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Research Article

Analysis of *Spirulina maxima* grown in CFTRI and Zarrouk's Media, Insights into nutraceutical enhancement

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Abstract
*Spirulina (*Cyanobacteria, *Arthrospira*) is the richest source of proteins, fatty acids, vitamin B12. It is used as a high-value health food, nutraceutical, functional food or dietary food supplement. It is considered a promising new-age food source with high nutraceutical value. In the present study the influence of different culture media on the growth and metabolite production of *Spirulina maxima* (Cyanobacteria, *Arthrospira*), was studied. Different media such as Central Food Technological Research Institute medium (CFTRI), Zarrouk's, Offer/A5 and distilled water (DW) as control were evaluated, with CFTRI (Central Food Technological Research Institute) media and Zarrouk's media yielding the highest biomass and metabolite levels. *S. maxima* cultured in CFTRI medium exhibited superior protein (55.79% ± 1.05) and carbohydrate (12.20% ± 1.01) contents compared to Zarrouk's medium (41.00% ± 1.74 and 8.90% ± 0.58, respectively). Total antioxidant capacity was comparable in both media (23.14 ± 0.13 µg/ml in CFTRI and 23.29 ± 2.00 µg/ml in Zarrouk's). Significant variations in biochemical composition were observed across media, particularly in fatty acid profiles, while flavonoid levels remained consistent. Notably, palmitic acid (hexadecanoic acid) levels were 21.38% in CFTRI and 22.80% in Zarrouk's medium. Gas chromatography-Mass spectrometry (GC-MS) analysis revealed that the biosynthesis of ω-6 fatty acids was unique to the CFTRI medium, suggesting its superior potential for enhancing the nutritional profile of *S. maxima*. Principal component analysis (PCA) further elucidated correlations and variances among metabolites across the tested media. Overall, CFTRI medium emerged as the most effective for optimizing both growth and metabolite production in *S. maxima*, offering valuable insights for its commercial cultivation and nutraceutical applications.

Keywords, Antioxidants, Fatty acids, Gas chromatography-Mass spectrometry (GC-MS), Microalgae, Principal component analysis (PCA), Proteins

INTRODUCTION

The global population is projected to reach 9.9 billion by 2050, necessitating the exploration of alternative food sources to meet the increasing nutritional demands (Kaneda *et al*. 2020; Lim *et al*. 2021). *Spirulina,* a blue-green microalga characterized by its filamentous, unicellular, and spiral-shaped morphology, is considered a promising future food due to its exceptional nutritional value (Kaneda *et al*. 2020; Saranraj *et al*. 2014; Sanchez *et al*. 2003). It is non-toxic, nutrient-dense, and exhibits health-promoting properties, including pro-

tective effects against anaemia, tumours, and viral infections (Sanchez *et al*. 2003; Mazo *et al*. 2004). *Spirulina* can be cultivated in simple pilot plants or large-scale industrial bioreactors (Sanchez *et al*. 2003) and is consumed both as a whole food and as a nutritional supplement in capsule form. Commercially, *Spirulina* is utilized in animal feed, nutraceuticals, cosmetics, and as a natural colorant (Oliveira *et al*. 2020). Owing to its diverse applications, the demand for *Spirulina* is rapidly increasing, with the market expected to grow at an annual rate of 10.56% by 2026 (Allied Market Research, 2019). Renowned as one of the richest sources of protein, *Spirulina* biomass yields 20 to 400 times more protein than conventional sources such as beef, corn, and soybeans (Lim *et al*. 2021). Beyond protein, it is incorporated into various therapeutic beverages and pastes used to manage conditions such as hypercholesterolemia, atherosclerosis, premenstrual tension, and arthritis (Prete *et al*., 2024; Sokary *et al*., 2024). Preclinical studies further highlight its immunological, antioxidant, and antimutagenic properties (Estrada *et al*. 2001). Additionally, *Spirulina* serves as a food supplement to enhance colouration in ornamental fish (Vasudevan *et al*. 2006), and phycocyanin—a blue pigment extracted from *Spirulina* is widely employed as a natural food colourant (Lim *et al*. 2021; ElFar *et al*. 2022). It is also regarded as a probiotic agent (Ramakrishnan *et al*. 2008). Apart from its high protein content, *Spirulina* is rich in carbohydrates, carotenes (notably beta-carotene), vitamins (B1, B2, tocopherols), minerals (Na, K, Ca, Mg, P, Fe), and essential fatty acids such as linoleic acid (LA, 18,2 cis-9,12) and gamma-linolenic acid (GLA, 18,3 cis-6,9,12) (Ötleş, 2001; Tokuşoglu, 2003; Gupta *et al*. 2008; Priyanka, 2023). It contains significant amounts of n-3 polyunsaturated fatty acids, particularly linolenic acid (a precursor for prostaglandins), eicosapentaenoic acid (EPA, 20,5 n-3), and docosahexaenoic acid (DHA, 22,6 n-3), which have been shown to exert protective effects against cardiovascular diseases (Sanchez *et al*. 2003; Dąbrowska, 2024).

