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

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8

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

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

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## 27        **1. Introduction**

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

56 The main consideration in the selection of the drying technology depends on the production scale and  
57 the final purpose for which the microalgae biomass is intended. Important factors to consider are also  
58 moisture content, quality requirements and maximum acceptable temperature for preventing thermal  
59 damage to the biomass. In this study, a prototype novel swirl flash dryer system, which was designed  
60 and constructed in one of our previous projects (Safafar 2017), was used for drying trials. In general,  
61 flash drying is achieved by rapid removal of moisture by injecting wet microalgae slurry into a hot air  
62 stream, which serves as a carrier for mass transfer of moisture from microalgae slurry to the gases  
63 (Debrand 1974). In the novel process design, liquid phase is being removed very fast, which requires  
64 less heat as a driving force so that the bioactive cell constituents are preserved. Swirl flash drying in  
65 theory is a combination of fluidized and flash drying and this technology has never been used for the  
66 drying of microalgae. In swirl flash dryer system, microalgae biomass is introduced to the drying  
67 chamber, which is distributed by the scraping paddles (which are moving close to the drying chamber  
68 walls) and fluidized in the stream of the spirally-flow stream of hot air. The energy consumption per  
69 unit of the drying by swirl flash dryer was estimated to be 28% less than spray drying for the same  
70 product (Safafar 2017). It uses less energy per kg of dry biomass, requires less investment and less area  
71 for the drying unit comparing to spray drying under the same condition (Katie 2000; Pertick et al.  
72 2013). Furthermore, this system could be operated in small scale (10-100 kg of the slurry), which may  
73 be desired for the microalgae cultivation systems.

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

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

89

## 90 **2. Materials and methods**

### 91 **2.1. Microalgae biomass**

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

### 101 **2.2. Biomass pretreatment – cell rupture**

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

## 112 2.3. Drying experiments

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

## 119 2.4. Analytical analysis

### 120 *Protein content*

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

130

### 131 *Total lipid content*

132 Lipid extraction was done as described by Bligh & Dyer (1959), but with a reduced amount of solvent  
133 (30 mL of each). Approximately 1 g of the dried microalgal biomass was weighted and placed into  
134 extraction glass. Before extraction samples were sonicated in sonication bath (Buch & Holm A/S,  
135 Herlev, Denmark) for 10 min at 10-15 °C in order to enhance cell rupture. Extraction was carried out  
136 by subsequent addition of methanol, chloroform and water while stirring. In order to separate  
137 methanol/water phase from chloroform/oil phase, samples were centrifuged at 1400 x g for 10 min.  
138 Bligh & Dyer extracts were used for analyses of oil content, fatty acids and tocopherols. Determination  
139 of total lipid content was done by weighing 15 g of extract in beakers and keeping it overnight in a  
140 fume hood in order to evaporate chloroform. Lipid content was calculated using the equation below (1).

141 
$$\% \text{ lipid} = \frac{g(\text{lipid}) \times \text{specified mass on chloroform phase (41 g)} \times 100}{(g(\text{extract}) - g(\text{lipid})) \times g(\text{sample})}$$
 [1]

142

143 *Fatty acids*

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

158

159 *Tocopherols*

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

169

## 170 *Pigments*

171 Pigment analysis was done according to the method described by Safafar et al. (2015). Approximately  
172 0.05 g of the dried algal biomass was weighted in centrifugation tubes. Extraction was carried out by  
173 methanol containing BHT in a sonication bath (Buch & Holm A/S, Herlev, Denmark) for 15 min at  
174  $5\pm 2$  °C. Pigment analysis was performed by HPLC using Agilent 1100 Liquid Chromatograph with  
175 diode array detector (DAD). Separation was carried out on a Zorbax Eclipse C8 column 150 mm x 46  
176 mm x 3.5  $\mu$ m (Phenomenex Inc. CA, USA) at 60°C. The mobile phase was a mixture of 70% methanol  
177 + 30% of 0.028 M tertiary butyl ammonium acetate in water and methanol at a flow rate of 1.1 mL  
178  $\text{min}^{-1}$  with total acquisition time of 40 min. DHI pigment standard mix (DHI LAB Products, Horsholm,  
179 Denmark) was used for identification of peaks. Detection of chlorophylls and carotenoids was done at  
180 660 nm and 440 nm, respectively, and for internal standard (BHT) at 280 nm. Pigments are reported as  
181  $\mu\text{g g}^{-1}$  dry biomass. Analyses were carried out in duplicate.

