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ORIGINAL PAPER

# Nutritional profiling of *Chlorella salina*, *Dunaliella salina*, and *Arthrospira platensis* as sustainable functional foods for human nutrition

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## Abstract

Microalgae offer significant potential for developing functional foods and nutraceuticals due to their exceptional nutritional and health-promoting properties. This study evaluated the nutritional profiles of *Chlorella salina*, *Dunaliella salina*, and *Arthrospira platensis*. Protein analysis revealed high content in *A. platensis* (65%) and *C. salina* (61%), surpassing traditional sources like soybeans and pulses, making them viable solutions to protein-energy malnutrition. *D. salina* exhibited an elevated total ash content (8.2%), contributing to its substantial mineral composition. The nucleic acid levels in *A. platensis* (1.8%), *D. salina* (1.7%), and *C. salina* (1.4%) were within the safe consumption range, emphasizing their role in supporting cellular functions and overall nutritional benefits. *C. salina* demonstrated a superior carotenoid content (2.3%), highlighting its potential as a rich source of bioactive compounds with significant antioxidant and nutritional properties. Lipid content was low, with *D. salina* exhibiting reduced cholesterol levels (0.78%) and high docosahexaenoic acid (DHA, 0.9%), contributing to cardiovascular health. Amino acid profiling highlighted elevated essential amino acids, with phenylalanine levels in *C. salina* (12.3%) and *A. platensis* (9.2%) exceeding FAO standards for high-quality proteins. Polyunsaturated fatty acids (PUFAs) dominated, particularly eicosapentaenoic acid (EPA) in *A. platensis* (0.58%) and docosahexaenoic acid (DHA) in *D. salina* (0.9%), with favorable omega-6/omega-3 ratios (4.0–6.4) and balanced PUFA/SFA ratios. Compared to plant-derived oils, *D. salina* offers higher DHA levels, supporting omega-3 requirements for vegetarians. These attributes, combined with low atherogenic and thrombogenic indices, highlight their potential cardioprotective effects. The findings position microalgae as sustainable, nutrient-dense options for promoting human health, warranting further exploration for food and nutraceutical applications.

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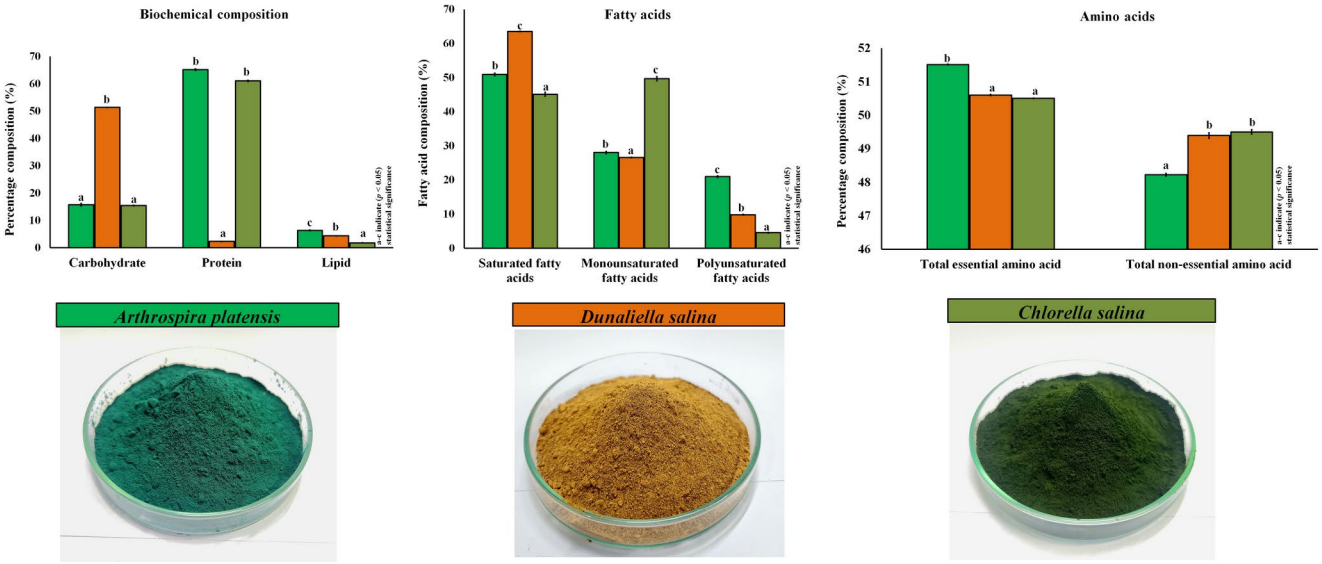
## Graphical Abstract




**Keywords** Microalgae · Fatty acid composition · Amino acid profiling · Carotenoid · Crude fiber and nucleic acid

## Introduction

Microalgae are emerging as the most nutrient-dense and sustainable food sources, gaining recognition as functional foods for their potential to enhance dietary options and mitigate nutritional deficiencies. Globally, microalgae production is steadily increasing, driven by its exceptional nutritional profile, which includes high-quality protein, vitamins, and essential fatty acids [1]. In recent years, microalgae have been increasingly incorporated into a wide range of applications, from dietary supplements to alternative protein. They are recognized not only for their dense nutritional profile but also for their role in advancing sustainable food systems. Minimal resource requirements and fast growth make microalgae a promising solution to meet the nutritional demands of a growing global population, while their unique bioactive compounds continue to attract attention for potential health benefits and therapeutic applications [2]. Microalgae production is predominantly centered in East and Southeast Asia, where they are widely incorporated into traditional foods [3–4].

Microalgae are unicellular, planktonic microorganisms that thrive in both marine and freshwater environments, consisting primarily of eukaryotic cells [5]. They often inhabit seawater regions with low light availability, such as the intertidal zone, and can be found at depths reaching up to 200 m. Microalgae exhibit the ability to thrive in mixotrophic environments, combining autotrophic and heterotrophic modes of growth, which enables them to utilize atmospheric $CO_2$ while absorbing organic substrates [5]. Their minimal land, water, and energy requirements compared to traditional crops result in reduced greenhouse gas emissions and significant contributions to carbon sequestration and climate change mitigation. Microalgae demonstrate high productivity in nutrient-rich environments, efficiently capturing $CO_2$ during photosynthesis. Studies have shown carbon capture efficiencies of up to 90% in open pond systems, with *Chlorella* species thriving in elevated $CO_2$ concentrations (up to 40%) [6]. In 2020, a logistic model projected that microalgae could sequester up to 2.35 gigatonne (Gt) $CO_2$ from 100,000 km² of cultivation, substantially contributing to global $CO_2$ reduction targets [7]. By addressing Sustainable Development Goals (SDG), 13 (Climate Action) and 14 (Life Below Water), microalgae promote climate change mitigation and the sustainable use of marine ecosystems, fostering a balance between environmental conservation and agricultural productivity. Additionally, their adaptability to extreme conditions, such as deserts and coastal areas, makes them suitable for areas unsuited to traditional agriculture. Cultivable in raceway ponds and photobioreactors with minimal freshwater, they rapidly produce biomass, offering a sustainable alternative to conventional crops [8].

Microalgal proteins, categorized as single-cell proteins, are highly sustainable and nutritionally rich alternatives to traditional protein sources. Driven by the high demand for *Chlorella vulgaris* and *Arthrospira platensis* due to their remarkable nutritional profiles, cultivation of these species has expanded considerably, achieving annual dry weight yields of approximately 2,000 and 5,000 tons, respectively. Both species have been classified- Generally Recognized As

Safe (GRAS) by the Food and Drug Administration (FDA) [9]. This growth can be attributed to their nutritional benefits and superior productivity compared to traditional crops. The inclusion of microalgae in food commodities such as breakfast cereals and pasta has been shown to increase their protein content, further demonstrating their potential in enhancing global nutrition [10]. *A. platensis* is abundant in proteins, essential fatty acids, vitamins E and B12, minerals, and $\beta$-carotene, giving it potent antioxidant, immune-enhancing, and antiviral effects. *Chlorella*, with a protein concentration of 59% by dry weight, surpasses the protein levels of soybeans (33%), making it a superior nutritional resource [11].

