

**PROXIMATE ANALYSIS, NUTRITIONAL VALUE AND AMINO ACID PROFILE OF
MICROALGAE BIOMASS OF STRAINS *Dictyosphaerium pulchellum* (DP) AND RED
ALGAE (R).**

**A Research Project Submitted to the Department of Biochemistry and Molecular Biology,
Usmanu Danfodiyo University Sokoto,
In Partial Fulfilment of the Requirements for the Award of B.Sc. (Hons) Biochemistry**

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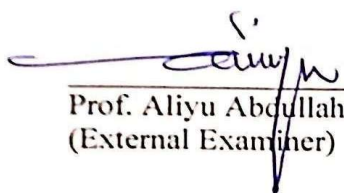
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DECEMBER, 2025

CERTIFICATION

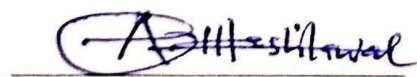
This is to certify that the research project have been conducted by MABERA, Mika'ilu Musa (2010304045) and has been approved as meeting part of the requirements for the award of Bachelor of Science (B.Sc.) degree in Biochemistry of Usmanu Danfodiyo University, Sokoto.


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DEDICATION

This work is wholeheartedly dedicated to the Almighty Allah, whose endless mercy, guidance, and protection have sustained me throughout the course of this research.

It is also dedicated to my beloved parents, whose prayers, sacrifices, and unwavering support have shaped every step of my academic journey.

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ABSTRACT

Microalgae are rapidly growing photosynthetic microorganisms with significant nutritional potential, particularly as sources of protein, lipids, and carbohydrates. This study assessed the proximate composition, nutritional value, and amino acid profile of two microalgae species: Red Algae and *Dictyosphaerium pulchellum* (DP). Microalgal biomass was harvested, oven-dried, and subjected to standard proximate analysis using AOAC (2016) procedures. Dry biomass yield was determined, and protein content was quantified using the Kjeldahl method. Results showed that DP produced a higher biomass yield (0.33 g) than Red Algae (0.12 g) and contained a higher crude protein content (5.98% vs 5.10%). Lipid, ash, moisture, and carbohydrate contents were also measured and compared between the two species. Amino acid profiling was prepared following standard HPLC procedures (acid hydrolysis with OPA/FMOC derivatization), though data collection is ongoing. This finding indicates that the microalgae Strain DP has superior nutritional potential compared to Strain (R), particularly in terms of protein content, supporting its use as a sustainable nutrient source for food, feed and biotechnological applications.

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CHAPTER ONE

1.0 INTRODUCTION

Microalgae are microscopic, photosynthetic organisms that inhabit freshwater and marine environments. They are a highly diverse group with the ability to produce biomass rich in proteins, lipids, carbohydrates, pigments, and other bioactive compounds (Becker, 2017; Pulz & Gross, 2024). Their fast growth rates, ability to thrive under controlled environmental conditions, and high nutrient density make them attractive as alternative sources of food, feed, and nutraceuticals. Among microalgae, *Dictyosphaerium pulchellum* (DP strain) and red algae (R strain) have attracted research interest due to their potential for high-protein biomass. Proteins from microalgae are often rich in essential amino acids, making them valuable for human and animal nutrition (Brown, 2017). Besides proteins, microalgae provide lipids and carbohydrates, contributing to overall nutritional value and energy content. Proximate analysis, which determines moisture, ash, crude protein, crude lipids, crude fiber, and carbohydrate content, is a primary step in evaluating the nutritional quality of microalgae (Spolaore, Joannis-Cassan, Duran, and Isambert, 2016). Protein content, in particular, is a critical determinant of biomass nutritional value, as it influences digestibility and overall applicability in food and feed formulations.

Despite extensive studies on widely used species like *Chlorella* and *Spirulina*, less-studied strains such as DP and R have limited data available. Investigating these strains provides essential baseline information that could facilitate their industrial and nutritional applications (Borowitzka, 2023).

1.2 Statement of the Problem

Global demand for sustainable, nutrient-dense protein sources is increasing due to population growth, food insecurity, and environmental constraints associated with conventional protein production. While microalgae are recognized as alternative protein sources, DP and R strains lack sufficient compositional and nutritional characterization, particularly regarding protein content and overall proximate composition. This knowledge gap limits their potential use in food, feed, and nutraceutical industries (Guedes *et al.*, 2021).

1.3 Justification of the Study

Microalgae are high in proteins and other macronutrients, making DP and R strains promising alternatives (Becker, 2017). There is a lack of data on proximate composition and protein content for DP and R strains, which this study seeks to fill. Microalgae cultivation requires less land and water compared to conventional crops, offering a sustainable protein source (Pulz and Gross, 2024). Information generated can support the use of these strains in food, feed, or nutraceutical formulations (Borowitzka, 2023).

1.4 Aim and Objectives

Aim of the Study was to determine the proximate composition and nutritional value, with emphasis on protein content, of *Dictyosphaerium pulchellum* (DP) and Red algae (R) biomass.

Objectives: The specific objectives were:

- To quantify moisture, ash, crude protein, lipids, fiber, and carbohydrate content of DP and R strains.
- To evaluate the nutritional value of the biomass based on proximate composition.