182

## 183 **2.5. Statistical analysis**

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

190

## 191 **3. Results**

### 192 **Effect of drying and high-pressure homogenization**

193 The effect of homogenization on the cell rupture of *C. pyrenoidosa* is shown in **Picture 1**. Detectable  
194 cell rupture can be seen within the *C. pyrenoidosa* cells after homogenization. Optical micrographs are  
195 indicating that there is a mixture of material associated with cellular fragments and extracellular  
196 polymeric substances (EPS) (**Picture 1b**), extracted by the cell under stress conditions (Rossi et al.  
197 2008). Results showed significantly higher lipid yield in dried and homogenized samples compared to  
198 non-dried and non-homogenized biomass (**Table 2**). On the other hand, protein content did not show  
199 any significant deviations between tested samples. The protein determination method that we used is

200 based on the complete combustion at a high temperature (900 °C) (section 2.4) so there is no extraction  
201 step included that may be affected by differently treated biomass. Effect of drying by swirl flash dryer  
202 on the fatty acid composition of *N. salina* is shown in **Table 3**. Results are showing that there was no  
203 significant adverse effect on the heat-sensitive polyunsaturated fatty acids, including EPA. The fatty  
204 acid profile of *C. pyrenoidosa* also showed no significant difference among applied treatments (**Table**  
205 **4**). Evaluation of the pigment composition of *N. salina* confirmed that there was no significant adverse  
206 effect due to the drying process (**Fig. 2**). There was no significant degradation in concentration of heat-  
207 sensitive carotenoids in the swirl flash dried biomass compared to the intact, non-dried biomass.  
208 Slightly lower concentration of violaxanthin was detected in the dried samples ( $3217 \pm 248$  compared  
209 to  $3882 \pm 21 \mu\text{g g}^{-1}$  DW), which may be the result of the heat-induced degradation. Ryckebosch et al.  
210 (2011) found that freeze dried microalgae were more susceptible to lipolysis than spray-dried  
211 microalgae, thus spray dried microalgae were more sensitive to pigment oxidation than freeze dried  
212 microalgae, possibly due to breakdown of protecting carotenoids upon spray drying. Leach et al. (1998)  
213 reported that spray drying affected  $\beta$ -carotene recoveries from *D. salina*, varying between 57% and  
214 91%, depending on the drying temperature. Chlorophylls can convert at high temperature to the  
215 undesirable degraded products (pheophytins) which function as a photoperoxidant resulting in  
216 reduction of the storage time of the dried microalgae (Hosikian et al. 2010). Concentrations of  
217 pheophytin and other chlorophyll degradation products were negligible in the present study, which  
218 demonstrate that the drying treatment did not alter the chlorophylls structure. Compared to *N. salina*,  
219 both freeze and flash drying had an adverse effect on pigment composition of *C. pyrenoidosa* (**Fig. 3**).  
220 The highest concentration of total pigments was in non-dried, non-homogenized biomass (**Table 5**).  
221 Chlorophylls showed significantly higher recovery in the intact biomass (non-dried, non-homogenized)  
222 compared to dried and homogenized samples, which may indicate that both drying and high pressure  
223 liquid shear methods reduce chlorophyll content in *C. pyrenoidosa*, specifically when both treatments  
224 are subsequently applied. On the other hand, recovery of total carotenoids showed no significant  
225 difference between dried and non-dried biomass. Carotenoids are thermo-sensitive and may decompose  
226 during the drying process, or any other heating process (Takaichi 2011). However, the concentration of  
227 carotenoids was found to be significantly lower in the non-dried biomass, which suggests that drying  
228 (both freeze drying and swirl flash drying) may enhance carotenoids extractability. Also, there was no  
229 significant reduction in carotenoid content after applying tested drying methods, for both homogenized

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

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

241

#### 242 **4. Discussion**

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

252

#### 253 *Fatty acids*

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

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

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

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

### 291 *Pigments*

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

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

318 explain significant difference in carotenoid concentration between freeze dried and non-dried  
319 homogenized samples ( $3853 \pm 162$  compared to  $2234 \pm 65 \mu\text{g g}^{-1}$  DW).

320

### 321 *Tocopherols*

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

329

## 330 **5. Conclusion**

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

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

345

346

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348 commercial, or not-for-profit sectors.

349 **Conflict of interest:** On behalf of all authors, the corresponding author states that there is no conflict of  
350 interest.

