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Seasonal biochemical fingerprints of outdoor cultivated Spirulina (*Limnospira platensis*) and *Microchloropsis gaditana*: insights into nutritional and functional shifts

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Abstract

Microalgae are increasingly recognized as sustainable bioresources with applications in food, feed, nutraceuticals, and aquaculture. Among them, Spirulina (*Limnospira platensis*) and *Microchloropsis gaditana* (formerly *Nannochloropsis gaditana*) stand out due to their rich biochemical composition with industrial applications and high market value. This study separately investigates the seasonal variability in the biochemical composition of *L. platensis* cultivated in open raceway ponds and *M. gaditana* grown in closed photobioreactors.

The results suggest a response to seasonal changes. *L. platensis* exhibited higher protein content in spring (47.82%) and summer (45.48%), while carbohydrate accumulation peaked in winter (50.85%). *M. gaditana* showed increased lipid (21.67%) and carbohydrate (14.56%) content in autumn compared to winter and summer, but with lower variations in response to seasonal environmental changes. This microalga exhibited higher FA 20:5 *n*-3 (EPA) levels in winter compared to summer, suggesting its potential as a sustainable EPA source during colder months. The dominant carbohydrate residue in both microalgae, present throughout all seasons, was glucose, which could be associated with the presence of β-glucans, in addition to glycogen, reinforcing their bioactive potential.

Pigment composition was also seasonally influenced in *L. platensis*, which accumulated more chlorophyll and phycocyanin in spring and summer, whereas *M. gaditana* showed elevated levels of neoxanthin in autumn and winter, and violaxanthin in spring.

Although limited by a single sampling per season and the different cultivation systems size, this study provides preliminary observations of seasonal variations in biomass composition, highlighting that biochemical composition varies with season in a species-specific manner. Understanding these variations improves our knowledge of microalgal biochemical plasticity under outdoor conditions.

Keywords: Cyanobacteria, Monodopsidaceae, aquaculture, meteorological factors, temperature, radiation

1. Introduction

Microalgae are widely recognized for their potential as sustainable bioresources, finding diverse applications in food, feed, cosmetics, and pharmaceuticals. They have the ability to produce valuable bioactive compounds and nutrient-rich biomasses [1]. Furthermore, microalgae play a significant role in carbon capture and climate change mitigation, underscoring their pivotal contribution to environmental sustainability [2,3].

Among the vast diversity of photosynthetic microalgae and cyanobacteria, the Monodopsidaceae family (Eustigmatophytes including *Nannochloropsis* sp. and *Microchloropsis* sp.) and the cyanobacteria Spirulina (*Limnospira* sp.) are two attractive groups with broad applications, such as food, feed, and nutraceuticals [4,5]. In this study, we focused on the widely used species, *Limnospira platensis* and *Microchloropsis gaditana* (formerly known as *Nannochloropsis gaditana* [6]).

Considering both microalgae and cyanobacteria, Spirulina is the most widely produced and consumed globally, with a global production of 56,208 tons in 2019 and a market size of €533 million in 2023 [7,8]. This cyanobacterium is renowned for its exceptional protein content, rich profile of macro- and micronutrients, including essential amino acids, lipids, vitamins, and minerals, as well as potent antioxidant capacity [9]. In Europe, the commercial uses of *Limnospira* sp. biomass are mainly for

human food, supplements, and nutraceuticals [10]. While Spirulina (*Limnospira* sp.) is approved for human consumption worldwide, *Nannochloropsis* sp. and *Microchloropsis* sp. are not yet authorised for use within the European Union. Nevertheless, *Nannochloropsis* sp. and *Microchloropsis* sp. are widely exploited in aquaculture feed to enhance the nutritional value of aquafeeds [11].

Monodopsidaceae species are highly valued for their lipid content and rich composition in omega-3 polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic acid (EPA, 20:5 *n*-3). EPA is widely used as a dietary supplement and is considered essential for human health [12]. Beyond its nutritional benefits, EPA derived from *Nannochloropsis* and *Microchloropsis* also enhances the oxidative stability of functional foods. Compared to PUFA supplements from fish or krill, *Nannochloropsis*- and *Microchloropsis*-based extracts exhibit greater stability and a longer shelf life, mainly due to the co-extraction of lipids and pigments with antioxidant activity [13–15]. The global market demand for *Nannochloropsis* is projected to reach USD 4.2 million by 2028, with an annual growth rate of 8.5% from 2021 to 2028 [15].

Both Spirulina and Monodopsidacea can be cultivated year-round using open raceway ponds (ORPs) and closed photobioreactors (PBRs). ORPs offer advantages such as ease of scale-up, low energy consumption, and relatively low construction and operating costs [16]. However, closed PBRs have gained increasing attention due to their ability to control growth conditions more effectively, minimise contamination risks, and achieve higher biomass-to-substrate conversion ratios [17]. In both cases, large-scale outdoor microalgae production faces seasonal challenges, particularly due to temperature and light fluctuations [18], which alter their biochemical composition and influence their value for final applications.

Seasonal environmental fluctuations can impact the growth of microalgae species, affecting the productivity and nutritional composition of the biomass [18]. Temperature shifts of ± 10 °C from the optimal conditions reduce growth rates or completely inhibit growth in *N. oceanica* [18]. For Spirulina, optimal growth occurs at 35 °C [9,19], with light intensities between 250–350 $W.m^{-2}$, maximising biomass production, whereas intensities above 400 $W.m^{-2}$ inhibit growth [19]. Indeed, *Limnospira* spp. can withstand a wide range of environments; however, growth and productivity are affected by temperature [9,19]. Growth was reported as practically nil at low temperatures (below 17 °C) [9]. Similarly, light intensity plays a crucial role in *Limnospira* growth and chemical composition — higher intensities can boost *Limnospira*'s growth. In contrast, lower intensities can enhance pigment and protein production, but at the expense of overall productivity [19]. Seasonal variations affect outdoor cultivation, with self-shading reducing productivity in summer and lower temperatures mitigating this effect in winter and spring [19]. For instance, Spirulina cultivated at 25 °C, 30 °C, and 37 °C showed a higher biomass production at higher temperatures, with cultivation at 30 °C enhancing the synthesis of pigments such as chlorophyll *a*, carotenoids, C-phycocyanin, and total phycobiliproteins, as well as proteins and carbohydrates [20]. Similarly, an average temperature of 30 °C, light irradiance of 100 µmol photons.m^2. s^{-1}, and orbital agitation of 136 rpm resulted in maximum biomass production and protein content after 7 days of cultivation [21].

Regarding *Nannochloropsis* species, such as *N. oceanica*, they thrive at 25–29 °C, with growth inhibited above 31 °C or below 9 °C [22]. In particular, *M. gaditana* reached its peak biomass production at 25 °C and 120 µmol photons.m^{-2}.s^{-1} under a 16h/8h light cycle [23,24]. Variations in temperature and light have a significant influence on the biochemical and nutritional profiles of Monodopsidacea species, particularly in their fatty acid (FA) composition. Suboptimal temperatures, especially

below 20 °C, have been associated with enhanced EPA production. For instance, *M. salina* exhibited the lowest growth rate but the highest EPA content at 5 °C [25], while *N. oculata* showed a 158% increase in EPA accumulation at 10 °C (12.8 $mg.L^{-1}$) compared to 25 °C [26]. In contrast, higher temperatures promoted lipid accumulation, with *M. gaditana* achieving a maximum lipid content at 30 °C [27]. Additionally, increased light intensity (500 µmol $photons.m^{-2}.s^{-1}$) stimulated lipid and carotenoid production in *N. oceanica*, but it reduced protein and sugar content [28].

Carbohydrate synthesis in microalgae is strongly influenced by external factors such as light intensity, temperature, and nutrient availability, and during colder or low-light conditions (e.g., winter) [29]. In particular, *Limnospira platensis* tends to accumulate a higher total carbohydrate content (up to 45% dw), acting as an energy reserve or cryoprotectant [30]. During warmer and high-light seasons, carbohydrate biosynthesis may decline in favor of protein or lipid synthesis. Glucose residues often dominate the carbohydrate profile, acting as structural components (cellulose) or storage polysaccharides, that correspond to glycogen in cyanobacteria such as *L. platensis* and β-glucans in eukaryotic microalgae. The β-glucans are recognized as bioactive compounds due to their immunomodulatory and prebiotic properties [31]. *Limnospira platensis* also synthesizes a complex sulphated heteropolysaccharide, mainly composed by rhamnose, xylose, and galactose, which has been reported to exhibit antiviral and immunomodulatory properties [32–34] . Similarly, *Nannochloropsis* species are described to have (β1→3)-glucans and smaller amounts of sulphated heterorhamnans. However, the seasonal variation in these polysaccharides remains poorly understood, with only the stimulation of total carbohydrate being described.

Although seasonal variations, particularly those related to temperature and light fluctuations, are well known to influence microalgae biomass production, their impact on biochemical composition remains poorly understood. To the best of the authors' knowledge, no studies have investigated these seasonal effects in *M. gaditana* and *L. platensis* with a comprehensive biochemical characterization. Addressing this gap is essential for characterising seasonal biochemical trends . A deeper understanding of seasonal shifts improves awareness of how environmental fluctuations impact biochemical composition.

This non-comparative study aims to investigate seasonal variations in the biochemical profiles of outdoor industrially produced *L. platensis* and *M. gaditana* subjected to ambient conditions of each season (autumn, winter, spring, and summer). The biochemical analyses include proximate composition, fatty acid profiles, pigments, carbohydrates, and amino acids to explore the potential of season-specific biochemical profiles.

2. Material and Methods

2.1. Outdoor industrial biomass production

The industrial production of *L. platensis* took place at the facilities of Allmicroalgae - Natural Products S.A. in Pataias, Portugal. The biomass was cultivated in ORPs (Supplementary Figure 1A), covering areas of 1000 m^2 (in autumn and winter) and 4000 m^2 (in spring and summer), within a transparent plastic greenhouse that allowed exposure to natural light. During autumn and winter, *L. platensis* cultures are kept in maintenance mode and thus kept in smaller ORPs. The culture medium used was organic medium [35] and pH was set at 10. Continuous agitation was maintained by

paddlewheels, with one operating in the 1000 m² ponds and two in the 4000 m² ponds. The culture depth varied between 15 and 20 cm, depending on seasonal fluctuations and evaporation rates. To compensate for water loss due to evaporation, fresh water was added daily. An internal ventilation system was activated when needed to maintain the culture's temperature under 35 °C, in order to avoid the collapse of the cultures. However, below 35 °C, the system allows temperature variations according to the external temperature. Samples were taken from the biomass harvested on October 24, 2023 (autumn); January 19, 2024 (winter); April 17, 2024 (spring); and July 10, 2024 (summer).

The production of *M. gaditana* occurred outdoors at Necton S.A. in Olhão, Portugal, in aerated Flat-Panel Photobioreactors (FP-PBRs) of 2700 L and horizontal flow-through TPBRs of 19000-27000 L (Supplementary Figure 1B). The culture medium used was Nutribloom® Plus (Necton S.A.) and the concentration of nitrates was kept between 2 and 4 mM by daily monitoring and adjustment with fresh medium. Throughout cultivation, pH was automatically maintained below 8.5 via the injection of pure CO_2, while culture temperature was maintained below 30 °C through the spray of underground water over the reactors. Samples were taken from the biomass harvested at 10 am on October 30, 2023 (autumn – TPBR); January 22, 2024 (winter – TPBR); April 22, 2024 (spring – TPBR); and July 17, 2024 (summer – FP-PBR).

Biomass concentration was inferred through daily measurements of culture's optical density. Culture's productivity ($g.L^{-1}.d^{-1}$) was calculated over the 7 days preceding harvesting. The harvest days corresponded to the peak of the 4 seasons of the year. The harvests were all carried out at the same time of the day, 10 am, so that the effect of biochemical fluctuations that occur during the day would not affect this comparison. Harvesting was done by a sieve (*L. platensis*) or by centrifugation (*M. gaditana*). The obtained paste was immediately frozen followed by freeze dried using a LyoMicron lyophilizer (Coolvacuum, Spain) operating at an absolute pressure of 0.04 mbar and a condenser temperature of −70 °C for 4 days. Samples were collected from industrial batches of each microalga, produced during each season. For biochemical analyses, analytical replicates were performed using different portions of each batch.

It is important to note that the two raceway pond systems used for *L. platensis* (1000 m² with a single paddle vs. 4000 m² with two paddles) may differ in hydrodynamics, which may lead to system-specific effects. Likewise, *M. gaditana* was cultivated in two photobioreactor configurations (FP-PBRs and horizontal flow-through TPBRs), which differ in geometry and mixing characteristics. These differences cannot be fully standardised and represent an intrinsic limitation of working under industrial cultivation conditions.

2.2 Elemental analysis and protein estimation

The CHN composition (Table S1) was determined by elemental analysis (Vario EL III®, Elemental Analyser System; GmbH, Hanau, Germany) according to the procedure provided by the manufacturer. The total protein was determined by multiplying the percentage of nitrogen by the factor 4.78 [36]. This conversion factor is widely accepted for microalgae and commonly applied in the scientific literature [37,38] ensuring comparability with previous studies. All analyses were performed in triplicate, and the average values were used for data interpretation.

2.3. Determination of ash content

Ash content was determined through gravimetry by burning 50 mg of the sample dry biomass at 550 °C for 4 h, in a muffle furnace (NABERTHERM, LE62K17N1). After the combustion of the biomass, the final weight corresponds to the ash content. All analyses were performed in triplicate, and the average values were used for data interpretation.

2.4. Amino acid analysis

Microalgae samples were subjected to acid hydrolysis to determine the total amino acid content. Approximately 100 mg of each sample was weighed into SPME glass vials, and 2 mL of 6 M HCl was added (≥37%, ACS grade, Sigma-Aldrich, USA). The mixture was vortexed for proper mixing. A needle was inserted into the vial septum to release oxygen, while another needle connected to a nitrogen stream was introduced. Nitrogen gas was bubbled through the sample for 1 min, after which the oxygen-release needle was removed, and nitrogen bubbling continued for an additional 3 min. The nitrogen inlet was removed, and the vial was tightly sealed with a PTFE tape. These vials were placed in an oven (Carbolite, UK) at 115 °C for 20 h to complete the hydrolysis. Afterwards, the vials were cooled to room temperature, diluted with 5 mL of ultra-pure water (18.2 MΩ·cm, Milli-Q, Merck Millipore, USA), and the pH was adjusted to 3.5 using 10 M NaOH (≥98%, Sigma-Aldrich, USA). The solution was transferred to a 10 mL Class-A volumetric flask, diluted to the mark with ultra-pure water, and filtered through a 0.22 µm PTFE syringe filter.