Given the increasing demand for sustainable food alternatives, this study explores the nutritional composition of *Chlorella salina* (*Chlorellaceae*), *Dunaliella salina* (*Dunaliellaceae*), and *Arthrospira platensis* (*Microcoleaceae*) biomass. These species were diligently selected for their exceptional protein content, essential amino acid richness, and bioactive compounds, which surpass conventional crops like soy and pulses in addressing nutritional deficiencies. Unlike many terrestrial crops or other microalgal species, these microalgae offer unique advantages such as scalability, resource efficiency, and a minimal environmental footprint. Their selection aligns with a paradigm shift toward sustainable and climate-resilient food systems that prioritize future food security. By comprehensively evaluating the nutritional and functional potential of *C. salina*, *D. salina*, and *A. platensis*, this study highlights their vital role as transformative, sustainable solutions to combat malnutrition and enhance global food security.

## Materials and methods

### Biochemical analyses

All reagents and chemicals used in this study were of analytical grade. $CuSO_4 \cdot 5\ H_2O$, sodium potassium tartrate, *O*-phthalaldehyde, glacial acetic acid, acetone, and isopropanol were procured from Sigma-Aldrich (St. Louis, MO, USA), HiMedia Laboratories (LLC, Kelton, PA, USA), and Sisco Research Laboratories Pvt. Ltd. (SRL, India).

#### Estimation of total carbohydrate

The total carbohydrate content was determined by phenol-sulfuric acid method, following the procedure outlined by Masuko et al. [12]. Briefly, 50 mg of the microalgal sample was treated with 150 $\mu$L of concentrated sulfuric acid (96%) and 30 $\mu$L of a 5% phenol solution. The reaction mixture was then subjected to an incubation for 5 min. After cooling, the absorbance was recorded at 488 nm (Agilent Cary® 50 UV-Vis spectrophotometer). Total carbohydrate content was expressed (as % w/w) and calibrated with a standard glucose solution. The total carbohydrate content was estimated by Eq. (1);

$$\%\ \text{Carbohydrate} = \frac{\text{XV}}{\text{W}} \times 100 \tag{1}$$

Where; X represents the mass of carbohydrates (g) obtained from the standard curve, V denotes the volume of the solution containing the carbohydrates (mL), and W signifies the weight of the sample (g).

#### Estimation of total protein

Protein content was determined following the methodology outlined by Lowry et al. [13]. The Lowry reagent was prepared by combining Solution A (0.5% $CuSO_4.5\ H_2O$), Solution B (1% sodium potassium tartrate in distilled water), and Solution C (2% $Na_2CO_3$ in 0.1 N NaOH) in distilled water in a volume ratio of 1:1:48 (v/v/v). The Folin reagent was formulated by diluting 5 mL of 2 N Folin-Ciocalteu Phenol reagent with 15 mL of distilled water. A standard bovine serum albumin (BSA) solution was made by dissolving 0.05 g of BSA in 500 mL of distilled water. Protein content was measured by adding 5 mL of Lowry reagent to 1 mL of the sample (1000 ppm). Subsequently, 0.5 mL of Folin reagent was introduced to the mixture, which was then incubated for 20 min. The absorbance was measured at 660 nm using an Agilent Cary® 50 UV-Vis spectrophotometer (Varian Cary 50, Agilent Technologies, Santa Clara, CA, USA). The protein concentration in the sample was determined by constructing a standard curve based on BSA and reported as percentage weight by weight (% w/w). The total protein content was estimated by Eq. (2);

$$\%\ \text{Protein} = \frac{\text{XV}}{\text{W}} \times 100 \tag{2}$$

where; X represents the mass of protein (g) obtained from the standard curve, V denotes the volume of the solution (mL) containing the protein, and W signifies the weight of the sample (g).

#### Estimation of cholesterol

Total cholesterol content was assessed spectrophotometrically (Varian Cary 50, Agilent Technologies, Inc., Santa Clara, CA) with modifications based on the method of Wanasundara and Shahidi [14]. The assay was conducted using *O*-phthalaldehyde at a concentration of 50 mg/dL in

glacial acetic acid, with cholesterol stock solutions (1000 ppm in ethanol) and working solutions (0-1000 $\mu$g/mL) prepared by serial dilution. For the assay, 0.1 mL of standard solution was mixed with 2 mL of *O*-phthalaldehyde and 1 mL of concentrated sulfuric acid. After 10 min of incubation, the absorbance was recorded at 550 nm. A standard curve was constructed by placing concentration values on the X-axis and corresponding absorbance readings on the Y-axis. For microalgae samples, 0.1 g was mixed with 3 ml of ethanol and 0.3 ml of 33% KOH, then heated in a water bath for 15 min at 60 °C. After cooling, 10 mL of hexane was added to the solution, followed by the addition of 3 mL of double-distilled water. The hexane layer was evaporated, and 1 mL of concentrated $H_2SO_4$ was added. After thorough homogenization, the absorbance was recorded at 550 nm followed by a 10 min incubation. The total cholesterol content was determined using a standard curve and expressed as mg/100 g of dry weight. The total cholesterol content was expressed (as % w/w) and calibrated with a standard cholesterol solution. The total cholesterol content was estimated by Eq. (3);

$$\% \text{ Cholesterol} = \frac{\text{XV}}{\text{W}} \times 100 \tag{3}$$

where; X represents the mass of cholesterol (g) obtained from the standard curve, V denotes the volume of the solution (mL) containing the cholesterol, and W signifies the weight of the sample (g).

### Estimation of lipid

The total lipid content was assessed using a revised Folch procedure [15]. Microalgal samples (20 g) were combined with methanol-chloroform mixture (2:1 v/v). After thorough shaking to ensure complete mixing, the homogenized sample was filtered and allowed to separate into distinct phases. The organic layer, rich in lipids, was carefully collected, and the solvent was evaporated to leave behind the lipid residue. The weight of this residue was quantified, and the lipid content was reported as g/100 g of dried microalgae.

### Estimation of crude fiber

The crude fiber content was determined following a sequential digestion process. A fat-free, oven-dried sample (0.5–0.8 g) was first treated with 1.25% sulfuric acid, followed by 1.25% NaOH. After each digestion step, the acid and alkali were drained, and the residue was thoroughly rinsed with distilled water. The remaining residue was transferred to pre-weighed crucibles, dried, and incinerated in a muffle furnace at 600 °C for 3 h. After cooling, the crucibles were weighed to calculate the crude fiber content.

### Estimation of ash

The ash content was determined by weighing 2–3 g of the sample into a pre-weighed silica crucible. The sample was then incinerated in a muffle furnace at 600 °C for 3 h. Following combustion, the crucible was cooled in a desiccator and reweighed. The crude ash content was calculated by Eq. (4);

$$\% \, Total \; ash \; = \frac{\text{weight of ash}}{\text{weight of the sample}} \times 100 \tag{4}$$

### Estimation of total carotenoid and chlorophyll

A 50 mg sample was extracted with 5 mL of acetone and subjected to vortex or sonication for 15 min. The extract was then centrifuged at 5000 rpm for 10 min, and the resulting supernatant was collected. Absorbance measurements were recorded at 470 nm, 645 nm, and 663 nm using an Agilent Cary® 50 UV-Vis spectrophotometer (Varian Cary 50, Agilent Technologies, Santa Clara, CA, USA). The total carotenoid and chlorophyll content were quantified using the equations outlined by Suwanaruang (2022) [16] in Eqs. (5, 6, and 7);

$$\text{Chlorophyll a} = \frac{12.7\,(\text{A}_{663}) - 2.69\,(\text{A}_{645})}{100 \times \text{W}} \times V \tag{5}$$

$$\text{Chlorophyll b} = \frac{22.9\,(\text{A}_{645}) - 4.68\,(\text{A}_{663})}{100 \times \text{W}} \times V \tag{6}$$

$$\text{Total Carotenoid} = 1000\,(\text{A}_{470}) + 3.27\frac{(\text{chlorophyll a}) - (\text{chlorophyll b})}{229 \times \text{W}} \times V \tag{7}$$