351

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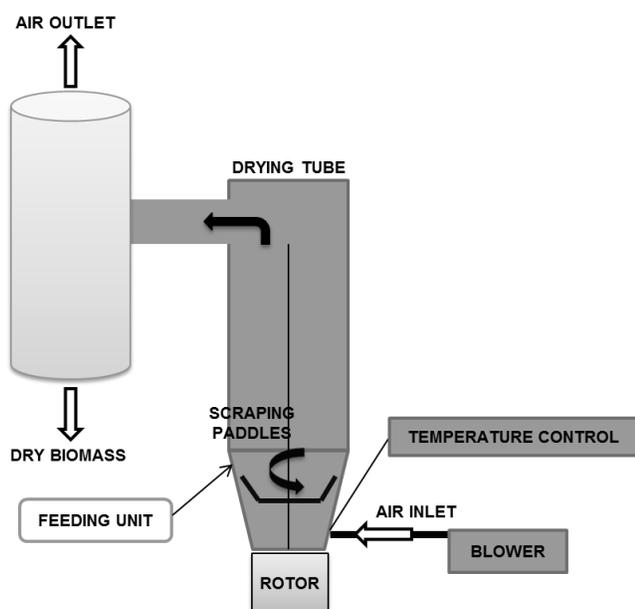
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435 **Tables and figures**



436

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

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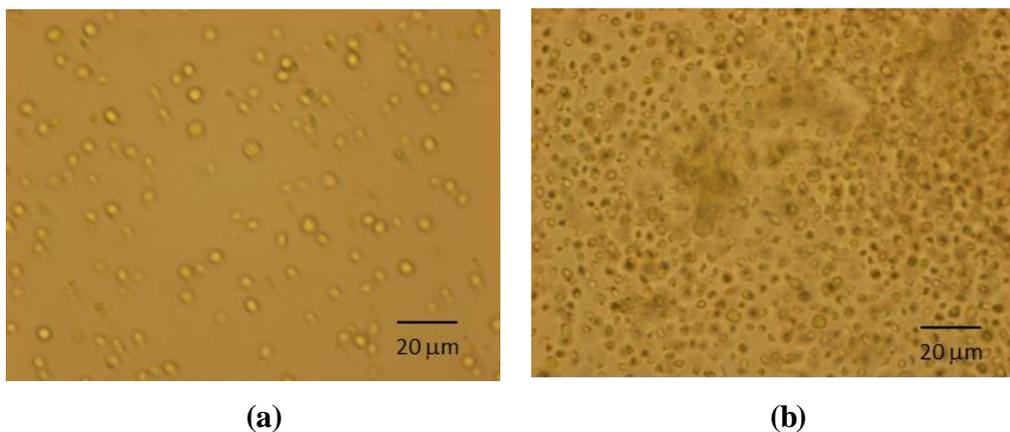
444 **Table 1.** Drying conditions and specifications for prototype novel swirl dryer system.

Parameters	Range	Unit
Feed rate	1-5	kg h <sup>-1</sup>
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 <sup>-1</sup> air
Air density	0.7	kg m <sup>-3</sup>
Volumetric air flow rate	18 (max 120)	m <sup>3</sup> h <sup>-1</sup>
Evaporation capacity	max 3.5	kg h <sup>-1</sup>
Drying tube length	100	cm
Drying tube diameter	12	cm
Scraping paddle speed	max 1000	rpm

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

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456 **Table 2.** Effect of drying and high-pressure homogenization on lipid and protein yield (%) from *C.*  
 457 *pyrenoidosa* biomass. The results are presented as the means of n = 2 measurements from two  
 458 replicates ± standard deviation. Different letters in the same column represent significant differences (p  
 459 < 0.01).

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

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

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

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

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

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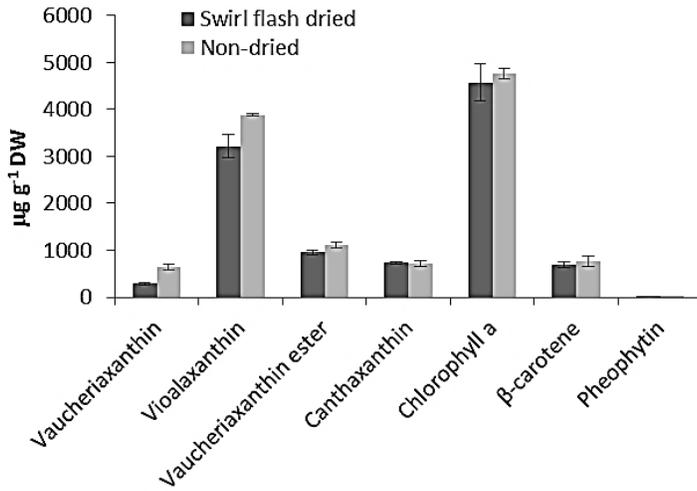
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469 **Table 4.** Fatty acid content (% of total FA) in the biomass of *C. pyrenoidosa* after applying different drying techniques with and  
 470 without pre-treatment by high-pressure homogenization. The results are presented as the means of n = 2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

