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Analysis of the Proximate Composition, Fatty Acid, and Mineral Profiling of Cultured Spirulina (*Arthrospira platensis*) in Modified Zarrouk's Media

Akshay P.[1,2], Preethy Treesa Paul[1,2], Rosemol Jacob M.[1,2], Amruth P.[1,2,4], Jean Mary Joy[1,2,3], Joshy C. G.[1], Shoji Joseph[5], George Ninan[1], and Suseela Mathew[1*]

[1] *ICAR-Central Institute of Fisheries Technology, Cochin 682029, Kerala, India*

[2] *Cochin University of Science and Technology, Cochin 682022, Kerala, India*

[3] *Department of Zoology, St. Teresa's College (Autonomous), Ernakulam, Cochin 682011, Kerala, India*

[4] *Department of Life Science, Christ University, Hosur Main Road, Bhavani Nagar, Bangalore, Karnataka, India - 560 029*

[5] *Mariculture Division, ICAR-Central Marine Fisheries Research Institute, Kochi 682 018*

Abstract

The nutritional and therapeutic potential of the filamentous cyanobacterium Spirulina (*Arthrospira platensis*) is remarkable, being rich in proteins, vitamins, and essential fatty acids, and is also recognized for its antioxidant properties. Spirulina's easy growth adaptability positions it as a top sustainable food source, especially in geographic regions with agricultural challenges. The current study emphasizes the significance of optimizing cultivation methods, focusing on a modified Zarrouk medium to enhance growth conditions. Key findings show that spirulina exhibited a high protein content of 69.74%, along with elevated levels of polyunsaturated and monounsaturated fatty acids, underscoring spirulina's role in addressing malnutrition and supporting metabolic health. Furthermore, the study highlights the need for in-depth research on spirulina's bioactive compounds to fully harness its benefits in the nutraceutical industry and promote innovative formulations in food and pharmaceuticals.

Keywords: Spirulina, Zarrouk medium, culture, proximate composition

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*Email: suseela1962@gmail.com

Introduction

Arthrospira platensis, commonly known as Spirulina, is a helical, multicellular cyanobacterium recognized for its high protein, vitamin, and essential fatty acid content, as well as its significant therapeutic potential due to antioxidant properties. These properties are attributed to bioactive compounds such as phenolics (e.g., salicylic acid, chlorogenic acid, and caffeic acid), carotenoids, chlorophyll, and phycobiliproteins (Bortolini et al., 2022). With its ability to thrive in diverse climates and minimal resource requirements, Spirulina emerges as a sustainable solution for food security, particularly in regions facing agricultural challenges (Arahou, Lijassi, Wahby, Rhazi, Arahou, & Wahby, 2023). The versatility of Spirulina has led to its application in human and animal nutrition (Holman & Malau Aduli, 2013), aquaculture (Chen, Leng, Lu, & Zhou, 2021), pharmaceuticals (Maddiboyina et al., 2023), cosmetics (Ikeda, Sydney, & Sydney, 2022), and environmental protection (Godlewska, Michalak, Pacyga, Bacœladyñska, & Chojnacka, 2019). It has demonstrated potential in carbon dioxide capture, heavy metal biosorption, wastewater treatment, and biofuel production. Despite its wide-ranging applications, its commercial use in aquaculture and other industries is limited by high production costs.

Cultivation of Spirulina is predominantly carried out in open systems, such as ponds and closed systems, like photobioreactors (PBRs). While open systems are cost-effective, their reliance on environmental conditions limits productivity. In contrast,

PBRs provide controlled environments that enhance biomass yield by optimizing factors such as light intensity, carbon dioxide levels, and nutrient availability (Celekli & Dönmez, 2006). However, the cost of traditional cultivation media, such as Zarrouk's medium, remains a barrier to large-scale production. Therefore, modifications to this medium using cost-effective components are critical to improving economic viability.

This study investigates the cultivation of spirulina in modified Zarrouk's medium, focusing on its proximate composition, mineral content, and fatty acid profile. The findings aim to contribute to the cost-effective production and wider application of this valuable resource in nutrition and beyond.

Materials and Methods

The algal strain of *A. platensis* was sourced from the algal culture laboratory at ICAR-Central Marine Fisheries Research Institute in Cochin, Kerala, and cultured under ambient conditions in the Biochemistry and Nutrition Division at ICAR-Central Institute of Fisheries Technology, Cochin, Kerala.