A = absorbance value (Abs)
V = acetone volume (mL)
W = sample weight (g)

### Estimation of nucleic acid

The nucleic acid content was quantified using a modified phenol-chloroform extraction method based on Muys et al. (2019) [17]. In brief, 100 mg of the sample was suspended in 10 mL of preheated lysis buffer (10 mM Tris, 1 mM EDTA, 0.1 M NaCl, 2% SDS, pH 8.0) and incubated at 65 °C for 30 min. Subsequently, an equal volume of phenol-chloroform (1:1) was added, and the mixture was gently vortexed. Following centrifugation at 12,000 rpm for

15 min at 4 °C, the aqueous phase was carefully separated and mixed with 0.7 volumes of ice-cold isopropanol. The sample was then incubated at -20 °C overnight to facilitate nucleic acid precipitation. After incubation, centrifugation was performed at 12,000 rpm for 15 min at 4 °C. The resulting pellet was washed with 70% ethanol and centrifuged again at 12,000 rpm for 5 min at 4 °C. Finally, the nucleic acid pellet was dissolved in 100 $\mu$L of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA), and its absorbance was recorded at 260 nm and 280 nm.

## Estimation of amino acid

The amino acid composition of the sample was analyzed by Accq Tag procedure with a Waters HPLC System. Approximately 0.1 g of the sample was hydrolyzed at 110 °C for 24 h using 6 N HCl in a nitrogen-sealed glass tube. Following hydrolysis, the acid was eliminated through three cycles of flash evaporation with a rotary vacuum evaporator. The remaining residue was diluted with 0.05 N HCl and incubated at 4 °C. The hydrolyzed samples were filtered using a syringe filter (Whatman 0.2 $\mu$m, nylon membrane) and reconstituted with AccQ-Tag Ultra reagent (borate buffer: derivatizing agent: standard/sample, 7:2:1 v/v/v). The samples were then analyzed using a Waters HPLC system (AccQ-Tag Amino Acid Analysis System) equipped with a reversed-phase column (2.5 $\mu$m Bridge BE+, 4.6 × 100 mm C18), maintained at 55 ± 1 °C. Detection was performed using a Waters 2489 dual absorbance detector at $\lambda_{max}$ 260 nm. Triplicate injections of the samples (AMQ amino acid derivatives) were performed, and data analysis was conducted using Empower software (version 3). The amino acid content was quantified by comparison with reference standards and expressed as a percentage of dry mass, with the findings presented as mean ± standard deviation (SD).

## Estimation of fatty acids

The fatty acid profile of the lipid was analyzed following the procedure outlined by Metcalfe et al. [18]. The lipid extract of the samples was initially mixed with methanolic KOH and refluxed for 30 min at 80 °C in a nitrogen atmosphere. Subsequently, 5 mL of $BF_3$ in methanol was incorporated, and the mixture was subjected to reflux for 5 min under nitrogen atmosphere. After cooling, the solution was placed in a separating funnel and mixed with petroleum ether (2:1) for phase separation. The upper organic phase was collected, washed with double distilled water for three to four times, and then passed through $Na_2SO_4$ to remove residual water. The petroleum ether layer was concentrated by rotary evaporator at 40 °C. The fatty acid ester was analyzed using a gas chromatography mass spectrometry system (GC-MS) (Nexis GC-2030, Shimadzu, Japan), equipped with a 30 m × 0.25 mm, 250 nm SH-Rxi-5Sil MS capillary column (Shimadzu, USA). The analysis was carried out using a splitless injection mode. The chromatographic oven was initially set at 60 °C and then heated at a rate of 15 °C per min until it reached 150 °C. This temperature was held for 5 min to allow for optimal separation and analysis. The temperature was ramped up to 280 °C at a rate of 25 °C/min and held for 15 min to ensure complete peak identification. The ion source and injector temperatures were set to 230 °C and 260 °C, respectively. Helium gas (purity > 99.99%) served as the carrier gas, flowing at a rate of 1.22 mL/min with a pressure of 74.4 kPa. Fatty acid methyl esters (FAMEs) were identified and quantified using the Supelco™ 37 Component FAME Mix standards. Results were expressed as the percentage of total fatty acids (% TFA), with sums of polyunsaturated (∑PUFA), monounsaturated (∑MUFA), and saturated fatty acids (∑SFA) calculated from the chromatographic data.

### Nutritional indices

The nutritional quality of the microalgal species was evaluated by analyzing their fatty acid composition and calculating health-related indices. These included the ratio of total polyunsaturated fatty acids to total saturated fatty acids (ΣPUFA/ΣSFA), the ratio of docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA), and the ratio of total n-6 to total n-3 polyunsaturated fatty acids (Σn-6/Σn-3 PUFAs). In addition, the atherogenicity index (AI) and thrombogenicity index (TI) were calculated using the methodology of Ulbricht and Southgate [19], while the hypocholesterolemic/hypercholesterolemic (h/H) ratio was determined following the approach outlined by Santos-Silva et al. [20]. The atherogenicity index (AI) and thrombogenicity index (TI) were determined according to the Eqs. (8 and 9):

$$\mathrm{AI} = \frac{(4 \times 14:0 + 12:0 + 16:0)}{(\mathrm{MUFA} + \Sigma\, n3\mathrm{PUFA} + \Sigma\, n6\mathrm{PUFA})} \tag{8}$$

$$\mathrm{TI} = \frac{(14:0 + 16:0 + 18:0)}{\begin{array}{c}(0.5 \times \mathrm{MUFA}) + (0.5 \times \mathrm{n6PUFA}) + \\ (3 \times \mathrm{n3PUFA}) + \left(\frac{\mathrm{n3PUFA}}{\mathrm{n6PUFA}}\right)\end{array}} \tag{9}$$

The hypocholesterolemia/ hypercholesterolemia (h/H) ratio was calculated by Eq. [10];

$$\frac{\mathrm{h}}{\mathrm{H}} = \frac{\begin{pmatrix}18:1\mathrm{n9} + 18:2\mathrm{n6} + 20:4\mathrm{n6} + 18:3\mathrm{n3} \\ +20:5\mathrm{n3} + 22:5\mathrm{n3} + 22:6\mathrm{n3}\end{pmatrix}}{(14:0 + 16:0)} \tag{10}$$

### Estimation of minerals

Mineral content analysis was conducted using two distinct methods for different minerals. Initially, samples were digested with nitric acid ($HNO_3$) to prepare them for analysis. For all minerals except sodium, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was utilized with a Thermo Scientific iCAP RQ instrument. This method involves aspirating the digested samples into the ICP-MS for precise measurement, with Helium Kinetic Energy Discrimination mode employed to enhance sensitivity and accuracy in mineral detection and quantification. Sodium content was measured separately using flame photometry with a Systronics 1027 instrument, which provides accurate quantification of sodium levels. Both macro and micro minerals were quantified and reported as mg/kg dry weight.

### Statistical analyses

Statistical analysis was carried out using Analysis of Variance (ANOVA) through the Statistical Program for Social Sciences (SPSS, version 20.0, USA). Each measurement was conducted in triplicate, and the results were reported as mean ± standard deviation (SD) and statistical significance determined at $p < 0.05$. To evaluate variations in fatty acid in microalgae, Principal Component Analysis (PCA) was conducted in R Studio (version 4.1.1). Two-dimensional scree plots and loading profiles of principal components (PCs) were utilized to depict the role of fatty acids in influencing the atherogenicity index (AI) and thrombogenicity index (TI).

