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Assessing different methods to preserve biochemical fractions in microalgal biomass for commercial applications

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ABSTRACT

Different methods exist for preserving microalgal biomass, but their relative effectiveness in maintaining the integrity of key biochemical constituents over an extended period of time remains unclear. This study compared the performance of different methods (refrigeration, freezing, freeze-drying, spray-drying, and oven-drying) for preserving different biochemical fractions (carbohydrates, lipids, proteins, chlorophyll *a* and carotenoids) of two commercially important microalgal species, *Scenedesmus (Tetradesmus) obliquus* and *Chlorella vulgaris*, over 43 days. Results show compound-specific and species-specific differences in degradation. In this study, carbohydrates in *S. obliquus* were best preserved by freezing, lipids by spray-drying, and proteins by freeze-drying. In contrast, carbohydrates and lipids in *C. vulgaris* were best preserved by oven-drying, and proteins by spray-drying. A decision chart based on different percent loss values aid operators to select the optimal preservation method, especially in cases where microalgal biomass is to be used to extract multiple chemical fractions. Beyond biochemical integrity, industrial-scale operations must also consider factors such as capital investment, energy consumption, labor and material costs, processing time and material loss. For example, oven-drying is inexpensive but has a long processing time, whereas spray-drying, while requiring higher capital and skill investment, has a fast throughput that may be more preferable in a competitive commercial space. This article provides practical recommendations for selecting appropriate preservation methods for algal biomass in both commercial and laboratory contexts, based on which a decision chart was formulated to aid operators in choosing the most appropriate preservation method.

1. Introduction

The global algal bioproduct market is worth more than 4 billion USD and is projected to grow to over 7 billion USD within the decade [1,2]. Microalgae are cultivated in industrial scale for the extraction of various bioactive compounds, such as proteins, lipids, carbohydrates and pigments, that are commonly used in food additives, nutraceuticals, pharmaceuticals, therapeutics, and other high-value products [3]. For example, algae of high protein content are used in animal feeds and food formulations [4]. Oil-rich algae have become the preferred and more sustainable source of omega-3 fatty acids than the traditional fish oil [5] and algal oils are also used to make biofuels [6]. Algal pigments such as chlorophylls and carotenoids are being promoted as antioxidants and health supplements [7].

Commercial algal farms grow algae in arrays of photobioreactors or open-ponds that reach thousands to millions of liters in total volume, and global algae production increased up to 35 million tonnes [8]. At the end of cultivation, the culture has to go through de-watering e.g. by filtration, to produce paste-like concentrated biomass, which still has a high water content that adds unnecessary volume and weight. The concentrated biomass may need to be stored and transported to other locations for downstream processing or consumption. Without appropriate treatment, the bioactive components of the biomass are susceptible to degradation due to bacterial growth, residual algal enzymatic activity, and oxidation by ambient light and air [9].

The aim of preservation is to protect the integrity of the algal biomass over time, ensuring that their nutritional and bioactive properties are retained for downstream processing and consumption. For commercial applications, operators need to balance the cost and effectiveness of the preservation methods according to their needs. For instance, refrigeration and freezing are preferred for their simplicity. However, their effectiveness in preserving bioactive compounds over an extended time

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is uncertain, and the requirement for constantly low temperatures adds to the cost for storage and transportation. Oven-drying removes the water content, which cuts down storage and transportation costs, but the process takes time, and the heating and drying may compromise the integrity of heat-sensitive compounds. The more advanced methods of freeze-drying and spray-drying are also popular. Freeze-drying involves slowly freezing the materials under low pressure in a specialised device, thereby removing the water content through sublimation [10]. Spray-drying works by spraying the materials under high pressure through a heated nozzle; the process removes the water content and convert the materials into a fine powder. Some researchers reported that freeze-drying and spray-drying do not compromise the chemical composition of algae in the short term [11,12] but longer-term effects still need to be tested.

Here, different long-term preservation methods: refrigeration, freezing, oven-drying, freeze-drying and spray-drying, were compared. The study focused on their effectiveness in preserving algal biochemicals that are of high values to the industries, including carbohydrates, lipids, proteins and pigments. Two algal species were studied: *Scenedesmus obliquus* and *Chlorella vulgaris,* which are widely relevant for commercial applications. For example, *Scenedesmus* species are commonly used in animal feeds and bioremediation [13], whereas *Chlorella* species are used as dietary supplements in food and feed products [14–16]. The goal was to assess which methods would be most suitable to preserve different biochemical fractions in concentrated algal biomass for downstream consumption or processing over time.

2. Methods

2.1. Algal species and cultivation

Inocula of the algal species *Scenedesmus (Tetradesmus) obliquus* CCAP 276/3 A) and *Chlorella vulgaris* (CCAP 211/11B) were obtained from CCAP collection in Scotland, UK. The inocula were added to f/2 media to establish stock cultures in a growth chamber (temperature of 18 °C and illumination of 100 μmol photons m^{-2} s^{-1} on a 16 h light: 8 h dark cycle). When the stock cultures reached exponential growth phase, they were used to inoculate photobioreactors (PBR) inside a greenhouse in Swansea, UK (51°36′29.1″ N, 3°58′53.1″ W). Two tubular PBR manufactured by Varicon Aqua LTD were inoculated at an initial OD_{750nm} of ~0.5 (ca. 1×10^6 cell mL^{-1}) for both species. Each PBR has a total capacity of 800 L with horizontally arranged 43 mm-diameter tubes. The PBRs were run for 18 days until the cultures reached a concentration of 200–250 $\times$ 10^6 cell mL^{-1} as measured with a Coulter counter (C3 Beckman Coulter GmBH, Drefield, Germany). The cultures were then harvested and dewatered by membrane microfiltration (0.2 μm pore size) at 50 L h^{-1} followed by centrifugation (8000 *g* at 4 °C, JA-2, Beckman, Germany) as described previously [17,18]. The concentrated biomass appeared as thick pastes with ~20 % water content. The algal pastes were then divided into different portions and subject to the different preservation methods.

2.2. Preservation methods

The algal pastes were subject to five preservation methods and stored for 43 days. A longer storage was not tested due to logistical constraints. Some commercial algal products claim to have a shelf-life of anywhere between 45 days (e.g., www.bernaqua.com/algae-paste/) to several months (e.g., reedmariculture.com/products/nannochloropsis-3600); therefore, we considered a 43-day period sufficient to test the chemical integrity of the algal biomass. Chemical analyses of the biomass were conducted on Day 1 and Day 43 to assess any changes in compositions or quality over time.

2.2.1. Refrigeration

The harvested pastes of the two algal species were stored in a refrigerator at a constant temperature of 4 °C.

2.2.2. Freezing

The algal pastes were stored at −80 °C (New Brunswick Green freezer model HEF U410).

2.2.3. Oven-drying

The paste was placed inside a drying oven at 45 °C for 48 h, where it turned into a dry, dark-brown cake.

2.2.4. Freeze-drying

The paste was dried at −110 °C under a vacuum using a bench-top freeze dryer (Scan Vac, Coolsafe basic 100–9). The process took 50 h and produced a dry, flake-like substance of deep-green colour.