- To compare protein content between DP and R strains.
- To provide baseline information for potential industrial and nutritional applications of the studied microalgae strains.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Microalgae

Microalgae are a diverse group of unicellular or simple multicellular organisms capable of photosynthesis. They are prolific producers of biomass rich in proteins, lipids, and carbohydrates, which vary depending on species, strain, and environmental conditions (Becker, 2017). These organisms are increasingly recognized as sustainable alternatives to terrestrial protein sources due to their high growth rates, nutrient density, and low environmental footprint (Pulz and Gross, 2024).

2.2 Classification of Microalgae

Microalgae are classified based on pigmentation, cell wall structure, storage compounds, and phylogeny:

2.2 Classification of Microalgae

Microalgae are a highly diverse group of photosynthetic organisms. They can be classified based on pigmentation, storage compounds, cell wall composition, and phylogenetic relationships. The major groups of microalgae relevant to this study are discussed below:

2.2.1 Green Algae (Chlorophyta)

Green algae are characterized by their green pigmentation due to the presence of chlorophyll a and b. They store carbohydrates as starch and have cell walls composed mainly of cellulose.

Species of *Chlorophyta*, including *Dictyosphaerium pulchellum* (DP strain), are known for high protein content, rapid growth rates, and adaptability to various culture conditions (Borowitzka, 2023). They are widely used in food, feed, and biofuel applications due to their nutritional richness and biomass productivity.

2.2.2 Red Algae (*Rhodophyta*)

Red algae are distinguished by their red pigmentation, mainly due to phycoerythrin, and store carbohydrates as floridean starch. The cell walls contain agar or carrageenan, which have industrial significance (Pulz and Gross, 2024). The R strain studied in this work belongs to this group. Red algae are particularly valued for their protein content, mineral composition, and use in nutraceuticals and functional foods.

2.2.3 Diatoms (*Bacillariophyta*)

Diatoms are unicellular algae with silica-based cell walls (frustules) that are often highly ornate. They store energy as lipids and chrysolaminarin, and are a rich source of polyunsaturated fatty acids, particularly EPA (eicosapentaenoic acid) (Becker, 2017). Diatoms are used in aquaculture feeds and are increasingly explored for biofuel production due to their high lipid content.

2.2.4 Brown Algae (*Phaeophyta*)

Brown algae contain fucoxanthin, giving them a characteristic brown color. They store carbohydrates as laminarin and mannitol. While mostly multicellular and macroscopic, some microscopic stages can be classified as microalgae. Brown algae are rich in bioactive compounds,

including antioxidants and polysaccharides with health-promoting properties (Spolaore *et al.*, 2016).

2.2.5 Cyanobacteria (Blue-Green Algae)

Cyanobacteria are prokaryotic microalgae capable of oxygenic photosynthesis. They store carbohydrates as glycogen and often produce bioactive compounds, including toxins in some species. Cyanobacteria such as *Spirulina* are commercially important for protein-rich biomass and nutraceutical products (Borowitzka, 2023). Each group exhibits variations in biochemical composition, which affects protein, lipid, and carbohydrate content, and consequently, their nutritional value.

2.3 Proximate Composition of Microalgae

Proximate analysis is a critical method to determine the nutritional quality of microalgal biomass. The components include:

2.3 Proximate Composition of Microalgae

Proximate analysis evaluates the major macronutrients and is essential for assessing the nutritional quality of microalgal biomass. The components typically analyzed include moisture, ash, crude protein, lipids, crude fiber, and carbohydrates (Spolaore *et al.*, 2016). Each component is discussed in detail below:

2.3.1 Moisture Content

Moisture content represents the water fraction of the biomass and is a key factor affecting shelf-life, storage, and processing. High moisture content may promote microbial growth and reduce stability, whereas lower moisture improves preservation (Becker, 2017). Moisture levels in microalgae can vary based on species, culture age, and harvesting techniques (Pulz & Gross, 2024). Maintaining consistent moisture during sample preparation ensures accuracy in subsequent analyses.

2.3.2 Ash Content

Ash content reflects the total mineral content of the biomass, including essential elements such as calcium, magnesium, potassium, and iron (Borowitzka, 2023). Minerals play vital roles in metabolic processes and contribute to the nutritional value of microalgae. Variations in ash content are influenced by growth medium composition, strain differences, and environmental conditions (Guedes *et al.*, 2021).

2.3.3 Crude Protein

Protein is one of the most important components of microalgae, often accounting for 30–70% of dry weight depending on species and culture conditions (Brown, 2017). Crude protein is determined using the Kjeldahl method, which measures total nitrogen and converts it to protein using a conversion factor (usually 6.25). Microalgal proteins are valued for their high-quality amino acid profiles, containing essential amino acids necessary for human and animal nutrition (Guedes *et al.*, 2021). Protein content also directly influences the energy and economic value of microalgae as a biomass source.

2.3.4 Lipid Content

Lipids in microalgae serve as energy reserves and contain essential fatty acids such as omega-3 and omega-6 (Becker, 2017). Lipid levels vary widely among species, culture conditions, and growth phases, with some species accumulating lipids up to 50% of dry weight under nutrient-limited conditions (Pulz & Gross, 2024). Besides energy provision, lipids have applications in nutraceuticals and biofuels, making them an important component of microalgal biomass.