DNA was extracted from the algal culture using the QIAGEN DNeasy Ultra Clean Microbial Kit, and its quality was assessed on 1% agarose gel. The amplification of the 16S rRNA gene was performed with specific primers (16SrRNA-F and 16SrRNA-R) under the following PCR conditions: initial denaturation at 95°C for 3 min, followed by 35 cycles of

Table 1. Composition of Zarrouk's medium

Constituents	Standard Zarrouk's Medium (SZM)	Modified Zarrouk's Medium (MZM)
	Composition (g L^{-1})	Composition (g L^{-1})
Sodium bicarbonate	18	54
Dipotassium hydrogen phosphate	0.5	1.5
Sodium nitrate	2.5	7.5
Potassium sulphate	1	3
Sodium chloride	1	3
Magnesium sulphate	0.2	0.6
Calcium chloride	0.04	0.12
Ferrous sulphate	0.01	0.03
Ethylene diamine tetra acetate	0.08	0.24
A_5 Solution	0.5	1.5
B_6 Solution	0.5	1.5
A_5 Solution		
Boric acid	1.43	4.29
Manganese chloride	0.905	2.715
Zinc sulphate	0.11	0.33
Sodium molybdate	0.09	0.27
Copper sulphate	0.04	0.12
B_6 Solution		
Ammonium nitrate	0.0115	0.0345
$K_2Cr_2(SO_4)_2.24H_2O$	0.048	0.144
$NiSO_4.7H_2O$	0.024	0.072
$Na_2WO_4.2H_2O$	0.009	0.027
$Ti_2(SO_4)_3$	0.02	0.06
$Co(NO_3)_2.6H_2O$	0.022	0.066

denaturation at 95ºC for 30 sec, annealing at 50ºC for 30 sec, and extension at 72ºC for 45 sec, with a final extension at 72ºC for 3 min. The purified PCR product was sequenced using forward and reverse primers, and the results were analyzed using the ABI 3730xl Genetic Analyzer. The primers used for amplification targeted the 16S rRNA gene region, with the forward primer 16SrRNA-F-27F (AGAGTTTGATCCTGGCTCAG) and the reverse primer 16SrRNA-R-1492R (CGGTTACCTTGTTACGACTT).

The mother culture of *A. platensis* was maintained in Zarrouk's medium and regularly sub-cultured. The cultivation was carried out at room temperature (28±2ºC) under a 12h light/dark cycle using standard white light, with the culture flasks receiving frequent aeration (Fig. 1). All reagents used to prepare the media were of food-grade quality. The mass culture of the microalgae was conducted using both the Standard Zarrouk Medium (SZM) and a Modified Zarrouk Medium (MZM), as detailed in Table 1.

The experimental setup for Spirulina cultivation utilized a laboratory-scale system with modified Zarrouk's media. Cultivation was performed in a 2-litre flask, capped with an aluminium lid to minimize the risk of contamination and maintain a sterile environment throughout the process (Fig. 1).

The growth parameters, including temperature, pH, water level, optical density, transmittance, and concentration, were monitored. Temperature was measured using a thermometer, pH was measured with a pH meter, and optical density, transmittance, and concentration were assessed using a spectrophotometer at 450 nm. These measurements were consistently maintained under controlled experimental conditions to effectively evaluate algal growth.

Inoculum: Both systems were inoculated with 10 ml L^{-1} of the mother culture.

Lighting: Spirulina requires significant sunlight for optimal growth and cell multiplication. The culture was exposed to a 12h light and 12h dark photoperiod. Illumination was provided with cool white fluorescent lights (2500 Lux).

Aeration system: Adequate aeration was provided by manual stirring using an air pump (buraq BL 710S) to meet Spirulina's CO_2 needs and prevent the algae from settling and forming a layer at the bottom.

pH and temperature measurement: Thermo Scientific Eutech instrument was used to monitor the pH value and temperature of the algal culture.

The concentration of cells (X) was determined daily using a UV-visible spectrophotometer SHIMADZU UV-1601 (Japan) at wavelengths between 400 nm to 500 nm, and a standard curve was plotted. The growth was monitored spectrophotometrically (mg g^{-1} biomass) from fresh biomass by measuring the optical density (OD) at 730 nm. The empirical relationship between biomass dry weight and optical density was established for *A. platensis* in this study, and growth curves were drawn for systematic quantification. Utilizing linear regression (Baker et al., 2021), the relationship was estimated to be:

$$\text{Biomass (g L}^{-1}) = OD_{730} \times 1.368 + 0.0691$$

Cell multiplication and growth were monitored under a LEICA DM750 microscope.

On the 30th day of cultivation, the total biomass was harvested by centrifugation (Remi C-24 Plus) at 5000 rpm for 15 min at 25ºC, resuspended in distilled water, and centrifuged again under the same conditions to remove salts, followed by membrane dialysis using PES membrane (Biomax® USA). The concentrated cell mass was subjected to lyophilization under ⁻80ºC.

Fig. 1. Mass culture of spirulina

The proximate composition (AOAC International, 2000) of the lyophilized Spirulina powder was evaluated, including moisture, protein, fat, and ash content.