2.2.5. Spray-drying

The paste was forced under high pressure (hot air at 150 bar) through a heated nozzle (60 °C) in a spray dryer (Buchi mini spray dryer S-300 with the evaporation capacity of 1 L h^{-1}; yield up to 70 %). The wet algal biomass only came into contact with the nozzle for a brief moment, and the biomass emerged as a dry, green powder.

The oven-dried, freeze-dried and spray-dried biomass was put inside sealed containers and left for 43 days at room temperature (18–20 °C) in the dark. On Day 1 and Day 43, ten replicates of the required amounts of the biomass for each of the preservation methods were taken to analyse carbohydrates, proteins, lipids and pigments.

2.3. Methods of biochemical analysis

2.3.1. Total carbohydrates

The protocol for quantifying total carbohydrates was modified from the DuBois assay [19]. 5 mg of algal dry biomass (for the regreferation and freezing treatments, biomass was lyofilised by freezedryer) [20] and 2 mL of 1 M H_2SO_4 were added to a centrifuge tube; the mixture was heated to 90 °C for 1 h in a water bath. Afterward, the tube was centrifuged at 5500 rpm for 10 min. 1 mL of the supernatant was pipetted into a test tube; 2.5 mL of 98 % sulphuric acid was added to the test tube, quickly followed by 0.5 mL of phenol. The mixture was left for 30 min to cool and carefully poured into a cuvette. The cuvette was measured for absorption at 485 nm in a spectrophotometer. The total carbohydrate content was calculated using the equation from a standard curve.

For standard curve, six test tubes were prepared with different amounts of a glucose solution (0.01 mg mL^{-1}): 1 mL, 0.8 mL, 0.6 mL, 0.4 mL, 0.2 mL and 0 mL; de-ionized water was added as necessary to make the final volume 1 mL. The tubes were then treated in the same manner as described earlier to create a standard curve of absorption at 485 nm.

2.3.2. Total lipids

Quantification of total lipids was based on standard protocol [21]. For this analysis, Teflon centrifuge tubes were pre-treated with Neutracon detergent at least one day before use. The pre-treated tubes were then rinsed with distilled water, dried (70 ± 5 °C), rinsed with methanol and dried again, then finally rinsed with chloroform and dried.

Samples of dry algal biomass (for the regreferation and freezing treatments, biomass was lyofilised by freeze dryer) were weighed (10–25 mg) into the Teflon tubes. 6 mL of chloroform and 3 mL of methanol were added for every 10 mg of biomass used. The samples were sonicated on ice again for 3 cycles of 15 min with 3 min intervals. The tubes were then left for at least 24 h at 4 °C in darkness to extract lipids from the biomass. 1 mL of 0.09 % NaCl was added to each of the tubes; the samples were centrifuged at 4000 rpm for 10–12 min at 4 °C (JA-2, Beckman, Germany). 10 mL glass vials were pre-weighed with the cap (Teflon lined), after having been dried and stored in a desiccator. The bottom chloroform-phase was carefully recovered with an inserted

pipette, and transferred into the pre-weighed glass vial. 2 mL of chloroform was added to the residual material in the teflon tube; this was then shaken vigorously and centrifuged again at 2000 rpm for 12 min at 4 °C (MSE Harrier centrifuge, swing-out 18/80R rotor) and left to settle for a further 20 min. The bottom phase was recovered in the same way as before and added to a another pre-weighed glass vial. The chloroform in the glass vials was evaporated at 40 °C under nitrogen gas inside a fume hood. The dry residuals after evaporation were stored in a nitrogen gas-filled desiccator until weighing. The percent lipid content of the samples was calculated as [22]:

$$\%lipids = \frac{Weight\ of\ tube\ with\ extracted\ lipids - Weight\ of\ tube}{Sample\ dry\ weight} \times 100\%$$

2.3.3. Total proteins

The nitrogen content of dried algal biomass was measured with CHN analyzer; afterward, a conversion factor of 6.28 was applied to estimate the protein content per the common practice in the industry [23,24] which was derived from the conventional Kjeldahl technique for determining protein content [25].

2.3.4. Chlorophyll and carotenoid pigments

The pigment extraction method was modified from Griffiths et al. [26] 0.2 mg of the biomass sample was weighed into an eppendorf tube with 1 mL of DMSO. The tube was agitated on a vortex and sonicated for 10 min. Afterward, the tube was centrifuged at 10,000 rpm for 3 min. The supernatant was removed and diluted with 1 mL of DMSO. The final solution was added to a cuvette and absorptions at 480, 649 and 665 nm were measured on a spectrophotometer. Total chlorophyll *a*nd carotenoid concentrations (μg mL^{-1}) were calculated according to Wellburn (1994) [27].

2.4. Statistical analysis

The measured chemical constituents were expressed as % dry mass. Statistical analyses were performed using SPSS. Data normality was assessed with the Shapiro–Wilk test. Two-way analyses of variance (ANOVA) with crossed factors were applied, and when significant effects were detected, Tukey's post hoc tests were conducted. For pairwise comparisons between Day 1 and Day 43 within each preservation treatment, Welch's *t*-tests were used to account for unequal variances. A significance threshold of $p < 0.05$ was adopted.

3. Results

3.1. Growth of S. obliquus and C. vulgaris in PBRs

The growth of *S. obliquus* and *C. vulgaris* in the PBRs over 18 days is presented in Fig. 1. Both species grew continuously throughout the 18-day cultivation period. *S. obliquus* increased from an initial concentration of 1.66×10^6 cells mL^{-1} to 2.40×10^8 cells mL^{-1}, resulting in an equivalent specific growth rate of 0.335 d^{-1}. *C. vulgaris* increased from 5.89×10^6 cells mL^{-1} to 2.89×10^8 cells mL^{-1}, with an equivalent specific growth rate of 0.275 d^{-1}.

3.2. Total carbohydrates

The initial (Day 1) carbohydrate contents in *S. obliquus* ranged from 36.6 % to 40.0 % dry weight across treatments. After 43 days (Fig. 2A), spray-dried biomass declined from 38.6 ± 3.0 % to 29.8 ± 6.3 % dry weight, representing a 23 % loss of the original carbohydrate content. This reduction was not statistically significant (Welch's $t(2.8) = 2.17$, $p = 0.123$, ns). Refrigerated samples decreased from 39.3 ± 6.4 % to 33.4 ± 4.5 % (Welch's $t(2.9) = 1.82$, $p = 0.164$, ns). Oven-dried biomass remained relatively stable, showing a minor change from 39.8 ± 2.8 % to 37.4 ± 2.8 % (Welch's $t(2.7) = 1.23$, $p = 0.308$, ns). Freeze-dried

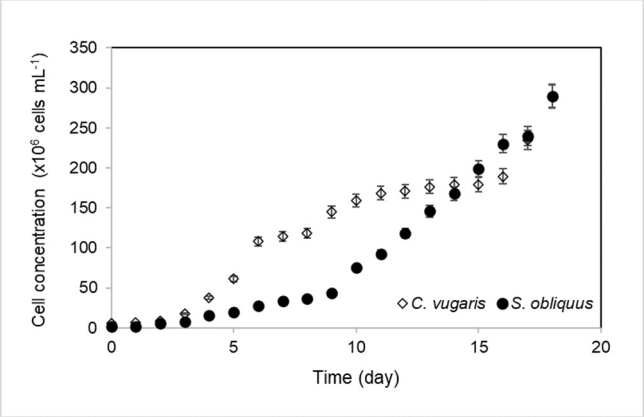

Fig. 1. Cell concentrations of *Scenedesmus obliquus* and *Chlorella vulgaris* in 800-L photobioreactors over an 18-days cultivation period.