2.3.5 Carbohydrate Content

Carbohydrates, calculated by difference, are the primary energy source in microalgae and include starches, sugars, and storage polysaccharides (Becker, 2017). Carbohydrate content varies depending on strain, light exposure, and nutrient availability. In addition to providing energy, carbohydrates are sometimes extracted for biofuel production or as prebiotics in food formulations. High carbohydrate content can enhance caloric value but may reduce relative protein percentage. Protein content in microalgae ranges widely (30–70% of dry weight) depending on strain and culture conditions, making it the most critical determinant of nutritional value (Guedes et al., 2021).

2.4 Protein and Nutritional Value

Microalgal protein is highly valued because it contains all essential amino acids, similar to animal proteins (Brown, 2017). Protein contributes not only to growth and maintenance of body tissues but also enhances the value of microalgae in feed and functional foods. Lipid content also provides caloric energy, while ash indicates important minerals, collectively defining the nutritional value of the biomass (Becker, 2017).

2.5 Amino Acid Profile

Although amino acid results are not yet available, literature suggests microalgae generally provide complete protein with essential amino acids in balanced proportions. Inclusion of amino acid data will strengthen assessment of protein quality and support nutritional recommendations (Brown, 1997; Guedes *et al.*, 2021).

2.6 Factors Affecting Proximate Composition

- Species/strain: Different strains inherently accumulate varying levels of protein, lipids, and carbohydrates.
- Culture conditions: Light intensity, temperature, nutrient availability, and growth phase influence nutrient accumulation (Pulz & Gross, 2024).
- Harvesting and processing: Methods such as centrifugation, drying, and storage can affect measured protein content and overall proximate composition (Borowitzka, 2023).

2.7 Applications of Microalgae

Microalgae are applied in human nutrition, animal feed, and nutraceutical industries due to their high protein and nutrient content. For example:

- Food applications: Protein-enriched supplements, fortified foods, and functional beverages.
- Animal feed: Sustainable protein replacement in aquaculture and livestock feed.
- Industrial applications: Source of pigments, bioactive compounds, and potential biofuels (Becker, 2007; Borowitzka, 2023).

CHAPTER THREE

3.0 Materials and Methods

3.1 Equipment and Apparatus

Incubator, Test tube racks, Culture tubes with screw cap, 10 mL sterile glass pipettes, 1 mL Glass pipettes, Bunsen burner, Autoclave, Measuring scale, Petri dish, Bunsen burner, Parafilm, Aseptic loop, Light microscope digital camera, Cover slip, Glass slides, Centrifuge tubes, Micropipette, Centrifuge machine, GCMSQP2010 Ultra gas-chromatograph (Shimadzu, Japan), Flame ionization detector (FID) 250°C; Column SP-2560, 100 m × 0.25 mm × 0.20 µm (Sigma-Aldrich); Carrier gas He, Micro centrifuge syringe-driven filter (13 mm, PVDF, 0.22 µm, Jet Biofil), Vortex mixer (XH-C Search tech Instruments China), Plant Growth Chamber, Digital Camera, PCR machine (Qiagen, Germany).

Table 3.1: Materials/Reagents and Chemicals

S/NO	COMPOUND	CHEMICAL FORMULA	SUPPLIER	%PURITY
1	Ferric chloride	FeCl ₃	Central drug house (CDH)	97.0%
2	Boric acid	H ₃ BO ₃	Molychem, India	99.5%
3	Zinc sulfate	ZnSO ₄ .7H ₂ O	LOBA Chemie	99%
4	Copper (ii) sulfate anhydrous	CuSO ₄	Guangdong guanghua Sci tech co. LTD. China	99%
5	Cobalt (ii) chloride	CoCl ₂ .6H ₂ O	Kermel	98%
6	Zinc chloride	ZnCl	LOBA Chemie	97%
7	Calcium chloride dehydrate	CaCl ₂ .2H ₂ O	LOBA Chemie	98%

8	Manganese chloride tetrahydrate	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	Molychem, India	Mumbai	98%
9	EDTA disodium salt	$\text{C}_{10}\text{H}_{14}\text{N}_2\text{NaO}_8 \cdot 2\text{H}_2\text{O}$	Molychem, India	Mumbai	98%
10	Sodium molybdate	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	Molychem, India	Mumbai	98%
11	Copper (ii) chloride dehydrate	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	Molychem, India	Mumbai	98%
12	Ammonium molybdate	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	Molychem, India	Mumbai	98%
13	Potassium nitrite	KNO_2	Molychem, India	Mumbai	97%
14	Sodium hydrogen carbonate	NaHCO_3	Molychem, India	Mumbai	99%
15	Manganese chloride tetrahydrate	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	Damao		99%
16	Magnesium chloride hexahydrate	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	Molychem, India	Mumbai	98%
17	Sodium nitrite	NaNO_2	Guangdong guanghua Sci tech co. LTD. China		99%
18	Magnesium sulfate heptahydrate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	Guangdong guanghua Sci tech co. LTD. China		99%
19	Dipotassium hydride phosphate	$\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$	Kermel		99%
20	Potassium chloride	KCl	Central drug house (CDH)		99%

All solvents used were of analytical grade.