The fatty acid methyl ester (FAME) analysis was conducted following the AOAC International (2000) method, using a Thermo Scientific ISQ 7000 Single Quadrupole Mass Spectrometer coupled with a TRACE 1300 Gas Chromatograph, a Thermo Scientific AI/AS 1310 autosampler, and a TG-POLAR column (105m x 0.25mm x 0.2 μm). The split injection method was employed, utilizing hydrogen as the carrier gas at a flow rate of 45 cm/sec, with a split flow of 90 ml/min. Detection was carried out using a flame ionization detector (FID). The fatty acid content from the dried *A. platensis* was quantified and expressed as g $100g^{-1}$ of total fatty acids.

Mineral content analysis was conducted using inductively coupled plasma mass spectrometry (ICP-MS) following acid digestion of the samples (AOAC International, 2015). Thirteen elements (Na, K, Ca, Mn, Fe, Cu, Zn, Cr, Cd, As, Hg, Mg, and Pb) were quantified in Spirulina samples. All samples were analysed in duplicate, with each sample measured in triplicate for accurate detection.

Chemical alterations in Spirulina powder were evaluated using Fourier Transform Infrared Spectroscopy (FTIR) following the methodology outlined by Dotto, Esquerdo, Vieira, and Pinto (2012). A Thermo Nicolet iS50 spectrophotometer with a scanning range of 4,000 to 400 cm^{-1} and a resolution of 0.4 cm^{-1} cell was employed.

Statistical analysis was performed on triplicate values, and the results were expressed as mean±SD. A level of $p<0.05$ was used to designate significant differences among the samples. The statistical analyses conducted using the SAS 9.3 software program for Windows.

Results and Discussion

The amplified product obtained from the Spirulina sample was sequenced, and the resulting nucleotide sequence (NR_125711) was analyzed using the Nucleotide Basic Local Alignment Search Tool (BLASTn) available on the US National Center for Biotechnology Information (NCBI) platform (www.ncbi.nlm.nih.gov).

The BLASTn search compared the sequence against the NCBI nucleotide database to identify homologous sequences and determine the genetic identity of the sample. The results revealed a 100% sequence similarity with multiple reference strains of *A. platensis*. This unequivocal match conclusively identifies the sample as *A. platensis*, highlighting the accuracy and specificity of the molecular analysis. This robust molecular identification provides a solid foundation for further exploration and understanding of the microbial composition within the studied sample (Zaki et al., 2021).

The growth kinetics analysis revealed significant differences ($p<0.05$) between the standard Zarrouk medium and the modified Zarrouk medium. In the standard medium, the maximum absorbance was observed at a wavelength of 400 nm. On day 1, with an initial algal concentration of 10 ml L^{-1}, the absorbance was recorded as 0.065, reflecting a slow initial growth rate. Subsequent days, specifically day 8, day 15, day 22, and day 29, showed absorbance values of 0.125, 0.240, 0.415, and 0.470, respectively, as illustrated in Fig. 2.

In contrast, the modified Zarrouk medium demonstrated superior growth performance under the same conditions. On day 1, the absorbance was 0.085, and subsequent values on day 8, day 15, day 22, and day 29 were 0.166, 0.360, 0.554, and 0.603, respectively. These values were significantly higher ($p<0.05$) than those recorded in the standard medium, indicating enhanced algal growth facilitated by the modifications in nutrient composition and cultivation conditions.

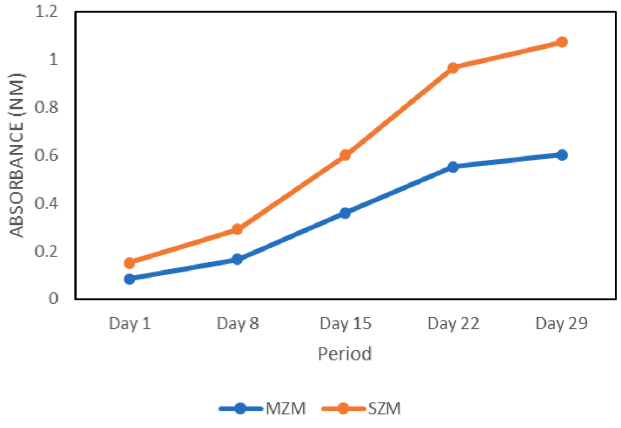

Fig. 2. Absorbance spectrum of spirulina biomass

Despite the reduced growth observed in the standard medium, the quality of the biomass remained consistent throughout the cultivation period, ensuring that the culture remained uncontaminated (Shimamatsu, 2004). The relationship between optical density and biomass concentration provided a valuable tool for estimating growth. However, the standard medium exhibited a weaker correlation between absorbance and biomass concentration compared to the modified medium, as shown in Fig. 3.

Over the 35-day cultivation period, a clear distinction was observed in the time-series data. In the standard medium, absorbance and biomass concentration increased steadily until day 25, after which the values plateaued earlier than in the modified medium. The maximum biomass yield recorded in the standard medium was 0.485 g L^{-1} after 30 days, which was significantly lower than the 0.665 g L^{-1} achieved in the modified medium.