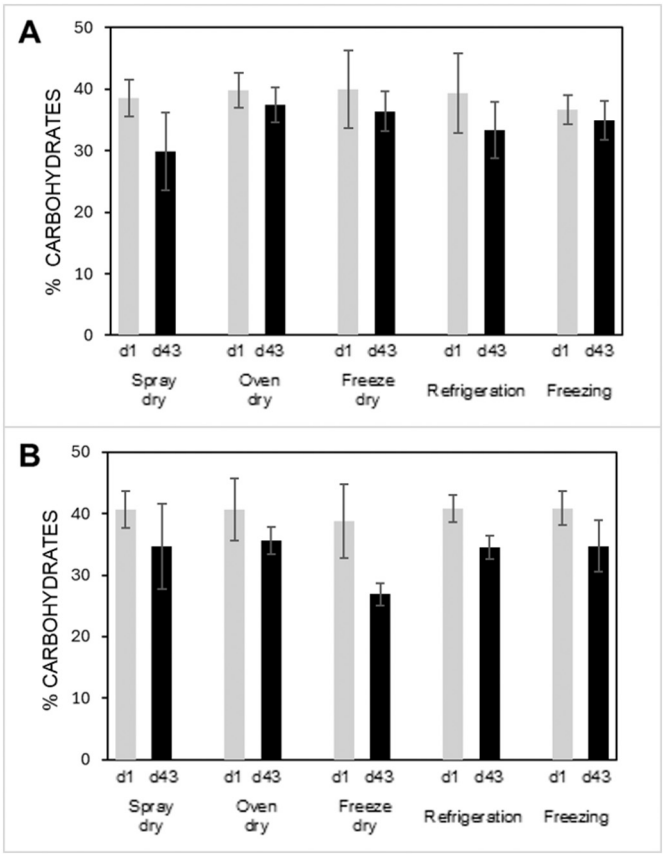

Fig. 2. Cellular carbohydrate content as % dry weight for *Scenedesmus obliquus* (A) and *Chlorella vulgaris* (B) on Day 1 (d1) and Day 43 (d43) with the different preservation methods. Data are presented as mean ± standard error ($n = 10$).

biomass declined moderately (~9 %), whilst frozen samples lost only ~4.7 %; both reductions were not statistically significant.

In *C. vulgaris* (Fig. 2B), initial carbohydrate contents ranged from 38.8 % to 40.9 % dry weight. Freeze-dried samples exhibited the most pronounced decline, from 38.8 ± 6.0 % to 26.9 ± 1.0 %, a 30.7 % loss that was statistically significant (Welch's $t(2.5) = 4.75$, $p = 0.018$, *). Refrigerated biomass decreased from 40.8 ± 2.0 % to 34.5 ± 1.0 % (Welch's $t(2.9) = 3.15$, $p = 0.048$, *), whilst spray-dried biomass declined from 40.7 ± 3.0 % to 34.7 ± 7.0 % (Welch's $t(2.8) = 2.92$, $p = 0.063$, ns). Frozen samples also decreased, from 40.9 ± 2.0 % to 34.7 ±

2.0 % (Welch's $t(2.7) = 3.41$, $p = 0.039$, *). Oven-dried samples showed the smallest loss, from 40.7 ± 5.0 % to 35.7 ± 2.0 %, equivalent to ~12.3 %, which was not statistically significant (Welch's $t(2.9) = 2.15$, $p = 0.091$, ns).

3.3. Total lipids

In *S. obliquus*, initial lipid contents ranged from 2.85 % to 3.60 % dry weight. After 43 days (Fig. 3A), frozen biomass declined from 3.15 ± 0.2 % to 2.40 ± 0.2 %, representing a 23.8 % reduction that was statistically significant (Welch's $t(2.7) = 3.28$, $p = 0.041$, *). Refrigerated samples decreased from 3.15 ± 0.2 % to 2.25 ± 0.1 % (28.6 % loss; Welch's $t(2.9) = 4.02$, $p = 0.029$, *). Freeze-dried samples fell from 3.60 ± 0.1 % to 2.55 ± 0.6 % (29.2 % reduction; Welch's $t(2.5) = 3.74$, $p = 0.033$, *). Oven-dried biomass declined more modestly, from 2.85 ± 0.1 % to 2.40 ± 0.2 % (15.8 %; Welch's $t(2.8) = 2.64$, $p = 0.048$, *). By contrast, spray-dried samples were comparatively stable, decreasing slightly from 3.45 ± 0.4 % to 3.30 ± 0.2 % (4.3 %; Welch's $t(2.9) = 1.11$, $p = 0.337$, ns).

In *C. vulgaris* (Fig. 3B), initial lipid contents ranged from 1.80 % to 2.30 % dry weight. Freeze-dried biomass declined most sharply, from 2.30 ± 0.2 % to 1.57 ± 0.2 %, a 31.6 % reduction that was significant (Welch's $t(2.8) = 4.51$, $p = 0.021$, *). Spray-dried biomass decreased from 2.28 ± 0.2 % to 1.80 ± 0.3 % (21.1 % loss; Welch's $t(2.6) = 3.62$, $p = 0.034$, *). Refrigerated biomass fell from 2.28 ± 0.2 % to 1.94 ± 0.1 % (15.2 %; Welch's $t(2.9) = 2.85$, $p = 0.047$, *), and frozen samples declined from 2.25 ± 0.2 % to 1.95 ± 0.1 % (13.3 %; Welch's $t(2.7) = 2.69$, $p = 0.052$, ns). Oven-dried biomass remained the most stable, decreasing from 2.10 ± 0.2 % to 1.90 ± 0.1 % (9.5 %; Welch's $t(2.9) = 1.97$, $p = 0.081$, ns).

3.4. Total proteins

The initial protein contents of *S. obliquus* samples ranged from 19.7 % to 24.7 %. After 43 days, all samples showed degradation, with the most occurred in the refrigerated samples, losing 31.1 % of the initial protein content (Fig. 4A). Spray-dried samples, oven-dried and frozen samples all showed considerable degradation, losing respectively 19.9 %, 19.7 % and 15.2 % of their protein contents. Freeze-dried samples maintained their protein contents better than the others, losing only 10.4 % over the 43-day period (Fig. 4A).