**Table 1** Biochemical composition (g/100 g dry sample) of *A. platensis*, *D. salina. and C. salina*

| Biochemical analyses | *A. platensis* | *D. salina* | *C. salina* |
|---|---|---|---|
| Lipid | $6.30^{c} \pm 0.15$ | $4.33^{b} \pm 0.02$ | $1.77^{a} \pm 0.02$ |
| Protein | $65.14^{b} \pm 0.31$ | $2.35^{a} \pm 0.01$ | $61.03^{b} \pm 2.5$ |
| Carbohydrate | $15.72^{a} \pm 0.50$ | $51.30^{b} \pm 0.01$ | $15.40^{a} \pm 0.11$ |
| Cholesterol[#] | $6.49^{c} \pm 0.38$ | $0.78^{a} \pm 0.78$ | $2.45^{b} \pm 0.38$ |
| Ash | $3.01^{a} \pm 0.11$ | $8.21^{c} \pm 0.09$ | $6.29^{b} \pm 0.1$ |
| Crude Fiber | $1.08^{a} \pm 0.15$ | $1.37^{b} \pm 0.19$ | $1.84^{c} \pm 0.12$ |
| Carotenoid | $0.32^{a} \pm 0.01$ | $0.91^{b} \pm 0.15$ | $2.33^{c} \pm 0.15$ |
| Chlorophyll a[*] | $5.42^{b} \pm 0.13$ | $2.24^{a} \pm 0.08$ | $18.25^{c} \pm 0.33$ |
| Chlorophyll b[*] | $6.05^{b} \pm 0.05$ | $2.65^{a} \pm 0.02$ | $20.68^{c} \pm 0.29$ |
| Nucleic acid | $1.87^{b} \pm 0.01$ | $1.74^{b} \pm 0.08$ | $1.41^{a} \pm 0.05$ |

Data are expressed in percentage with mean ± standard deviation ($n = 3$)

Means followed by the different superscripts (a-c) within same row indicate significant difference ($p < 0.05$)

[#] Total cholesterol content is expressed in mg/100 g dry sample

*Chlorophyll content is expressed in mg/g dry sample

## Result and discussion

### General

Microalgae-based functional foods and nutraceuticals offer significant potential for enhancing human health. These microalgae, known for their high protein content and abundance of essential nutrients, offer substantial potential for establishing a robust algae-driven food sector centered on the production of nutritious foods [21]. The current study demonstrated the nutritional composition of three economically significant microalgae species, *Arthrospira platensis*, *Dunaliella salina*, and *Chlorella salina*, highlighting their potential as health-promoting foods. The analysis confirms that these microalgae can be consumed safely, offering nutritional benefits without posing risks related to lifestyle diseases.

### Biochemical composition

The biochemical analysis demonstrated that microalgae exhibited a significantly higher protein content compared to other evaluated parameters. *A. platensis* demonstrated the highest protein concentration, measuring 65.1%, followed by *C. salina* at 61%, while *D. salina* showed the lowest protein content at 2.35% (Table 1). These results align with existing literature that identifies *A. platensis* as a rich source of dietary protein [22]. The protein content observed in *A. platensis* and *C. salina* surpasses that of conventional plant-based protein sources, including lentils (26.1%) and peas (13 −38%), as previously reported [23, 24]. The total lipid content of *A. platensis* (6.3%), *D. salina* (4.3%), and *C. salina* (1.7%) was found to be lower compared to other microalgae species [25]. This lower lipid content is particularly significant, as excess lipid accumulation on arterial walls can contribute to the formation of atherosclerotic plaques, leading to the gradual narrowing and hardening of the arteries. *D. salina* exhibited the lowest cholesterol content (0.78%), followed by *C. salina* (2.4%) and *A. platensis* (6.3%), highlighting its potential suitability for low-cholesterol diets. Low-cholesterol diets offer substantial health benefits, including support for cardiovascular health, enhanced lipid metabolism, and reduced inflammation [26]. Studies conducted by Brown et al. [26] demonstrated a significant reduction in total cholesterol levels in individuals after six weeks on a Healthy Plant-Based Diet (hPBD).

*D. salina* exhibited a notably higher ash content (8.21%) compared to *A. platensis* (3.01%) and *C. salina* (6.29%) (Table 1). This elevated ash content is likely attributable to the accumulation of surface salts on the dried biomass, a characteristic feature of halotolerant species such as *D. salina*, which thrives in hypersaline environments. Ash

content serves as an indicator of essential mineral composition, including calcium, magnesium, iron, zinc, phosphorus, and potassium, which play vital roles in human nutrition and metabolic processes [27].

The fiber content of the analyzed microalgae varied significantly, with *C. salina* exhibiting the highest fiber content (1.84%), followed by *D. salina* (1.37%) and *A. platensis* (1.08%) (Table 1). Dietary fiber supports digestive health by promoting gut motility, preventing constipation, and acting as a prebiotic to enhance beneficial gut microbiota. It also aids in blood sugar regulation by slowing glucose absorption and contributes to lipid metabolism by reducing LDL cholesterol levels [28].

The analysis of total carotenoid content revealed considerable variation among the examined microalgae, with *C. salina* (2.33%) exhibiting the highest concentration, followed by *A. platensis* (0.32%) and *D. salina* (0.91%) (Table 1). These findings are consistent with prior research by Bazarnova et al., which also reported elevated carotenoid levels in *C. salina*, reinforcing its potential as a rich source of these bioactive compounds [29]. Carotenoids play a crucial role as antioxidants, contributing to photoprotection, oxidative stress reduction, and human health promotion. Additionally, these pigments have been identified as natural inhibitors of NF-$\kappa$B signaling, exhibiting significant anti-inflammatory properties [30]. *C. salina* exhibited the highest concentrations of chlorophyll a (18.25 mg/g) and chlorophyll b (20.68 mg/g), followed by *A. platensis* (chlorophyll a: 5.42 mg/g, chlorophyll b: 6.05 mg/g) and *D. salina* (chlorophyll a: 2.24 mg/g, chlorophyll b: 2.65 mg/g). These values align with previous reports by D'Alessandro et al., which indicated that microalgae typically contain 0.5–1.0% chlorophyll [31]. The elevated chlorophyll content in *Chlorella* sp. is further corroborated by findings from Sandgruber et al., while the values obtained for *D. salina* are consistent with earlier studies [32]. Chlorophyll is a key bioactive molecule with antioxidant, anti-inflammatory, and detoxifying properties. Its capacity to chelate heavy metals and facilitate their excretion helps mitigate metal-induced toxicity and oxidative stress. Additionally, due to its structural resemblance to haemoglobin, chlorophyll has been proposed to enhance oxygen transport and support erythropoiesis, contributing to physiological well-being [33].

The analysis of nucleic acid content revealed that *A. platensis* (1.87%), *D. salina* (1.74%), and *C. salina* (1.41%) contain nucleic acids within the acceptable threshold for human consumption. These values align with previous findings by Janssen et al., which reported nucleic acid levels below 2% for *Chlorella* and *Arthrospira*, supporting the potential direct utilization of their biomass in food applications [34]. Nucleic acids are essential biomolecules involved in genetic information storage, protein synthesis regulation, and cellular processes. However, an excessive intake of nucleic acids, particularly purine-rich compounds, may lead to increased uric acid levels in the blood and elevated urinary excretion, posing potential health risks. Given that the tolerable daily intake of nucleic acids from alternative food sources is estimated at 2 g, the observed nucleic acid levels in these microalgae suggest they are within the safe consumption range [17].

