube by a peristaltic pump. At the conically shaped bottom of the drying tube there is an air inlet, through which the hot air stream flows spirally into the drying chamber. Rotating scraping paddles with specific aerodynamic design prevent the feed from adhering to the walls and disperse the feed to smaller particles by providing the fluidizing spiral flow while moving the dried microalgal biomass to the outlet.

Table 1. Drying conditions and specifications for prototype novel swirl dryer system.

Parameters	**Range**	**Unit**
Feed rate	1-5	kg h^{-1}
Feed relative moisture content	60-88	%
Feed viscosity	20-50	Pa s
Product moisture content	8	%
Product particle size range	200-1500	nm

Drying air temperature	100-120	°C
Humidity of inlet air	0.003	kg kg^{-1} air
Air density	0.7	kg m^{-3}
Volumetric air flow rate	18 (max 120)	m^3 h^{-1}
Evaporation capacity	max 3.5	kg h^{-1}
Drying tube length	100	cm
Drying tube diameter	12	cm
Scraping paddle speed	max 1000	rpm



(a) **(b)**

Picture 1. *Chlorella pyrenoidosa* (400x); (**a**) non-homogenized biomass, no cell rupture observed (**b**) homogenized biomass, moderate cell rupture observed.

Table 2. Effect of drying and high-pressure homogenization on lipid and protein yield (%) from *C. pyrenoidosa* biomass. The results are presented as the means of n = 2 measurements from two replicates ± standard deviation. Different letters in the same column represent significant differences ($p < 0.01$).

	Total lipid (%)	Protein (%)
Flash dried, homogenized	12.12 ± 1.05[a]	38.49 ± 1.51[a]
Flash dried, non-homogenized	11.02 ± 0.15[a]	37.59 ± 0.70[a]
Freeze dried, homogenized	12.25 ± 0.46[a]	39.51 ± 0.15[a]
Freeze dried, non-homogenized	11.38 ± 0.17[a]	39.98 ± 1.28[a]
Non-dried, homogenized	12.29 ± 0.01[a]	39.07 ± 2.27[a]
Non-dried, non-homogenized	9.04 ± 0.02[b]	38.98 ± 0.61[a]

Table 3. Fatty acid composition (% of total FA) of swirl flash-dried and non-dried *N. salina*. The results are presented as the means of n = 2 measurements from two replicates ± standard deviation. Different letters in the same row represent significant differences ($p < 0.01$).

Fatty acid	Non-dried	Swirl flash dried
14:00	2.84 ± 0.42[a]	3.62 ± 0.32[a]
15:00	0.37 ± 0.02[a]	0.43 ± 0.00[a]
16:00	24.00 ± 0.75[a]	21.14 ± 1.09[a]
16:1 (n-7)	31.23 ± 0.93[a]	35.70 ± 1.87[a]
16:2 (n-4)	1.59 ± 0.07[a]	1.57 ± 0.10[a]
16:3 (n-4)	0.51 ± 0.06[a]	0.30 ± 0.06[a]
16:4 (n-1)	0.00 ± 0.03[a]	0.48 ± 0.08[b]
18:00	0.91 ± 0.01[a]	0.63 ± 0.03[b]
18:1 (n-9)	4.58 ± 0.25[a]	4.14 ± 0.11[a]
18:1 (n-7)	1.64 ± 0.02[a]	0.78 ± 0.03[b]
18:2 (n-6)	2.18 ± 0.05[a]	2.13 ± 0.01[a]
18:3 (n-3)	0.90 ± 0.12[a]	0.00 ± 0.23[b]
20:1 (n-9)	0.34 ± 0.10[a]	0.00 ± 0.02[b]

20:4 (n-6)	1.71	±	0.06 [a]	2.00	±	0.05 [b]
20:3 (n-3)	0.26	±	0.02 [a]	0.00	±	0.02 [b]
20:5 (n-3)	25.92	±	0.69 [a]	25.63	±	1.17 [a]
22:5 (n-3)	0.48	±	0.05 [a]	0.16	±	0.03 [b]
22:6 (n-3)	0.23	±	0.01 [a]	0.12	±	0.10 [a]
Σ SAFA	28.12	±	1.20 [a]	25.82	±	1.44 [a]
Σ MUFA	37.79	±	1.30 [a]	40.62	±	2.03 [a]
Σ PUFA	33.77	±	1.16 [a]	32.40	±	1.85 [a]

SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Table 4. Fatty acid content (% of total FA) in the biomass of *C. pyrenoidosa* after applying different drying techniques with and without pre-treatment by high-pressure homogenization. The results are presented as the means of n = 2 measurements from two replicates ± standard deviation. Different letters in the same row represent significant differences ($p < 0.01$).