C. vulgaris samples had an initial protein content of 20.2–24.0 %. After 43 days, freeze-dried and refrigerated samples experienced the most severe protein losses, at 37.0 % and 38.2 %, respectively (Fig. 4B). Moderate loss was observed in frozen samples and oven-dried samples, at 25.9 % and 20.5 %, respectively. Spray-drying appeared to work the best, with the samples losing only 9.0 % of their protein content over the storage period (Fig. 4B).

The *t*-test analysis confirmed statistically significant reductions in protein content between Day 1 and Day 43 for most preservation methods. In *Scenedesmus obliquus*, significant decreases were observed following refrigeration ($t(2.8) = 5.12$, $p = 0.015$, *), spray-drying ($t(2.9) = 3.87$, $p = 0.031$, *), oven-drying ($t(2.8) = 3.65$, $p = 0.037$, *), and freezing ($t(2.9) = 2.95$, $p = 0.048$, *), whilst freeze-drying did not result in a significant difference ($p > 0.05$). In *Chlorella vulgaris*, highly significant declines were detected under freeze-drying ($t(2.8) = 6.02$, $p = 0.009$, **), refrigeration ($t(2.7) = 6.24$, $p = 0.008$, **), and moderate but significant losses were also noted in frozen ($t(2.8) = 4.21$, $p = 0.024$, *) and oven-dried ($t(2.9) = 3.28$, $p = 0.041$, *) samples. Spray-drying, by contrast, resulted in no significant reduction ($t(2.8) = 1.45$, $p = 0.225$, ns). Collectively, these results demonstrate that protein stability in both microalgal species is strongly influenced by the preservation method, with refrigeration and freeze-drying showing the greatest degradation

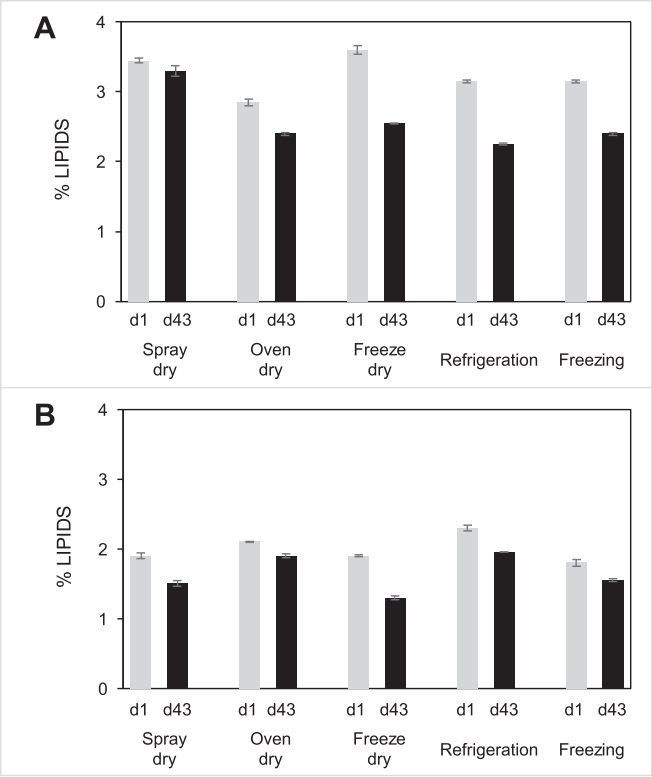

Fig. 3. Cellular lipid content as % dry weight for *Scenedesmus obliquus* (A) and *Chlorella vulgaris* (B) on Day 1 (d1) and Day 43 (d43) with the different preservation methods. Data are presented as mean ± standard error ($n = 10$).

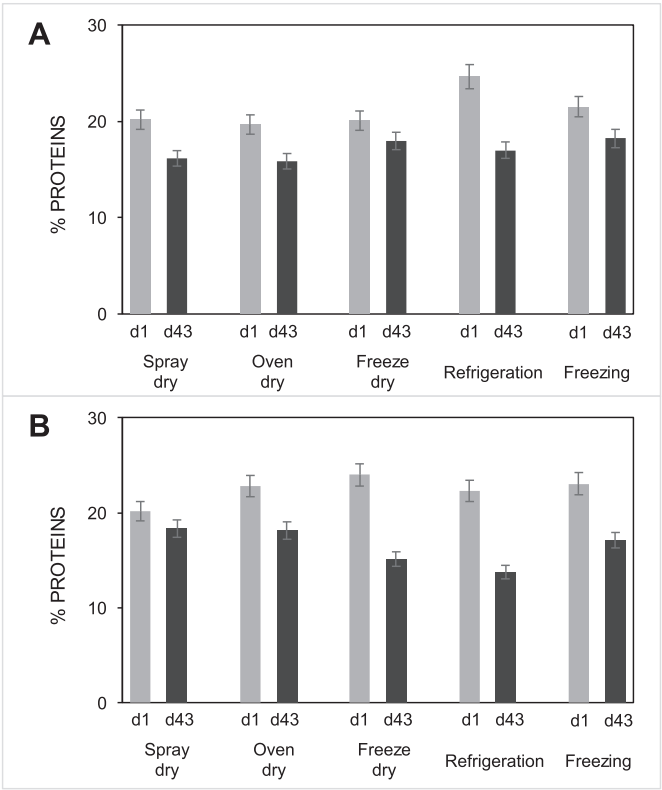

Fig. 4. Cellular protein content as % dry weight for *Scenedesmus obliquus* (A) and *Chlorella vulgaris* (B) on Day 1 (d1) and Day 43 (d43) with the different preservation methods. Data are presented as mean ± standard error ($n = 10$).

over the storage period.

3.5. Chlorophyll a

The chlorophyll *a* data for *S. obliquus* are presented in Fig. 5A. The initial chlorophyll *a* content varied from 0.014 % to 0.018 %. At the end of the storage period, refrigerated samples showed the largest decline, losing more than 50 % of their chlorophyll *a* content. Frozen samples lost ~37 %. Oven-dried and spray-dried samples fared better, losing 21–26 % of their chlorophyll *a*. The best method to preserve chlorophyll *a* in *S. obliquus* samples was freeze-drying, resulting in only 10 % loss over 43 days.

The initial chlorophyll *a* content of *C. vulgaris* samples varied very little, between 0.014 and 0.015 % (Fig. 5B). Refrigerated samples lost the most chlorophyll *a* content during storage, at 33.3 %. Oven-dried, spray-dried and frozen samples lost respectively 26.7, 21.4 and 13.3 % of their initial chlorophyll *a* contents. Overall, freeze-dried samples maintained the most chlorophyll *a*, losing only 7.1 % after 43 days.

A significant reduction in chlorophyll *a* content was observed between d1 and d43 in most treatments for both *Scenedesmus* and *Chlorella*. In *S. obliquus*, spray-drying, oven-drying, refrigeration, and freezing resulted in statistically significant declines ($p < 0.05$), while freeze-drying showed no significant change. In *C. vulgaris*, all treatments led to a significant decrease in chlorophyll *a* levels over time.