## Amino acid analysis

Microalgae-derived amino acids are recognized for their enhanced bioavailability, elevated nutrient density, and greater environmental sustainability, positioning microalgae as a superior alternative to traditional protein sources [35]. The amino acid profile analysis indicated that phenylalanine was the predominant amino acid across the three species examined. Among these, *C. salina* exhibited the highest concentration of phenylalanine, reaching 12.3%, followed by *D. salina* at 11.0%, and *A. platensis* at 9.2%. (Table 2). These findings surpass previously reported values [36, 37]. Phenylalanine plays a vital role as a precursor in the synthesis of neurotransmitters, making it essential for various physiological processes. Arginine, the second most abundant amino acid in *D. salina* (10.6%), followed by *C. salina* (9.1%) and *A. platensis* (6.8%), was observed in higher concentrations. Arginine is integral to nitric oxide production, which enhances blood flow, supports cardiovascular health, and promotes wound healing. Tyrosine, found in the highest concentrations relative to other amino acids, was most abundant in *C. salina* (11.9%), followed by *A. platensis* (10.9%) and *D. salina* (10.6%), surpassing levels reported in earlier studies [32].

The total essential amino acid (EAA) content exceeded that of non-essential amino acids (NEAA), highlighting the contribution of these microalgae as valuable nutritional supplements. The EAA values obtained were significantly higher than those reported in earlier studies for plant-based protein isolates [38]. However, microalgae are generally characterized by lower levels of sulfur-containing amino acids, such as cysteine and methionine. In contrast, *C. salina* demonstrated significant concentrations of cystine (8.1%) and methionine (2%), which are crucial for various biological functions (Table 2). Methionine acts as a primary methyl donor through the S-adenosylmethionine (SAM) pathway, influencing DNA methylation, gene expression, and lipid metabolism [39]. It also plays a key role in protein synthesis as the initiating amino acid in protein chains. Cysteine, a precursor to glutathione, serves as a critical antioxidant that protects cells from oxidative stress and supports immune function [40].

**Table 2** Amino acid composition (g/100 g protein) of the microalgae species

| | *A. platensis* | *D. salina* | *C. salina* | FAO/WHO recommended values |
|---|---|---|---|---|
| **Essential amino acids** | | | | |
| Thr | $6.16^{a}\pm0.01$ | $6.97^{c}\pm0.02$ | $6.44^{b}\pm0.01$ | 1.5 |
| Val | $6.24^{c}\pm0.02$ | $6.18^{b}\pm0.02$ | $5.68^{a}\pm0.01$ | 2.6 |
| His | $4.59^{b}\pm0.01$ | $1.01^{a}\pm0.07$ | $1.51^{a}\pm0.05$ | 1.0 |
| Lys | $6.18^{b}\pm0.03$ | $1.77^{a}\pm0.03$ | $1.67^{a}\pm0.08$ | 3.0 |
| Met | $1.01^{a}\pm0.04$ | $1.86^{b}\pm0.06$ | $2.09^{c}\pm0.08$ | 1.0 |
| Leu | $6.05^{a}\pm0.01$ | $6.44^{b}\pm0.04$ | $6.53^{c}\pm0.01$ | 3.9 |
| Ile | $4.84^{b}\pm0.08$ | $4.69^{a}\pm0.01$ | $5.09^{c}\pm0.02$ | 2.0 |
| Phe | $9.23^{a}\pm0.03$ | $11.02^{b}\pm0.01$ | $12.37^{c}\pm0.01$ | 3.0 |
| Arg | $6.85^{a}\pm0.09$ | $10.66^{c}\pm0.09$ | $9.12^{b}\pm0.04$ | - |
| **TEAA** | $51.15^{b}\pm0.01$ | $50.60^{a}\pm0.02$ | $50.50^{a}\pm0.01$ | - |
| **Non-Essential amino acids** | | | | |
| Glu | $5.20^{a}\pm0.02$ | $5.75^{b}\pm0.02$ | $5.12^{a}\pm0.09$ | |
| Asp | $2.52^{b}\pm0.03$ | $3.30^{c}\pm0.01$ | $2.08^{a}\pm0.02$ | - |
| Ser | $6.28^{b}\pm0.02$ | $2.85^{a}\pm0.01$ | $2.36^{a}\pm0.03$ | - |
| Gly | $10.71^{c}\pm0.01$ | $8.06^{b}\pm0.08$ | $6.67^{a}\pm0.03$ | - |
| Ala | $6.32^{a}\pm0.08$ | $6.67^{b}\pm0.06$ | $6.62^{b}\pm0.03$ | - |
| Pro | $3.94^{a}\pm0.01$ | $5.42^{b}\pm0.06$ | $6.55^{c}\pm0.05$ | - |
| Tyr | $10.93^{b}\pm0.04$ | $10.63^{a}\pm0.04$ | $11.94^{c}\pm0.01$ | - |
| Cys | $2.32^{a}\pm0.05$ | $6.71^{b}\pm0.01$ | $8.16^{c}\pm0.01$ | - |
| **TNEAA** | $48.22^{a}\pm0.04$ | $49.39^{b}\pm0.08$ | $49.5^{b}\pm0.07$ | - |
| **Ratio of amino acid** | | | | |
| TAA | 99.37 | 99.99 | 100 | 27.70 |
| TEAA/TAA | 0.51 | 0.50 | 0.50 | - |
| TNEAA/TAA | 0.48 | 0.49 | 0.49 | - |
| TEAA/TNEAA | 1.06 | 1.01 | 1.02 | - |
| Arg/Lys | 1.10 | 6.02 | 5.46 | - |
| Leu/Ileu | 1.24 | 1.37 | 1.28 | - |

TEAA-Total essential amino acids; TNEAA-Total non-essential amino acids; TAA-Total amino acids

Data are expressed as mean ± standard deviation ($n=3$)

Means followed by different superscripts (a-c) within same row indicate significant difference ($p<0.05$)

According to the FAO/WHO/UNU guidelines, a recommended daily intake of 13–15 mg/kg of essential amino acids (EAAs) can be achieved by consuming approximately 20 g of algal protein per day for an average adult [41]. The EAA profile of microalgae generally aligns with the FAO criteria for optimal protein sources, such as eggs and soybeans [42]. Additionally, microalgae exhibit the presence of branched-chain amino acids including leucine, isoleucine, and valine, that are comparable to those found in soybeans and eggs, underlining their potential as a valuable protein source for supporting muscle health. *A. platensis* demonstrated elevated levels of histidine (4.5%) and lysine (6.1%), exceeding those present in eggs and soybeans. The EAA profiles of *D. salina* and *C. salina* were comparable to those found in eggs and soybeans [43, 44]. The arginine to lysine ratio exhibited variability across different species, ranging from 1 to 6, with *D. salina* showing a dominant presence within this range (Table 2). Previous studies have indicated an optimal arginine: lysine ratio between 0.90 and 1.18 [45]. The consistently high arginine: lysine ratio across these species suggests a potential cardiovascular health benefit associated with the elevated arginine content [46].

## Fatty acid analysis

### Saturated fatty acid (SFA)

Saturated fatty acids (SFAs) are pivotal in shaping microalgal growth, significantly influencing membrane stability, energy reserves, and metabolic adaptation to changing environmental conditions [47]. The triglyceride fractions of the analyzed microalgae exhibited a notable dominance of saturated fatty acids (SFA) (Table 3). Specifically, *D. salina* exhibited a significantly higher palmitic acid (16:0) level (59.4%) compared to *A. platensis* (47.7%) and *C. salina* (22.8%). The reported value was notably higher than the previously reported by Sahu et al. [48]. This elevated palmitic acid content likely reflects its functional role in