Given these extensive health benefits, *Spirulina* is increasingly cultivated at an industrial scale under varying culture conditions and media compositions. Therefore, it is crucial to standardize optimal growth conditions to maximize biomass production for commercial processing. Different culture media were employed to evaluate *Spirulina maxima's* growth under varying nutrient conditions. This comparative approach allowed us to identify which medium supports optimal biomass production, protein yield, pigment synthesis, assessment of antioxidant activities etc. while also assessing cost-effectiveness for potential industrial-scale cultivation.

MATERIALS AND METHODS

To assess the influence of different culture media on *S. maxima*, 10 mL of suspended culture was inoculated into CFTRI medium (Venkataraman *et al*., 1995), Zarrouk's medium (Zarrouk, 1966; Raoof *et al*., 2006), and Offer/A5 medium (Dubois *et al*., 1951), with distilled water (DW) serving as the control. Zarrouk's medium is widely used as a standard reference owing to its nutrient-rich formulation, composed of $NaHCO_3$, Na_2CO_3, $NaNO_3$, K_2SO_4, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, EDTA, and trace elements. CFTRI medium, containing $NaHCO_3$, NaCl, $NaNO_3$, K_2SO_4, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, and trace elements, is a cost-effective alternative characterized by lower bicarbonate levels. Offer/A5 medium ($NaHCO_3$, Na_2CO_3, $NaNO_3$, K_2HPO_4, K_2SO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, and trace elements) is a classical algal culture medium formulated originally by Dubois *et al*. (1951). 100 ml of each medium was inoculated with 10 ml of a *Spirulina maxima* stock culture (procured from Banaras Hindu University,Varanasi) and maintained under aseptic conditions at 25 ± 2 °C and light intensity of 78 µmol m^{-2} s^{-1} under 12/12-hour (Light, Dark) cycle. Manual shaking was done frequently at regular intervals. To study the effect on the growth of different media on *S. maxima,* optical density at 680 nm was measured every 12 hrs. For microscopic examination, the suspended media was properly shaken before sampling. A 50 µl culture sample was taken using micropipettes and observed under a microscope at 40X. The growth curve was prepared by recording the optical density at 680 nm of algal cultures using a UV-Vis spectrophotometer (Shimadzu, UV-1900). All experiments were performed in triplicate.

Biochemical profiling

Quantitative measurements of *S. maxima* were performed only in CFTRI and Zarrouk's media, as growth performance was best in these two media. Analysis of various biochemical constituents was performed in triplicate on lyophilized algal samples.

Total carbohydrate estimation was performed using the Phenol–sulfuric acid method with glucose as the standard, in which H_2SO_4 (conc.) cleaves all poly-, di-, and oligosaccharides into monosaccharides. These are further dehydrated to furfural or hydroxymethylfurfurals, which react with phenol to give an orange-coloured complex (Dubois *et al*. 1951). The optical density (O.D.) of the complex was measured at 490 nm through a spectrophotometer (Shimadzu, UV-1900). The reducing sugars have been estimated using the Dinitrosalicylic acid (DNSA) method (Miller, 1959). Dinitrosalicylic acid gets reduced by reducing sugars and a reddish-orange coloured complex is formed. The O.D. was measured by a spectrophotometer (Shimadzu, UV-1900) at 540 nm (Miller, 1959). Total proteins were estimated with BSA as a standard (Bradford MM (1976). Optical density was measured

through a spectrophotometer (Shimadzu, UV-1900). The presence of proteins in the sample changes the colour of the solution containing Coomassie Brilliant Blue G-250 (CBB), which can be measured by a spectrophotometer at 595 nm (Bradford, 1976).