3.6. Carotenoids

Fig. 6 present the carotenoid data for *S. obliquus* and *C. vulgaris* samples. The different samples of *S. obliquus* biomass (Fig. 6A) all had comparable initial amounts of carotenoids, at 0.015 %. Storage led to the most loss of carotenoids in spray-dried samples, at 45.6 %. Refrigerated samples, freeze-dried samples and oven-dried samples all lost carotenoids to a similar extent, at 17.8, 15.9 and 14.9 %, respectively.

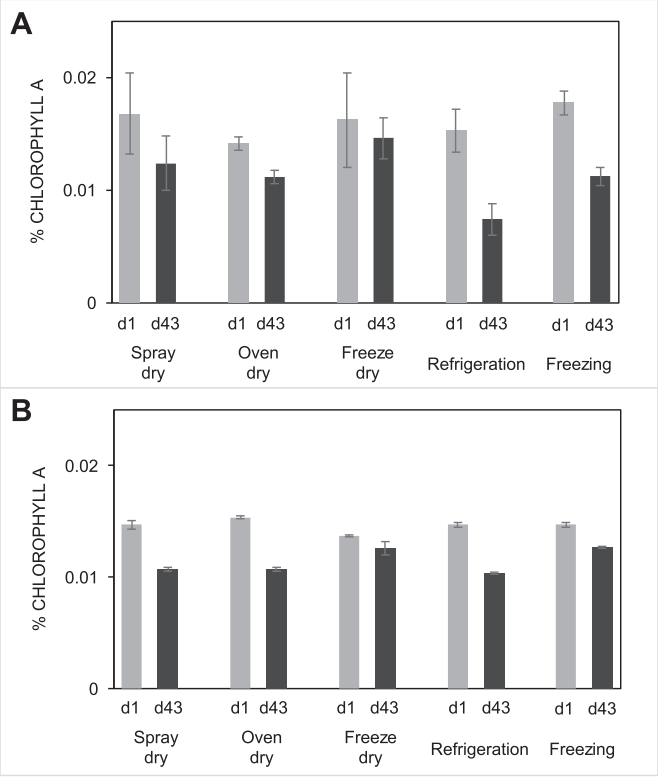

Fig. 5. Cellular Chlorophyll *a* content as % dry weight for *Scenedesmus obliquus* (A) and *Chlorella vulgaris* (B) on Day 1 (d1) and Day 43 (d43) with the different preservation methods. Data are presented as mean ± standard error ($n = 10$).

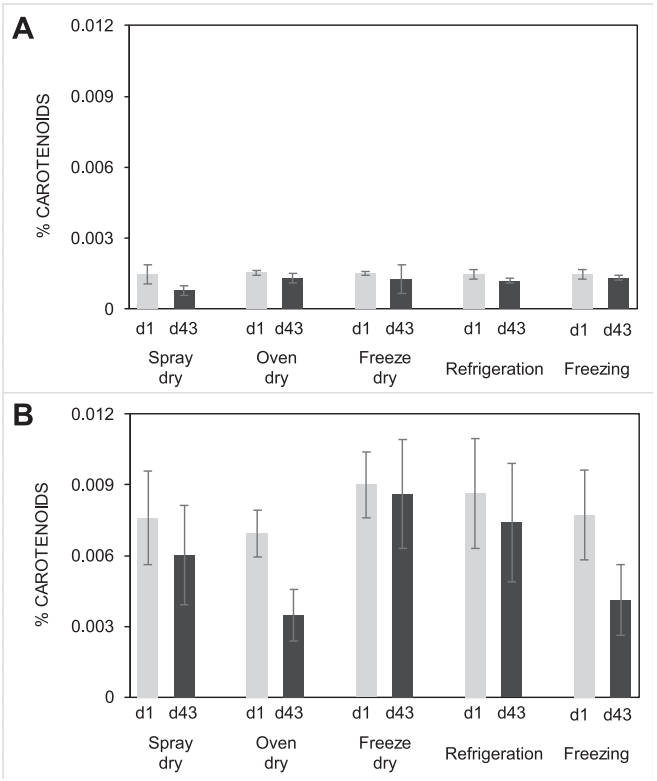

Fig. 6. Cellular carotenoid content as % dry weight for *Scenedesmus obliquus* (A) and *Chlorella vulgaris* (B) on Day 1 (d1) and Day 43 (d43) with the different preservation methods. Data are presented as mean ± standard error (n = 10).

Frozen samples lost the minimal amount of carotenoids, at 9.5 %.

The initial carotenoid content of *C. vulgaris* samples varied little, between 0.007 and 0.009 % (Fig. 6B). Storage led to large losses of carotenoids in both oven-dried samples and frozen samples, at 49.9 % and 46.6 %, respectively. Spray-dried samples lost 20.6 %, whereas refrigerated samples lost 14.3 % of their initial carotenoid contents. By comparison, freeze-dried samples had the most stable carotenoid content, losing only 4.4 % over the 43-day period.

Statistical analysis ($n = 10$) revealed significant reductions in carotenoid content ($p < 0.05$) between d1 and d43 across multiple treatments. In *S. obliquus*, spray-drying, oven-drying, freeze-drying, refrigeration, and freezing all resulted in significant declines. Similarly, in *C. vulgaris*, significant reductions were observed in all treatments, with the most pronounced effects under oven-drying and freezing conditions.

4. Discussion

The global algal bioproduct market value is estimated to exceed 7 billion dollars by 2028 (marketsandmarkets.com, 2025) [2]. Algal biomass are used in a vast range of products, from animal feeds to fertilizers, food additives, nutraceuticals, pharmaceuticals, cosmetics, biofuel, and more [28]. The life-cycle of the production begins with cultivation of algae at scales. The so-produced biomass may then need to be stored for an extended time and/or transported to other locations for downstream processing and consumption. However, many of the valuable biochemical constituents of the biomass can be susceptible to degradation without proper preservation. For example, the algal taxa *Nanochloropsis* is often sold in frozen or refrigerated wet pastes as aquaculture feeds, but the long-term integrity of the biochemical composition is unclear. Also, the wet pastes still retain a high water content that add to storage and transportation costs [29].

Although drying may extend the shelf life of the biomass and save

cost for storage and transportation, the drying process inevitably adds to the operation time and expense. There are different drying methods on the market, but few studies have been done to compare their effectiveness in preserving the different biochemical fractions of different algal species. Algal cells are damaged by the preservation process [30] such that they do not remain viable [31]. Ultimately, the preservation methods of choice would depend on the trade-off between cost and benefit for the algae producers as well as the downstream users.

This study compared different common methods for preserving biochemical compositions of concentrated algal biomass for long-term storage. The effectiveness of the methods was found to vary between species and the different biochemical fractions. For *S. obliquus*, freezing was the most effective method to preserve the carbohydrate content of the biomass, whereas for *C. vulgaris* it was oven-drying. Algal carbohydrates are mostly associated with the structural (e.g. cellulose) and storage components of the cells (e.g. starch), which tend to be more resistant to degradation [32].