Fatty acid	Flash dried, homogenized	Flash dried, non homogenized	Freeze dried, homogenized	Freeze dried,non homogenized	Non-dried, homogenized	Non-dried, non homogenized
14:0	0.28 ± 0.06^{a}	1.57 ± 0.07^{b}	1.75 ± 0.06^{b}	0.30 ± 0.09^{a}	0.58 ± 0.38^{a}	1.35 ± 0.01^{b}
15:0	0.11 ± 0.01^{a}	0.39 ± 0.01^{b}	0.36 ± 0.01^{b}	0.09 ± 0.01^{a}	0.10 ± 0.00^{a}	0.27 ± 0.01^{b}
16:0	20.74 ± 0.36^{ab}	19.87 ± 0.02^{b}	20.08 ± 0.05^{b}	20.85 ± 0.37^{b}	21.70 ± 0.07^{ac}	19.63 ± 0.17^{b}
16:1 (n-7)	2.26 ± 0.16^{a}	2.45 ± 0.01^{a}	2.44 ± 0.00^{a}	2.28 ± 0.17^{a}	2.39 ± 0.02^{a}	2.47 ± 0.03^{a}
16:2 (n-4)	8.39 ± 0.22^{a}	8.62 ± 0.45^{a}	8.78 ± 0.01^{a}	8.46 ± 0.20^{a}	8.47 ± 0.06^{a}	8.90 ± 0.03^{a}
16:3 (n-4)	12.37 ± 0.34^{a}	13.20 ± 0.17^{a}	12.95 ± 0.04^{a}	12.46 ± 0.30^{a}	12.33 ± 0.20^{a}	13.14 ± 0.05^{a}
18:0	0.86 ± 0.24^{a}	0.57 ± 0.01^{a}	0.64 ± 0.05^{a}	0.86 ± 0.25^{a}	0.66 ± 0.02^{a}	0.59 ± 0.00^{a}
18:1 (n-7)	6.38 ± 0.72^{a}	5.66 ± 0.48^{a}	5.71 ± 0.05^{a}	6.43 ± 0.74^{a}	5.76 ± 0.02^{a}	5.63 ± 0.16^{a}
18:2 (n-6)	21.29 ± 0.09^{ab}	20.83 ± 0.10^{a}	20.72 ± 0.08^{a}	21.45 ± 0.02^{b}	21.33 ± 0.04^{ab}	20.94 ± 0.03^{ab}
18:3 (n-3)	26.65 ± 0.58^{a}	26.84 ± 0.06^{a}	26.58 ± 0.04^{a}	26.78 ± 0.42^{a}	26.68 ± 0.18^{a}	27.08 ± 0.01^{a}
Σ SAFA	21.98 ± 0.67^{a}	22.40 ± 0.10^{a}	22.83 ± 0.17^{a}	22.11 ± 0.72^{a}	23.04 ± 0.48^{a}	21.84 ± 0.19^{a}
Σ MUFA	8.63 ± 0.87^{a}	8.11 ± 0.49^{a}	8.14 ± 0.05^{a}	8.70 ± 0.91^{a}	8.15 ± 0.05^{a}	8.11 ± 0.20^{a}
Σ PUFA	68.70 ± 1.24^{a}	69.49 ± 0.78^{a}	69.03 ± 0.16^{a}	69.16 ± 0.93^{a}	68.80 ± 0.48^{a}	70.06 ± 0.12^{a}

SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

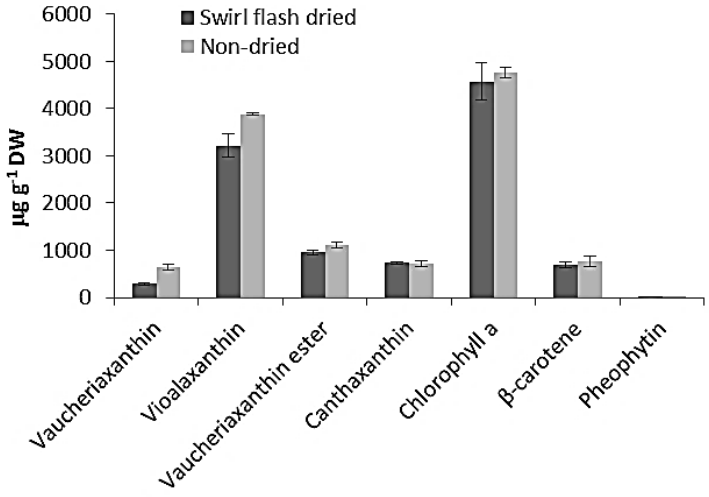


Figure 2. Pigment composition (µg g^{-1} dry biomass) of swirl flash-dried and non-dried *N. salina*. The results are presented as the means of n = 2 measurements from two replicates; error bars represent standard deviation.