A liquid chromatograph (Thermo Scientific Vanquish, USA) equipped with Vanquish Pump, Vanquish Autosampler, Vanquish Diode Array Detector (DAD), and Vanquish Fluorescence Detector (FLD) was used for the detection and quantification of amino acids. The chromatographic separation was performed on a Chromolith Performance RP18e column (100-4.6 mm, Merck, Germany). Two eluents were prepared for HPLC analysis: i) eluent A: a solution of 2.8 g Na_2HPO_4 (≥99%, analytical grade, Merck, Germany), 7.6 g $Na_2B_4O_7{\cdot}10\ H_2O$ (≥99.5%, Merck,Germany), and 0.064 g NaN_3 (≥99%) dissolved in ultra-pure water, adjusted to pH 7.5 with HCl, and made up to 2 L; eluent B: a mixture of 450 mL methanol (HPLC grade, Merck, Germany), 450 mL acetonitrile (HPLC grade, Merck, Germany), and 100 mL ultra-pure water, prepared to a total volume of 1 L. The elution program included a flow rate increasing from 0.9 $mL.min^{-1}$ to 1.2 $mL.min^{-1}$ at set intervals and a gradient starting at 10% eluent B, increasing to 100% eluent B, and returning to the initial conditions. The program duration was 55 min, including stabilization phases. Fluorescence detection was conducted at an excitation wavelength of 356 nm and an emission wavelength of 445 nm, with a sensitivity factor of 1. The data collection rate was 2 Hz, and the column temperature was maintained at 25 °C.

To ensure proper amino acid derivatization, additional reagents were prepared: i) borate buffer (pH 9.5): 6.2 g of H_3BO_3 (≥99.5%, Merck, Germany) was dissolved in ultra-pure water, pH adjusted to 9.5 with 4 M NaOH (≥98%) and diluted to 1 L; ii) internal standard (IS): a stock solution of 200 $mg.L^{-1}$ homoserine (≥98%, VWR, USA) in 0.1 M HCl was prepared by dissolving 0.01 g of homoserine in 50 mL 0.1 M HCl, then diluted tenfold to a working solution of 20 $mg.L^{-1}$; iii) reagent A: a mixture of 3 mL IS solution (20 $mg.L^{-1}$), 100 mL mercaptoethanol (Fluka Analytical, USA), and 0.5 g sodium tetraphenylborate (Merck, Germany), diluted to 25 mL with borate buffer and stored cold for up to a week; iv) reagent B: a solution of 0.6 g iodoacetic acid (Sigma-Aldrich, USA) in 15 mL borate buffer, pH adjusted to 9.5 with 4 M NaOH, and diluted to 20 mL; v) reagent C: 0.175 g o-Phthalaldehyde (OPA, ≥99%, Millipore Sigma, Germany)

dissolved in 5 mL methanol (HPLC grade), mixed with 0.5 mL mercaptoethanol, diluted to 25 mL with borate buffer, and bubbled with nitrogen gas. This solution was stored cold and protected from light. A calibration curve was created using a 100 $mg.L^{-1}$ amino acid standard mixture (MilliporeSigma, Germany) prepared in 0.1 M HCl. Standard solutions (1-30 $mg.L^{-1}$) were prepared by diluting the stock solution in 0.1 M HCl.

2.5. Carbohydrate analysis

The total carbohydrate content and profile were determined in the different microalgae biomass. Neutral sugars were analysed by gas chromatography-flame ionization detection (GC-FID) (Perkin Elmer Clarus 400), after conversion into their alditol acetates, as previously reported by Ferreira et al. [39]. The samples (1-2 mg) were pre-hydrolysed with H_2SO_4 (0.2 mL, 72% w/w, 95-97%; PanReac, Spain) for 3 h at room temperature, with occasional stirring, followed by a hydrolysis in H_2SO_4 (2.2 mL, 1 M, 95-97%; PanReac, Spain) for 2.5 h at 100 °C to release the monosaccharides from the polysaccharides. 2-Deoxyglucose (0.2 mL, 1.0 mg/mL, >99%; Sigma-Aldrich, USA) was used as internal standard. Monosaccharides were reduced with $NaBH_4$ (0.1 mL, 15% w/v in 3 M NH_3, >99%; Scharlau, Spain) for 1 h at 30 °C and acetylated with acetic anhydride (3 mL, >99%; Carlo Erba, Italy), using methyl-imidazole (0.45 mL, >99%; Thermo Scientific, USA) as a catalyst, for 30 min at 30 °C.
Free alditols in dry biomass were also analysed by converting them into their respective alditol acetates through acetylation, using 2-deoxyglucitol (0.2 mL, 1.0 mg/mL, >99%; Sigma-Aldrich, USA) as internal standard.
The alditol acetates were analysed using a GC-FID Perkin Elmer Clarus 400 equipped with a DB-225 column (30 m × 0.25 mm and 0.15 μm of film thickness (J&W Scientific). The operating temperatures of the injector and the detector were 220 °C and 240 °C, respectively. The oven temperature program was used as follows: the initial temperature was set to 220 °C, hold for 7 min, and then raised to 240 °C at 10 °C. min^{-1}. The carrier gas (H_2) had a flow rate of 1.7 $mL.min^{-1}$. Uronic acids (UAs) were determined using the *m*-phenylphenol colorimetric method [40]. Briefly, after pre-hydrolysis with H_2SO_4 (0.2 mL, 72% w/w, 95-97%; PanReac, Spain) for 3 h at room temperature, the samples were hydrolysed in 1 M H_2SO_4 for 1 h at 100 °C. The hydrolysed samples were diluted (1:3, v/v) and boric acid (1 mL, 200 mM, >99%; Sigma-Aldrich in H_2SO_4 98% w/v) was added to 0.1 mL of each sample. The mixture was heated at 100 °C for 10 min, cooled on ice, and m-phenylphenol solution (20 μL; 0.15% w/v; 90%, Acros Organics, USA) was added. Absorbance was measured at 520 nm (BioTek - Eon Microplate Reader). A calibration curve was prepared with D-galacturonic acid (0–100 μg/mL, 7 points, >97%; Sigma-Aldrich, USA), and results were expressed as GalA equivalents.

2.6. Lipid analysis

2.6.1. Lipid extraction

Lipids of *L. platensis* and *M. gaditana* were extracted from 25 mg of freeze-dried biomass using a modified Folch's method [41,42]. All the reagents used for lipid extraction are HPLC grade. The biomass was mixed with dichloromethane (DCM):methanol (MeOH) (2:1, *v/v*), vortexed, and incubated for 30 min. After centrifugation (Pro-Analytical C400R; centurion Scientific, UK), the organic phase was collected, and the process was repeated three times. The extract was dried under nitrogen and re-dissolved in DCM:MeOH (2:1, *v/v*). Phase separation was achieved by

adding Milli-Q water, vortexing, and centrifugation, with the organic phase collected and the aqueous phase re-extracted twice using DCM. Combined organic phases were filtered, dried under nitrogen, transferred to pre-weighed amber vials, and stored at -20 °C. The lipid content was determined gravimetrically as a percentage of dry biomass.

2.6.2. Fatty acids analysis

Fatty acid methyl esters (FAME) were prepared from lipid extracts of *L. platensis* and *M. gaditana* using alkaline trans-esterification [43]. Lipid extracts were combined with an internal standard solution (methyl nonadecanoate in *n*-hexane 99%), followed by KOH solution (2.0 M in methanol), and vortexed. A saturated NaCl solution (1 g in 100 mL of Milli-Q water) was added to induce phase separation, and the organic phase was collected, dried under nitrogen, and dissolved in *n*-hexane 99% for GC-MS analysis (Agilent Technologies 8860 GC System, USA). For analysis, 2 µL of the prepared FAME solution was injected into a GC-MS equipped with a DB-FFAP column with the following specifications: 30 m of length, 0.32 mm internal diameter, and 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA) [44]. The injector and detector temperatures were set at 220 °C and 230 °C, respectively. The oven temperature followed a programmed gradient, starting at 58 °C (held for 2 min), increasing to 160 °C at a rate of 25 °C min^{-1}, then to 210 °C at 2 °C min^{-1}, and finally reaching 225 °C at 20 °C min^{-1}. The system operated in electron impact mode with helium as the carrier gas. FAME were identified by comparing their retention times and mass spectra with standard references (Supelco 37 Component FAME Mix) and databases (NIST Library, "Lipid Web"). Results were processed using Agilent MassHunter software.

2.7. Pigment analysis

2.7.1 Quantification of carotenoids and chlorophyll content

Carotenoids (i.e. beta-carotene, canthaxanthin, zeaxanthin, fucoxanthin, neoxanthin, violaxanthin, astaxanthin, and lutein) and chlorophyll were extracted from 5 mg of freeze-dried biomass using organic solvents (i.e. methanol for carotenoids and acetone for chlorophyll) following the methods described by Couso et al. (2012) and Schüler et al. (2020) [45,46]. These solvents were added until complete extraction was achieved, aided by cell disruption through tungsten bead milling. The quantification of carotenoids was performed using high-performance liquid chromatography (HPLC) on a Chromaster System (Hitachi, VWR), equipped with a diode array detector (5430 DAD, Hitachi, VWR) and a Purospher® STAR RP-18 chromatographic column (Merck, 250 × 2.1 mm, 5 µm). The analysis was conducted at a temperature of 27 °C, with a flow rate of 1 $mL.min^{-1}$ over 40 minutes, and an injection volume of 50 µL. Compound identification took place at a wavelength of 450 nm, using standards for comparisonThe concentrations of the carotenoids were calculated based on their respective calibration curves [45,46]. Additionally, the chlorophyll content was determined spectrophotometrically from acetone extracts, with concentrations calculated according to the equations established by Lichtenthaler & Wellburn [47] using a HITACHI spectrophotometer (model UH5300). All analyses were performed in triplicate, and the average values were used for data interpretation.

2.7.2 Phycocyanin content

Phycocyanin was extracted using distilled water from the dry biomass of various Spirulina samples. The extraction conditions were consistent across all three biomasses, with a solid-liquid ratio (SLR) of 0.03 g of dry biomass *per* mL of solvent, distilled water, at a constant temperature of 25 °C, and stirring for 30 min in an orbital rotor at 80 rpm. After extraction, the samples were centrifuged (Hettich® MIKRO 200/200R) for 15 min at 15,000 rpm. The supernatant was then collected for analysis. The solvent was added to the recovered pellet, and the extraction process was repeated until the supernatant became colorless. The absorbance spectra of the various extracts were measured between 230-700 nm using a UV-Vis microplate reader (SpectraMax, USA). The calibration curve was prepared using a pure standard of phycocyanin (lyophilized powder) from Sigma-Aldrich, measured at 615 nm.

2.8 Statistical analysis

The statistical analysis was performed to identify significant differences between seasons for each microalga. Univariate statistical analyses were performed using GraphPad Prism 8.0.1. software. The Shapiro−Wilk test was performed to verify normality of the dataset. When normality was observed, significant differences between groups were evaluated using One-Way ANOVA with Tukey's post hoc test, considering differences significant at p-value < 0.05. Whenever normality was not verified, the Kruskal-Wallis test, followed by Dunn's post-hoc comparisons was employed.

3. Results and discussion

3.1 Meteorological and productivity data

To enhance the understanding and correlation between the biochemical profile and environmental factors, meteorological and productivity data were obtained from the producers of the microalgae under study. Specifically, temperature and external radiation levels recorded on the day of harvest (Supplementary Figures S2 and S3) were compared across the different seasons. Because each species was grown in the industrial system in which it is routinely cultivated, system-specific effects may overlap with seasonal responses. For this reason, the present work does not aim to compare cultivation systems or species, but to describe seasonal biochemical trends within each system.

For *L. platensis*, temperature variations were observed across the seasons, with the lowest values recorded in winter (18.4 °C) and spring (19.0 °C). Autumn presented a moderate temperature of 23.1 °C, while summer exhibited the highest temperature at 33.3 °C (Figure S2). On the other hand, the external radiation recorded during production of *L. platensis* was higher in spring (483.1 $W.m^{-2}$) and summer (321.0 $W.m^{-2}$), and lower in winter (30.5 $W \cdot m^{-2}$) and autumn (90.9 $W \cdot m^{-2}$) (Figure S2).

A similar seasonal trend was observed for *M. gaditana*, though with less pronounced fluctuations. The lowest temperatures occurred during winter (20.9 °C) and spring (22.2 °C), whereas autumn and summer recorded slightly higher values of 24.8 °C and 26.1 °C, respectively (Figure S3). The external radiation recorded during production of *M. gaditana* (Figure S3) increased from winter (627.6 $W.m^{-2}$) to summer (987.5 $W.m^{-2}$), with intermediate values recorded in autumn (830.4 $W.m^{-2}$) and spring (917.0 $W.m^{-2}$).

In terms of culture's productivity, for *L. platensis* the highest values were achieved in spring (0.04 $g.L^{-1}.d^{-1}$) and summer (0.03 $g.L^{-1}.d^{-1}$), while productivity declined to 0.01 $g.L^{-1}.d^{-1}$ in autumn and was null in winter. During autumn and winter, *L. platensis* cultures are kept in maintenance mode, and no actual production was performed. Because no biomass growth occurred in autumn and winter, the biochemical

composition reported for these seasons reflects maintenance biomass and cannot be interpreted in a production context.

M. gaditana presented higher productivity values in autumn (0.22 $g.L^{-1}.d^{-1}$) and spring (0.25 $g.L^{-1}.d^{-1}$) compared to winter (0.14 $g.L^{-1}.d^{-1}$). The productivity of *M. gaditana* during the summer was null due to several constraints. Therefore, summer biochemical profile may not be representative.

Microalgae growth is affected by temperature and light. The lower (or null) productivity values observed in winter could be correlated with lower radiation levels, shorter photoperiods, and colder temperatures. A previous work demonstrated the influence of these parameters on *L. platensis* growth and productivity [48]. Similarly, the productivity of *Nannochloropsis oculata* cultivated in an outdoor pilot-scale OPR was influenced by seasonal variations, resulting in increases of 264% and 90% during summer compared to winter and autumn-spring, respectively [49].

3.2 Proximal analysis of microalgae biomass grown across the different seasons

To evaluate the effects of seasonality on the biochemical composition, we first investigated the changes in the proximal composition. In Spirulina (*L. platensis*), the proximal analysis (Figure 1 and Table S2) revealed that proteins were the predominant component during autumn, spring, and summer, ranging from 43.34 ± 0.13% in autumn to 47.82 ± 0.74% in spring. In contrast, protein content declined markedly in winter, reaching 27.01%, suggesting a seasonal influence on protein accumulation. Spirulina is recognized for its high protein content, which generally ranges between 46-63%, and can reach as high as 70% in nitrogen-supplemented media [50]. The influence of temperature and light intensity, two environmental factors that fluctuate across the four seasons, can impact the protein content of Spirulina. A decrease in the protein content of Spirulina under high light (500 $\mu E.m^{-2}.s^{-1}$) and high temperature (35 ˚C) was reported [51], while the protein content of Spirulina grown in thermostated PBRs between May and September was higher at 35 ˚C (64-68%) when compared to 25 ˚C (57-62%) [52]. In the present study, protein levels remained stable in spring (47.82 ± 0.74%) and summer (45.48 ± 1.24%), reflecting more favourable growth conditions during these seasons. A possible explanation for the highest protein content observed in spring and summer may be linked to higher external radiation recorded during these seasons (Supplementary Figure S2). Previous studies with Spirulina demonstrated that high light can reduce glutamine synthetase (GS) activity, which in turn enhance the activity of nitrate reductase (NR) and proteases involved in nitrogen assimilation. This higher nitrogen assimilation may contribute to protein production and could partly explain the protein accumulation observed in our study [53].