**Table 3** Fatty acid composition (% total fatty acids) of microalgae species

| Fatty acid | *A. platensis* | *D. salina* | *C. salina* |
|---|---|---|---|
| Saturated fatty acids | | | |
| 12:0 | $0.73^{a}\pm0.03$ | $1.74^{b}\pm0.02$ | $1.98^{c}\pm0.04$ |
| 14:0 | - | $0.47^{b}\pm0.01$ | $1.99^{c}\pm0.02$ |
| 15:0 | $1.09^{a}\pm0.03$ | $1.30^{a}\pm0.01$ | $12.07^{b}\pm0.16$ |
| 16:0 | $47.75^{b}\pm0.17$ | $59.41^{c}\pm0.06$ | $22.48^{a}\pm0.29$ |
| 17:0 | $0.66^{b}\pm0.01$ | $0.35^{a}\pm0.01$ | $4.11^{c}\pm0.05$ |
| 18:0 | $0.68^{b}\pm0.02$ | $0.21^{a}\pm0.02$ | $2.46^{c}\pm0.03$ |
| 20:0 | - | - | - |
| 22:0 | - | - | - |
| 24:0 | - | - | - |
| **∑SFA*** | $50.92^{b}\pm0.13$ | $63.51^{c}\pm0.05$ | $45.11^{a}\pm0.57$ |
| **Monounsaturated fatty acids (MUFA)** | | | |
| 14:1*n*-7 | - | - | - |
| 15:1*n*-7 | $3.78^{a}\pm0.04$ | $3.81^{a}\pm0.01$ | $8.18^{b}\pm1.12$ |
| 16:1*n*-7 | $4.30^{a}\pm0.04$ | $6.28^{b}\pm0.05$ | $6.21^{b}\pm0.11$ |
| 18:1*n*-7 | - | - | - |
| 18:1*n*-9 | $19.95^{b}\pm0.04$ | $16.48^{a}\pm0.06$ | $32.77^{c}\pm0.59$ |
| 22:1*n*-9 | - | - | $2.50\pm0.03$ |
| **∑MUFA**** | $28.04^{b}\pm0.12$ | $26.57^{a}\pm0.10$ | $49.67^{c}\pm0.62$ |
| **Polyunsaturated fatty acids (PUFA)** | | | |
| 18:2*n*-6 | $14.71^{c}\pm0.01$ | $4.70^{b}\pm0.09$ | - |
| 18:3*n*-6 | $3.43^{b}\pm0.02$ | $3.16^{a}\pm0.12$ | $3.37^{a}\pm0.08$ |
| 18:3*n*-3 | $2.25^{c}\pm0.03$ | $0.79^{b}\pm0.01$ | - |
| 20:4*n*-6 | - | - | $1.01^{b}\pm0.13$ |
| 20:5*n*-3 EPA | $0.58^{b}\pm0.02$ | $0.20^{a}\pm0.01$ | $0.17^{a}\pm0.06$ |
| 22:6*n*-3 DHA | - | $0.95^{b}\pm0.03$ | - |
| **∑PUFA***** | $20.98^{c}\pm0.04$ | $9.81^{b}\pm0.11$ | $4.54^{a}\pm0.10$ |
| ∑*n*-3 | $2.83^{c}\pm0.05$ | $1.95^{b}\pm0.03$ | $0.17^{a}\pm0.06$ |
| ∑*n*-6 | $18.15^{c}\pm0.01$ | $7.86^{b}\pm0.08$ | $4.37^{a}\pm0.04$ |
| ∑*n*-6/∑*n*-3 | $6.41^{a}\pm0.11$ | $4.03^{a}\pm0.06$ | $25.70^{b}\pm0.88$ |
| DHA + EPA | $0.58^{b}\pm0.02$ | $1.15^{c}\pm0.40$ | $0.17^{a}\pm0.06$ |
| ∑PUFA/∑SFA | $0.41^{c}\pm0.01$ | $0.15^{b}\pm0.01$ | $0.10^{a}\pm0.01$ |
| AI | $0.98^{b}\pm0.01$ | $1.73^{c}\pm0.01$ | $0.59^{a}\pm0.01$ |
| TI | $1.52^{c}\pm0.01$ | $0.38^{a}\pm0.01$ | $0.97^{b}\pm0.02$ |
| h/H ratio | $0.78^{b}\pm0.01$ | $0.38^{a}\pm0.01$ | $1.38^{c}\pm0.01$ |

*Total saturated fatty acids; **total monounsaturated fatty acids; ***total polyunsaturated fatty acids. Data are presented as mean values of three samples (mean ± SD). Different superscripts (a-c) within same row indicate significant difference ($p < 0.05$)

microalgal cellular structure and energy storage, consistent with its role in marine algal lipids [49]. The absence of long-chain SFAs such as lignoceric (C24:0), behenic (C22:0) and arachidic acids(C20:0) across all samples is noteworthy and aligns with typical microalgal fatty acid profiles [48]. Furthermore, trace amounts of lauric (C12:0) and myristic (C14:0) acids were identified, each constituting less than 2% of the total fatty acids.

### Monounsaturated fatty acid

Monounsaturated fatty acid was found to be higher in *C. salina* with oleic acid (18:1n-9) being the prevalent fatty acid (32%) which was in comparison with the early reported value by Otleş & Pire [49], followed by *A. platensis* (19.9%) and *D. salina* (16.5%) (Table 3). Monounsaturated fatty acids (MUFA) are essential for cellular function, significantly enhancing membrane fluidity and stability [50]. The ability of microalgae to accumulate elevated levels of MUFAs is attributed to their unique metabolic pathways and adaptive responses to environmental factors such as light, temperature, and nutrient availability [51]. MUFAs play a key role in improving cardiovascular health by lowering LDL cholesterol and increasing HDL cholesterol, reducing the risk of heart disease and stroke [52].

### Polyunsaturated fatty acid (PUFA)

Microalgae are increasingly recognized as a sustainable and promising alternative source of essential fatty acids,

particularly omega-3s, offering a viable substitute for fish oil [53]. *A. platensis* exhibited a polyunsaturated fatty acid content of 21%, surpassing previous findings [54], followed by *D. salina* (9.8%) and *C. salina* (4.5%) (Table 3). Notably, *A. platensis* showed a significant concentration of eicosapentaenoic acid (EPA, 20:5n-3) at 0.58%, while *D. salina* emerged as a prominent source of docosahexaenoic acid (DHA, 22:6n-3) at 0.9%. The levels of EPA in *A. platensis* and DHA in *D. salina* were found to be higher than those in commonly recognized plant sources, such as chia seeds and flaxseed, as well as in nut sources like walnuts, which are renowned for their richness in polyunsaturated fatty acids [55]. Elevated levels of EPA in *A. platensis* offer significant cardiovascular benefits by exerting potent anti-inflammatory effects that help reduce chronic inflammation [56]. EPA supports brain health by offering neuroprotective effects, enhancing cognitive function, and potentially alleviating symptoms of depression and anxiety [57]. Meanwhile, the DHA content in *D. salina* serves as a fundamental structural component of neuronal membranes, contributing to the fluidity and functionality of brain cells [58]. *C. salina* is distinguished by its substantial arachidonic acid (20:4*n*-6) concentration (1%), which notably surpasses the levels found in a range of common plant-based dietary sources. The concentration of gamma-linolenic acid (18:3n-6) across the microalgal samples ranged from 3 to 3.5%, reflecting a prominent consistency (Table 3). Importantly, these levels exceeded those reported for various plant and nut sources, as highlighted in earlier findings [55]. Gamma-linolenic acid (GLA) functions as an important precursor in pathways that mitigate inflammation and its involvement in the synthesis of prostaglandins, which are signaling molecules that regulate various physiological processes [59].

## Nutritional and health indices

According to the World Health Organization, maintaining an omega-6 to omega-3 ratio of 5:1 to 10:1 is essential, as this balance plays a critical role in modulating the inflammatory response of body and supporting overall health [60]. *A. platensis* (6.4) and *D. salina* (4.0) exhibited moderate levels of the omega-6 to omega-3 ratio. Human diets had a nearly balanced omega-6/omega-3 ratio of about 1:1 to 4:1, which supported optimal health and a balanced immune response (Table 3). Modern diets, particularly those high in processed foods and vegetable oils, often exhibit an elevated omega-6 to omega-3 ratio, shifting the balance towards pro-inflammatory responses. This imbalance has been linked to an increased risk of chronic inflammatory conditions, including cardiovascular disease, obesity, diabetes, and arthritis [61].