Total lipids in the samples were determined using chloroform (Bligh, 1959). The Chlorophyll a and carotenoids were estimated following Yang *et al*. (1998) using acetone-water mixture (4:1) as a solvent. The absorbance maxima were read at 663.6 nm and 470 nm for Chlorophyll a and carotenoids respectively (Yang *et al*. 1998).

Phenolic compounds measurements were done as per Malik and Singh (1980) using Folin-Ciacalteu reagent (FCR) as a blank. The O.D. was measured at 650 nm using a spectrophotometer (Shimadzu, UV-1900). Total flavonoids were measured through aluminium chloride colorimetric method (Chang *et al*. 2002). Antioxidant capacity was measured as per Prieto *et al*. 1999 using gallic acid as standard. The antioxidant extraction was modified after Safafer *et al*. (2015). Dried, powdered algal material (0.2 g) was sonicated with 5 ml solvent (ethanol/ethyl acetate) for 30 min, followed by centrifugation at 2164xg (10 min). The supernatant was collected separately, and the pellets were resuspended in the same solvent. The procedure was replicated twice with the same sample. The material was dried under a nitrogen flow, and the extracts were stored at -20°C until final analysis. The dried extracts were dissolved in 1 mL of ethanol/ethyl acetate, as previously used, for final analysis.

During GC–MS FAMES the samples were first homogenized with 1 ml of 2 % methanolic HCl in a glass tube and incubated for 1 hr at 80°C in a water bath. One ml of 0.9% NaCl and 2 ml of hexane were added to the samples, which were then centrifuged at 2000 rpm for 2 minutes. The upper layer was discarded and samples were dried under nitrogen flow. 50 µl of hexane was added to the dried sample, and 1-10 µl was injected into the gas chromatography–mass spectrometer. The GC–MS analysis of FAMEs was done using a gas chromatography-mass spectrometer (GC-7890B coupled with GC–MS5977A MSD).

Statistical analysis

Data is expressed as mean ± standard deviation. A One-Way ANOVA was performed in SPSS to determine statistical significance among the study groups, with $p < 0.05$. All the values were calculated using MS Office (Excel) 2022. The differences in the mean values for various parameters across different media were determined by calculating t-values at p=0.05. Mean and standard deviation (SD) were obtained by descriptive statistics. Principal component analysis (PCA) was performed in OriginPro 9.0. The Score plot, and the loading plot have been prepared to explain the distribution and possible grouping of samples. Agglomerative hierarchical clustering (AHC) using squared Euclidean distance was used to prepare the dendrograms (Ward Jr JH, 1963; Verma *et al*. 2017).

RESULTS AND DISCUSSION

Spirulina, a filamentous cyanobacterium, is renowned for its rich nutritional profile comprising high levels of proteins, carbohydrates, vitamins, minerals, and essential fatty acids. Often classified as a single-cell protein, *Spirulina is* valued for its exceptionally high protein content and broad spectrum of bioactive compounds. Its global cultivation is driven by its abundance of pigments, flavonoids, phenolic compounds, and carbohydrates, which contribute significantly to human health and make it a valuable supplement in aquaculture feed (Fedekar *et al*., 2012).

The present study optimized cultivation conditions to maximize biomass yield while enhancing the nutraceutical value of *S. maxima*. Algal growth is strongly influenced by the composition of the culture medium, which plays a crucial role in regulating cellular metabolism and physiological activity. *Spirulina* exhibits robust growth in alkaline environments that are rich in carbonates and bicarbonates (Nyabuto *et al*. 2015). In this context, both CFTRI and Zarrouk's media were found to support substantial growth of *S. maxima*, owing to their high content of sodium bicarbonate and essential mineral salts that collectively promote enhanced algal proliferation.

The optimal pH for *Spirulina maxima* growth was observed to be 9.0, a value conducive to maximal biomass accumulation. This pH level aligned with the typical alkaline conditions favorable for *Spirulina* cultivation. A gradual increase in pH during growth can be attributed to the decomposition of bicarbonate, which releases carbon dioxide and hydroxide ions, thus altering the medium's alkalinity (Moberg *et al*. 2012). Previous studies have consistently reported an optimal pH range of 9.0–9.5, beyond which algal growth declines significantly as the pH nears 11.0 (Ismaiel *et al*., 2016; Pandey *et al*. 2010). pH not only influences the availability of inorganic carbon but also affects nutrient uptake efficiency and the permeability of cell membranes (Nyabuto *et al*. 2015; Zhang *et al*., 2021).