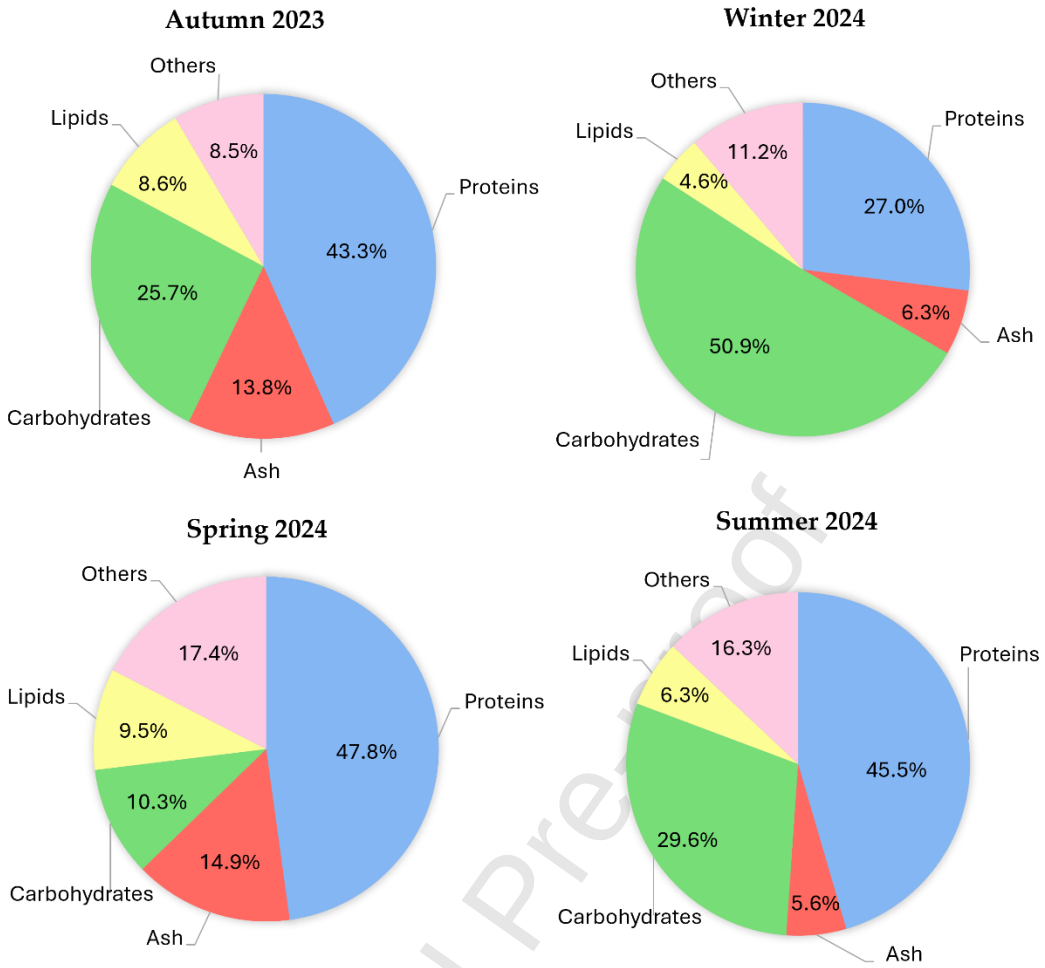

Figure 1. Proximal composition of *L. platensis* biomass across the seasons (autumn, winter, spring, and summer), including the percentage (% of dried biomass) of proteins, ashes, carbohydrates and lipids content. The results of statistical analysis are presented in Table S2.

The carbohydrate content, quantified by the sum of neutral sugars, uronic acids and alditols, was also affected by seasonality (Figure 1 and Table S2), with winter exhibiting the highest levels (50.85 ± 1.47%) and spring the lowest (10.32 ± 0.05%). Previous studies indicate that the carbohydrate content of Spirulina is typically between 10-27% [54].

The marked increase during winter may reflect metabolic adjustments to low-temperature stress. In plants, cold acclimation triggers reprogramming of the transcriptome, proteome, and metabolome, with carbohydrates playing a central role in this process [55]. A study in *Spirulina platensis* demonstrated an accumulation of soluble sugars under low temperatures, associated with the up-regulation of enzymes involved in gluconeogenesis and carbohydrate metabolism, including fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase (PEPCK) [56]. The enhancement of gluconeogenesis under cold stress thus contributes to increased soluble sugar production. The increase of soluble carbohydrates under lower temperature may have contributed to the increase of total carbohydrates observed during the winter season.

In addition, other study with outdoor cultivation has reported that carbohydrate content in *Arthrospira platensis* can rise to 44–45% of the dry weight during winter, which is associated with reduced protein synthesis, promoting carbon storage in polysaccharides due to low temperatures, decreased light intensity, and nutrient limitation [30]. Overall, these findings suggest that the elevated carbohydrate content observed in our study during winter represents an adaptive metabolic strategy to cope with combined environmental stresses.

Regarding the lipid content of *L. platensis*, fluctuations were observed across the different seasons, with lower values found in winter (4.56 ± 0.49%) and summer (6.30 ± 1.54%) and higher values in autumn (8.63 ± 0.52) and spring (9.51 ± 0.35%). The lipid content in *L. platensis* typically varies between 4-9% [50], and can also be affected by changes in environmental factors across different seasons. In a study with *Spirulina plantensis*, lipid accumulation was observed by increasing the temperature (from 25 to 30 °C) and light intensity (from 2500 to 5000 lux) [57]. However, many microalgae species accumulate lipids in response to low light and temperatures [58]. In fact, studies have reported a decrease in transcript levels associated with lipid metabolism, namely gpsA gene (GO:0004367) and glpA gene (GO:0004368), during exposure to high temperature, which in turn can result in a decrease in lipid content [59]. This effect in gene expression related to lipid metabolism may corroborate the decrease of lipids between spring and summer.

The ash content of *L. platensis* also varied seasonally, peaking in spring (14.92 ± 0.13%) and dropping significantly in winter (6.34 ± 0.11%) and summer (5.63 ± 0.08%). The "others" fraction (Figure 1), estimated by difference, also changed across the four seasons, ranging from 8.53 ± 0.89% in autumn to 17.43 ± 0.33% in spring. In *M. gaditana* (Figure 2 and Table S2), proteins represented always the predominant fraction, ranging from 30.71 ± 2.99% in winter to 37.36 ± 0.75% in spring. The protein content reported for *M. gaditana* varies between 20-45% [60]. Similar to what has been observed in Spirulina, the higher protein content recorded in spring may be associated with the increased external radiation during this season (Supplementary Figure S2). Elevated radiation has been shown to reduce GS activity, thereby inducing NR and protease activities involved in nitrogen assimilation, which could contribute to enhanced protein accumulation.

The total carbohydrate content ranged from $11.23 \pm 0.12\%$ in winter to a maximum of $14.56 \pm 0.69\%$ in autumn. This range is in accordance with values previously reported for the *Microchloropsis* (formerly *Nannochloropsis*) genus (5-30 %) [61,62]. Light intensity can affect carbohydrate content, as higher light intensities promote carbohydrate accumulation in *N. gaditana* grown under different light intensities [63]. A previous study on *Nannochloropsis oceanica* also revealed an up-regulation of proteins involved in glycolysis under high light intensity [64], Accordingly, we hypothesize that the increase in light intensity registered between winter and spring (Supplementary Figure S3) contributed to the higher amounts of carbohydrates found in spring than in winter (Figure 2 and Table S2).

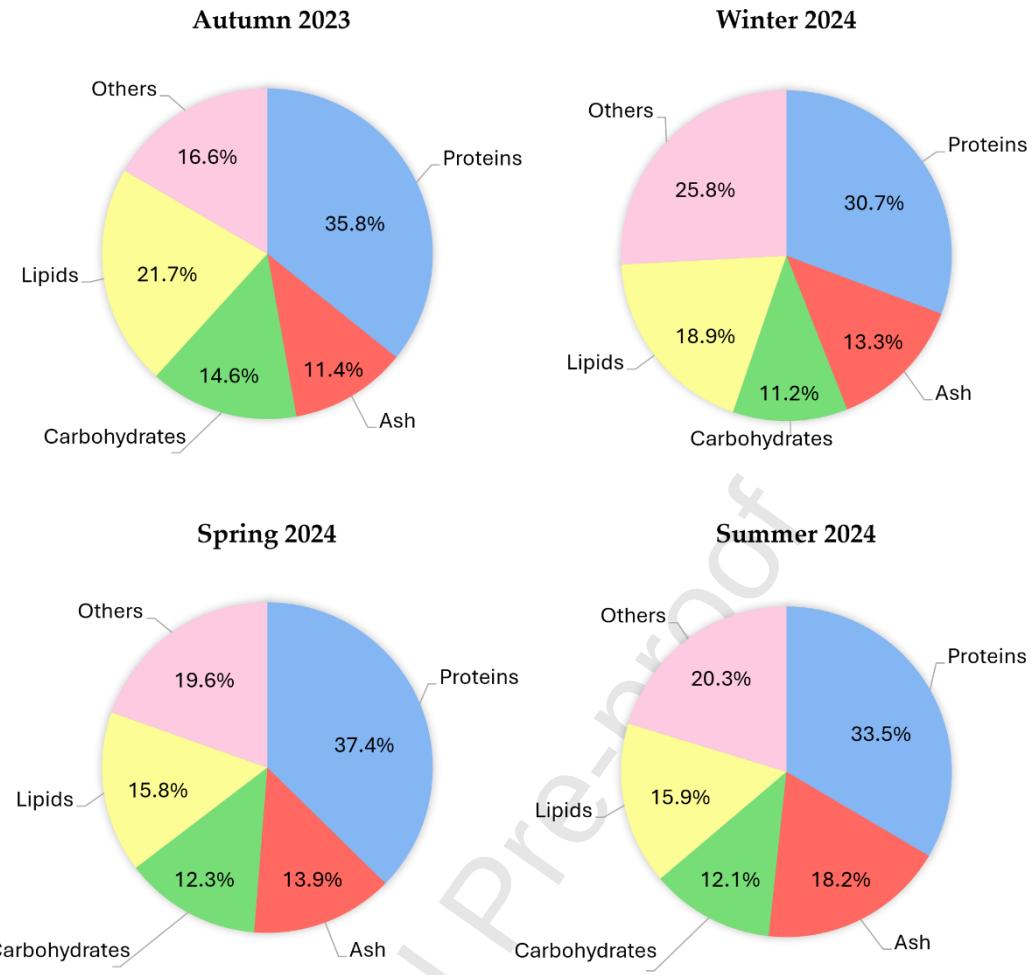

Figure 2. Proximal composition of *M. gaditana* biomass across the seasons (autumn, winter, spring, and summer), including the percentage (% of dried biomass) of proteins, ashes, carbohydrates and lipids content. The results of statistical analysis are presented in Table S2.

The lipid content in *M. gaditana* varied between seasons, with higher levels found in autumn (21.67 ± 1.35%) and winter (18.92 ± 0.42%). The lower values recorded in spring and summer were 15.80 ± 0.96% and 15.93 ± 0.60%, respectively. These values are in range with those previously reported for *M. gaditana* (13.50% and 15.45%) [65]. Several studies have shown that lipid accumulation in microalgae can increase under low-temperature conditions, as this helps maintain membrane fluidity and ensures normal cellular function [66,67]. Stress conditions such as low temperature can trigger the formation of reactive oxygen species (ROS). At moderate levels, ROS act as signaling molecules that activate pathways regulating lipid biosynthesis, thereby promoting lipid accumulation [68]. Furthermore, under low-temperature stress, the antioxidant defense system of algal cells is stimulated, leading to up-regulation of antioxidant-related enzymes and increased amino acid biosynthesis, which can provide additional precursors for lipid synthesis [69]. Overall, these mechanisms likely contribute to the higher lipid content observed in *M. gaditana* during winter (colder season) compared to spring and summer seasons.

The ash content varied between 11.41 ± 0.12% in autumn and 18.23 ± 0.74% in summer, representing a lower value compared to a previous report for spray-dried *M.*

gaditana (21.26%) [70]. The "others" fraction (estimated by difference) ranged from 16.59 ± 1.28% in autumn to 25.84 ± 3.79 % in winter.
It is important to emphasise that not all seasons supported growth for both species. *L. platensis* did not grow in autumn and winter, and *M. gaditana* exhibited no productivity during summer. Therefore, biochemical data from these non-productive seasons do not reflect feasible production outputs and cannot be used to identify optimal cultivation periods.

3.3. Amino acids profile of microalgae biomass grown across the different seasons

The amino acid content of *L. platensis* and *M. gaditana* was also analysed across the four seasons. Regarding *L. platensis* (Figure 3 and Supplementary Table S3), the most abundant amino acids identified were methionine, threonine and tyrosine. Spring exhibited the highest total amino acid content (47.15 ± 0.11 g.100 g^{-1} DW biomass), followed by summer (46.09 ± 0.32 g.100 g^{-1} DW biomass). This is in accordance with the highest total protein contents found in these seasons. The higher abundance in spring and summer was particularly observed in essential amino acids, such as methionine and threonine. The increased metabolic activity due to higher temperatures and prolonged sunlight exposure enhances Spirulina's protein production [71]. This was observed when *Spirulina* sp. was exposed to temperatures ranging from 25-40˚C, where protein content was higher at 30 ˚C followed by 35 ˚C [72], which were in the range of the temperature recorded in summer (33.3 ˚C) (Supplementary Figure S2). However, excessively high temperatures could also impact specific amino acids due to thermal degradation [73]. Contrarily, during winter, the total amino acid content was the lowest (30.94 ± 0.17 g.100 g^{-1} DW biomass) (Table S8). The reduced metabolic activity of *L. platensis* in colder temperatures likely contributes to this decrease in amino acid synthesis. *L. platensis* relies on photosynthesis for energy, and in colder conditions, its growth rate decreases because lower temperatures can impair photosynthetic efficiency. This means that less energy and fewer resources are available for amino acid and protein synthesis [71].

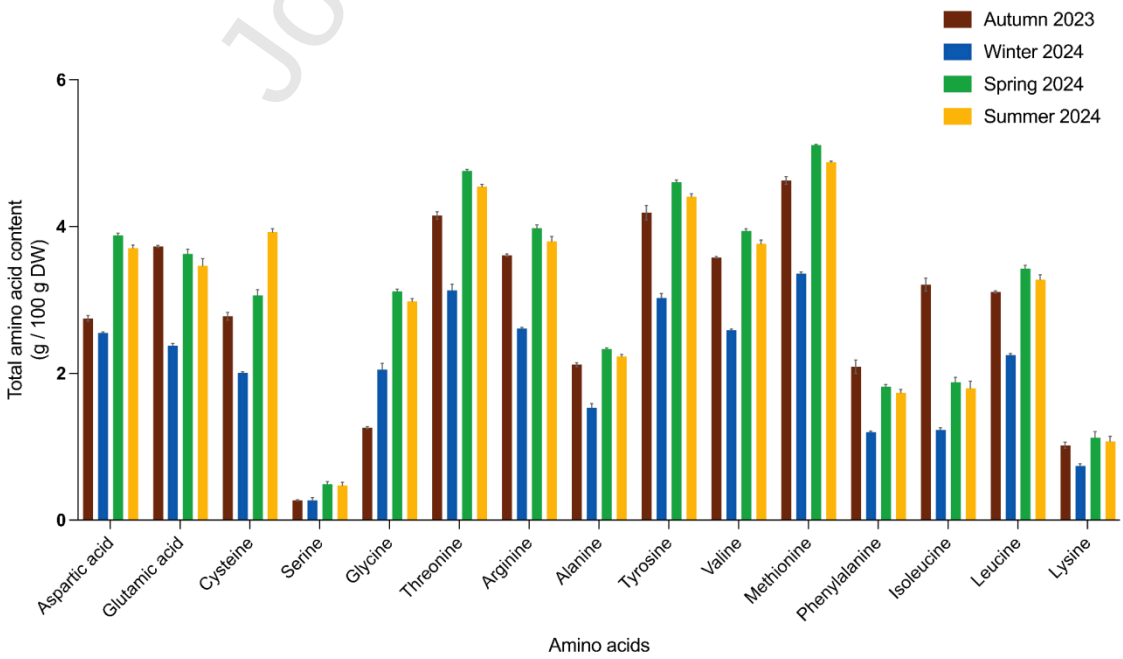

Figure 3. Amino acids content (g.100 g^{-1} DW biomass) of *L. platensis* along the seasons. This analysis includes both essential (threonine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine) and non-essential (aspartic acid, glutamic acid, cysteine, asparagine, glutamine, glycine, arginine, alanine, and tyrosine) amino acids. Values are three replicates' mean ± standard deviation (SD) (*n*=3). Results of statistical analysis are presented in Supplementary Table S8.