The period between day 21 and day 28 in the modified medium showed a substantial rise in absorbance and biomass concentration, attributed to the improved nutrient availability and optimized growth conditions. In contrast, the standard medium exhibited a slower growth rate and an earlier plateau phase, suggesting potential nutrient limitations or other environmental stress factors. These findings underscore the importance of modifying nutrient compositions in standard media to enhance algal growth. The superior performance of the modified Zarrouk medium highlights its potential for large-scale algal biomass production.

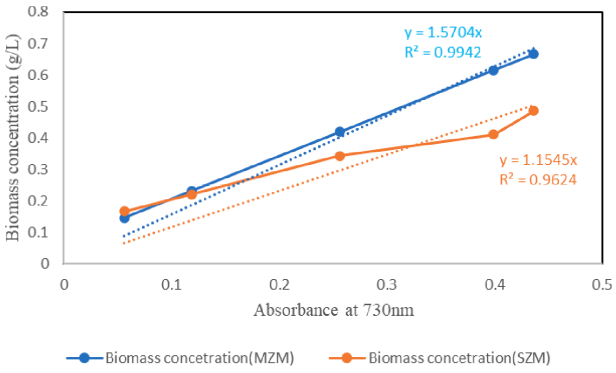

Fig. 3. Biomass concentration

The proximate composition of *A. platensis* cultured in both Standard Zarrouk Medium (SZM) and Modified Zarrouk Medium (MZM) revealed significant variations ($p<0.05$) (Table 2). The protein content in MZM (69.74±0.30%) was markedly higher than in SZM (55.10±0.40%), highlighting the impact of media modification on protein yield, as also noted by Joshi, Kaur, Mishra, and Singh (2014), who emphasized the role of optimized nutrient availability in boosting protein levels.

Comparatively, studies have demonstrated variability in the proximate composition of spirulina influenced by culture conditions, media formulations, and environmental factors. According to

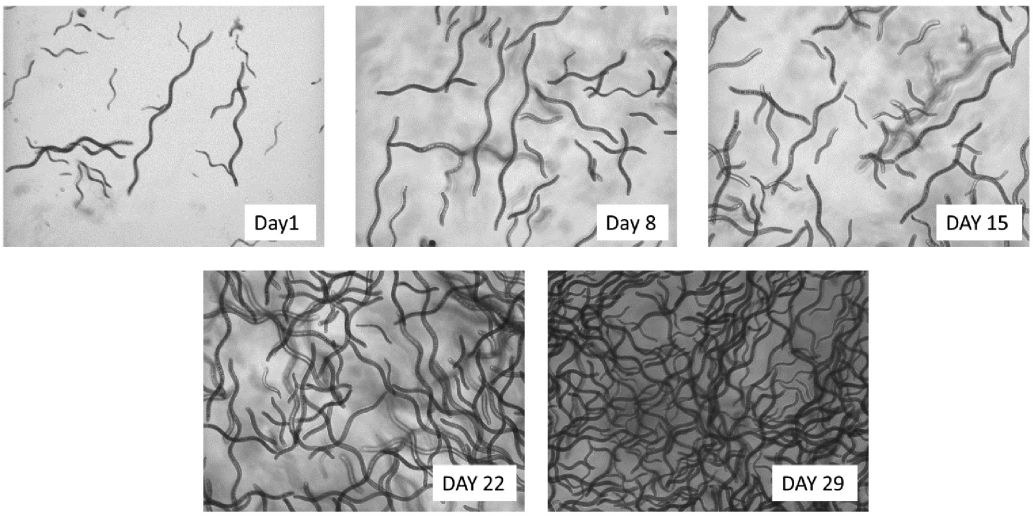

Fig. 4. Growth of spirulina (Microscopic view at 100x)

Madkour, Kamil, and Nasr (2012), protein content in Spirulina typically ranges between 50-70%, depending on nutrient availability and culture methods.

Table 2. Proximate composition

Components (%)	Standard Zarrouk Medium (SZM)	Modified Zarrouk Medium (MZM)
Moisture	5.95 ± 0.10	6.25 ± 0.05
Crude Protein	55.10 ± 0.40	69.74± 0.30
Crude Fat	1.10 ± 0.25	1.33 ± 0.20
Ash	7.00 ±0.35	7.70 ± 0.31

Values are represented as mean ± SD (n=3).

The mineral composition of the species was also analyzed, complementing the proximate analysis (Table 3). Among the major minerals, potassium (0.84%) and sodium (0.72%) were prominent in MZM, with magnesium at 0.25%. Calcium was particularly notable, with a concentration of 481 ppm. Trace minerals, including chromium, iron, copper, manganese, and zinc, were found in lower concentrations, ranging from 1.07 to 20.53 ppm. Importantly, arsenic and selenium were not detected (ND), indicating that these elements were either absent or present at undetectable levels. Overall, Spirulina exhibited a diverse mineral profile, characterized by significant levels of essential minerals and low levels of heavy metals, highlighting its potential as a valuable dietary supplement with health benefits (Al-Dhabi, 2013; Rutar et al., 2022). Modification of the nutrient composition of Zarrouk's medium can significantly impact the mineral content of Spirulina by enhancing its growth and nutrient uptake. Increased availability of key nutrients such as phosphorus and potassium may lead to higher biomass production and accumulation of essential minerals like calcium, magnesium, and iron. While higher nutrient concentrations might boost mineral content and protein synthesis, optimizing nutrient levels through controlled experiments is essential to achieve a balance between improved productivity and economic feasibility.