Spray-drying preserved the lipid content of *S. obliquus* more effectively, likely due to its thick, rigid cell wall and possibly more stable lipid profile. The brief exposure to high temperature in spray-drying may have limited oxidation in this species, which can better withstand thermal stress.

In contrast, *C. vulgaris* responded less well to spray-drying, possibly because its thinner cell wall and higher proportion of heat-sensitive polyunsaturated fatty acids made it more vulnerable to exposure to high-pressure and intense heat in spray-drying. By comparison, the lower sustained temperature of oven-drying may have minimised lipid oxidation and better preserved its lipid content.

Freeze-drying is recommended for *S. obliquus* because its proteins are likely more sensitive to heat, and the species' rigid cell wall may hinder moisture removal, increasing the risk of thermal denaturation during other drying methods. In contrast, *C. vulgaris* contains thermotolerant proteins, making it is less sensitive to heat-based drying methods (spray-drying and oven-drying) than *S. obliquus*, and its less rigid cell structure allows efficient water removal during the brief heat exposure in spray-drying and still maintains biochemical integrity [33–36]. As a result, spray-drying can effectively preserve protein content in *C. vulgaris* while offering faster and more cost-efficient processing.

Algal pigments are very sensitive to heat [37]. As such, preservation methods that involve heating (oven-drying, spray-drying) should be avoided. Instead, freeze-drying was the preferred method for chlorophyll *a* in both species as well as carotenoids in *C. vulgaris*, whereas freezing was recommended for preserving carotenoids in *S. obliquus* [38].

In addition to differences among the preservation methods, our results also revealed different susceptibility to degradation between the two algal species. *C. vulgaris* experienced a larger loss of carbohydrates than *S. obliquus* during storage in most cases. Proteins in *C. vulgaris* biomass also appeared to be more prone to degration than that in *S. obliquus*. Conversely, *C. vulgaris* biomass maintained its chlorophyll *a* and carotenoid contents better than *S. obliquus* biomass. These species-specific differences in degradation susceptibility likely stem from variations in cell wall composition, and intracellular structure [39]. *C. vulgaris* generally has a thinner and less complex cell wall compared to *S. obliquus*, which may make its carbohydrates and proteins more susceptible to degradation during storage [40]. In contrast, the more robust cell structure of *S. obliquus* may offer better protection against enzymatic or microbial breakdown of these macromolecules [41]. On the other hand, *C. vulgaris* may possess more stable pigment-protein complexes or higher concentrations of antioxidant compounds, which could explain its better retention of chlorophyll *a* and carotenoids. Additionally, differences in post-harvest metabolic activity or stress responses triggered by preservation conditions may influence how cellular components degrade in each species. Future study may investigate the change in the algal biomass composition at a higher temporal resolution, and characterise the breakdown products in more detail.

Based on these results, a 'decision chart' is proposed to guide operators in selecting the most appropriate preservation methods according to the algal species and which biochemical fractions are being targeted (Table 1). Such a decision tool would be particularly useful in cases where the preserved algal biomass is used to extract multiple biochemical fractions. For example, if the producer grows *S. obliquus*, ideally they should preserve the biomass with different methods for extracting different fractions: freezing for carbohydrates, spray-drying for lipids, and freeze-drying for proteins. This inevitably increases the operational complexity and costs. Instead, based on the % loss values illustrated in the decision chart, oven-drying turned out to be the optimal method for preserving all three fractions.

For the industry, in addition to preserving the integrity of the chemical constituents, operators also have to consider the costs in terms of capital investments (e.g. Does it require specialised equipment and installations?), operational expenses (e.g. How much electricity is consumed?), labor and material costs (e.g. Does it require specially trained operators? Does it require specialised consumables?), processing time (e.g. How fast can it process the biomass?) and material loss. Semi-quantitative information is compiled in Table 2 to compare the costs associated with the different preservation methods.

Freeze-drying has the highest capital cost, ranging from £20,000 to £50,000 for an industrial scale freeze-drier (and up to £5000 for laboratory scale). It also has a high operating cost, consuming approximately 4.5–18 kWh per day, which amounts to about £2.50 daily or nearly 1000 kWh annually. Spray-drying also has a high capital cost, with industrial systems ranging from £15,000 to £40,000 and laboratory systems between £3000 and £5000. Its operating cost is high as well, estimated at around 408 kWh annually. Oven-drying has a comparatively low capital cost, with laboratory setups costing less than £1000 and industrial systems ranging from £10,000 to £20,000. Its operating cost is moderate, with energy consumption estimated between 100 and 200 kWh annually. Refrigeration is the least capital-intensive, while its energy consumption is about 200 kWh per year. Freezing, on the other hand, has a moderate capital cost but relatively high operating costs, requiring approximately 500 kWh annually.

In terms of labour and material costs, both freeze-drying and spray-drying require some training and specialised operational consumables. Oven-drying, refrigeration and freezing require no special training or consumables. Regarding processing time, freeze-drying takes 24 h or more, spray-drying requires approximately 1–3 h, and oven-drying takes about 6–12 h. Refrigeration and freezing require virtually no processing time. Material loss is negligible in freeze-drying, but it ranges from 10 to 18 % in spray-drying and 5–10 % in oven-drying. Additionally, the elevated temperatures in spary-drying, even for a short duration, can degrade sensitive compounds in the algal biomass, leading to a loss of valuable constituents such as lipids and antioxidants [42]. Furthermore, algal cultures may need to be pre-concentrated to optimise time and energy use, as this helps improve the efficiency of the spray-drying process [43]. Refrigeration and freezing have minimal material loss.

By presenting the different cost categories, Table 2 complements the 'decision chart' (Table 1) to allow operators to arrive at an economically viable option. For example, oven-drying requires relatively inexpensive installation, low energy consumption, and no special skills, whereas spray-drying requires a higher monetary and skill investment. However, spray-drying can process the algal materials in a fraction of the time needed for oven-drying. In a competitive commercial space, a shorter processing time may be far more favorable in the long run.

5. Conclusion

Proper preservation is crucial for maintaining the integrity of algal biomass compositions for downstream processing or consumption. Here, different methods for preserving different biochemical fractions of two common algal species over a 43-day period were tested. While there was no single best method for every situation, by comparing the % loss of

Table 1
Comparison of different preservation methods for preserving different biochemical fractions of *S. obliquus* and *C. vulgaris* cells. Numbers are % loss over 43-day storage. Colored triangles highlight the best preservation methods for the specific chemical constituents (blue for *S. obliquus*; green for *C. vulgaris*).

	Refrigeration	Freezing	Oven-drying	Freeze-drying	Spray-drying
Carbohydrates	15 / 15.5	4.7 / 14.3	6 / 12.3	9 / 30.7	23 / 14.7
Lipids	28.6 / 15.2	23.8 / 13.3	15.8 / 9.5	29.2 / 31.6	4.3 / 21.1
Proteins	31.1 / 38.2	15.2 / 25.9	19.7 / 20.5	10.4 / 37	19.9 / 9
Chlorophyll *a*	50 / 33.3	37 / 13.3	21 / 26.7	10 / 7.1	26 / 21.4
Carotenoids	17.8 / 14.3	9.5 / 46.6	14.9 / 49.9	15.9 / 4.4	45.6 / 20.6

Scenedesmus obliquus *Chlorella vulgaris*

Table 2
Semi-quantitative information on costs associated with the different preservation methods.