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

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

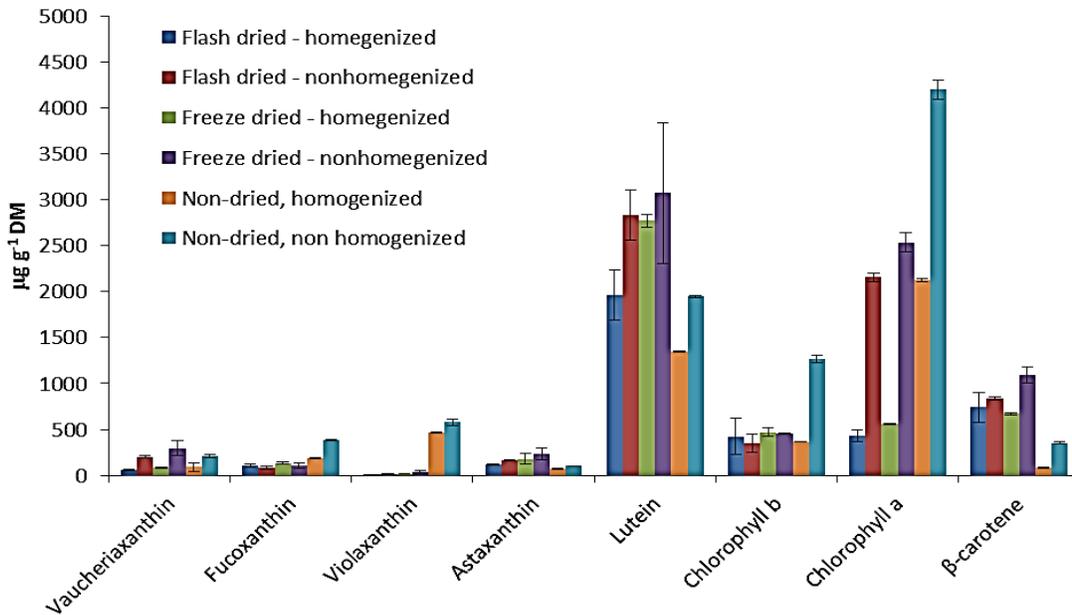
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475 **Figure 2.** Pigment composition ( $\mu\text{g g}^{-1}$  dry biomass) of swirl flash-dried and non-dried *N. salina*. The  
476 results are presented as the means of  $n = 2$  measurements from two replicates; error bars represent  
477 standard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

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479

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

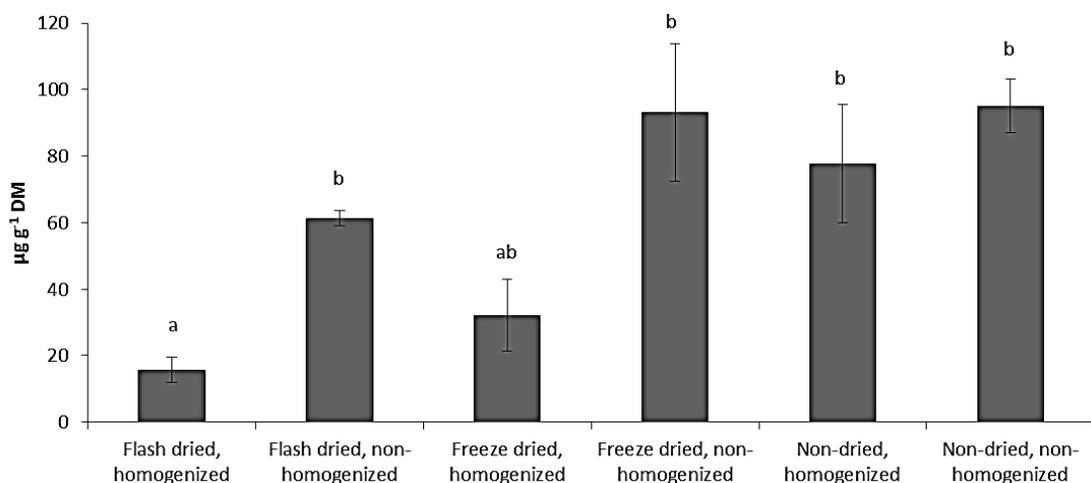
482 presented as the means of  $n = 2$  measurements from two replicates; error bars represent standard  
 483 deviation.

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

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

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