3.2 Microalgae Sample Collection

Microalgae samples were collected from Rivers and ponds, along BUA cement also from Hushi village where the Sokoto-Rima River flows through in Wamakko Local Government Area,

Sokoto State, Nigeria (12.21°N, 5.05°E). Water samples (50 mL) containing visible microalgal species were collected at 7:00 am using aseptic bottles from top to bottom at a depth of 30 cm, stored in Ziploc bags, and transported in an ice box to the Centre of Biotechnology and Plant Tissue Culture, Usmanu Danfodiyo University, Sokoto, Nigeria for further analysis.

3.3 Culture Media

One (1) litre of modified Johnson culture media (J/1) by Borowitska (2018) will be prepared and used for enrichment and culture of microalgal strains.

Table 3.2: Modified Johnson media (1L) composition

No	Component	Amount(mL)	Stock concentration (g/mL)	Solution
	Stock 1	980 mL		
1	MgCl ₂ .6H ₂ O		1.5	
2	MgSO ₄ .7H ₂ O		0.5	
3	KCl		0.2	
4	CaCl ₂ .2H ₂ O		0.2	
5	KNO ₃		1.0	
6	NaHCO ₃		0.043	
7	KH ₂ PO ₄		0.035	
	Stock 2	Fe solution (10 mL)		
1	Na ₂ EDTA		189 mg	
2	FeCl ₃ .6H ₂ O		244 mg	
	dH ₂ O 1000ml			
	Stock 3	Trace element solution (10 mL)		

1	H ₃ BO ₃		61.0 mg
2	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O		38.0 mg
3	CuSO ₄ ·5H ₂ O		6.0 mg
4	CoCl ₂ ·6H ₂ O		5.1 mg
5	ZnCl ₂		4.1 mg
6	MnCl ₂ ·4H ₂ O		4.1 mg
7	dH ₂ O	To	1000 mL

3.3.1 Procedure for Preparation of Johnson's Medium

Stock 1 was prepared in 980 mL of distilled water using pre-measured components in the order specified, with continuous stirring on a magnetic stirrer. The total volume was brought to 1 L by adding 10 mL of Stock 2 and 10 mL of Stock 3, after which it was autoclaved at 15 psi for 30 minutes at 121 °C. After cooling, the pH was adjusted to 7.5 using 1.0 M HCl or NaOH. The medium was then dispensed into 250 mL flasks, covered with cotton wool or stoppers, and stored at a cool temperature. The pH was rechecked prior to use to maintain consistency (Johnson *et al.*, 2018). Note: For solid media, 3.75 g of agar was added to 250 mL of Johnson's medium.

3.4 Microalgae Cultivation and Harvesting

Microalgae cultures were maintained under controlled light and temperature conditions. Biomass was routinely harvested using centrifugation at 3,000 rpm for 8 minutes (Abubakar, 2014). Harvested biomass was used for subsequent analyses.

3.5 Determination of Biomass Dry Weight

Cells in the log phase of growth were collected by centrifugation at 3,000 g for 10 minutes at room temperature. The pellets were transferred to crucibles and dried in an oven at 105°C for 24 hours to determine the dry weight.

The oven-dried samples were then ashed in a furnace at 500°C for 6 hours. After cooling in a desiccator, the ash-free dry weight (AFDW) was calculated using:

Ash Free Dry Weight (AFDW) = Cells Dry Weight – Ashed Weight

This measurement provided an accurate estimate of the actual biomass content of the microalgae cultures.

3.6 Determination of Nutritional Composition (Proximate Analysis)

3.6.1 Determination of Moisture Content

PRINCIPLE

Moisture content was determined by drying the sample to constant weight at 105°C. The weight loss was taken as the moisture present in the sample.

PROCEDURE

one grams (1 g) of the powdered sample was weighed into a pre-weighed crucible and dried in a hot-air oven at 105 °C for 24 hours. The sample was cooled in a desiccator and reweighed. The drying and weighing were repeated until a constant weight was obtained.

CALCULATION:

$$\% \text{ Moisture Content} = (W1 - W2) \times 100 / W3$$

Where:

W1 = initial weight of crucible + sample.

W2 = final weight of crucible + sample.

W3= weight of sample

3.6.2 Determination of Ash Content

PRINCIPLE

Ash represents the total inorganic mineral content. It was determined by incinerating the sample at 550°C until light grey ash was obtained.

PROCEDURE

one grams (1g) of the powdered sample was placed in a pre-weighed porcelain crucible and charred on a low flame. It was then placed in a muffle furnace at 550°C for 5 hours. The crucible was cooled in a desiccator and reweighed.

CALCULATION

$$\% \text{ Ash content} = \frac{W1 - W2}{W3} \times 100$$

Where:

W2 = weight of crucible + ash

W1 = weight of empty crucible

W3 = weight of sample

3.6.3 Determination of Crude Protein (Kjeldahl Method)

PRINCIPLE

In the determination of protein content in a sample, amount of nitrogen is determined initially, the sample is digested by boiling with concentrated H_2SO_4 catalyst, during digestion, ammonia is formed as ammonium sulfate, the concentration of ammonia was liberated by distillation with NaOH and boric acid, followed by titration using standard solution

PROCEDURE

The procedure involves three steps

Digestion:

One gram (1 g) of the dried sample was placed in test tube 3g of catalyst were added and 10ml of H_2SO_4 was added carefully, the solution was then digested until a clear solution was obtained, distilled water was used to dilute the solution to 50ml solution.