The ratio of PUFA to MUFA is a critical nutritional factor that influences lipid metabolism, heart health, and the regulation of inflammation [62]. A PUFA/SFA ratio of 0.45 or higher is advised to encourage the intake of health-promoting polyunsaturated fats while reducing the consumption of detrimental saturated fats. *A. platensis* demonstrates a PUFA/SFA ratio of 0.41 (Fig. 1) (Table 1), indicating its potential as a health-promoting supplement [63]. Atherogenicity Index (AI) and Thrombogenicity Index (TI) serves as vital metrics for assessing cardiovascular risk. Ideally, AI and TI values should be below 1, indicating a healthier profile. The fatty acid profile analysis indicated a Thrombogenicity Index (TI) value of *D. salina* (0.38) as by the required limit, suggesting a healthier lipid profile [64]. Furthermore, the Atherogenicity Index (AI) was found to be within the recommended limits for *A. platensis* (0.98%) and *C. salina* (0.6%). These findings highlight the considerable potential

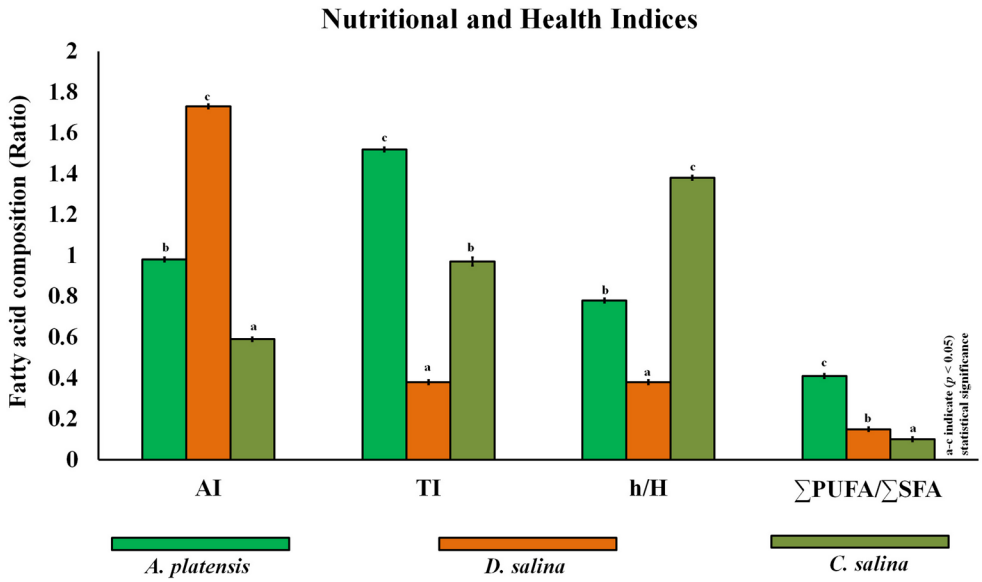



**Fig. 1** Comparative representation of nutrition and health indices

of these microalgae in mitigating cardiovascular disease risk. The hypocholesterolemia to hypercholesterolemia fatty acid ratio (h/H index) is an important indicator for assessing fatty acid profiles concerning lipid metabolism. Nutritionally, an elevated h/H index is linked to positive health effects, particularly in managing cholesterol levels and promoting cardiovascular well-being [65]. *C. salina* has demonstrated a promising h/H ratio of 1.38, indicating its potential to positively influence cholesterol levels. These findings highlight the importance of integrating microalgal sources into the diet, as they provide a beneficial fatty acid profile that promotes healthy lipid metabolism and may help reduce the risk of cardiovascular disease.

### Principle component analysis

The principal component analysis (PCA) of the fatty acid dataset reveals that the first two components (PC1 and PC2) encapsulate nearly all the variability, with PC1 accounting for 79.13% and PC2 adding another 20.45%, resulting in a combined explanation of 99.59% of the total variance (Fig. 2). This indicates that the inherent structure of the dataset is effectively captured by these two components, rendering additional components largely insignificant. The result is advantageous for interpreting the major sources of variability in fatty acid composition across samples, which can simplify further analyses, such as clustering or pattern recognition. The loadings on PC1 and PC2 provide a deeper understanding of the structure within the data. Fatty acids like C14, C15, C17, C18, n918, and MUFA show strong loadings on PC1, suggesting that this component captures a primary dimension of variability tied to these fatty acids shared structural or functional attributes. This could reflect overall fatty acid chain length, degree of saturation, or related biochemical properties. Conversely, PC2 is characterized by high loadings for n6182, PUFA, TI, n6pufa, and pufasfar (PUFA/ SFA), which might indicate a secondary dimension of variability, potentially distinguishing between polyunsaturated and saturated fatty acids or specific fatty acid families. This differentiation is valuable in applications where the particular characteristics are known to impact health outcomes, as it aids in identifying key fatty acids

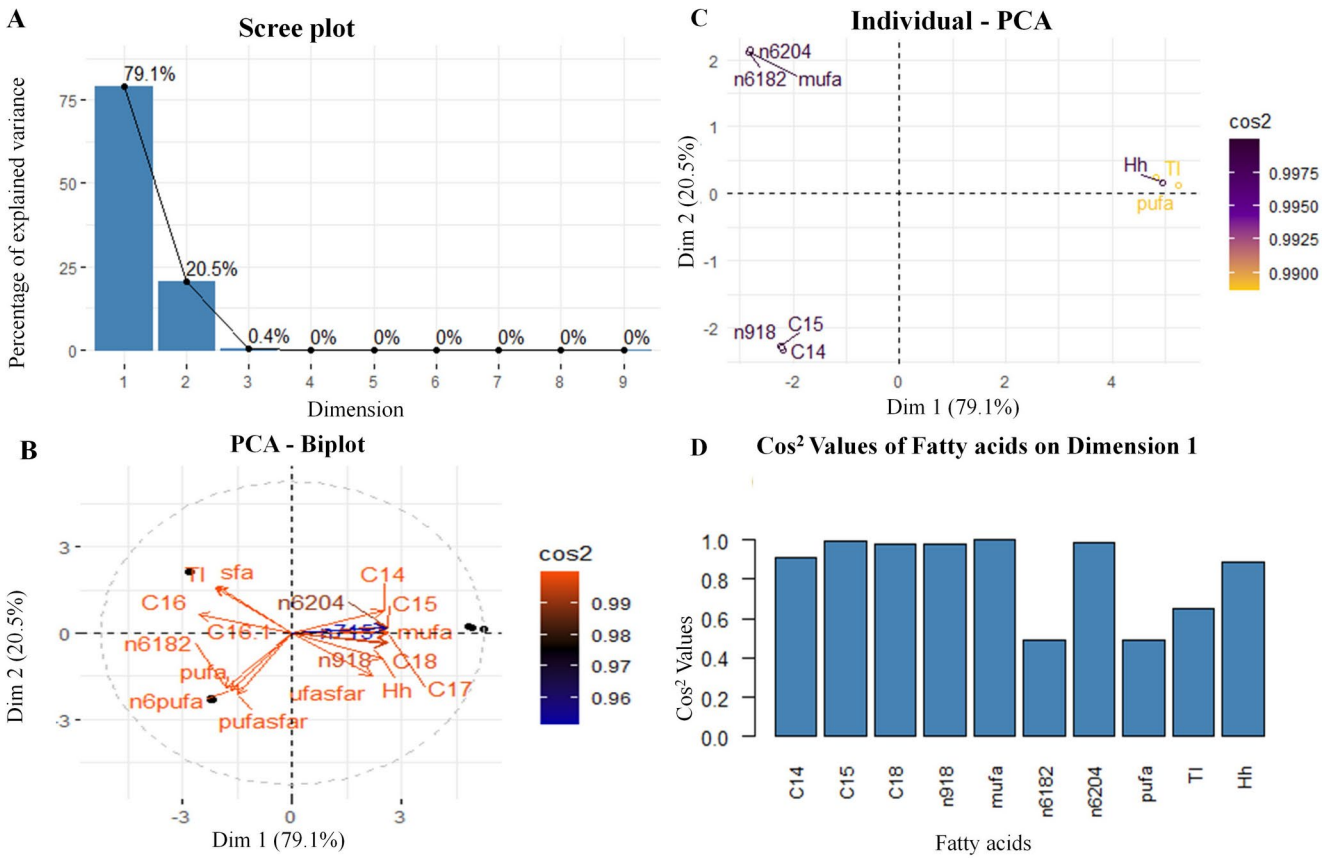



**Fig. 2** Principal Component Analysis (PCA) of fatty acid profiles is presented as follows: (**A**) A scree plot showcasing the percentage of variance attributed to each principal components, with the first two components collectively accounting for 79.1% and 20.5% of the total variance. (**B**) A biplot illustrating the correlation of fatty acids with the first two principal components. (**C**) An individual PCA plot depicting the distribution of fatty acids across the first two principal components, with Dimension 1 (Dim1) explaining 79.1% and Dimension 2 (Dim2) explaining 20.5% of the variance. (**D**) $Cos^2$ values reflecting the quality of representation for variables in Dimension 1