Both CFTRI and Zarrouk's media supplied essential macro- and micronutrients—including carbon, nitrogen, phosphorus, potassium, and hydrogen—which collectively contributed to enhanced algal biomass production (Xin *et al*. 2010, Li *et al*., 2022). Among these, nitrogen plays a pivotal role in lipid biosynthesis, while carbon, comprising nearly 50% of *Spirulina's* dry weight, is mainly supplied through sodium bicarbonate ($NaHCO_3$), which is known to substantially improve biomass productivity (Nyabuto *et al*. 2015; Moberg *et al*. 2012). Phosphorus, critical for photosynthetic pro-

cesses and intracellular energy transfer, was provided in the form of dipotassium hydrogen phosphate (K_2HPO_4). Interestingly, while moderate phosphorus concentrations support growth, elevated levels may exert inhibitory effects on algal proliferation (Navarro *et al*. 2008; Chu *et al*. 2013).

The present study demonstrates that *Spirulina maxima* cultivated in CFTRI medium exhibited superior biochemical and nutritional profiles compared to cultures grown in Zarrouk's medium. *Spirulina maxima* cultivated in CFTRI medium demonstrated significantly higher carbohydrate content (12.20 ± 1.01%) compared to that grown in Zarrouk's medium (8.90 ± 0.58%), with a statistically significant difference (t = 4.54) (Table 1). The results align with previous reports that CFTRI medium may enhance the nutraceutical potential of *Spirulina by* promoting carbohydrate accumulation. *Spirulina* primarily stores carbohydrates as polysaccharides, which have been associated with diverse bioactivities, including antiviral effects against Herpes Simplex Virus type 1 (Chaiklahan, *et al*. 2013; Chirasuwan, *et al*. 2007). Polysaccharides from *Spirulina* have also been linked to immunomodulatory and anti-inflammatory effects, reinforcing their nutraceutical potential (Spínola *et al*., 2024).

Protein yield was markedly elevated in cultures grown in CFTRI medium (55%) relative to those in Zarrouk's medium (41%) (t = 11.89). This finding reinforces the recognition of *Spirulina* as a rich, complete, and highly digestible protein source (Morsy *et al*., 2014). The significantly higher protein yield (55.79±1.05%) in CFTRI medium further highlights its nutritional superiority (Table 1). This observation is consistent with CFTRI's own reports, which highlight *Spirulina* as one of the richest vegetarian sources of protein and vitamin B12 (CFTRI, 2025). Similar findings have been reported in modified Zarrouk's medium, where protein accumulation was also high, though lower than CFTRI (Akshay *et al*., 2025).

Similarly, lipid content was higher in CFTRI-grown cultures (6.16 ± 0.20%) in comparison to Zarrouk's medium (3.61 ± 0.19%) (t = 11.89). Typically, lipid accumulation in microalgae shows an inverse relationship with biomass, however, under nutrient-optimized conditions, a balance can be achieved between biomass productivity and lipid biosynthesis (Bhakar *et al*. 2013; Soni *et al*., 2019). Recent studies confirm that nutrient optimization can enhance both lipid and biomass yields simultaneously (Mercy Bai *et al*., 2023).

The comparative analysis of pigment content revealed that chlorophyll concentration was significantly higher in *Spirulina* cultured in CFTRI medium (18.53 ± 1.00 µg/ml) than in Zarrouk's medium (12.03 ± 1.62 µg/ml). This suggests that the nutrient composition of CFTRI medium, particularly its balance of bicarbonate and nitrate, may be more conducive to chlorophyll biosynthesis and photosynthetic efficiency. Elevated chlorophyll levels are indicative of enhanced light-harvesting capacity, which could translate into improved biomass productivity under controlled conditions (Akshay *et al*., 2025). In contrast, carotenoid concentrations remained similar across both media, implying that carotenoid biosynthesis is less sensitive to medium composition and may be more strongly regulated by light intensity or stress responses (Table 1).