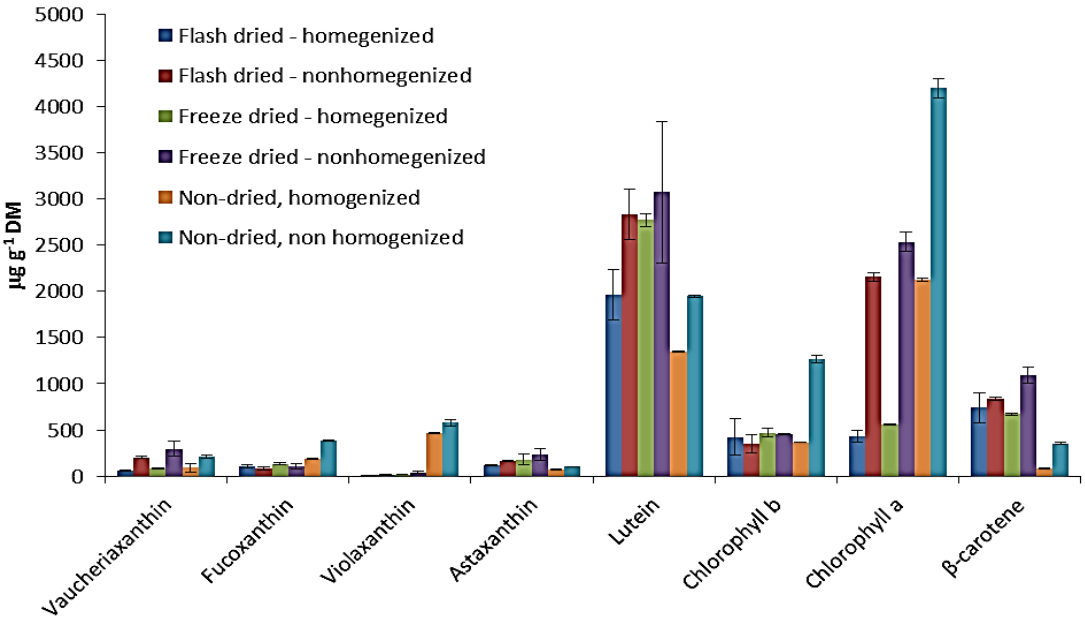


Figure 3. Pigment composition (µg g^{-1} dry biomass) of *C. pyrenoidosa* after applying two different drying techniques with and without pre-treatment by high-pressure homogenization. The results are

presented as the means of n = 2 measurements from two replicates; error bars represent standard deviation.

Table 5. Content of carotenoids and chlorophylls (µg g^{-1} dry biomass) in *C. pyrenoidosa* after applying two different drying techniques with and without pre-treatment by high-pressure homogenization. The results are presented as the means of n = 2 measurements from two replicates ± standard deviation. Different letters in the same column represent significant differences ($p < 0.01$).

Microalgal biomass	Carotenoids	Chlorophylls	Total pigments
Flash dried, homogenized	3001 ± 469^{ab}	852 ± 260^{a}	3854 ± 729^{a}
Flash dried, non-homogenized	4127 ± 320^{b}	2504 ± 142^{b}	6631 ± 463^{b}
Freeze dried, homogenized	3853 ± 162^{b}	1032 ± 46^{a}	4885 ± 209^{a}
Freeze dried, non-homogenized	4836 ± 1093^{b}	2990 ± 104^{b}	7826 ± 1197^{bc}
Non-dried, homogenized	2234 ± 65^{a}	2490 ± 16^{b}	4725 ± 81^{a}
Non-dried, non-homogenized	3576 ± 84^{b}	5458 ± 141^{c}	9034 ± 225^{c}

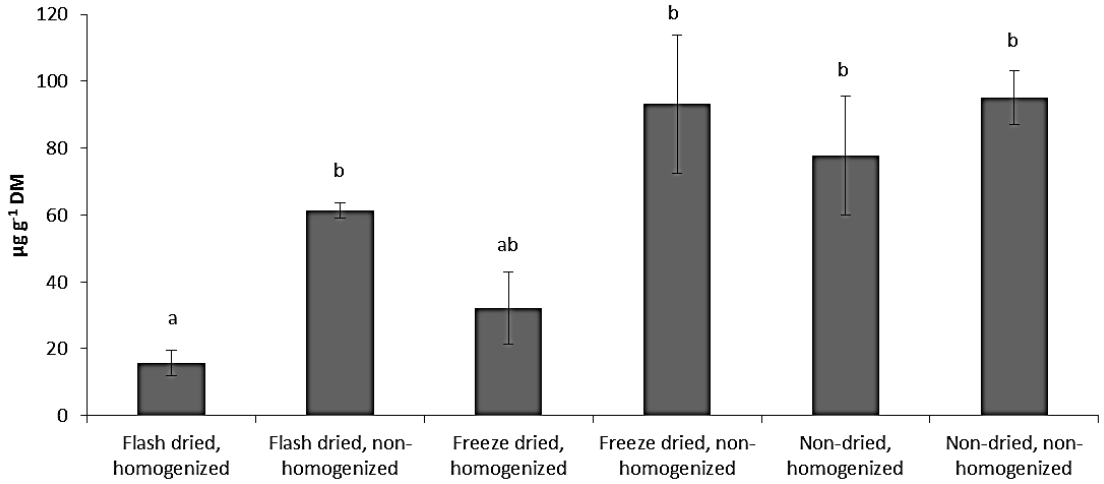


Figure 4. Tocopherol content (µg g^{-1} dry biomass) in *C. pyrenoidosa* after applying different drying techniques with and without pre-treatment by high-pressure homogenization. The results are presented as the means of n = 2 measurements from two replicates; error bars represent standard deviation. Different letters represent significant differences ($p < 0.01$).