Although the impact of seasonal environmental changes on *L. platensis* amino acids has not been previously addressed, several studies have reported these changes in plants and algae in response to changes in factors such as temperature and radiation. For instance, one study addressed the impact of different temperature regimes on *Chlorella* and *Nannochloropsis*, and an increase in amino acids content was observed from 25 to 35˚C [74]. Another study, focusing on the diatom *Skeletonema costatum*, demonstrated that environmental conditions, including temperature and light, significantly impact its growth and biochemical composition. This work noted that exposure to high levels of UVB radiation led to a decrease in amino acid content, indicating that environmental factors can influence amino acid concentrations in algae [75]. This suggests that amino acids biosynthesis is particularly sensitive to environmental conditions, with higher temperatures (30-35˚C), and prolonged light exposure favouring it. From a nutritional perspective, *L. platensis* harvested in spring provides a richer source of essential amino acids, making it the ideal season for maximizing its protein content.

The most abundant amino acids detected in *M. gaditana* were also methionine, threonine and tyrosine (Figure 4 and Table S3). The data revealed that while seasonal differences exist, the fluctuations in total amino acid content for M. *gaditana* are less pronounced compared to *L. platensis*. The highest total amino acid content was observed for *M. gaditana* produced in spring (35.56 ± 0.11 g.100 g^{-1} DW biomass), followed by summer (33.67 ± 0.09 g.100 g^{-1} DW biomass) and autumn (33.86 ± 0.25 g.100 g^{-1} DW biomass). The lowest content found in winter (32.13 ± 0.14 g.100 g^{-1} DW biomass) may be attributed to lower metabolic activity due to decreased temperature and light availability, which could impact protein biosynthesis [76]. However, the extent of reduction is not as extensive as observed in *L. platensis*, suggesting relatively stable protein metabolism in *M. gaditana* even under less favorable environmental conditions. The observed differences in amino acid variability between *L. platensis* and *M. gaditana* can be attributed to their distinct metabolic strategies and ecological adaptations. *L. platensis*, as a cyanobacterium, relies predominantly on photoautotrophic growth, making its metabolic processes highly responsive to fluctuations in environmental factors such as light intensity and temperature. This dependence results in significant variations in amino acid composition under different environmental conditions. For instance, studies have shown that the amino acid content in Spirulina varies widely, with total amino acids ranging from 11.49 to 56.14 mg.100 g^{-1} DW biomass across different samples [77]. In contrast, *M. gaditana*, a eukaryotic microalga, exhibits greater metabolic plasticity, particularly through its ability to modulate lipid metabolism under stress conditions. Under nutrient-replete conditions, certain mutants of *M. gaditana* have demonstrated improved lipid productivity, indicating a capacity to adjust metabolic pathways to enhance lipid accumulation without compromising growth [78]. This metabolic flexibility likely contributes to a more stable amino acid profile, as the organism can reallocate resources to maintain essential functions despite environmental fluctuations. A more stable amino acid profile in *M. gaditana* implies a more robust metabolic regulation, allowing it to sustain protein synthesis more evenly throughout the year.

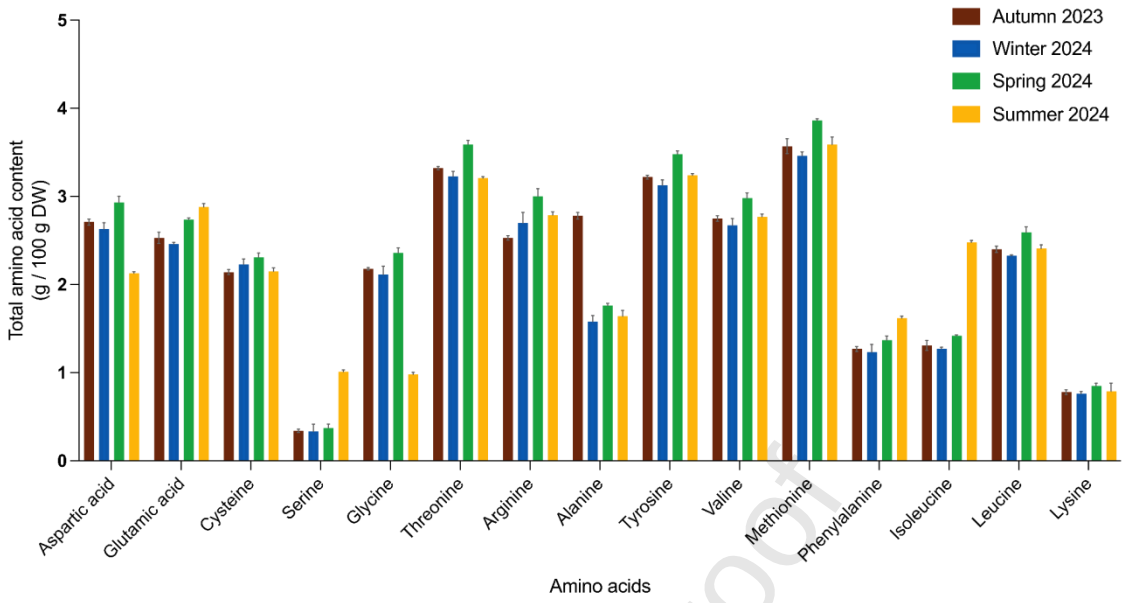

Figure 4. Amino acids content and profile (g.100 g^{-1} DW biomass) of *Microchloropsis gaditana* along the seasons. This analysis includes both essential (threonine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine) and non-essential (aspartic acid, glutamic acid, cysteine, asparagine, glutamine, glycine, arginine, alanine, and tyrosine) amino acids. Values are three replicates' mean ± standard deviation (SD) (*n*=3).

From a nutritional perspective, while *L. platensis* harvested in spring offers the highest total amino acid content, *M. gaditana* provides a more consistent protein profile throughout the year. This stability may be advantageous for applications where a steady protein composition is desired, regardless of seasonal variations. The findings suggest that while both species respond to environmental changes, *M. gaditana* exhibits greater resilience and metabolic balance across seasons, making it a reliable source of amino acids in varying conditions.

These findings suggest that while external factors influence both microalgae, *L. platensis* demonstrates greater susceptibility to environmental changes in amino acid levels, whereas *M. gaditana* maintains a more stable biochemical composition. Understanding these differences is crucial for optimising cultivation strategies, particularly in biotechnological applications where consistent protein yields are desirable. Further research exploring the molecular mechanisms underlying these metabolic adjustments could provide deeper insights into how microalgae regulate amino acid biosynthesis in response to environmental stressors.

3.4. Carbohydrates profile of microalgae biomass grown across the different seasons

The carbohydrate profile was determined for *L. platensis* and *M. gaditana* biomass cultivated throughout the different seasons of the year. In addition to the variation observed in the total content (Figure 1 and Table S2), *L. platensis* showed a significant variation on the carbohydrate profile depending on the time of the year (Table 1). In winter, *L. platensis* was predominantly composed by glucose (90.12 ± 0.79 mol%), with minor amounts (< 5 mol%) of uronic acids, galactose, rhamnose, xylose, ribose, fucose, arabinose, and mannose residues. Contrastingly, in spring, the glucose residues only

represented half of the total carbohydrates (48.08 ± 2.33 mol%), increasing the relative percentage of the other sugar residues, mainly galactose (15.04 ± 1.03 mol%), uronic acids (10.72 ± 1.15 mol%), xylose (7.06 ± 0.27 mol%), and rhamnose (6.37 ± 0.51 mol%). In autumn and summer, this cyanobacterium presented a carbohydrate profile similar to winter profile´s, with glucose as major monosaccharide representing 79.52 ± 1.81 mol% and 81.82 ± 1.03 mol%, respectively, and with other sugar residues appearing in minor amounts (< 7 mol%). Despite this similar trend, significant differences were found between these seasons (Table 1).

Table 1. Carbohydrate profile (mol%) of *L. platensis* biomass across autumn, winter, spring, and summer seasons. Values are the mean ± standard deviation (SD) of three replicates (*n*=3). Values in the same row followed by the same letter are significantly different at $p < 0.05$.

Monosaccharides (mol%)	**Autumn 2023**	**Winter 2024**	**Spring 2024**	**Summer 2024**
Rha	1.96 ± 0.09 ^{a,b,c}	1.22 ± 0.16 ^{a,d,e}	6.37 ± 0.51 ^{b,d,f}	2.76 ± 0.05 ^{c,e,f}
Fuc	0.64 ± 0.07 ^{a}	0.48 ± 0.01 ^{b}	2.42 ± 0.21 ^{a,b,c}	0.73 ± 0.04 ^{c}
Rib	1.91 ± 0.2 ^{a,b}	0.93 ± 0.08 ^{a,c}	5.82 ± 0.35 ^{b,c}	1.3 ± 0.32
Ara	0.74 ± 0.11 ^{a,b}	0.49 ± 0.03 ^{a,c,d}	2.3 ± 0.35 ^{b,c,e}	0.95 ± 0.06 ^{d,e}
Xyl	1.26 ± 0.09 ^{a,b}	1.13 ± 0.04 ^{a,c,d}	7.06 ± 0.27 ^{b,c,e}	2.75 ± 0.12 ^{d,e}
Man	1.63 ± 0.16 ^{a,b}	0.25 ± 0.02 ^{a,c,d}	2.2 ± 0.27 ^{c,e}	0.59 ± 0.22 ^{b,d,e}
Gal	5.08 ± 0.27 ^{a,b}	1.75 ± 0.1 ^{a,c,d}	15.04 ± 1.03 ^{b,c,e}	5.45 ± 0.12 ^{d,e}
Glc	79.52 ± 1.81 ^{a,b}	90.12 ± 0.79 ^{a,c,d}	48.08 ± 2.33 ^{b,c,e}	81.82 ± 1.03 ^{d,e}
UA	7.26 ± 1.77 ^{a}	3.63 ± 1.08 ^{a,b}	10.72 ± 1.15 ^{b,c}	3.64 ± 0.4 ^{c}

Abbreviations: Rha, ramnose; Fuc, fucose; Rib, ribose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acids.

Glucose residues are associated with the presence of storage carbohydrates, namely glycogen and 1,3/1,6-linked β-glucans [31,79]. Similar to other prokaryotes, cyanobacteria accumulate glycogen, a highly branched glucose polymer with (α1,4) glycosidic bonds for linear chains and (α1,6) at branch points. *L. platensis* and other cyanobacteria can also produce 1,3:1,6-β-glucans, β-linked glucose molecules linked by 1–3 glycosidic bonds with 1–6 glycosidic branches, similarly to some microalgae, mushrooms, yeast, and bacteria [79,80]. β-glucans are well known for their bioactive properties, such as antitumor, antioxidant, immunomodulatory, and antibacterial activities, and are therefore highly interesting as bioactive ingredients for the development of functional foods [31].

The higher cells' accumulation of carbohydrates, namely glucans, was observed in winter, where lower light intensity and lower temperatures (18.4 °C) were registered (Supplementary Figure S2). This increase in carbohydrate synthesis was previously observed for L. platensis grown at a suboptimal temperature of 25 °C, compared with the optimal temperature of 35 °C (in summer), where the productivity of *L. platensis* was superior [81]. According to the literature, high light irradiance has a positive influence on the synthesis of carbohydrates in *L. platensis* [81,82]. However, seasonal

environmental stress in winter, manifested in colder temperatures, reduced light, and nutrient limitations, could lead *L. platensis* to redirect carbon into carbohydrate reserves, significantly elevating its content (~44–45% DW), as observed for other cyanobacteria. Conversely, the more favorable spring and summer conditions support growth-focused metabolism, resulting in lower carbohydrate accumulation (~15–25% DW) [30].

The existence of other sugar residues (*e.g.*, galactose, uronic acids, xylose, rhamnose, fucose, and mannose) with a higher production ratio in spring in relation to glucans could be related to the presence of sulphated polysaccharides [32]. These polysaccharides have been associated with bioactive properties, namely antioxidant and antimicrobial activities , from sulphated polysaccharides isolated from *L. platensis* [83], and calcium spirulan, which exhibits antiviral properties [84].

Regarding *M. gaditana*, the biomass of each season showed little change in its total carbohydrate content (Figure 2 and Table S2), but also differences in the amount of each individual monosaccharide (Table 2). Glucose residues were predominant in all seasons (60.45–66.04 mol%), in accordance with the literature for Eustigmatophytes (e.g., *Microchloropsis* and *Nannochloropsis*) [62,85]. Glucose residues found in these genera can be associated with the presence of β-glucans, namely laminaran in *N. oceanica*, β-1,3/β1,4-glucans [29] and β-1,3-/β1,6-glucans were described in *N. oculata* [86] and a soluble and low molecular weight β-1,3-glucan with limited β-1,6-branching in *M. gaditana* [87].

Table 2. Carbohydrate profile (mol%) and free alditols (mg.g^{-1} DW biomass)) of *M. gaditana* biomass across autumn, winter, spring, and summer seasons. Values are the mean ± standard deviation (SD) of three replicates (n=3). Values in the same row followed by the same letter are significantly different at $p < 0.05$.

Monosaccharides (mol%)	**Autumn 2023**	**Winter 2024**	**Spring 2024**	**Summer 2024**
Rha	6.12 ± 0.19	5.85 ± 0.32	6.35 ± 0.16	5.71 ± 0.38
Fuc	1.62 ± 0.05	1.46 ± 0.03	1.31 ± 0.19	2.51 ± 1.58
Rib	4.35 ± 0.22 [a]	2.67 ± 0.8 [a,b]	6.29 ± 0.22 [b,c]	3.16 ± 0.10 [c]
Ara	1.57 ± 0.05	4.33 ± 2.01 [a]	2.22 ± 1.19	0.85 ± 0.06 [a]
Xyl	1.43 ± 0.26 [a]	2.04 ± 0.28 [b]	4.10 ± 0.43	1.73 ± 0.07
Man	0.54 ± 0.11 [a]	3.00 ± 0.75	2.13 ± 0.40	3.46 ± 0.78
Gal	19.50 ± 0.44	20.19 ± 0.79	16.79 ± 0.72	16.53 ± 1.23
Glc	64.87 ± 0.49	60.45 ± 1.31	60.81 ± 1.04	66.04 ± 0.87

Abbreviations: Rha, ramnose; Fuc, fucose; Rib, ribose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.