Antioxidants interact with free radicals and prevent chain reaction thus stop the production of highly reactive oxygen products. Antioxidant activity was high in cultures from both CFTRI (23.14 ± 0.13) and Zarrouk's (23.29 ± 2.10) media, with a statistically significant difference (t = 10.65) (Table 1). Despite having lower total phenolic content than higher plants, *Spirulina* exhibits substantial antioxidant potential (El-Baky *et al*., 2009; Uebel *et al*., 2019; Richa *et al*., 2011; Ratnam *et al*., 2006). Phenolic compounds not only contribute to antioxidative capacity but also offer additional benefits such as antifungal activity (Pagnussatt *et al*. 2014). Recent work confirms that *Spirulina* phenolics contribute signifi-

Table 1: Biochemical composition of *Spirulina maxima* in CFTRI and Zarrouk's medium

S. No.	Biochemical composition	CFTRI	Zarrouk's
1	Total carbohydrates (%)	12.20 ± 1.01	8.90 ± 0.58
2	Reducing sugar (%)	7.87 ± 0.54	5.76 ± 0.61
3	Total Protein (%)	55.79 ± 1.05	41.00 ± 1.74
4	Total Lipid (%)	6.16 ± 0.20	3.61 ± 0.19
5	Ash Content (%)	23.05 ± 0.5	45.67 ± 0.78
6	Chlorophyll a (µg/ml)	18.53 ±1.00	12.03 ± 1.62
7	Carotenoids (µg/ml)	4.43 ± 0.16	4.65 ± 0.44
8	Total antioxidant capacity (µg/ml)	23.14 ± 0.13	23.29 ± 2.00
9	Phenol (mg GAE/g DW)	0.28 ± 0.01	0.14 ± 0.00
10	Flavonoids (mg QE/g DE)	0.17 ± 0.00	0.14 ± 0.00

Table 2. Fatty acid profile of *Spirulina maxima* (%) in CFTRI and Zarrouk's media

Compound name (CFTRI)	**Retention time**	**Peak area Relative abundance (%)**	**Common name**	**Fatty acid chain**	**% Composition**
8,11,14-Eicosatrienoic acid, methyl ester	19.967	3.606883	**Dihomo-γ-linolenic acid (DGLA)**	*20:3* (n-6) (ω−6)	4
9,12-octadecadienoic acid, methyl Ester, (e,e)	15.475	11.22877	Linolenic acid	18:2(n-6)	11
6,9,12-octadecatrienoic acid, methyl ester	15.993	16.25855	Gamma linolenic acid (GLA)	18:3(n-6)	16
9-octadecenoic acid (z)-, methyl ester	14.715	5.779837	Elaidic acid	18:1(n-9)	6
9-hexadecenoic acid, methyl ester	12.009	3.430399	Palmitoleic acid, or-hexadec-9-enoic acid, is an omega-7	16:1(n-9)	3
11-Hexadecenoic acid, methyl ester	11.933	1.136113	Palmitvaccenic acid	16:1(n-11)	1
9-octadecenoic acid, methyl ester e	14.808	10.94198	Oleic acid	18:1	11
9-hexadecenoic acid, methyl ester, Z	11.941	1.047871	Palmitoleic acid Or hexadec-9-enoic acid, is an omega-7	16:1	1
Octadecane	7.629	11.49349	Stearate	C18:0	11
Heptadecane	7.629	11.49349	Margaric acid	C17:0	11
Hexadecanoic acid, methyl ester	11.678	23.58262	Palmitic acid	16:0	24
Methyl stearate	14.358	2.30	Methyl ester		
Pentadecanoic acid, 14-methyl-, methyl ester	11.678	21.38	Fatty acid methyl ester		
Pentadecanoic acid, 14-methyl-, methyl ester	11.669	35.38	Methyl esters of fatty acids		
Compound name (Zarrouk's)	**Retention time(min)**	**Peak area relative abundance %**	**Common name**	**Fatty acids chain**	**% Composition**
6,9,12-Octadecatrienoic acid, methyl ester	16.006	18.14	*Gamma*-linolenic acid; (all *cis*-6,9,12) Fatty acid	C18:3	26
9,12-octadecadienoic acid, methyl Ester, (e,e)-	15.475	8.64	Linolenic acid	18:2(n-6)	12
9-hexadecenoic acid, methyl ester	12.018	4.18	Palmitoleic acid, or-hexadec-9-enoic acid, is an omega-7	16:1(n-9)	6
9-octadecenoic acid, methyl ester E	14.823	15.60	Oleic acid	18:1	22
Hexadecanoic acid, methyl ester	11.678	22.80	Palmitic acid	16:0	33
Tridecanoic acid, 12-methyl-, methyl ester	9.712	0.97	Sarcinic acid	15:0	1
Heptadecane	7.633	12.52	Methyl esters of fatty acids.		
Pentadecanoic acid, 14-methyl-, methyl ester	11.678	22.80	Methyl esters of fatty acids.		