**Table 4** Minerals compositions (g/100 g) of various microalgae

| | *A. platensis* | *D. salina* | *C. salina* |
|---|---|---|---|
| **Minerals composition** | | | |
| **Macro elements** | | | |
| Na | $1.0^{a}\pm0.04$ | $9.1^{b}\pm0.05$ | $0.3^{a}\pm0.08$ |
| Ca | $0.7^{a}\pm0.06$ | $6.5^{b}\pm0.06$ | $0.2^{a}\pm0.05$ |
| K | $1.3^{c}\pm0.11$ | $0.5^{a}\pm0.07$ | $0.9^{b}\pm0.12$ |
| Mg | $0.4^{a}\pm0.05$ | $1.9^{b}\pm0.06$ | $0.6^{a}\pm0.07$ |
| **Micro elements** | | | |
| Zn* | $29.7^{b}\pm0.06$ | $18.8^{a}\pm0.05$ | $43.3^{c}\pm0.06$ |
| Mn* | $22.6^{b}\pm0.06$ | $12.8^{a}\pm0.6$ | $34.3^{c}\pm0.05$ |
| Cu | ND | ND | ND |
| Fe | $0.1^{a}\pm0.04$ | $0.1^{a}\pm0.05$ | $0.1^{a}\pm0.08$ |
| P | $1.0^{b}\pm0.11$ | $0.3^{a}\pm0.04$ | $1.1^{b}\pm0.03$ |
| Pb* | $0.7^{a}\pm0.01$ | $1.4^{c}\pm0.05$ | $1.1^{b}\pm0.01$ |
| Se* | BDL | $99.6^{c}\pm0.05$ | BDL |

Data are expressed as mean ± standard deviation ($n=3$). Means followed by the different superscripts (a-c) within same row indicate significant difference ($p < 0.05$)

*Microelements are represented as $10^{-4}$g/100 g

BDL represents below detectable level, ND represents not detectable

responsible for desired functional or therapeutic properties. PC1 and PC2 could streamline efforts in nutraceutical development, quality control, or dietary formulation by emphasizing fatty acids that contribute most significantly to product variability and potential bioactivity.

## Mineral content analysis

*D. salina*, adapted to thrive in hypersaline environments, exhibited the highest sodium level (9.1 g/100 g dry mass) (Table 4), significantly surpassing *A. platensis* (1.0 g/100 g) and *C. salina* (0.3 g/100 g). Furthermore, *D. salina* demonstrated a substantially higher calcium level (6.5 g/100 g), which surpasses the previously reported values [66]. The elevated sodium and calcium concentrations in *D. salina* are likely attributable to its cellular adaptations and the specific culture medium employed. With its notably high calcium levels, *D. salina* shows promise as a natural calcium supplement to promote bone health. *A. platensis* was found to have the highest potassium content (1.3 g/100 g), followed by *C. salina* (0.9 g/100 g) and *D. salina* (0.5 g/100 g). These findings align with previous studies by Costa et al., further supporting the comparative analysis of potassium concentrations in these microalgal species [67]. Potassium is essential for regulating bodily fluids and maintaining the electrochemical gradients across cell membranes. *D. salina* stands out with the highest magnesium content (1.9 g/100 g), followed by *C. salina* (0.6 g /100 g), and *A. platensis* (0.4 g /100 g). These findings are in accordance with previous reports by Tertychnaya et al. (2020), confirming the rich magnesium content of these algae species [66]. Magnesium is an essential mineral, constituting approximately 30–40% of the body's total magnesium content in muscles and soft tissues. Magnesium acts as a cofactor in more than 300 enzymatic processes, playing a key role in protein synthesis, muscle and nerve function, glucose metabolism, and regulation of blood pressure [68].

The micromineral analysis revealed a substantial concentration of zinc in *C. salina* ($43.3\times10^{-4}$ g/100 g), surpassing previously documented levels [69]. Zinc plays an essential role as a component of enzymes that drive the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids. It is also vital for the metabolism of numerous micronutrients. The iron content was found to be consistent across all analyzed microalgae (0.1 g/100 g), aligns with earlier reports for *A. platensis* and *Chlorella* species [67, 70]. Iron is essential for various physiological processes, including oxygen transport by hemoglobin in red blood cell, electron transport within cellular system, and as a key component of enzyme systems across different tissues [71]. *C. salina* exhibited a substantial phosphorus content (1.1 g/100 g), surpassing that of *A. platensis* (1.0 g/100 g) and *D. salina* (0.3 g/100 g), with results for *D. salina* being comparable to previous reports [70]. Lead content in *A. platensis* ($0.7\times10^{-4}$ g/100 g), *D. salina* ($1.4\times10^{-4}$ g/100 g), and *C. salina* ($1.1\times10^{-4}$ g/100 g) was found to be minimal, indicating that these microalgae can be considered safe for consumption. Selenium levels were below detectable limits in *A. platensis* and *C. salina*, while *D. salina* ($99.6\times10^{-4}$ mg/100 g) showed a trace amount. Selenium is typically present in trace amounts in aquatic environments, with its concentration varying geographically. Manganese content in *A. platensis* ($22.6\times10^{-4}$ g/100 g), *D. salina* ($12.8\times10^{-4}$ g/100 g), and *C. salina* ($34.3\times10^{-4}$ g/100 g) was also found to be comparatively low compared to earlier reports [7, 66, 67].

## Conclusions

This study provides a comprehensive assessment of the nutritional profile of widely available microalgae from the classes Chlorophyceae, Cyanophyceae, and Trebouxiophyceae, sourced from southern part of India. The biochemical composition, mineral content, fatty acid and amino acid profiles collectively position these microalgae as rich sources of essential nutrients vital for human metabolic and dietary functions. These species are particularly notable for their elevated levels of essential amino acids, highlighting their superior protein quality. Additionally, the higher total essential amino acid (EAA) content compared to non-essential amino acids (NEAAs) highlights the contribution of microalgae as valuable supplements for human nutrition. The findings align with FAO/WHO/UNU guidelines, supporting

the feasibility of microalgae as a sustainable, plant-based protein source. Furthermore, the significant presence of C20-22 polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), reinforces their potential as a well-balanced dietary option. The observed favorable atherogenicity and thrombogenicity indices suggest these microalgae may contribute positively to cardiovascular health. These findings signifies the potential of these microalgal species as valuable nutritional resources. However, additional research including in vivo studies are warranted to fully explore the pharmacological properties and therapeutic potentials of these microalgae and their bioactive compounds.

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**Data availability** All data generated or examined in the course of this study are comprehensively presented within this published article.

## Declarations

**Ethical approval** This article does not include any research involving human participants or animals conducted by the authors.

**Conflict of interest** The authors state that there are no conflicts of interest, whether financial, personal, or otherwise, that could influence or appear to compromise the integrity of this study.

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