The fatty acid profile in Table 4 includes both saturated and polyunsaturated fatty acids (PUFA). α-Linolenic acid (ALA) was detected at concentrations of 62.6% in microalgae cultured in MZM and 42.6% in SZM. Andrade et al. (2019) reported the linoleic acid (C18:2ω6) content to range from 37.58% to 47.40% across five commercial samples cultured in Zarrouk media.

Arachidonic acid, another omega-6 fatty acid, was found in small quantities, 0.17% in MZM. Eicosapentaenoic acid (EPA), an omega-3 fatty acid known for its cardiovascular benefits, was present at concentrations of 0.15% in the SZM and 0.32% in the MZM. Lauric acid, a saturated fatty acid, was present at 0.22%, while myristic acid was found at 1.02% in MZM. Stearic acid was more abundant, with a concentration of 5.36%, whereas only trace amounts of elaidic (0.12%), arachidic (0.21%), and lignoceric acids (0.18%) were detected. The table underscores the presence of essential fatty acids (omega-6 and omega-3) and other fatty acids. Essential fatty acids are crucial for various physiological processes and must be obtained from the diet, as the body cannot synthesize them. Palmitic acid was the most abundant saturated fatty acid, with a concentration of 14.91%, and earlier studies

Table 3. Mineral composition

Minerals (ppm)	Standard Zarrouk Medium (SZM)	Modified Zarrouk Medium (MZM)
Magnesium (Mg)	1300 ± 0.1^{A}	2500 ± 0.21^{B}
Potassium (K)	5600 ± 0.32^{A}	8400 ± 0.11^{B}
Sodium (Na)	5200 ± 1.01^{A}	7200 ± 0.1^{B}
Calcium (Ca)	301.2 ± 2.1^{A}	481.03 ± 1.04^{A}
Chromium (Cr)	0.95 ± 0.02^{A}	1.07± 0.11^{A}
Iron (Fe)	145.14 ± 0.98^{A}	246.18 ± 0.85^{A}
Copper (Cu)	3.65 ± 0.22^{A}	4.21 ± 0.98^{A}
Manganese (Mn)	10.6 ± 0.29^{A}	14.53± 0.74^{A}
Zinc (Zn)	17.5 ± 0.21^{A}	20.53 ± 0.68^{A}
Arsenic (As)	ND	ND
Selenium (Se)	ND	ND
Lead (Pb)	0.54 ± 1.02^{A}	0.39 ± 0.87^{A}
Cadmium (Cd)	0.21± 0.39^{A}	0.40± 0.54^{A}
Mercury (Hg)	0.31± 0.44^{A}	0.26± 1.02^{A}

Values are represented as Mean±SD (n=3)

A different alphabet superscript (A and B) in the column indicates a significantly different percentage value than others by a Tukey test at $p<0.05$

Table 4. Fatty acid composition

Saturated fatty acids Fatty acids	SZM (%)	MZM (%)
C12:0 (Lauric)	0.69±0.1	0.22±0.01
C14:0 (Myristic)	2.24±1.2	1.02±0.1
C16:0 (Palmitic)	28.77±0.14	14.91±0.08
C18:0 (Stearic)	13.63±0.33	5.36±0.21
C18:1 n9t (Elaidic)	0.73±1.21	0.12±0.01
C20:0 (Arachidic)	0.13±0.96	0.21±0.01
C24:0 (Lignoceric)	0.08±0.65	0.18±0.31
Polyunsaturated fatty acids		
C18:3n6 (α-Linolenic)	42.62±0.33	6.65±0.11
C20:4n6 (Arachidonic)	ND	0.17±0.01
C20:5n3 (cis-5.8.11.14.17-Eicosapentaenoic)	0.15±0.3	0.32±0.01
Monounsaturated fatty acids		
C16:1 (Palmitoleic)	8.76±0.32	11.59±0.08
C18:1 n9c (Oleic)	1.95±0.35	2.55±0.01
C24:1 n9 (Nervonic)	1.12±0.1	0.46±0.04
Other fatty acids		
C15:0 (Pentadecanoic)	0.14±0.2	0.24±0.30
C20:3n3 (cis-11,14,17-Eicosatrienoic)	ND	ND

Values are represented as Mean±SD (n=3)

have reported total saturated fatty acid levels ranging from 23% to 60% (Gupta, Bhadauriya, Chauhan, & Bisen, 2008; Mühling, Belay, & Whitton, 2005). The excess carbon in MZM, such as sodium bicarbonate, can promote the synthesis of polyunsaturated fatty acids (PUFAs), which enhance membrane fluidity under high nutrient conditions. This surplus carbon also increases desaturation rates, converting saturated fatty acids (SFAs) into unsaturated ones as the cells adapt to maintain membrane stability under stress, resulting in lower SFA levels (Vonshak, 1997).