Table 3. One-way analyses of variance (ANOVA)_ of Biochemical Compounds

ANOVA						
Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	4589.971	9	509.9968	12.83666	0.000216	3.020383
Within groups	397.2973	10	39.72973			
Total	4987.268	19				

cantly to antioxidative capacity and can be harnessed in functional foods (Boyle & Verghese, 2024; Dimitrova & Zhelev, 2025).

Fatty acid profiling revealed distinct differences between the two media (Table 2). *S. maxima* cultured in CFTRI medium synthesized a broader spectrum of fatty acids—eleven in total, including three methyl esters—compared to eight fatty acids and two methyl esters identified in cultures grown in Zarrouk's medium. The predominant fatty acid in CFTRI medium was palmitic acid (C16,0) at 23.58%, whereas gamma-linolenic acid (GLA; C18,3n-6) was dominant in Zarrouk's medium at 25.79%.

CFTRI medium supported the biosynthesis of several nutritionally important fatty acids, including dihomo-γ-linolenic acid (DGLA; 3.60%), elaidic acid (5.77%), linolenic acid (11.22%), and palmitoleic acid (3.43%). In contrast, Zarrouk's medium was characterized by a higher concentration of oleic acid (22.18%), palmitic acid (32.41%), and the unique presence of sarcinic acid (C15,0).

Monounsaturated fatty acids (MUFAs), such as palmitoleic acid (C16,1) and oleic acid (C18,1), were present in both media. Notably, elaidic acid was exclusive to CFTRI-grown cultures, while GLA and linolenic acid were more prominent in Zarrouk's. Importantly, polyunsaturated fatty acids (PUFAs), including DGLA, were absent in cultures grown in Zarrouk's medium but were present in those cultivated in CFTRI.

Overall, palmitic acid (C16,0) was the most abundant fatty acid, followed by PUFAs such as linoleic acid (C18,2n-6) and GLA (C18,3n-6), which is consistent with findings from earlier studies (Dibeklioglu *et al*. 2009; Jafari *et al*. 2014; Teimouri *et al*. 2013). The general pattern of fatty acid abundance followed the order, saturated fatty acids (SFAs; C16,0 > C18,0 > C17,0), followed by PUFAs (C20,3, C18,3, C18,2), and MUFAs (C16,1, C18,1). Taken together, these findings demonstrate that CFTRI medium not only enhances carbohydrate, protein, lipid, and chlorophyll yields but also supports the biosynthesis of a broader range of nutritionally relevant fatty acids. This highlights its potential as a cost-effective and nutritionally superior alternative to Zarrouk's medium for *Spirulina* cultivation. Future studies should explore the scalability of CFTRI medium under outdoor or photobioreactor conditions, as well as its impact on other bioactive compounds such as phycocyanin, to fully establish its industrial applicability.

Analysis of variance (ANOVA) analysis

The ANOVA test compared the variance between groups to the variance within groups to determine if there were statistically significant differences among group means. The calculated F-value was approximately 12.84, which was much larger than the critical F -value of 3.02 at the given degrees of freedom (df between groups = 9, df within groups = 10). The corresponding p-value was 0.000216, which was far below the common significance threshold of 0.05. This indicated that the data was significant (Table 3).

Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) of biochemical compounds and fatty acids

Principal Component Analysis (PCA) is a statistical technique that transforms a dataset with many potentially correlated variables into a set of uncorrelated principal components, each a linear combination of the original variables and ranked by the amount of variance they explain. By computing the eigenvectors and eigenvalues of the covariance matrix, PCA identifies new axes (principal components) that maximize data variance, projects the dataset onto them, and reduces dimensionality for more efficient analysis while preserving most of the important information. This method is widely used to simplify complex datasets, visualize high -dimensional data, address multicollinearity, filter noise, and enable faster, more accurate downstream analysis (Kumar *et al*. 2023; Kumar and Sahoo, 2024 and 2025).