Preservation Method	Capital Cost per unit	Operating Cost In KWh annually	Labor & Material Costs	Processing Time	Material Loss	Algal biomass process (kg)	Key Considerations
Freeze-Drying	High. Industrial scale £20-50 K; lab scale up to £5 K	High. ~ 1000	High	~24 h	Negligible	Up to 1000	Preserves most proteins and sensitive compounds; high energy consumption; not appropriate for low-value products.
Spray-drying	High. Industrial scale £15-40 K; lab scale £3-5 K per unit	High. ~408	Moderate	~1–3 h	~10–18 %	Up to 800	Suitable for high-value products; rapid drying; potential nutrient loss due to high temperatures.
Oven-drying	Low. Industrial scale £10-20 K per unit; lab scale < £1 K per unit	Moderate. 100–200	Low	~6–12 h	~5–10 %	Up to 500	Simple setup; risk of nutrient degradation at high temperatures; not suitable for heat-intensive materials.
Refrigeration	Low	Moderate. ~200	Low	n/a	Minimal	~200	Suitable for temporary preservation; not ideal for long-term storage; continuous energy use.
Freezing	Moderate	High. ~500	Low	n/a	Minimal	Up to 600	Preserves biomass effectively; high energy costs for maintenance; potential cell damage due to ice crystal formation.

initial biomass contents using the decision chart, operators would be able to select the most appropriate method based on algal species and target biochemical compounds. The decision chart also helps operators to find an optimal preservation method when multiple biochemical components are targeted. Additional considerations are needed with regards to capital investment, operational costs, labor and material, processing time and material loss. A short processing time, such as with spray-drying, is preferable in a competitive commercial space and may more than compensate for the other associated costs.

CRediT authorship contribution statement

Alla Silkina: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **José Ignacio Gayo-Peláez:** Writing – original draft, Validation, Formal analysis, Data curation. **Kam W. Tang:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] P. Loke Show, Global market and economic analysis of microalgae technology: status and perspectives, Bioresour. Technol. 357 (2022) 127329, https://doi.org/10.1016/j.biortech.2022.127329.
[2] Marketsandmarkets.com, Market-Reports/algae-product-market, Https://Www.Marketsandmarkets.Com/Market-Reports/Algae-Product-Market-250538721.Html (2025).
[3] M.T. Guarnieri, P.T. Pienkos, Algal omics: unlocking bioproduct diversity in algae cell factories, Photosynth. Res. 123 (2015) 255–263, https://doi.org/10.1007/s11120-014-9989-4.
[4] E.W. Becker, Micro-algae as a source of protein, Biotechnol. Adv. 25 (2007) 207–210, https://doi.org/10.1016/j.biotechadv.2006.11.002.
[5] G. Lenihan-Geels, K. Bishop, L. Ferguson, Alternative sources of Omega-3 fats: can we find a sustainable substitute for fish? Nutrients 5 (2013) 1301–1315, https://doi.org/10.3390/nu5041301.
[6] M.F. Demirbas, Biofuels from algae for sustainable development, Appl. Energy 88 (2011) 3473–3480, https://doi.org/10.1016/j.apenergy.2011.01.059.
[7] R. Pangestuti, S.-K. Kim, Biological activities and health benefit effects of natural pigments derived from marine algae, J. Funct. Foods 3 (2011) 255–266, https://doi.org/10.1016/j.jff.2011.07.001.
[8] Kuech A., Breuer M., Popescu I., Research for PECH Committee – The Future of the EU Algae Sector, in: European Parliament, Policy Department for Structural and Cohesion Policies, 2023. Brussels.
[9] A. Sarwer, S.M. Hamed, A.I. Osman, F. Jamil, A.H. Al-Muhtaseb, N.S. Alhajeri, D. W. Rooney, Algal biomass valorization for biofuel production and carbon sequestration: a review, Environ. Chem. Lett. 20 (2022) 2797–2851, https://doi.org/10.1007/s10311-022-01458-1.
[10] B. Cordero, D. Voltolina, Viability of mass algal cultures preserved by freezing and freeze-drying, Aquac. Eng. 16 (1997) 205–211, https://doi.org/10.1016/S0144-8609(97)00001-0.
[11] E. Ryckebosch, M. Drouillon, H. Vervaeren, Techniques for transformation of biogas to biomethane, Biomass Bioenergy 35 (2011) 1633–1645, https://doi.org/10.1016/j.biombioe.2011.02.033.
[12] E. Montaini, G. Chini Zittelli, M.R. Tredici, E. Molina Grima, J.M. Fernández Sevilla, J.A. Sánchez Pérez, Long-term preservation of *Tetraselmis suecica*: influence