The infrared spectrum is characterized by various bands corresponding to different functional groups in its proteins, saccharides, and other nutrients. The interpretation of these bands using FTIR spectroscopy sheds light on the functional groups present on the surface of Spirulina (Berthomieu & Hienerwadel, 2009). In the FTIR spectrum of *Spirulina* sp. (Fig. 5), bands corresponding to O–H, N–H, and C=O groups were identified. These findings suggest the presence of hydroxyl (OH) and carbonyl (C=O) groups, confirming the existence of carboxyl (COOH) groups in the microalgae. Additionally, detecting -NH and -OH groups was linked to amino acid groups.

As mentioned in Table 5, the peak observed at 3410 cm^{-1} corresponds to the stretching vibrations of O-H, indicative of hydroxyl groups. This strong band is commonly associated with water, alcohol, and carbohydrates in spirulina, reflecting the hydrogen bonding interactions often observed in its polysaccharides and water content. The peak at 2924 cm^{-1} is attributed to the stretching vibrations of C-H, representing carbon-hydrogen bonds. This is consistent with the presence of fatty acids and proteins within Spirulina (Michalak, Mironiuk, Godlewska, Trynda, & Marycz, 2020). The peak at 1651 cm^{-1} primarily arises from the C=O stretching vibration of amide groups, confirming the proteinaceous nature of spirulina, while the amide II band at 1545 cm^{-1} results from N–H bending and C–N stretching vibrations, further supporting the presence of proteins. The strong presence of the C=O

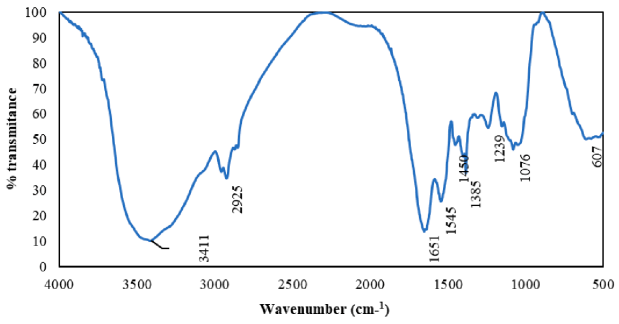

Fig. 5. FTIR Spectrum of spirulina

bond is attributed to the high protein content in Spirulina, which comprises about 60% of its composition (Liu et al., 2013). The peak identified at 1545 cm^{-1} corresponds to amide II, linked to protein peptide bonds. The peak at 1449 cm^{-1} is related to the asymmetric CH_3 bending of methyl groups, confirming the presence of methyl groups in proteins and lipids. The peak at 1384 cm^{-1} indicates stretching C-O, C-H deformation, and N-H deformation vibrations, which are the characteristics of polysaccharides and proteins. The peak at 1239 cm^{-1} signifies PO_2 asymmetric vibrations associated with phosphate groups essential for nucleic acids and phospholipids. (Michalak et al., 2020). The 1075 cm^{-1} peak is linked to the amide III band, reflecting the presence of amide groups and further confirming the protein structure of Spirulina as described by Eckel et al. (2001). Lastly, the peak at 606 cm^{-1} corresponds to CH out-of-plane bending vibrations, which are commonly seen in aromatic compounds, further supporting the complex molecular composition of Spirulina.

The research examined the growth patterns and biochemical composition of *A. platensis* when cultured in a modified Zarrouk medium, a widely used nutrient medium for microalgae cultivation. By altering the standard composition of the Zarrouk medium, the study optimized the growth conditions for *A. platensis*, resulting in a microalga with an improved nutritional profile. One of the key findings was the significant increase in protein content from 55.10% to 69.74%. This enhancement in protein content is particularly noteworthy, as *A. platensis* is already known for its high protein levels, making it a popular supplement in various nutritional products. Additionally, the study observed elevated levels of polyunsaturated and monounsaturated fatty acids (PUFAs and MUFAs) in the modified cultures. These beneficial fatty acids in higher concentrations strengthen the case for *A. platensis* as a valuable dietary supplement, offering more than just protein but also essential fatty acids that contribute to a balanced diet. *A. platensis* contains many bioactive compounds, including pigments like phycocyanin, antioxidants, vitamins, and minerals. Identifying and characterizing these compounds in the context of the modified culture conditions could reveal additional health benefits, enhancing the potential of *A. platensis* as a comprehensive dietary supplement. Future studies should focus on a detailed metabolomic analysis to explore the full spectrum of bioactive compounds and their potential health benefits. This could lead to the development of targeted nutritional interventions and novel applications in the nutraceutical industry.