The analyses presented in Fig. 1 collectively highlight clear biochemical differences in microalgae grown in CFTRI and Zarrouk's media. In Fig. 1a, the boxplot shows that cultures grown in Zarrouk's medium exhibit a wider range of metabolite values, indicating greater variability and broader metabolic adjustments than the more consistent biochemical responses observed in CFTRI. The PCA results in Fig. 1b and 1d further illus-

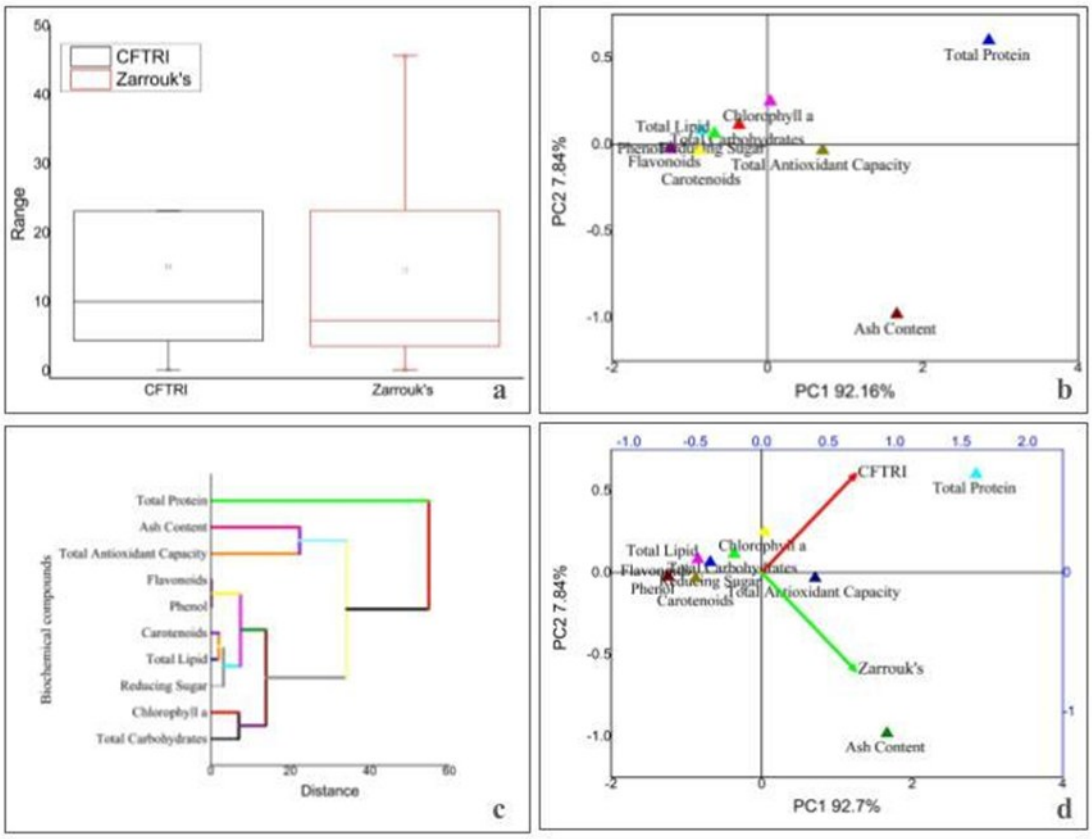

Fig. 1. *Principal component analysis of biochemical constituent of Spirulina maxima (A: Box plot; B: Score plot; C: Agglomerative Hierarchical Clustering; D: Biplot)*