Distillation:

10ml of boric acid was added in kjeldahl flask, 30ml of 40% NaOH was added, the flask then connected to kjedahl distillation machine for 20mins, the ammonia produced was collected in a receiving flask containing 10ml of 2% boric acid. A color change was observed from purple to green

Titration:

The distilled solution were titrated against 0.1M solution until the appearance of pink color as the end point was observed, the titre value were recorded

CALCULATION

$$\% \text{Nitrogen} = \frac{\text{TV} \times 0.1 \times N \times \text{DF} \times 100}{\text{Weight of sample} \times \text{unit of digest}}$$

$$\% \text{Crude protein} = \% \text{nitrogen} \times 6.25$$

Where:

TV= titer value

0.1= normality of acid

N= nitrogen factor (0.014)

DF= dilution factor

6.25= conversion factor

3.6.4 Determination of Crude Fat (Soxhlet Method)

PRINCIPLE

The lipid fraction was extracted using petroleum ether as solvent and determined gravimetrically.

PROCEDURE

Two grams 1 g of dried sample was placed in a thimble and extracted with petroleum ether in a Soxhlet extractor for 24 hours. The solvent was evaporated, and the residue was oven-dried at 105°C, cooled in a desiccator, and weighed.

CALCULATION

$$\% \text{Crude Fat} = \frac{W_2 - W_1 \times 100}{W_3}$$

Where:

W1= weight of empty beaker

W2= weight of empty beaker + oil after drying

W3= weight of sample

3.6.5 Determination of Crude Fiber

PRINCIPLE

Crude fiber is the residue remaining after successive digestion with dilute acid and alkali, representing cellulose and lignin.

PROCEDURE

Two grams (2g) of defatted sample was boiled with 200 ml of 1.25% H₂SO₄ for 30 minutes, filtered, and washed with hot water. The residue was boiled again with 1.25% NaOH for 30 minutes, filtered, washed, dried, weighed, and ashed at 550°C.

CALCULATION

$$\% \text{Crude Fiber} = \frac{W2 - W1 \times 100}{W3}$$

Where:

W1= weight of empty crucible

W2= weight of empty crucible+ sample after ashing

W3= weight of sample

3.6.6 Determination of Carbohydrate

The total carbohydrate content was determined by estimation using arithmetic difference method (Sodeke 2005).

CALCULATION

$$\% \text{Carbohydrate} = 100\% - (\% \text{Moisture} + \% \text{Protein} + \% \text{Fat} + \% \text{Ash} + \% \text{Fiber})$$

3.7 Determination of Amino Acid Profile

The amino acid composition of the microalgal biomass was determined according to the standard acid hydrolysis and high-performance liquid chromatography (HPLC) procedure described by

AOAC (2005) with slight modifications. Approximately 5–10 mg of the oven-dried and homogenized microalgal biomass was weighed into clean hydrolysis tubes, after which 6 N hydrochloric acid containing 0.1% phenol was added. The tubes were flushed with nitrogen, sealed, and hydrolysed at 110°C for 24 hours to release the amino acids.

After hydrolysis, the samples were allowed to cool to room temperature, filtered to remove particulate matter, and evaporated to dryness under reduced pressure. The hydrolysates were then reconstituted in citrate buffer in preparation for derivatization.

Determination of sulfur-containing amino acids required an oxidation step. For cysteine and methionine, samples were pre-oxidized using performic acid, which converted them to cysteic acid and methionine sulfone before acid hydrolysis. Tryptophan was determined separately using alkaline hydrolysis because it is destroyed by strong acid.

Derivatization of amino acids was carried out using o-phthalaldehyde (OPA) for primary amino acids, while FMOC-Cl was used for secondary amino acids such as proline. The derivatized samples were injected into a reverse-phase HPLC system equipped with a C18 analytical column and fluorescence detector. The mobile phase composition, flow rates, and gradient conditions followed the standard OPA/FMOC method. Amino acids were identified by comparing their retention times with certified amino acid standards. Quantification was achieved using calibration curves prepared from known standard concentrations, with norleucine included as an internal standard to correct for injection and analytical variability. Final results were expressed as mg amino acid per gram of dry biomass and as percentage composition of total amino acids.

3.7 Data Analysis

All analyses were performed in triplicate, and Results are expressed as mean $\pm$ standard deviation. Comparative analysis of protein content between DP and R strains was performed using students t-test, with statistical significance defined at ($p < 0.05$) while Graphpad instant software (version 3.0) was used for the t-test. Kolmogrov-Smirnov and Anderson Darling tests were performed using stats model libraries to confirm the data meets the assumptions required for t-test.

CHAPTER FOUR

4.0 RESULTS, DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 RESULTS

This chapter presents the findings of the study, including the dry biomass weight of the two microalgae species (Red Algae and *Dictyosphaerium pulchellum*), followed by their proximate composition. All analyses were carried out in triplicates, and the mean $\pm$ standard deviation values were calculated accordingly.

4.1.1 Biomass Yield of Microalgae

The dry biomass weight of the microalgae samples was determined after oven-drying at 105 °C for 24 hours. The results revealed a clear difference in biomass accumulation between the two species are:

1.Red (R): 2.42 g

DP: 3.51 g

Strain DP produced almost three times more biomass than the red algae, indicating stronger growth performance and a higher potential for nutrient accumulation. Increased biomass yield is often associated with efficient nutrient uptake and favorable growth physiology (Abubakar, 2014; Johnson *et al.*, 1968).