The production cost of Modified Zarrouk's Medium is slightly higher than the standard medium, but its

Table 5. Interpretation of FTIR bands (Michalak et al., 2020)

Sl. No.	Peak	Functional Groups
1	3410 cm^{-1}	Stretching O-H asymmetric
2	2924 cm^{-1}	Stretching C-H
3	1651cm^{-1}	Amide I absorption (predominantly the C55O stretching vibration of the amideC55O)
4	1545 cm^{-1}	Peptide amide II
5	1449 cm^{-1}	Asymmetric CH3 bending of the methyl groups of proteins
6	1384 cm^{-1}	Stretching C-O, deformation C-H, deformation N-H
7	1239 cm^{-1}	PO_2 asymmetric (phosphate I)
8	1075 cm^{-1}	Amide III band region
9	606 cm^{-1}	CH out-of-plane bending vibrations

superior biochemical output justifies the expense. The enhanced medium yields higher levels of polyunsaturated fatty acids (PUFAs), proteins, and essential minerals, significantly improving the nutritional and commercial value of the final product. These attributes make it highly valuable for applications in the nutraceutical, pharmaceutical, and feed industries, where bioactive compounds command premium prices.

Despite the initial cost, the superior product quality leads to better marketability and higher revenue. The improved nutrient profile enhances processing efficiency and reduces the need for additional supplementation, optimizing overall production costs. Thus, the Modified Zarrouk Medium presents a cost-effective option with significant economic benefits for commercial microalgae cultivation.

References

Al-Dhabi, N. A. (2013). Heavy metal analysis in commercial Spirulina products for human consumption. *Saudi Journal of Biological Sciences*, *20*(4), 383-388. https://doi.org/10.1016/j.sjbs.2013.04.006.

Andrade, B. B., Cardoso, L. G., Assis, D. J., Costa, J. A. V., Druzian, J. I., & Lima, S. T. C. (2019). Production and characterization of *Spirulina* sp. LEB 18 cultured in reused Zarrouk's medium in a raceway-type bioreactor. *Bioresource Technology*, *284*, 340-348. https://doi.org/10.1016/j.biortech.2019.03.144.

AOAC International. (2000). *Official methods of analysis of AOAC International* (17th ed.). AOAC International.

AOAC International. (2015). Official Method 2015.01: Heavy metals in food—Inductively coupled plasma mass spectrometry (ICP-MS) method. *Official Methods of Analysis of AOAC International*. AOAC International.

Arahou, F., Lijassi, I., Wahby, A., Rhazi, L., Arahou, M., & Wahby, I. (2023). Spirulina-based biostimulants for sustainable agriculture: Yield improvement and market trends. *BioEnergy Research*, *16*(3), 1401-1416. https://doi.org/10.1007/s12155-022-10537-8.

Baker, M., Blackman, S., Cooper, E., Smartt, K., Walser, D., Boland, M., Kolar, P., Beck, A. E., & Chinn, M. S. (2021). Exploratory analysis of *Spirulina platensis* LB 2340 growth in varied concentrations of anaerobically digested pig effluent (ADPE). *Heliyon*, *7*(9), Article e08065. https://doi.org/10.1016/j.heliyon.2021.e08065.

Berthomieu, C., & Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. *Photosynthesis Research*, *101*, 157-170. https://doi.org/10.1007/s11120-009-9439-x.

Bortolini, D. G., Maciel, G. M., Fernandes, I. A. A., Pedro, A. C., Rubio, F. T. V., Branco, I. G., & Haminiuk, C. W. I. (2022). Functional properties of bioactive compounds from *Spirulina* spp.: Current status and future trends. *Food Chemistry: Molecular Sciences*, *5*, Article 100134. https://doi.org/10.1016/j.fochms.2022.100134.

Çelekli, A., & Dönmez, G. (2006). Effect of pH, light intensity, salt and nitrogen concentration on growth and â-carotene accumulation by a new isolate *Dunaliella* sp. *World Journal of Microbiology and Biotechnology*, *22*, 183-189. https://doi.org/10.1007/s11274-005-9017-0.

Chen, F., Leng, Y., Lu, Q., & Zhou, W. (2021). The application of microalgae biomass and bio-products as aquafeed for aquaculture. *Algal Research*, *60*, Article 102541. https://doi.org/10.1016/j.algal.2021.102541.

Dotto, G. L., Esquerdo, V. M., Vieira, M. L. G., & Pinto, L. A. A. (2012). Optimization and kinetic analysis of food dyes biosorption by *Spirulina platensis*. *Colloids and Surfaces B: Biointerfaces*, *91*, 234-241. https://doi.org/10.1016/j.colsurfb.2011.11.008.