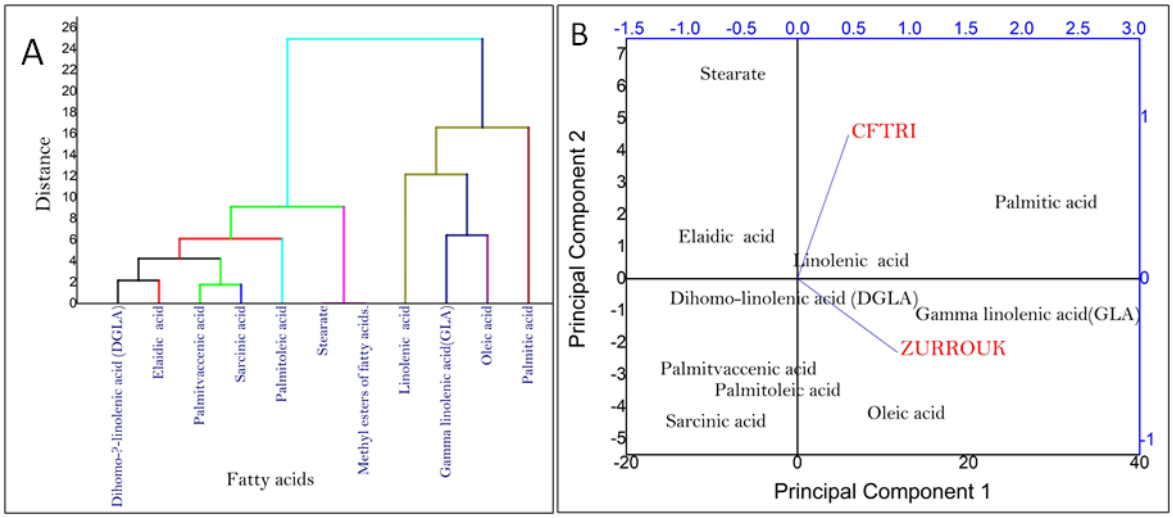

Fig. 2. *Principal component analysis of fatty acid profile of Spirulina maxima (A: Dendrogram; B: Biplot)*

trate distinct clustering patterns between media types. PC1 is driven largely by protein content, which is strongly associated with CFTRI, whereas ash content and antioxidant related traits contribute more to variation in Zarrouk's medium. The PCA biplots also reveal that protein aligns positively with CFTRI, while ash content and antioxidant capacity align more closely with Zarrouk's, and most other metabolites cluster tightly, indicating strong intercorrelations and relatively low variance across conditions. Fig. 1c, the hierarchical clustering dendrogram, supports these relationships by grouping pigments, lipids, and antioxidant associated metabolites together, while protein and phenolic compounds form separate, more distant clusters, reflecting their distinct biochemical behavior and importance in distinguishing the two media. Overall, Fig. 1 shows that CFTRI medium favours the production of protein-rich biomass, whereas Zarrouk's medium induces greater

metabolic variability, with comparatively higher levels of ash and antioxidant-related components.

Fig. 2 illustrates how the fatty acid profiles of microalgae respond to different culture media, revealing distinct metabolic preferences under CFTRI and Zarrouk's conditions. The hierarchical clustering in Fig. 2a shows that saturated and monounsaturated fatty acids, including palmitic, stearic, elaidic, and palmitoleic acids, cluster closely together, indicating their similar metabolic regulation and consistent expression across both media. In contrast, polyunsaturated fatty acids such as linolenic acid, gamma linolenic acid, and dihomo linolenic acid form separate clusters, reflecting their more specialized biosynthetic pathways and sensitivity to culture conditions. The PCA biplot in Fig. 2b reinforces these distinctions, separating the two media along PC1 and PC2. CFTRI-grown cultures align strongly with linolenic acid and certain saturated fatty acids, suggesting that this medium promotes the synthesis of higher-value polyunsaturated lipids. Conversely, Zarrouk's medium is more closely associated with oleic, palmitoleic, and long-chain polyunsaturated fatty acids, such as GLA and DGLA, indicating a metabolic shift toward desaturation and elongation pathways. The strong loading of palmitic acid on PC1 shows its major influence on overall lipid variance. Together, these analyses suggest that CFTRI favours the accumulation of select saturated and omega-3 polyunsaturated fatty acids, while Zarrouk's medium supports a broader, more diverse fatty acid profile enriched in omega-6 derivatives, highlighting the role of culture medium in steering lipid metabolism and in potential bioactive lipid production.

Conclusion

Optimising culture conditions is essential to maximise bioactive compound production in Spirulina, particularly as its commercial and nutraceutical applications expand. Among the evaluated media, CFTRI and Zarrouk's proved most effective in supporting strong biomass growth and metabolite accumulation, while also remaining practical and economical for large-scale cultivation. Notable differences emerged in the biochemical composition of *S. maxima* grown in the two media. Cultures grown at CFTRI exhibited substantially higher protein, carbohydrate, and lipid levels, along with valuable fatty acids, including dihomo-γ-linolenic acid (DGLA), an important omega-6 PUFA. Conversely, Zarrouk's medium enhanced antioxidant activity and ash content, indicating its potential for improving the functional quality of the biomass. Despite these distinctions, both media supported the production of key nutraceutical compounds, confirming their suitability for sustainable algal bioprocessing. The findings highlight the significance of selecting and fine-tuning media formulations to achieve targeted metabolite enrichment. Overall, *S. maxima* remains a promising source of high-value biomolecules, including proteins, essential fatty acids, and antioxidants. Further optimization of culture parameters could yield even greater improvements in specific compound production, benefiting the food, pharmaceutical, and aquaculture sectors.

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Conflict of interest

The authors declare that they have no conflict of interest.

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