The sugar residues, galactose (16.53 – 20.19 mol%), rhamnose (5.71 – 6.35 mol%), mannose (0.55 – 3.46 mol%), ribose (2.67 – 6.29 mol%), xylose (1.43 – 4.10 mol%), fucose (1.31 – 2.51 mol%), and arabinose (0.85 - 4.33 mol%) are also present in minor amounts in *M. gaditana* across all seasons, in accordance with the literature for Eustigmatophytes [29,62,85]. In contrast to glucose, galactose, rhamnose, and fucose, other sugar residues showed significant changes throughout the year (Table 2).

According to literature, total carbohydrates production can be stimulated increasing the temperature from 15 to 30 °C in *N. oculata* [88], which could explain the lower carbohydrate production in winter (112.27±1.25 mg.g^{-1} DW biomass) in relation to the autumn (145.55 ± 6.92 mg.g^{-1} DW biomass) and spring (133.52 ± 5.57 mg.g^{-1} DW biomass) and summer (120.8 ± 2.49 mg.g^{-1} DW biomass) seasons (Figure 2). Higher

light intensities (160 µmol $m^{-2}.s^{-1}$) promoted the carbohydrate content in *M. gaditana* [23], which agrees with the higher carbohydrate content in autumn, spring and summer in relation to winter, where the light availability was lower (Figure S2).
The free polyol mannitol also significantly decreased in summer (2.80 ± 0.08 $mg.g^{-1}$ DW biomass), when compared with autumn (28.25 ± 0.69 $mg.g^{-1}$ DW biomass), winter (20.00 ± 3.51 $mg.g^{-1}$ DW biomass), and spring (33.65 ± 2.65 $mg.g^{-1}$ DW biomass). Mannitol is an osmoregulatory compound found in Eustigmatophytes, that allows a rapid acclimation to variable osmolarity [89]. Besides salinity-driven changes in the intracellular content, high light intensity also reduces the content of osmoprotectants, such as mannitol, in *N. oceanica* [90], consistent with the lower mannitol levels observed in this study during summer.

3.5. Fatty acids profile of microalgae biomass grown across the different seasons

The profile of esterified FA was identified for *L. platensis* and *M. gaditana* to determine changes across the seasons (Supplementary Table S4).
In *L. platensis*, the most abundant FAs throughout each season were FA 16:0, FA 18:2 *n*-6, and FA 18:3 *n*-6 (Figure 5), which is in agreement with previous studies [91,92]. The total content of saturated fatty acids (SFA) was higher in autumn (15.31 ± 0.41 $mg.g^{-1}$ biomass) compared to winter (5.32 ± 0.46 $mg.g^{-1}$ biomass). SFA levels in spring (12.72 ± 2.60 mg $FA.g^{-1}$ biomass) and summer (9.79 ± 2.05 mg $FA.g^{-1}$ biomass) were similar. Regarding the impact of seasonality in the SFA, a previous study with *L. platensis* under controlled conditions indicated that an increase in temperature from 25 to 38 ˚C potentially increases the proportion of SFA, while an increase in light intensity from 170 to 1400 µmol photons.$m^{-2}.s^{-1}$ is associated with a decrease in the relative abundance of SFA [93]. In the present study, the decrease in SFA observed between autumn and winter could be connected to the decrease in the temperatures registered (23.1 ˚C in autumn and 18.4 ˚C in winter).

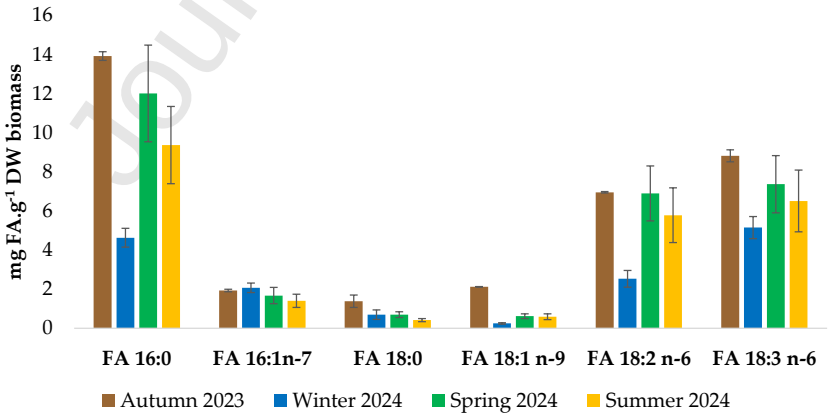

Figure 5. Composition of the most abundant fatty acids (mg·g^{-1} DW biomass) of *L. platensis* determined by gas chromatography–mass spectrometry (GC–MS). Values are the mean ± standard deviation (SD) of three replicates (*n*=3). Results of statistical analysis are presented in Supplementary Table S3.

Regarding the PUFA, namely FA 18:2 *n*-6 (linoleic acid, LA) and FA 18:3 *n*-6 (gamma-linolenic acid, GLA), they exhibited their lowest levels in winter (2.53 ± 0.43 and 5.15 ± 0.06 mg $FA.g^{-1}$ biomass, respectively). In contrast, their levels were higher and

comparable across autumn (6.95 ± 0.03 and 8.82 ± 0.30 mg FA.g^{-1} biomass, respectively), spring (6.90 ± 1.41 and 7.37 ± 1.46 mg FA.g^{-1} biomass, respectively), and summer (5.78 ± 1.40 and 6.51 ± 1.58 mg FA.g^{-1} biomass, respectively). The study with *L. platensis* subjected to different controlled conditions of temperature and light intensity indicated that, contrary to SFA, the proportion of PUFA decreases at higher temperatures, while higher light intensities seem to stimulate higher relative abundances of PUFA [93]. While the absolute amount of GLA was lower in winter compared to the other seasons (Supplementary Table S3), the relative abundance of this PUFA was higher in comparison (Supplementary Table S4). Thus, the changes in temperature and light intensity across the different seasons promoted a decrease in the proportion of GLA in biomasses grown under warmer seasons.

Both omega-6 FA (LA and GLA) are considered high-value compounds and are sought out as dietary components [94,95]. GLA was the most abundant PUFA reported in *L. platensis* in the present study. This omega-6 PUFA is obtained through dietary intake, typically from milk, and can play an essential role in the regulation of inflammation. In particular, supplementation of GLA is associated with anti-inflammatory properties [95], as GLA helps balance arachidonic acid (ARA, FA 20:4*n*-6) levels, and can be converted to dihomo-γ-linolenic acid (FA 20:3*n*-6), a precursor for the anti-inflammatory prostaglandin PGE1 [96]. Another study indicated that dietary supplementation with GLA can improve the skin's protective barrier, preventing dry skin and managing mild atopic dermatitis [94]. On the other hand, LA (FA 18:2*n*-6) is an essential FA that mammals must obtain from the diet, as it serves as a precursor to longer-chain, more unsaturated fatty acids such as ARA (FA 20:4*n*-6), which plays a role in cell function and the biosynthesis of eicosanoids with pro-inflammatory and vasomodulatory actions [97]. Observational data further support an inverse relationship between LA intake and the incidence of cardiovascular diseases (particularly coronary artery disease) and metabolic disorders such as type 2 diabetes [98]. Given its role in human health, an adequate dietary supply of LA is essential. The seasonal variation observed in *L. platensis* suggests that biomass collected during autumn, spring, and summer, when LA and GLA levels are highest, could serve as a valuable natural source of LA and GLA for the production of nutraceuticals with anti-inflammatory and skin-improvement properties.

In *M. gaditana* (Figure 6 and Supplementary Table S6), the most abundant FA across all seasons were FA 16:0, FA 16:1 *n*-7, and FA 20:5 *n*-3. This is in accordance with the FA profile reported for this microalga [99]. Regarding seasonal variations, the SFA content, namely FA 14:0, FA 16:0, and FA 18:0, remained relatively stable throughout the year. In contrast, the levels of unsaturated FA, especially of FA 20:5 *n*-3, exhibited seasonal fluctuations. The levels of FA 20:5 decreased significantly between winter (31.64 ± 3.69 mg FA.g^{-1} biomass) and summer (11.44 ± 1.19 mg FA.g^{-1} biomass), which corresponded to the seasons with the lowest (20.9 °C) and highest (26.1 °C) registered temperatures (Figure S2).

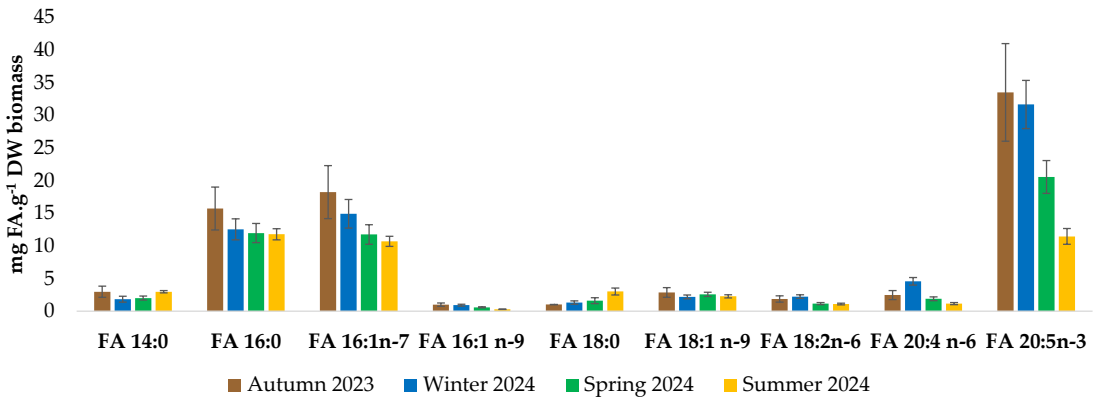

Figure 6. Composition of the most abundant fatty acids (mg·g^{-1} DW biomass) of *M. gaditana* determined by gas chromatography–mass spectrometry (GC–MS). Values are the mean ± standard deviation (SD) of three replicates (*n*=3). Results of statistical analysis are presented in Supplementary Table S4.

This pattern aligns with previous results with *M. gaditana*, where higher temperatures (33 ˚C) promoted a substantial decrease in PUFA content, particularly in EPA (FA 20:5 *n*-3) [100]. Other studies have indicated that light also affects EPA accumulation in *Nannochloropsis* species, with low light intensity being associated with higher yields of EPA [101]. Thus, it was expected that seasons with lower light intensities and lower temperatures, such as winter, would provide biomass enriched in this omega-3 FA. At lower temperatures, microalgae typically increase PUFA production to maintain membrane fluidity, ensuring optimal cellular function under colder conditions [102–104]. The seasonal variations in *M. gaditana* indicated that EPA levels were higher in winter.

This study highlights the high EPA content in *M. gaditana*, surpassing levels reported in other microalgae species such as *Pavlova gyrans* and *Tetraselmis striata*, both of which are also used as sources of PUFA and EPA for supplements and aquafeed [105,106]. Notably, the EPA concentrations observed here are consistent with values reported for the same species [107] and other *Nannochloropsis* sp. [92,108,109], reinforcing its potential as a sustainable and efficient source of this essential FA. The health benefits of EPA are well established, with numerous studies highlighting their role in reducing the risk of coronary diseases, lowering triglyceride levels, and supporting overall well-being [110]. While *M. gaditana* has potential as a food supplement, it is still not approved for human consumption within the European Union. However, its primary application has been in the aquaculture sector due to its high nutritional value, particularly in terms of protein and EPA content [111]. The aquaculture industry heavily depends on fish oil as a primary source of omega-3 PUFA, including EPA, which are essential for many fish species and critical for maintaining the nutritional value of farmed seafood, supporting growth performance and ensuring fillet quality for consumers [112]. The supplementation of aquafeed with EPA-enriched sources is fundamental to increase the available EPA in the produced fish, thus *M. gaditana* grown in winter can support the requirements of feed-enriched in this healthy FA.

3.6.Pigments of microalgae biomass grown across the different seasons

The pigment profiles, including chlorophylls and carotenoids, of *L. platensis* (Figure 7 and Table S8) and *M. gaditana* (Figure 8 and Table S9), and phycocyanin levels of *L. platensis* (Supplementary Table S7) were evaluated across the four seasons.

For *L. platensis* (Figure 7 and Table S8), *c*hlorophyll *a* was the most abundant pigment identified across all four seasons, with levels ranging between 2.29 ± 0.05 mg.g^{-1} DW biomass in winter to 12.35 ± 0.38 mg.g^{-1} DW biomass in summer. Chlorophyll *a*, with its blue-green color is valuable as natural food colorants (E140 and E141) —a trend driven by consumer demand for clean-label products [113]. Furthermore, these pigments offer a range of health benefits, including antioxidant, anti-cancer, and anti-inflammatory properties [113]. The results from our study suggest that chlorophyll concentrations were higher in summer.

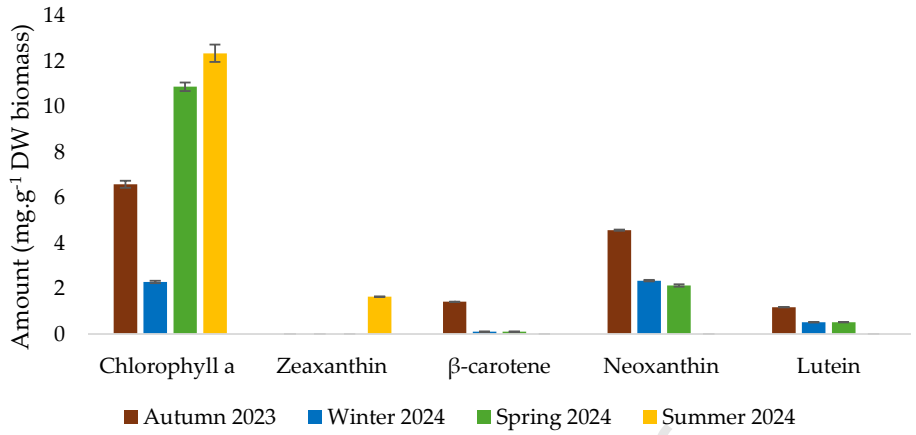

Figure 7. Profile of chlorophylls and carotenoids (mg.g^{-1} DW biomass) in *L. platensis* along the seasons determined by HPLC. Values are the mean ± standard deviation (SD) of three replicates (*n*=3). Results of statistical analysis are included in Supplementary Table S5.

Carotenoid levels were also affected by seasonality. Neoxanthin was the most abundant carotenoid species identified in *L. platensis*, reaching peak production in autumn (4.55 ± 0.02 mg.g^{-1} DW biomass) and being completely absent in summer. Similarly, β-carotene and lutein followed a similar pattern by peaking in autumn (1.42 ± 0.01 and 1.18 ± 0.00 mg.g^{-1} DW biomass) and disappearing in summer. These carotenoids provide not only interesting antioxidant compounds for applications as nutraceuticals, pharmaceuticals, and cosmeceuticals, but also a wide colour spectrum of natural dyes from yellow to purple, while exhibiting excellent stability under reducing conditions, positioning them as potential natural alternatives to synthetic dyes [33]. Thus, autumn samples exhibited higher carotenoid levels.