4.2 Proximate Composition of Microalgae Biomass

Proximate analysis was conducted to determine moisture, ash, lipid, crude protein, and carbohydrate contents. Results for each parameter are shown as mean $\pm$ standard deviation (SD).

Table 4.1: Proximate Composition of Microalgae Biomass (DP & R Strains)

Parameter	Strain Red (R) (Mean $\pm$ SD)	Strain DP (Mean $\pm$ SD)
Moisture (%)	4.33 $\pm$ 2.52	4.00 $\pm$ 1.00
Ash (%)	4.00 $\pm$ 2.00	1.66 $\pm$ 0.58
Lipid (%)	13.33 $\pm$ 2.62	7.33 $\pm$ 2.08
Protein (%)	5.10 $\pm$ 0.66	5.98 $\pm$ 0.25
Carbohydrates (%)	73.23 $\pm$ 0.88	81.03 $\pm$ 0.97
Dry Biomass (g)	2.42	3.51

4.3 Amino Acid Profile of the Samples

The amino acid concentrations of the DP and R strains were quantified and expressed in mg/g. The results revealed variations in both essential and non-essential amino acids across the two samples. These variations may be attributed to differences in processing, biochemical composition, or environmental conditions influencing biomass formation (FAO/WHO, 2023; Loveday, 2019).

4.3.1 Amino Acid Composition of Strain DP

Strain DP contained relatively higher concentrations of most amino acids compared with the R sample. Glycine (5.128 mg/g) and histidine (5.052 mg/g) were the most abundant amino acids in the sample. Moderate amino acids included alanine (1.501 mg/g), serine (1.685 mg/g), and glutamic acid (1.344 mg/g).

Essential amino acids such as valine (0.101 mg/g), leucine (0.106 mg/g), isoleucine (0.102 mg/g),

lysine (0.109 mg/g), and threonine (0.183 mg/g) were present in lower concentrations compared to non-essential amino acids.

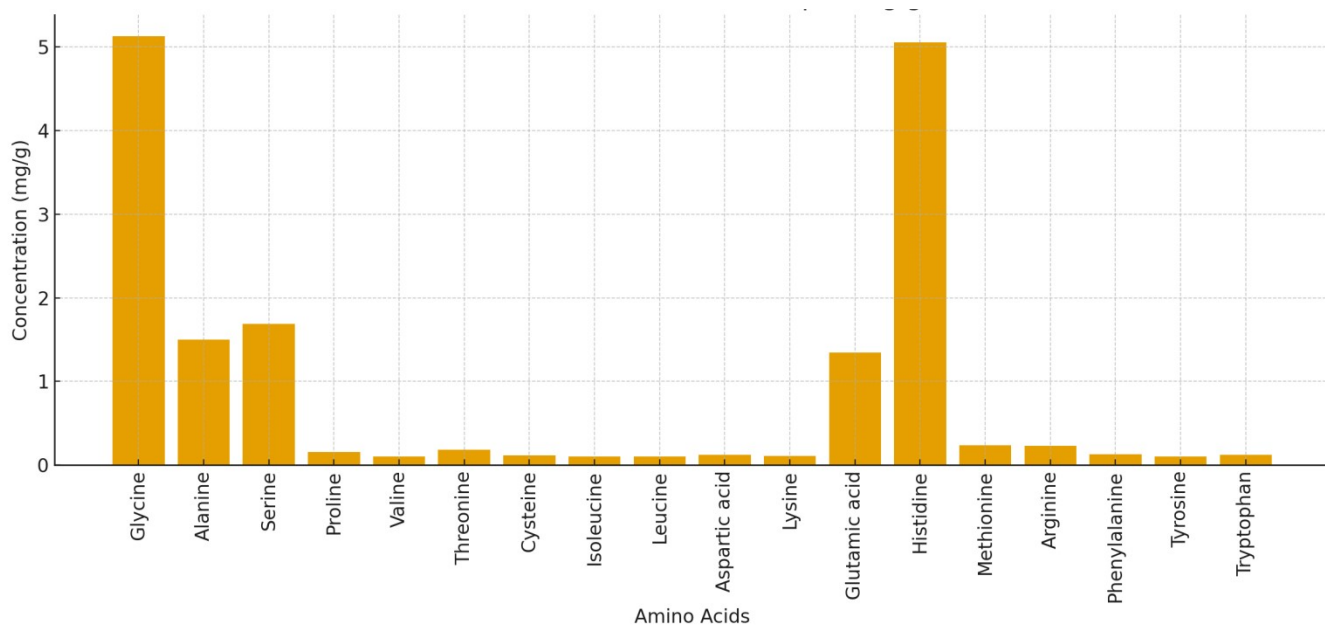


Figure 4.1: Amino Acid Profile of strain Dp (mg/g)

4.3.2 Amino Acid Composition of R Sample

Strain R generally exhibited lower amino acid levels than DP. Glycine (2.798 mg/g) and histidine (3.879 mg/g) remained the dominant amino acids, similar to the pattern seen in DP. Other amino acids such as alanine (0.844 mg/g), serine (1.371 mg/g), and glutamic acid (0.829 mg/g) were present in moderate quantities. Essential amino acids showed low concentrations, with methionine (0.082 mg/g) being notably low, followed by valine (0.083 mg/g), leucine (0.092 mg/g), and tryptophan (0.086 mg/g).