of storage on viability and fatty acid profile, Aquaculture 134 (1995) 81–90, https://doi.org/10.1016/0044-8486(95)00034-Y.
[13] Y. Ma, W. Zhou, P. Chen, P.E. Urriola, G.C. Shurson, R. Ruan, C. Chen, Metabolomic evaluation of Scenedesmus sp. as a feed ingredient revealed dose-dependent effects on redox balance, intermediary and microbial metabolism in a mouse model, Nutrients 11 (2019) 1971, https://doi.org/10.3390/nu11091971.
[14] M. Sakarika, M. Kornaros, Chlorella vulgaris as a green biofuel factory: comparison between biodiesel, biogas and combustible biomass production, Bioresour. Technol. 273 (2019) 237–243, https://doi.org/10.1016/j.biortech.2018.11.017.
[15] S. Je, Y. Yamaoka, Biotechnological approaches for biomass and lipid production using microalgae *Chlorella* and its future perspectives, J. Microbiol. Biotechnol. 32 (2022) 1357–1372, https://doi.org/10.4014/jmb.2209.09012.
[16] T. Bito, E. Okumura, M. Fujishima, F. Watanabe, Potential of Chlorella as a dietary supplement to promote human health, Nutrients 12 (2020) 2524, https://doi.org/10.3390/nu12092524.
[17] A. Silkina, J.I. Gayo-Peláez, F. Fernandes, C. Fuentes-Grünewald, R.V. Kapoore, K. W. Tang, From waste to wealth: coupling different nutritional modes of Scenedesmus obliquus for waste remediation and algal product development, J. Appl. Phycol. 37 (2025) 1003–1012, https://doi.org/10.1007/s10811-024-03438-0.
[18] A. Silkina, N.E. Ginnever, F. Fernandes, C. Fuentes-Grünewald, Large-scale waste bio-remediation using microalgae cultivation as a platform, Energies (Basel) 12 (2019), https://doi.org/10.3390/en12142772.
[19] M. Dubois, K. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, A colorimetric method for the determination of sugars, Nature 168 (1951) 167, https://doi.org/10.1038/168167a0.
[20] J.J. Mayers, K.J. Flynn, R.J. Shields, Rapid determination of bulk microalgal biochemical composition by Fourier-transform infrared spectroscopy, Bioresour. Technol. 148 (2013) 215–220, https://doi.org/10.1016/j.biortech.2013.08.133.
[21] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917, https://doi.org/10.1139/o59-099.
[22] J. Folch, M. Lees, G.H.S. Stanley, A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226 (1957) 497–509, https://doi.org/10.1016/S0021-9258(18)64849-5.
[23] C. Safi, M. Charton, O. Pignolet, F. Silvestre, C. Vaca-Garcia, P.-Y. Pontalier, Influence of microalgae cell wall characteristics on protein extractability and determination of nitrogen-to-protein conversion factors, J. Appl. Phycol. 25 (2013) 523–529, https://doi.org/10.1007/s10811-012-9886-1.
[24] J.J. Mayers, K.J. Flynn, R.J. Shields, Rapid determination of bulk microalgal biochemical composition by Fourier-transform infrared spectroscopy, Bioresour. Technol. 148 (2013) 215–220, https://doi.org/10.1016/j.biortech.2013.08.133.
[25] C. Safi, M. Charton, O. Pignolet, F. Silvestre, C. Vaca-Garcia, P.-Y. Pontalier, Influence of microalgae cell wall characteristics on protein extractability and determination of nitrogen-to-protein conversion factors, J. Appl. Phycol. 25 (2013) 523–529, https://doi.org/10.1007/s10811-012-9886-1.
[26] M.J. Griffiths, C. Garcin, R.P. van Hille, S.T.L. Harrison, Interference by pigment in the estimation of microalgal biomass concentration by optical density, J. Microbiol. Methods 85 (2011) 119–123, https://doi.org/10.1016/j.mimet.2011.02.005.
[27] A.R. Wellburn, The spectral determination of chlorophylls a and b, as well as Total carotenoids, using various solvents with spectrophotometers of different resolution, J. Plant Physiol. 144 (1994) 307–313, https://doi.org/10.1016/S0176-1617(11)81192-2.
[28] E.S. Okeke, O. Ejeromedoghene, C.O. Okoye, T.P.C. Ezeorba, R. Nyaruaba, C. K. Ikechukwu, A. Oladipo, J.I. Orege, Microalgae biorefinery: an integrated route for the sustainable production of high-value-added products, Energy Convers. Manag.: X 16 (2022) 100323, https://doi.org/10.1016/j.ecmx.2022.100323.
[29] C. Shene, M.T. Monsalve, D. Vergara, M.E. Lienqueo, M. Rubilar, High pressure homogenization of *Nannochloropsis oculata* for the extraction of intracellular components: effect of process conditions and culture age, Eur. J. Lipid Sci. Technol. 118 (2016) 631–639, https://doi.org/10.1002/ejlt.201500011.
[30] R. Seghiri, J. Legrand, R. Hsissou, A. Essamri, Comparative study of the impact of conventional and unconventional drying processes on phycobiliproteins from Arthrospira platensis, Algal Res. 53 (2021) 102165, https://doi.org/10.1016/j.algal.2020.102165.
[31] M. Stramarkou, S. Papadaki, K. Kyriakopoulou, I. Tzovenis, M. Chronis, M. Krokida, Comparative analysis of different drying techniques based on the qualitative characteristics of *Spirulina platensis* biomass, J. Aquatic Food Prod. Technol. 30 (2021) 498–516, https://doi.org/10.1080/10498850.2021.1900969.
[32] J.G. Ortiz-Tena, B. Rühmann, D. Schieder, V. Sieber, Revealing the diversity of algal monosaccharides: fast carbohydrate fingerprinting of microalgae using crude biomass and showcasing sugar distribution in Chlorella vulgaris by biomass fractionation, Algal Res. 17 (2016) 227–235, https://doi.org/10.1016/j.algal.2016.05.008.
[33] W.M. Brück, E. Alfonso, M. Rienth, W. Andlauer, Heat stress resistance in *Chlorella vulgaris* enhanced by hydrolyzed whey proteins, Agronomy 14 (2024) 2854, https://doi.org/10.3390/agronomy14122854.
[34] D. Karmakar, N. Tasnim, Md.R. Hasan, Md.S. Hossain, P. Arefin, D. Bhowmik, Y. M. Nibir, K.B. Kabir, R. Karim, Kinetic study and modelling of drying of Chlorella vulgaris, Heliyon 10 (2024) e38441, https://doi.org/10.1016/j.heliyon.2024.e38441.
[35] H. Shen, Y.K. Lee, Thermotolerance induced by heat shock in Chlorella, J. Appl. Phycol. 9 (1997) 471–475, https://doi.org/10.1023/A:1007956507345.
[36] T. Viswanathan, S. Mani, K.C. Das, S. Chinnasamy, A. Bhatnagar, Drying characteristics of a microalgae consortium developed for biofuels production, Trans. ASABE 54 (2011) 2245–2252, https://doi.org/10.13031/2013.40637.
[37] S.P. Singh, P. Singh, Effect of temperature and light on the growth of algae species: A review, Renew. Sust. Energ. Rev. 50 (2015) 431–444, https://doi.org/10.1016/j.rser.2015.05.024.
[38] A. Hernández, M. González-Moya, A. Márquez, L. Acevedo, Review microalgae drying: A comprehensive exploration from conventional air drying to microwave drying methods, Future Foods 10 (2024) 100420, https://doi.org/10.1016/j.fufo.2024.100420.
[39] M. de Jesus Oliveira Santos, C. Oliveira de Souza, H.R. Marcelino, Blue technology for a sustainable pharmaceutical industry: microalgae for bioremediation and pharmaceutical production, Algal Res. 69 (2023) 102931, https://doi.org/10.1016/j.algal.2022.102931.
[40] V. Okoro, U. Azimov, J. Munoz, H.H. Hernandez, A.N. Phan, Microalgae cultivation and harvesting: growth performance and use of flocculants - A review, Renew. Sust. Energ. Rev. 115 (2019) 109364, https://doi.org/10.1016/j.rser.2019.109364.
[41] M. He, Y. Yan, F. Pei, M. Wu, T. Gebreluel, S. Zou, C. Wang, Improvement on lipid production by Scenedesmus obliquus triggered by low dose exposure to nanoparticles, Sci. Rep. 7 (2017) 15526, https://doi.org/10.1038/s41598-017-15667-0.
[42] A. Vilatte, X. Spencer-Milnes, H.O. Jackson, S. Purton, B. Parker, Spray drying is a viable Technology for the Preservation of recombinant proteins in microalgae, Microorganisms 11 (2023) 512, https://doi.org/10.3390/microorganisms11020512.
[43] N. Attri, S. Kaur, P. Aggarwal, Spray Drying: A Promising Technique in Food Processing – Concept, Applications, and Latest Advancements, in: Novel Drying Technologies in Food Science [Working Title], IntechOpen, 2025, https://doi.org/10.5772/intechopen.1009780.