On the other hand, zeaxanthin was only detected in the summer (1.64 ± 0.02 mg.g^{-1} DW biomass), indicating its synthesis under high-light and high-temperature conditions. Although it has not been reported in microalgae yet, studies in *Arabidopsis thaliana* have reported the accumulation of zeaxanthin under excess light [34]. This carotenoid is known to enhance photoprotection and mitigate light-induced stress [34], and has a robust antioxidant activity that can protect eye cells from light-induced damage and help prevent retinal impairment [114].

For *L. platensis* we have also quantified phycocyanin (Supplementary Table S10), a pigment-protein complex that is highly abundant in this cyanobacterium [115]. We observed a similar seasonal trend to chlorophylls, with a significantly higher concentration produced during summer (110.06 ± 1.22 mg.g^{-1} DW biomass) and a lower concentration obtained in winter (36.1 ± 1.59 mg.g^{-1} DW biomass) (Supplementary Table S8). The coordinated accumulation of both chlorophylls and phycocyanin is expected, as this fluorescent protein acts as an antenna, capturing light and directing it towards the chloroplasts, where chlorophylls are located, thereby contributing directly to biomass growth [116]. The higher light irradiance during the summer season stimulates production of phycocyanin, resulting in higher phycocyanin concentrations. Phycocyanin exhibits similar beneficial properties to chlorophyll *a*, including anti-obesity, anti-melanogenic, and anti-neurodegenerative effects, making it valuable across various industries, from food as a natural colourant to pharmaceuticals as a

biofluorescent marker or an active anti-tumoral agent. However, the stability of this pigment is often considered a drawback [115].
Regarding the pigment profile of *M. gaditana* (Figure 8 and Supplementary Table S9), chlorophyll *a* peaked at 12.70 ± 0.01 mg.g^{-1} DW during autumn.

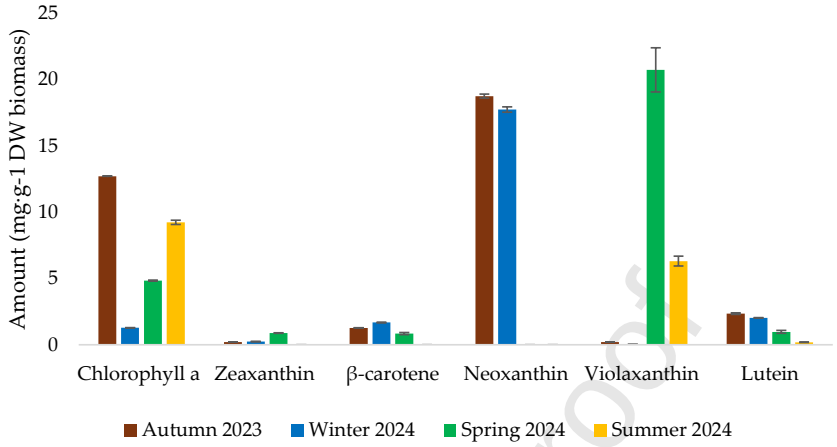

Figure 8. Profile of chlorophylls and carotenoids (mg.g^{-1} DW biomass) in *M. gaditana* along the seasons determined by HPLC. Values are the mean ± standard deviation (SD) of three replicates (*n*=3). Results of statistical analysis are included in Supplementary Table S6.

Neoxanthin was especially abundant in autumn (18.75 ± 0.15 mg.g^{-1} DW biomass) and winter (17.73 ± 0.16 mg.g^{-1} DW biomass), but it was absent in spring and summer. In contrast, violaxanthin peaked in spring (20.73 ± 1.36 mg.g^{-1} DW biomass), indicating optimal production conditions during this period. Elevated temperatures, especially when combined with high light intensity, can enhance the activity of the xanthophyll cycle, leading to an increase in the conversion of violaxanthin into other pigments [117], which can explain the decrease in this carotenoid from spring to summer. Regarding neoxanthin, further studies are still required to understand how this carotenoid is produced during seasons associated with lower temperatures and light intensities. The remaining carotenoids, zeaxanthin, β-carotene, and lutein were found in lower concentrations throughout the year, with lutein steadily decreasing from 2.34 ± 0.06 mg.g^{-1} DW in autumn to 0.17 ± 0.00 mg.g^{-1} DW in summer.
The seasonal pigment profile of *M. gaditana* highlights its potential as a source of commercially valuable carotenoids, particularly violaxanthin and neoxanthin, which are currently underutilized in industrial applications. While carotenoids, such as β-carotene, lutein, and zeaxanthin, are widely used as colorants in food and nutraceuticals in human health, violaxanthin and neoxanthin remain largely unavailable on the market, despite their presence in higher plants and their potential health benefits [118–121]. Violaxanthin, in particular, has been identified in various microalgae, and has exhibited bioactive properties, including antioxidant, anti-proliferative, anti-inflammatory, and pro-apoptotic effects against human cancer cells, reinforcing its potential for medical, nutraceutical, and functional food applications [118,119]. Similarly, neoxanthin has shown biological activities, including a suppressive effect on the differentiation of 3T3-L1 adipose cells, leading to reduced lipid accumulation, an effect not observed with other carotenoids evaluated in the study [120]. Neoxanthin also demonstrated anticancer potential by inducing apoptosis in PC-3 human prostate cancer cells [121]. These findings highlight the relevance of *M. gaditana* as a promising source of bioactive

carotenoids with potential applications in health-promoting and therapeutic products, positioning all year-round produced biomasses as sources of high-value compounds.

4. Conclusions

This study suggests that seasonal variations may have a significant influence on the biochemical composition of microalgae and cyanobacteria, reflecting their metabolic plasticity and adaptability to changing environmental conditions.

L. platensis exhibited seasonal fluctuations, particularly in protein, carbohydrate, and ash contents. Protein levels peaked in spring and summer, with essential and non-essential amino acids showing pronounced increases during this season. On the other hand, carbohydrate accumulation was highest in winter, likely as a response to the stress of lower temperatures. Glucose was the dominant carbohydrate residue, indicating the presence of storage polysaccharides, such as glycogen and β-glucans. These polysaccharides are also predominant in autumn and summer. Furthermore, spring featured a more diverse sugar profile, including galactose, uronic acids, and rhamnose, potentially indicative of bioactive sulfated polysaccharides. Lipid content remained relatively stable, with elevated LA levels in autumn, spring, and summer, reinforcing its potential as a dietary source of essential FA.

M. gaditana maintained a more stable biochemical profile throughout the year, with lipid and carbohydrate content increasing in colder seasons. PUFA content, especially EPA, peaked in autumn and winter. This microalga also showed glucose as the main sugar associated to the presence of β-glucans and cellulose (structural component). The protein content of *M. gaditana* was relatively stable throughout the year, which is reflected in its amino acid profile.

L. platensis exhibited higher chlorophyll and phycocyanin concentrations in spring and summer, and carotenoids such as β-carotene, neoxanthin, and violaxanthin peaked in autumn, while *M. gaditana* displayed significant seasonal variations in violaxanthin and neoxanthin.

Overall, these preliminary findings highlight that seasonal variation should be considered when interpreting biochemical composition of outdoor cultures. However, these results cannot be used to determine optimal production seasons, as productivity was not sustained in all periods for either species. Seasons with no growth (autumn/winter for *L. platensis* and summer for *M. gaditana*) provide biochemical information but do not represent viable production scenarios.

Further studies with different sampling times within each season would also be valuable to determine whether the observations presented here, based on a single sampling per season, are consistent across seasons and to evaluate possible intra-seasonal variations. Also, larger datasets combined with multivariate analyses would allow for a more robust and comprehensive evaluation of the seasonal variations. Finally, as each species was cultivated in its standard industrial system, system-specific effects cannot be completely separated from seasonal influences, and no comparison between cultivation systems is intended.

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Declaration of competing interest

The authors declare no conflict of interest.

References:

[1] V. Sousa, R.N. Pereira, A.A. Vicente, O. Dias, P. Geada, Microalgae biomass as an alternative source of biocompounds: New insights and future perspectives of extraction methodologies, Food Research International 173 (2023) 113282. https://doi.org/10.1016/j.foodres.2023.113282.

[2] D. Nagarajan, D.-J. Lee, J.-S. Chang, Integration of anaerobic digestion and microalgal cultivation for digestate bioremediation and biogas upgrading, Bioresource Technology 290 (2019) 121804. https://doi.org/10.1016/j.biortech.2019.121804.

[3] P.A. Diankristanti, N. Hei Ernest Ho, J.-H. Chen, D. Nagarajan, C.-Y. Chen, Y.-M. Hsieh, I.-S. Ng, J.-S. Chang, Unlocking the potential of microalgae as sustainable bioresources from up to downstream processing: A critical review, Chemical Engineering Journal 488 (2024) 151124. https://doi.org/10.1016/j.cej.2024.151124.

[4] S.M. Hoseini, K. Khosravi-Darani, M.R. Mozafari, Nutritional and Medical Applications of Spirulina Microalgae, Mini-Reviews in Medicinal Chemistry 13 (2013) 1231–1237. https://doi.org/10.2174/1389557511313080009.

[5] M. Matos, M.A. Fernandes, I. Costa, N. Coelho, T.F. Santos, V. Rossetto, J. Varela, I. Sá-Correia, Culturable Yeast Diversity Associated with Industrial Cultures of the Microalga Microchloropsis gaditana and Their Ability to Produce Lipids and Biosurfactants, Journal of Fungi 11 (2025). https://doi.org/10.3390/jof11030228.

[6] M.W. Fawley, Jameson ,Ian, K.P. and Fawley, The phylogeny of the genus Nannochloropsis (Monodopsidaceae, Eustigmatophyceae), with descriptions of N. australis sp. nov. and Microchloropsis gen. nov., Phycologia 54 (2015) 545–552. https://doi.org/10.2216/15-60.1.

[7] Global seaweeds and microalgae production, (n.d.). https://openknowledge.fao.org/items/5f24c0c2-3a0d-44f9-8b85-fbb775a86735 (accessed January 17, 2025).

[8] M.B. Zrimec, E. Sforza, L. Pattaro, D. Carecci, E. Ficara, A. Idà, N. Ferrer-Ledo, S. Canziani, S. Mangini, B. Lazar, S. Papadaki, G. Markou, I. Tzovenis, R. Reinhardt, M.B. Zrimec, E. Sforza, L. Pattaro, D. Carecci, E. Ficara, A. Idà, N. Ferrer-Ledo, S. Canziani, S. Mangini, B. Lazar, S. Papadaki, G. Markou, I. Tzovenis, R. Reinhardt, Advances in Spirulina Cultivation: Techniques, Challenges, and Applications, in: Insights Into Algae - Fundamentals, Culture Techniques and Biotechnological Uses of Microalgae and Cyanobacteria, IntechOpen, 2024. https://doi.org/10.5772/intechopen.1005474.

[9] R.A. Soni, K. Sudhakar, R.S. Rana, *Spirulina* – From growth to nutritional product: A review, Trends in Food Science & Technology 69 (2017) 157–171. https://doi.org/10.1016/j.tifs.2017.09.010.

[10] T. Lafarga, J.M. Fernández-Sevilla, C. González-López, F.G. Acién-Fernández, *Spirulina* for the food and functional food industries, Food Research International 137 (2020) 109356. https://doi.org/10.1016/j.foodres.2020.109356.

[11] L. Zanella, F. Vianello, Microalgae of the genus *Nannochloropsis*: Chemical composition and functional implications for human nutrition, Journal of Functional Foods 68 (2020) 103919. https://doi.org/10.1016/j.jff.2020.103919.

[12] X.-N. Ma, T.-P. Chen, B. Yang, J. Liu, F. Chen, Lipid Production from Nannochloropsis, Mar Drugs 14 (2016) 61. https://doi.org/10.3390/md14040061.

[13] L. Gheysen, N. Lagae, J. Devaere, K. Goiris, P. Goos, T. Bernaerts, A. Van Loey, L. De Cooman, I. Foubert, Impact of Nannochloropsis sp. dosage form on the oxidative stability of n-3 LC-PUFA enriched tomato purees, Food Chemistry 279 (2019) 389–400.

[14] E. Ryckebosch, C. Bruneel, R. Termote-Verhalle, C. Lemahieu, K. Muylaert, J. Van Durme, K. Goiris, I. Foubert, Stability of omega-3 LC-PUFA-rich photoautotrophic microalgal oils compared to commercially available omega-3 LC-PUFA oils, Journal of Agricultural and Food Chemistry 61 (2013) 10145–10155.

[15] S. Piyatilleke, B. Thevarajah, P. Nimarshana, T.U. Ariyadasa, Large-scale production of Nannochloropsis-derived EPA: Current status and perspectives via a biorefinery context, Food and Bioproducts Processing (2024).

[16] H. Wang, W. Zhang, L. Chen, J. Wang, T. Liu, The contamination and control of biological pollutants in mass cultivation of microalgae, Bioresource Technology 128 (2013) 745–750.

[17] N.-H. Norsker, M.J. Barbosa, M.H. Vermuë, R.H. Wijffels, Microalgal production — A close look at the economics, Biotechnology Advances 29 (2011) 24–27. https://doi.org/10.1016/j.biotechadv.2010.08.005.

[18] M. Huesemann, B. Crowe, P. Waller, A. Chavis, S. Hobbs, S. Edmundson, M. Wigmosta, A validated model to predict microalgae growth in outdoor pond cultures subjected to fluctuating light intensities and water temperatures, Algal Research 13 (2016) 195–206.

[19] N.K.Z. AlFadhly, N. Alhelfi, A.B. Altemimi, D.K. Verma, F. Cacciola, Tendencies Affecting the Growth and Cultivation of Genus Spirulina: An Investigative Review on Current Trends, Plants (Basel) 11 (2022) 3063. https://doi.org/10.3390/plants11223063.

[20] M. Ismaiel, M. Piercey-Normore, Gene transcription and antioxidants production in Arthrospira (Spirulina) platensis grown under temperature variation, Journal of Applied Microbiology 130 (2021) 891–900.

[21] S.P. de Souza da Silva, D. Perrone, A.F. do Valle, Optimization of Arthrospira maxima cultivation for biomass and protein production and biomass technological treatment to color, flavor, and aroma masking for addition to food products, J Appl Phycol 34 (2022) 65–80. https://doi.org/10.1007/s10811-021-02601-1.

[22] N. Ferrer-Ledo, L. Stegemüller, M. Janssen, R.H. Wijffels, M.J. Barbosa, Growth and fatty acid distribution over lipid classes in Nannochloropsis oceanica acclimated to different temperatures, Frontiers in Plant Science 14 (2023) 1078998.

[23] M. Onay, Enhancing carbohydrate productivity from Nannochloropsis gaditana for bio-butanol production, Energy Reports 6 (2020) 63–67.

[24] J. Camacho-Rodríguez, M.C. Cerón-García, J.M. Fernández-Sevilla, E. Molina-Grima, The influence of culture conditions on biomass and high value product generation by Nannochloropsis gaditana in aquaculture, Algal Research 11 (2015) 63–73. https://doi.org/10.1016/j.algal.2015.05.017.

[25] S.S. Gill, S. Willette, B. Dungan, J.M. Jarvis, T. Schaub, D.M. VanLeeuwen, R. St Hilaire, F.O. Holguin, Suboptimal temperature acclimation affects kennedy pathway gene expression, lipidome and metabolite profile of Nannochloropsis salina during PUFA enriched TAG synthesis, Marine Drugs 16 (2018) 425.