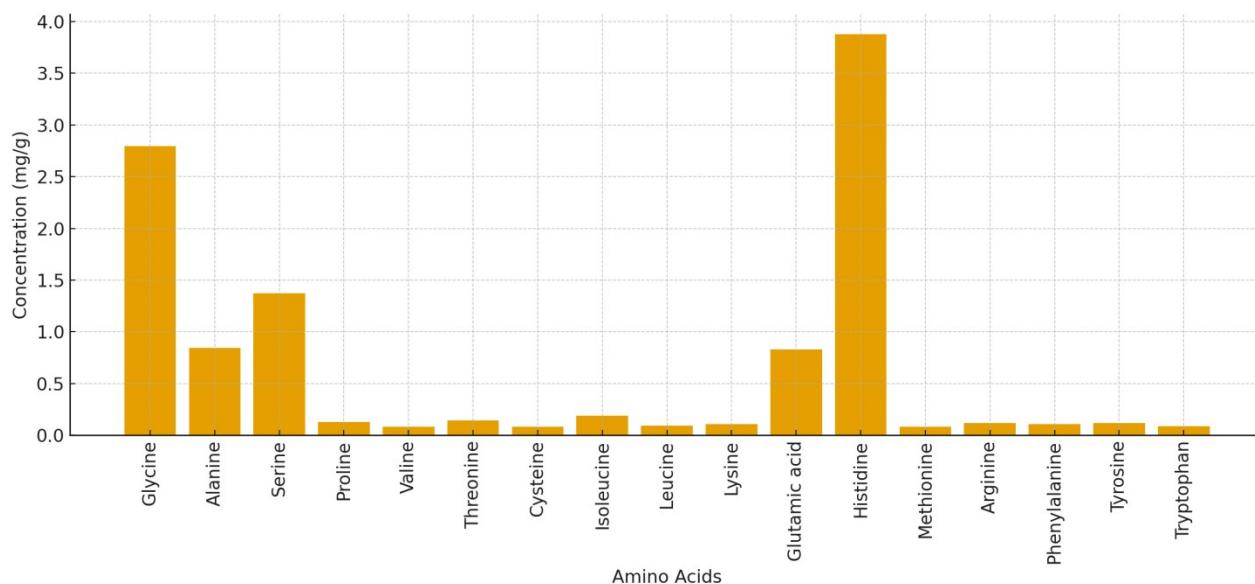


Figure 4.2: Amino Acid Profile of Strain R(mg/g)

4.3.3 Comparative Analysis of the Two Samples

Overall, Strain DP showed higher concentrations for nearly all amino acids when compared with the R sample. This suggests that Strain DP may have undergone processes or conditions that preserved or enhanced its amino acid content relative to the Strain R. Essential amino acids (e.g., Lysine, Methionine, Isoleucine, Leucine, Phenylalanine, Valine) were present in the biomass both Strains, though consistently lower than non-essential amino acids. Histidine and Glycine remained dominant in both, which may indicate their significant roles or prevalence in the sample source materials. The comparative trends support the nutritional relevance of Strain DP, showing potentially higher biological value.

4.3 Discussion

The amino acid profile of Strains DP and R provides important insight into their nutritional quality and potential applications. In both samples, a mixture of essential and non-essential

amino acids was detected, but their concentrations varied considerably. Overall, Strain DP exhibited higher amino acid levels across nearly all components compared to the R. This difference suggests that the strain DP may possess superior protein quality or may have undergone less degradation during processing. Glycine and Histidine were the dominant amino acids in both samples. Their high abundance indicates that the protein source in both samples is rich in these amino acids. Histidine, being an essential amino acid, is particularly relevant for metabolic functions and tissue repair. The elevated levels in DP suggest enhanced nutritional value, especially for growth-related applications. Non-essential amino acids such as Alanine, Serine, and Glutamic acid also appeared at moderate concentrations in both strains, though again higher in DP. These amino acids play central roles in energy metabolism and cellular function, meaning their higher levels further support the nutritional advantage of the DP specie.

The essential amino acids Leucine, Isoleucine, Valine, Lysine, Methionine, Phenylalanine, and Threonine were present at relatively low levels in both samples. However, DP consistently contained slightly higher concentrations. Since essential amino acids cannot be synthesized by the body, their presence contributes directly to the biological value of a protein source. The low levels in both samples indicate that while nutritionally relevant, these samples may require supplementation to meet full dietary requirements.

Comparatively, Strain R showed reduced amino acid concentrations across nearly all categories. This reduction may be due to processing conditions, environmental factors, or compositional differences in the raw material. The lower levels suggest potential loss of nitrogenous compounds, protein denaturation, or structural breakdown during drying or extraction. Overall, the results indicate that Strain DP has superior protein quality and amino acid density. This

aligns with the broader objective of evaluating the nutritional potential of the samples ((FAO/WHO, 2023). The findings imply that DP specie may be more suitable for applications requiring higher amino acid availability, such as enriched food formulations or nutrient fortification.