Eckel, R., Huo, H., Guan, H. W., Hu, X., Che, X., & Huang, W. D. (2001). Characteristic infrared spectroscopic patterns in the protein bands of human breast cancer tissue. *Vibrational Spectroscopy*, *27*(2), 165-173. https://doi.org/10.1016/S0924-2031(01)00134-5.

Godlewska, K., Michalak, I., Pacyga, P., Bacœladyñska, S., & Chojnacka, K. (2019). Potential applications of cyanobacteria: *Spirulina platensis* filtrates and homogenates in agriculture. *World Journal of Microbiology and Biotechnology*, *35*, Article 80. https://doi.org/10.1007/s11274-019-2653-6.

Gupta, R., Bhadauriya, P., Chauhan, V. S., & Bisen, P. S. (2008). Impact of UV-B radiation on thylakoid membrane and fatty acid profile of *Spirulina platensis*. *Current Microbiology*, *56*, 156-161. https://doi.org/10.1007/s00284-007-9049-9.

Holman, B. W. B., & Malau Aduli, A. E. O. (2013). *Spirulina* as a livestock supplement and animal feed. *Journal of Animal Physiology and Animal Nutrition*, *97*(4), 615-623. https://doi.org/10.1111/j.1439-0396.2012.01328.x.

Ikeda, I. K., Sydney, E. B., & Sydney, A. C. N. (2022). Potential application of *Spirulina* in dermatology. *Journal of Cosmetic Dermatology*, *21*(10), 4205-4214. https://doi.org/10.1111/jocd.14997.

Joshi, M., Kaur, K., Mishra, T., & Singh, S. (2014). To evaluate lab scale cultivation of *Spirulina* by using different substrates and to evaluate its chlorophyll and protein content. *International Research Journal of Biological Sciences*, *3*(1), 22-30. https://doi.org/10.13140/RG.2.2.35275.23847.

Liu, H. J., Xu, C. H., Zhou, Q., Wang, F., Li, W. M., Ha, Y. M., & Sun, S. Q. (2013). Analysis and identification of irradiated *Spirulina* powder by a three-step infrared

macro-fingerprint spectroscopy. *Radiation Physics and Chemistry*, *85*, 210-217. https://doi.org/10.1016/j.radphyschem.2012.12.001.

Maddiboyina, B., Vanamamalai, H. K., Roy, H., Ramaiah, Gandhi, S., Kavisri, M., & Moovendhan, M. (2023). Food and drug industry applications of microalgae *Spirulina platensis*: A review. *Journal of Basic Microbiology*, *63*(6), 573-583. https://doi.org/10.1002/jobm.202200704.

Madkour, F. F., Kamil, A. W., & Nasr, H. S. (2012). Production and nutritive value of *Spirulina platensis* in reduced cost media. *The Egyptian Journal of Aquatic Research*, *38*(1), 51-57. https://doi.org/10.1016/j.ejar.2012.09.003.

Michalak, I., Mironiuk, M., Godlewska, K., Trynda, J., & Marycz, K. (2020). *Arthrospira* (*Spirulina*) *platensis*: An effective biosorbent for nutrients. *Process Biochemistry*, *88*, 129-137. https://doi.org/10.1016/j.procbio.2019.10.004.

Mühling, M., Belay, A., & Whitton, B. A. (2005). Variation in fatty acid composition of *Arthrospira* (*Spirulina*) strains. *Journal of Applied Phycology*, *17*, 137-146. https://doi.org/10.1007/s10811-005=-7213-9.

NCBI. (n.d.). *BLAST: Basic Local Alignment Search Tool.* National Center for Biotechnology Information. Retrieved from https://www.ncbi.nlm.nih.gov/BLAST/.

Rutar, J. M., Hudobivnik, M. J., Neèemer, M., Mikuš, K. V., Arèon, I., & Ogrinc, N. (2022). Nutritional quality and safety of the Spirulina dietary supplements sold in the Slovenian market. *Foods*, *11*(6), Article 849. https://doi.org/10.3390/foods11060849.

Shimamatsu, H. (2004). Mass production of *Spirulina*, an edible microalga. *Hydrobiologia*, *512*(1), 39-44. https://doi.org/10.1023/B:HYDR.0000020364.23796.04.

Vonshak, A. (1997). *Spirulina platensis* (*Arthrospira*): *Physiology, cell-biology and biotechnology*. Taylor & Francis Ltd, London.

Zaki, M. A., Ashour, M., Heneash, A. M. M., Mabrouk, M. M., Alprol, A. E., Khairy, H. M., Nour, A. M., Mansour, A. T., Hassanien, H. A., Gaber, A., & Elshobary, M. E. (2021). Potential applications of native cyanobacterium isolate (*Arthrospira platensis* NIOF17/003) for biodiesel production and utilization of its byproduct in marine rotifer (*Brachionus plicatilis*) production. *Sustainability*, *13*(4), Article 1769. https://doi.org/10.3390/su13041769.