[26] S. Sousa, A.C. Freitas, A.M. Gomes, A.P. Carvalho, Modulated stress to balance Nannochloropsis oculata growth and eicosapentaenoic acid production, Applied Microbiology and Biotechnology 106 (2022) 4017–4027.

[27] S. MarKose, A. Chellappan, P. Thangamani, S. George, S. Thangaswamy, C. Thavasimuthu, M. Mariavincent, Optimization of physical parameters for the growth and lipid production in Nannochloropsis gaditana (Lubian, 1982), J. Appl. Biol. Biotechnol 8 (2020) 6–12.

[28] Y. Ye, M. Liu, L. Yu, H. Sun, J. Liu, Nannochloropsis as an Emerging Algal Chassis for Light-Driven Synthesis of Lipids and High-Value Products, Marine Drugs 22 (2024) 54.

[29] C.O. Pandeirada, É. Maricato, S.S. Ferreira, V.G. Correia, B.A. Pinheiro, D.V. Evtuguin, A.S. Palma, A. Correia, M. Vilanova, M.A. Coimbra, C. Nunes, Structural analysis and potential immunostimulatory activity of Nannochloropsis oculata polysaccharides, Carbohydr Polym 222 (2019) 114962. https://doi.org/10.1016/j.carbpol.2019.06.001.

[30] H. Wu, T. Li, J. Lv, Z. Chen, J. Wu, N. Wang, H. Wu, W. Xiang, Growth and Biochemical Composition Characteristics of Arthrospira platensis Induced by Simultaneous Nitrogen Deficiency and Seawater-Supplemented Medium in an Outdoor Raceway Pond in Winter, Foods 10 (2021) 2974. https://doi.org/10.3390/foods10122974.

[31] V. Kumar, M.S. Bhoyar, C.S. Mohanty, P.S. Chauhan, K. Toppo, S.K. Ratha, Untapping the potential of algae for β-glucan production: A review of biological properties, strategies for enhanced production and future perspectives, Carbohydrate Polymers 348 (2025) 122895. https://doi.org/10.1016/j.carbpol.2024.122895.

[32] Y. Chen, X. Wan, D. Wu, Y. Ouyang, L. Gao, Z. Chen, H.R. El-Seedi, M. Wang, X. Chen, C. Zhao, Characterization of the structure and analysis of the anti-oxidant effect of microalga Spirulina platensis polysaccharide on Caenorhabditis elegans mediated by modulating microRNAs and gut microbiota, International Journal of Biological Macromolecules 163 (2020) 2295–2305. https://doi.org/10.1016/j.ijbiomac.2020.09.041.

[33] T. Singh, V.K. Pandey, K.K. Dash, S. Zanwar, R. Singh, Natural bio-colorant and pigments: Sources and applications in food processing, Journal of Agriculture and Food Research 12 (2023) 100628. https://doi.org/10.1016/j.jafr.2023.100628.

[34] L. Dall'Osto, N.E. Holt, S. Kaligotla, M. Fuciman, S. Cazzaniga, D. Carbonera, H.A. Frank, J. Alric, R. Bassi, Zeaxanthin protects plant photosynthesis by modulating chlorophyll triplet yield in specific light-harvesting antenna subunits, J Biol Chem 287 (2012) 41820–41834. https://doi.org/10.1074/jbc.M112.405498.

[35] A. Machado, H. Pereira, M. Costa, T. Santos, B. Carvalho, M. Soares, P. Quelhas, J.T. Silva, M. Trovão, A. Barros, J. Varela, A.A. Vicente, J. Silva, Development of an Organic Culture Medium for Autotrophic Production of Chlorella vulgaris Biomass, Applied Sciences 10 (2020) 2156. https://doi.org/10.3390/app10062156.

[36] S.O. Lourenço, E. Barbarino, P.L. Lavín, U.M. Lanfer Marquez, E. Aidar, Distribution of intracellular nitrogen in marine microalgae: Calculation of new nitrogen-to-protein conversion factors, European Journal of Phycology 39 (2004) 17–32. https://doi.org/10.1080/0967026032000157156.

[37] C. Sägesser, J.M. Kallfelz, S. Boulos, L. Hammer, L. Böcker, R. Portmann, L. Nyström, A. Mathys, A novel approach for the protein determination in food-relevant microalgae, Bioresource Technology 390 (2023) 129849. https://doi.org/10.1016/j.biortech.2023.129849.

[38] S.M. Tibbetts, C.G. Whitney, M.J. MacPherson, S. Bhatti, A.H. Banskota, R. Stefanova, P.J. McGinn, Biochemical characterization of microalgal

biomass from freshwater species isolated in Alberta, Canada for animal feed applications, Algal Research 11 (2015) 435–447. https://doi.org/10.1016/j.algal.2014.11.011.

[39] A.S. Ferreira, S.S. Ferreira, A. Correia, M. Vilanova, T.H. Silva, M.A. Coimbra, C. Nunes, Reserve, structural and extracellular polysaccharides of Chlorella vulgaris: A holistic approach, Algal Research 45 (2020) 101757.

[40] S.P. Silva, P. Ferreira-Santos, G.R. Lopes, S.F. Reis, A. González, C. Nobre, V. Freitas, M.A. Coimbra, E. Coelho, Industrial byproduct pine nut skin factorial design optimization for production of subcritical water extracts rich in pectic polysaccharides, xyloglucans, and phenolic compounds by microwave extraction, Carbohydrate Polymer Technologies and Applications 7 (2024) 100508.

[41] J. Folch, M. Lees, G.H.S. Stanley, A Simple Method For the Isolation and Purification of Total Lipids From Animal Tissues, Journal of Biological Chemistry 226 (1957) 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5.

[42] D. Lopes, F. Rey, A. Gomes, L. Duarte, J. Pereira, M. Pinho, T. Melo, R. Domingues, Tracing the Impact of Domestic Storage Conditions on Antioxidant Activity and Lipid Profiles in the Edible Microalgae Chlorella vulgaris and Tetraselmis chui, Marine Drugs 22 (2024) 254. https://doi.org/10.3390/md22060254.

[43] S. Aued-Pimentel, J.H.G. Lago, M.H. Chaves, E.E. Kumagai, Evaluation of a methylation procedure to determine cyclopropenoids fatty acids from Sterculia striata St. Hil. Et Nauds seed oil, Journal of Chromatography A 1054 (2004) 235–239.

[44] A.S. Moreira, E. da Costa, T. Melo, D. Lopes, A.C. Pais, S.A. Santos, B. Pitarma, M. Mendes, M.H. Abreu, P.N. Collén, Polar lipids of commercial Ulva spp. of different origins: Profiling and relevance for seaweed valorization, Foods 10 (2021) 914.

[45] I. Couso, Vila ,Marta, Vigara ,Javier, Cordero ,Baldo F., Vargas ,Maria Ángeles, Rodríguez ,Herminia, R. and León, Synthesis of carotenoids and regulation of the carotenoid biosynthesis pathway in response to high light stress in the unicellular microalga Chlamydomonas reinhardtii, European Journal of Phycology 47 (2012) 223–232. https://doi.org/10.1080/09670262.2012.692816.

[46] L.M. Schüler, K.N. Gangadhar, P. Duarte, C. Placines, A.M. Molina-Márquez, R. Léon-Bañares, V.S. Sousa, J. Varela, L. Barreira, Improvement of carotenoid extraction from a recently isolated, robust microalga, Tetraselmis sp. CTP4 (chlorophyta), Bioprocess and Biosystems Engineering 43 (2020) 785–796. https://doi.org/10.1007/s00449-019-02273-9.

[47] H.K. Lichtenthaler, A.R. Wellburn, Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents, Biochemical Society Transactions 11 (1983) 591–592. https://doi.org/10.1042/bst0110591.

[48] N.K.Z. AlFadhly, N. Alhelfi, A.B. Altemimi, D.K. Verma, F. Cacciola, Tendencies Affecting the Growth and Cultivation of Genus Spirulina: An Investigative Review on Current Trends, Plants 11 (2022). https://doi.org/10.3390/plants11223063.

[49] A. Parsy, L. Bidoire, M. Saadouni, M. Bahuaud, T. Elan, F. Périé, C. Sambusiti, Impact of seasonal variations on Nannochloropsis oculata phototrophic productivity in an outdoor pilot scale raceway, Algal Research 58 (2021) 102375. https://doi.org/10.1016/j.algal.2021.102375.

[50] A.K. Koyande, K.W. Chew, K. Rambabu, Y. Tao, D.-T. Chu, P.-L. Show, Microalgae: A potential alternative to health supplementation for humans, Food Science and Human Wellness 8 (2019) 16–24. https://doi.org/10.1016/j.fshw.2019.03.001.

[51] R. Chaiklahan, N. Khonsarn, N. Chirasuwan, M. Ruengjitchatchawalya, B. Bunnag, M. Tanticharoen, Response of Spirulina platensis C1 to High Temperature and High Light Intensity, Kasetsart Journal - Natural Science 41 (2007) 123–129.

[52] G. Torzillo, A. Sacchi, R. Materassi, Temperature as an important factor affecting productivity and night biomass loss in Spirulina platensis grown outdoors in tubular photobioreactors, Bioresource Technology 38 (1991) 95–100. https://doi.org/10.1016/0960-8524(91)90137-9.

[53] D.P. Singh, N. Singh, Calcium and Phosphate Regulation of Nitrogen Metabolism in the Cyanobacterium Spirulina platensis Under the High Light Stress, Current Microbiology 41 (2000) 368–373. https://doi.org/10.1007/s002840010151.

[54] R. Seghiri, M. Kharbach, A. Essamri, Functional Composition, Nutritional Properties, and Biological Activities of Moroccan Spirulina Microalga, Journal of Food Quality 2019 (2019) 3707219. https://doi.org/10.1155/2019/3707219.

[55] L. Fürtauer, J. Weiszmann, W. Weckwerth, T. Nägele, Dynamics of Plant Metabolism during Cold Acclimation, International Journal of Molecular Sciences 20 (2019). https://doi.org/10.3390/ijms20215411.

[56] Q. Li, R. Chang, Y. Sun, B. Li, iTRAQ-Based Quantitative Proteomic Analysis of Spirulina platensis in Response to Low Temperature Stress, PloS One 11 (2016) e0166876. https://doi.org/10.1371/journal.pone.0166876.

[57] M. Hamidi, A. Mohammadi, H. Mashhadi, F. Mahmoudnia, Evaluation of effective environmental parameters on lipid, protein and beta-carotene production in Spirulina platensis microalga, Results in Engineering 18 (2023) 101102. https://doi.org/10.1016/j.rineng.2023.101102.

[58] S. Bellou, M.N. Baeshen, A.M. Elazzazy, D. Aggeli, F. Sayegh, G. Aggelis, Microalgal lipids biochemistry and biotechnological perspectives, Biotechnology Advances 32 (2014) 1476–1493.

[59] J. Panyakampol, S. Cheevadhanarak, S. Sutheeworapong, J. Chaijaruwanich, J. Senachak, W. Siangdung, W. Jeamton, M. Tanticharoen, K. Paithoonrangsarid, Physiological and Transcriptional Responses to High Temperature in Arthrospira (Spirulina) platensis C1, Plant and Cell Physiology 56 (2015) 481–496. https://doi.org/10.1093/pcp/pcu192.

[60] Â.P. Matos, M.G. Cavanholi, E.H.S. Moecke, E.S. Sant'Anna, Effects of different photoperiod and trophic conditions on biomass, protein and lipid production by the marine alga Nannochloropsis gaditana at optimal concentration of desalination concentrate, Bioresource Technology 224 (2017) 490–497. https://doi.org/10.1016/j.biortech.2016.11.004.

[61] J. Fábregas, A. Maseda, A. Domínguez, A. Otero, The cell composition of Nannochloropsis sp. changes under different irradiances in semicontinuous culture, World Journal of Microbiology and Biotechnology 20 (2004) 31–35. https://doi.org/10.1023/B:WIBI.0000013288.67536.ed.

[62] J.K. Volkman, M.R. Brown, G.A. Dunstan, S. Jeffrey, The biochemical composition of marine microalgae from the class Eustigmatophyceae 1, Journal of Phycology 29 (1993) 69–78.

[63] M. Onay, Enhancing carbohydrate productivity from Nannochloropsis gaditana for bio-butanol production, Energy Reports 6 (2020) 63–67. https://doi.org/10.1016/j.egyr.2019.08.019.

[64] B. Wang, J. Jia, Photoprotection mechanisms of Nannochloropsis oceanica in response to light stress, Algal Research 46 (2020) 101784. https://doi.org/10.1016/j.algal.2019.101784.

[65] G. Cauchie, G. Delfau-Bonnet, G. Caulier, A.-L. Hantson, J.-H. Renault, P. Gerbaux, Comprehensive lipid profiling of Microchloropsis gaditana by liquid chromatography - (tandem) mass spectrometry: Bead milling and extraction solvent effects, Algal Research 58 (2021) 102388. https://doi.org/10.1016/j.algal.2021.102388.

[66] S.M. Renaud, L.-V. Thinh, G. Lambrinidis, D.L. Parry, Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures, Aquaculture 211 (2002) 195–214. https://doi.org/10.1016/S0044-8486(01)00875-4.

[67] X. Song, F. Kong, B.-F. Liu, Q. Song, N.-Q. Ren, H.-Y. Ren, Combined transcriptomic and metabolomic analyses of temperature response of microalgae using waste activated sludge extracts for promising biodiesel production, Water Research 251 (2024) 121120. https://doi.org/10.1016/j.watres.2024.121120.

[68] Y. Zhao, H.-P. Wang, B. Han, X. Yu, Coupling of abiotic stresses and phytohormones for the production of lipids and high-value by-products by microalgae: A review, Bioresource Technology 274 (2019) 549–556. https://doi.org/10.1016/j.biortech.2018.12.030.

[69] J.-Y. Cao, Z.-Y. Kong, M.-W. Ye, T. Ling, K. Chen, J.-L. Xu, C.-X. Zhou, K. Liao, L. Zhang, X.-J. Yan, Comprehensive comparable study of metabolomic and transcriptomic profiling of Isochrysis galbana exposed to high temperature, an important diet microalgal species, Aquaculture 521 (2020) 735034. https://doi.org/10.1016/j.aquaculture.2020.735034.

[70] N. Díaz, S. Muñoz, A. Medina, C. Riquelme, I. Lozano-Muñoz, Microchloropsis gaditana as a Natural Antimicrobial with a One Health Approach to Food Safety in Farmed Salmon, Life 15 (2025). https://doi.org/10.3390/life15030455.

[71] Y. Maltsev, K. Maltseva, M. Kulikovskiy, S. Maltseva, Influence of Light Conditions on Microalgae Growth and Content of Lipids, Carotenoids, and Fatty Acid Composition, Biology 10 (2021). https://doi.org/10.3390/biology10101060.

[72] K.H. Ogbonda, R.E. Aminigo, G.O. Abu, Influence of temperature and pH on biomass production and protein biosynthesis in a putative Spirulina sp., Bioresource Technology 98 (2007) 2207–2211. https://doi.org/10.1016/j.biortech.2006.08.028.

[73] R. Serra-Maia, O. Bernard, A. Gonçalves, S. Bensalem, F. Lopes, Influence of temperature on Chlorella vulgaris growth and mortality rates in a photobioreactor, Algal Research 18 (2016) 352–359. https://doi.org/10.1016/j.algal.2016.06.016.