4.4 Conclusion

The amino acid profiling revealed that both DP and R strains contain essential and non-essential amino acids, though in varying concentrations. DP demonstrated generally higher amino acid content, suggesting superior nutritional quality. These findings further complement the proximate and mineral composition results previously discussed.

4.5 Recommendations

Based on the observed amino acid profile and the initial study objectives:

1. Quality Optimization: The processing technique used in producing strain DP is recommended for adoption, as it appears to retain higher amino acid levels.
2. Further Research: Additional analysis on the bioavailability of the amino acids is advisable to determine the actual nutritional benefits when consumed.
3. Application in Food Formulation: DP strain may be more suitable for enriched nutritional products due to its higher amino acid concentration.

REFERENCE

AACC International. (2023). Approved methods of the American Association of Cereal Chemists (10th ed.). AACC Press.

Adebayo, E. A., Abideen, O. D., Ezenobi, N. O., Adedokun, S. A., & Agubana, N. S. (2024). Kinetic modeling of drug release from polymeric systems: A critical review. *European Journal of Pharmaceutical Sciences*, 194, 106720. <https://doi.org/10.1016/j.ejps.2024.106720>

AOAC International. (2019). Official methods of analysis of AOAC International (21st ed.). AOAC International.

Arora, D. R., & Arora, B. B. (2024). Textbook of microbiology (4th ed.). CBS Publishers.

FAO/WHO. (2017). Joint FAO/WHO/UNU Expert Consultation on protein and amino acid requirements in human nutrition. FAO/WHO.

Fawole, M. O., & Oso, B. A. (2017). Laboratory manual of microbiology. Spectrum Books.

Gilani, G. S., Xiao, C. W., & Lee, N. (2018). Need for accurate and standardized determination of amino acids and bioactive peptides for evaluating protein quality and potential health effects of foods and dietary supplements. *Journal of AOAC International*, 91(4), 894–900.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2018). Modern food microbiology (7th ed.). Springer.

Kumar, V., Rani, A., Dixit, A. K., Pratap, D., & Bhatnagar, D. (2020). A comparative assessment of the nutritional composition of locally available and high-yielding soybean varieties. *Food Chemistry*, 122(3), 738–744.

Nelson, D. L., & Cox, M. M. (2017). Lehninger principles of biochemistry (7th ed.). W. H. Freeman.

Oko, A. O., Ubi, B. E., Efisue, A. A., & Dambaba, N. (2022). Comparative analysis of the chemical nutrient composition of selected local and newly introduced rice varieties grown in Ebonyi State of Nigeria. *International Journal of Agriculture and Forestry*, 2(2), 16–23.

Peace, R. W., & Gilani, G. S. (2015). Chromatographic determination of amino acids in foods. *Journal of AOAC International*, 88(3), 877–887.

- Pelczar, M. J., Chan, E. C. S., & Krieg, N. R. (2018). *Microbiology* (5th ed.). McGraw-Hill.
- Rutherford, S. M., & Gilani, G. S. (2019). Amino acid analysis. In *Current protocols in protein science* (58th ed., pp. 11–36). Wiley.
- Singleton, V. L., & Rossi, J. A. (2016). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158.
- Suliman, A. M. E. (2020). Proximate composition and amino acid profile of *Cicer arietinum* (chickpea) seed. *Pakistan Journal of Nutrition*, 9(11), 1074–1079.
- Ahmed, T., Hossain, M., & Sanin, K. I. (2022). Global burden of maternal and child undernutrition and micronutrient deficiencies. *Annals of Nutrition and Metabolism*, 61(Suppl 1), 8–17.
- Briend, A., Collins, S., Golden, M., Manary, M., Myatt, M., & Walsh, A. (2017). Ready-to-use therapeutic food for treating severe acute malnutrition: Principles, results, and future directions. *Food and Nutrition Bulletin*, 36(1), S49–S54.
- Ijarotimi, O. S., & Keshinro, O. O. (2012). Determination of microbial quality, shelf life and suitability of a developed weaning food from local food materials. *Journal of Microbiology Research*, 2(6), 169–176.
- Manary, M. J., & Sandige, H. L. (2018). Management of acute moderate and severe childhood malnutrition. *BMJ*, 337, a2180.
- World Health Organization. (2023). *Updates on severe acute malnutrition management: Quality standards for RUTF production*. WHO Press.
- Olorode, O. A., Idowu, O. A., & Bamiro, O. M. (2015). Microbiological assessment of weaning foods produced in selected markets in Ogun State, Nigeria. *Nigerian Food Journal*, 33(1), 8–13.
- Abebe, T., Wodajo, K. E., & Tirfie, M. (2020). Microbial safety and quality of complementary foods in developing countries. *International Journal of Food Science*, 2020, 1–10.
- Bamgboye, O. O., & Ogunjimi, M. O. (2021). Assessment of bacteriological quality of ready-to-eat and ready-to-use food products sold in Nigeria. *African Journal of Clinical and Experimental Microbiology*, 22(2), 177–184.
- AOAC International. (2016). *Official Methods of Analysis* (20th ed.). AOAC. (Relevant for standard plating methods, moisture analysis, and microbial enumeration.)
- EFSA Panel on Biological Hazards. (2020). Microbial hazards in low-moisture foods. *EFSA Journal*, 18(4), e06038.

Appendix