[74] C.M. James, S. Al-Hinty, A.E. Salman, Growth and ω3 fatty acid and amino acid composition of microalgae under different temperature regimes, Aquaculture 77 (1989) 337–351. https://doi.org/10.1016/0044-8486(89)90218-4.

[75] S. Nahon, F. Charles, F. Lantoine, G. Vétion, K. Escoubeyrou, M. Desmalades, A.M. Pruski, Ultraviolet radiation negatively affects growth and food quality of the pelagic diatom Skeletonema costatum, Journal of Experimental Marine Biology and Ecology 383 (2010) 164–170. https://doi.org/10.1016/j.jembe.2009.12.006.

[76] Y. Maltsev, K. Maltseva, M. Kulikovskiy, S. Maltseva, Influence of Light Conditions on Microalgae Growth and Content of Lipids, Carotenoids, and Fatty Acid Composition, Biology 10 (2021). https://doi.org/10.3390/biology10101060.

[77] N.A. Al-Dhabi, M. Valan Arasu, Quantification of Phytochemicals from Commercial Spirulina Products and Their Antioxidant Activities, Evidence-Based Complementary and Alternative Medicine 2016 (2016) 7631864. https://doi.org/10.1155/2016/7631864.

[78] M. Cecchin, S. Berteotti, S. Paltrinieri, I. Vigliante, B. Iadarola, B. Giovannone, M.E. Maffei, M. Delledonne, M. Ballottari, Improved lipid productivity in Nannochloropsis gaditana in nitrogen-replete conditions by selection of pale green mutants, Biotechnology for Biofuels 13 (2020) 78. https://doi.org/10.1186/s13068-020-01718-8.

[79] M.F.A. Anny, T.R. Tuhin, X. Liu, K. Yoshiyama, S. Akizuki, T. Toda, S. Ban, Enhancement of β-glucan production in two Arthrospira species cultivating in anaerobic digestion effluent of water hyacinth, Biochemical Engineering Journal 203 (2024) 109206.

[80] K.M.I. Bashir, J.-S. Choi, Clinical and physiological perspectives of β-glucans: the past, present, and future, International Journal of Molecular Sciences 18 (2017) 1906.

[81] G. Torzillo, A. Sacchi, R. Materassi, Temperature as an important factor affecting productivity and night biomass loss in Spirulina platensis grown outdoors in tubular photobioreactors, Bioresource Technology 38 (1991) 95–100.

[82] S. Aikawa, Y. Izumi, F. Matsuda, T. Hasunuma, J.-S. Chang, A. Kondo, Synergistic enhancement of glycogen production in Arthrospira platensis by optimization of light intensity and nitrate supply, Bioresource Technology 108 (2012) 211–215.

[83] P. Rajasekar, S. Palanisamy, R. Anjali, M. Vinosha, M. Elakkiya, T. Marudhupandi, M. Tabarsa, S. You, N.M. Prabhu, Isolation and structural characterization of sulfated polysaccharide from Spirulina platensis and its bioactive potential: In vitro antioxidant, antibacterial activity and Zebrafish growth and reproductive performance, International Journal of Biological Macromolecules 141 (2019) 809–821.

[84] J.-B. Lee, T. Hayashi, K. Hayashi, U. Sankawa, M. Maeda, T. Nemoto, H. Nakanishi, Further purification and structural analysis of calcium spirulan from Spirulina platensis, Journal of Natural Products 61 (1998) 1101–1104.

[85] M.R. Brown, The amino-acid and sugar composition of 16 species of microalgae used in mariculture, Journal of Experimental Marine Biology and Ecology 145 (1991) 79–99.

[86] A. Rojo-Cebreros, M.E. Morales-Plasencia, L. Quijano, L. Ibarra-Castro, J.M. Martínez-Brown, A. Escamilla, Molecular Structure Analysis of Water-Soluble Polysaccharides From Nannochloropsis Oculata, Available at SSRN 5116440 (n.d.).

[87] B.W. Vogler, J. Brannum, J.W. Chung, M. Seger, M.C. Posewitz, Characterization of the Nannochloropsis gaditana storage carbohydrate: A 1, 3-beta glucan with limited 1, 6-branching, Algal Research 36 (2018) 152–158.

[88] M.E. Morales-Plasencia, L. Ibarra-Castro, J.M. Martínez-Brown, M. Nieves-Soto, J.F. Bermúdez-Lizárraga, A.H. Rojo-Cebreros, The effect of nitrogen limitation on carbohydrates and β-glucan accumulation in *Nannochloropsis oculata*, Algal Research 72 (2023) 103125. https://doi.org/10.1016/j.algal.2023.103125.

[89] D. Pal, I. Khozin-Goldberg, S. Didi-Cohen, A. Solovchenko, A. Batushansky, Y. Kaye, N. Sikron, T. Samani, A. Fait, S. Boussiba, Growth, lipid production and metabolic adjustments in the euryhaline eustigmatophyte Nannochloropsis oceanica CCALA 804 in response to osmotic downshift, Applied Microbiology and Biotechnology 97 (2013) 8291–8306.

[90] Y. Xiao, J. Zhang, J. Cui, X. Yao, Z. Sun, Y. Feng, Q. Cui, Simultaneous accumulation of neutral lipids and biomass in Nannochloropsis oceanica IMET1 under high light intensity and nitrogen replete conditions, Algal Research 11 (2015) 55–62.

[91] Z. Cohen, A. Vonshak, A. Richmond, Fatty acid composition of Spirulina strains grown under various environmental conditions, Phytochemistry 26 (1987) 2255–2258. https://doi.org/10.1016/S0031-9422(00)84694-4.

[92] T.A. Conde, B.F. Neves, D. Couto, T. Melo, B. Neves, M. Costa, J. Silva, P. Domingues, M.R. Domingues, Microalgae as Sustainable Bio-Factories of Healthy Lipids: Evaluating Fatty Acid Content and Antioxidant Activity, Marine Drugs 19 (2021). https://doi.org/10.3390/md19070357.

[93] M.A. Tedesco, E.O. Duerr, Light, temperature and nitrogen starvation effects on the total lipid and fatty acid content and composition ofSpirulina platensis UTEX 1928, Journal of Applied Phycology 1 (1989) 201–209. https://doi.org/10.1007/BF00003646.

[94] A. Kawamura, K. Ooyama, K. Kojima, H. Kachi, T. Abe, K. Amano, T. Aoyama, Dietary Supplementation of Gamma-Linolenic Acid Improves Skin Parameters in Subjects with Dry Skin and Mild Atopic Dermatitis, Journal of Oleo Science 60 (2011) 597–607. https://doi.org/10.5650/jos.60.597.

[95] M.M. Johnson, D.D. Swan, M.E. Surette, J. Stegner, T. Chilton, A.N. Fonteh, F.H. Chilton, Dietary Supplementation with γ-Linolenic Acid Alters Fatty Acid Content and Eicosanoid Production in Healthy Humans1,2, The Journal of Nutrition 127 (1997) 1435–1444. https://doi.org/10.1093/jn/127.8.1435.

[96] S. Sergeant, E. Rahbar, F.H. Chilton, Gamma-linolenic acid, Dihommo-gamma linolenic, Eicosanoids and Inflammatory Processes, European Journal of Pharmacology 785 (2016) 77–86. https://doi.org/10.1016/j.ejphar.2016.04.020.

[97] T. Sonnweber, A. Pizzini, M. Nairz, G. Weiss, I. Tancevski, Arachidonic acid metabolites in cardiovascular and metabolic diseases, International Journal of Molecular Sciences 19 (2018) 3285.

[98] F. Marangoni, C. Agostoni, C. Borghi, A.L. Catapano, H. Cena, A. Ghiselli, C. La Vecchia, G. Lercker, E. Manzato, A. Pirillo, G. Riccardi, P. Risé, F. Visioli, A. Poli, Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects, Atherosclerosis 292 (2020) 90–98. https://doi.org/10.1016/j.atherosclerosis.2019.11.018.

[99] Simionato Diana, Block Maryse A., La Rocca Nicoletta, Jouhet Juliette, Maréchal Eric, Finazzi Giovanni, Morosinotto Tomas, The Response of Nannochloropsis gaditana to Nitrogen Starvation Includes De Novo Biosynthesis of Triacylglycerols, a Decrease of Chloroplast Galactolipids, and Reorganization of the Photosynthetic Apparatus, Eukaryotic Cell 12 (2013) 665–676. https://doi.org/10.1128/ec.00363-12.

[100] A. San Pedro, C.V. González-López, F.G. Acién, E. Molina-Grima, Outdoor pilot production of Nannochloropsis gaditana: Influence of culture parameters and lipid production rates in flat-panel photobioreactors, Algal Research 18 (2016) 156–165. https://doi.org/10.1016/j.algal.2016.06.011.

[101] Y. Xiao, J. Zhang, J. Cui, X. Yao, Z. Sun, Y. Feng, Q. Cui, Simultaneous accumulation of neutral lipids and biomass in Nannochloropsis oceanica IMET1 under high light intensity and nitrogen replete conditions, Algal Research 11 (2015) 55–62. https://doi.org/10.1016/j.algal.2015.05.019.

[102] I.A. Guschina, J.L. Harwood, Algal lipids and their metabolism, in: Algae for Biofuels and Energy, Springer, 2012: pp. 17–36.

[103] X. Ma, L. Zhang, B. Zhu, K. Pan, S. Li, G. Yang, Low-temperature affected LC-PUFA conversion and associated gene transcript level in Nannochloropsis oculata CS-179, Journal of Ocean University of China 10 (2011) 270–274.

[104] M.F. Zakaria, F.N.A. Mat Zaidi, A.H. Ahmad Kamal, N.A. Aleng, M.E. Abd Wahid, T. Katayama, M. Jusoh, PUFA from microalgae: Challenges, factors affecting high production and industrial application, Aquaculture and Fisheries (2025). https://doi.org/10.1016/j.aaf.2025.01.004.

[105] T. Conde, D. Lopes, M. Pinho, T. Melo, N. Coelho, A.M. Rodrigues, H. Pereira, P. Domingues, R. Domingues, Unraveling the lipidome of Pavlova gyrans, a natural reservoir of bioactive lipids for biotechnological applications, Journal of Applied Phycology (2025) 1–17.

[106] T. Conde, S. Aveiro, T. Melo, T. Santos, B. Neves, P. Domingues, J. Varela, H. Pereira, M.R. Domingues, Cross-stress lipid response of Tetraselmis striata CTP4 to temperature and salinity variation, Algal Research 74 (2023) 103218.

[107] J. Camacho-Rodríguez, A. González-Céspedes, M. Cerón-García, J. Fernández-Sevilla, F. Acién-Fernández, E. Molina-Grima, A quantitative study of eicosapentaenoic acid (EPA) production by Nannochloropsis gaditana for aquaculture as a function of dilution rate, temperature and average irradiance, Applied Microbiology and Biotechnology 98 (2014) 2429–2440.

[108] E. Rodriguez De Marco, M.E. Steffolani, M. Martínez, A.E. León, The use of Nannochloropsis sp. as a source of omega-3 fatty acids in dry pasta: chemical, technological and sensory evaluation, International Journal of Food Science & Technology 53 (2018) 499–507.

[109] C.J. Hulatt, R.H. Wijffels, S. Bolla, V. Kiron, Production of fatty acids and protein by Nannochloropsis in flat-plate photobioreactors, PloS One 12 (2017) e0170440.

[110] T.-T. Zhang, J. Xu, Y.-M. Wang, C.-H. Xue, Health benefits of dietary marine DHA/EPA-enriched glycerophospholipids, Progress in Lipid Research 75 (2019) 100997.

[111] J. Camacho-Rodríguez, A. Vizcaíno-Torres, M.D. Macías-Sánchez, E. Navarro-López, Y. Soriano-Jerez, F.J. Alarcón-López, M.C. Cerón-García, Economically viable bioprocess for inclusion of microalga Nannochloropsis gaditana in aquaculture feeds: Evaluation of antioxidant addition in preventing lipid oxidation during storage, Bioresource Technology 406 (2024) 131024. https://doi.org/10.1016/j.biortech.2024.131024.

[112] J.A. Emery, F. Norambuena, J. Trushenski, G.M. Turchini, Uncoupling EPA and DHA in Fish Nutrition: Dietary Demand is Limited in Atlantic Salmon and Effectively Met by DHA Alone, Lipids 51 (2016) 399–412. https://doi.org/10.1007/s11745-016-4136-y.

[113] K. Solymosi, B. Mysliwa-Kurdziel, Chlorophylls and their Derivatives Used in Food Industry and Medicine, Mini Rev Med Chem 17 (2017) 1194–1222. https://doi.org/10.2174/1389557516666161004161411.

[114] Q. Bian, S. Gao, J. Zhou, J. Qin, A. Taylor, E.J. Johnson, G. Tang, J.R. Sparrow, D. Gierhart, F. Shang, Lutein and zeaxanthin supplementation reduces photooxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells, Free Radical Biology and Medicine 53 (2012) 1298–1307. https://doi.org/10.1016/j.freeradbiomed.2012.06.024.

[115] K.D. Athiyappan, W. Routray, B. Paramasivan, Phycocyanin from Spirulina: A comprehensive review on cultivation, extraction, purification, and its application in food and allied industries, Food and Humanity 2 (2024) 100235. https://doi.org/10.1016/j.foohum.2024.100235.

[116] M. Zeece, Chapter Eight - Food colorants, in: M. Zeece (Ed.), Introduction to the Chemistry of Food, Academic Press, 2020: pp. 313–344. https://doi.org/10.1016/B978-0-12-809434-1.00008-6.

[117] M.-P. Gentile, H.W. Blanch, Physiology and xanthophyll cycle activity of Nannochloropsis gaditana, Biotechnology and Bioengineering 75 (2001) 1–12. https://doi.org/10.1002/bit.1158.

[118] H. Fu, B. Xie, S. Ma, X. Zhu, G. Fan, S. Pan, Evaluation of antioxidant activities of principal carotenoids available in water spinach (Ipomoea aquatica), Journal of Food Composition and Analysis 24 (2011) 288–297.

[119] E. Talero, S. García-Mauriño, J. Ávila-Román, A. Rodríguez-Luna, A. Alcaide, V. Motilva, Bioactive compounds isolated from microalgae in chronic inflammation and cancer, Marine Drugs 13 (2015) 6152–6209.

[120] T. Okada, M. Nakai, H. Maeda, M. Hosokawa, T. Sashima, K. Miyashita, Suppressive effect of neoxanthin on the differentiation of 3T3-L1 adipose cells, J Oleo Sci 57 (2008) 345–351. https://doi.org/10.5650/jos.57.345.

[121] E. Kotake-Nara, A. Asai, A. Nagao, Neoxanthin and fucoxanthin induce apoptosis in PC-3 human prostate cancer cells, Cancer Letters 220 (2005) 75–84. https://doi.org/10.1016/j.canlet.2004.07.048.

Highlights

- Spirulina and *M. gaditana* showed species-specific responses to season variations
- Protein content was lower in Spirulina biomass grown in winter
- *M. gaditana* showed higher amounts of EPA in winter and autumn
- Glucose was the most abundant sugar residue in both microalgae
- Summer and spring Spirulina had higher levels of phycocyanin