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Study of amino acids and the functional properties of two types of algae (*Arthrospira platensis* and a local *Arthrospira sp*)

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Abstract

Objective

Algae have the ability to grow rapidly, utilize light energy and carbon dioxide from the atmosphere, and produce a greater amount of biomass per hectare compared to vascular plants. The bioactive compounds have antioxidant, antimicrobial, and antiviral properties, as well as the prevention of stomach ulcers, constipation, anemia, diabetes, and hypertension. The aim of this study was to compare the proximate composition, GC-MS volatile profile, amino acid composition, and selected functional properties (water- and oil-holding capacities) of *S. major* and *S. platensis* collected from Basrah, Iraq.

Materials and methods

This study was conducted on two types of algae, *Spirulina paltensis* and *Spirulina major*, collected from water bodies in the Karma Ali area of Basra Governorate, southern Iraq. Preliminary analyses of the algal extracts were performed, including the analysis of proteins, carbohydrates, phenols, flavonoids, and glycosides, as well as their chemical composition.

Results

The protein content in *S. major* was 55.6%, and in *S. paltensis*, it was 53.11%. The content of bioactive compounds was determined using gas chromatography-mass spectrometry (GC-MS). A total of 39 bioactive compounds were identified in both algae. The highest concentration in *Spirulina major* was 32, represented by hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, followed by 13, represented by hexadecanoic acid, methyl ester. The bioactive compounds in *Spirulina paltensis* were not specified. The alga *S. paltensis* showed that the highest concentration was at peak 34, represented by the compound Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, followed by peak 13, represented by the compound Hexadecanoic acid, methyl ester. The amino acid profile of the two algae was also studied, identifying 13 amino

acids in both species. Serine was the most abundant, reaching concentrations of 52.1 µg/gm and 62.4 µg/gm in *S. major* and *S. paltensis*.

Conclusion

Regarding functional properties such as water-holding capacity and lipid binding, *S. major* exhibited superiority over *S.paltensis*.

Keywords: Algae, Amino Acids, Bioactive Chemicals, Functional Properties, GC MS

Paper Type: Research Paper.

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Introduction

Algae are an important source of a variety of essential nutrients for human health and are widespread throughout the world, thriving in many different environments. They are a diverse group of microscopic photosynthetic organisms found in both marine and freshwater environments (microalgae) and multicellular organisms (macroalgae). These organisms have the ability to grow rapidly, utilize light energy and carbon dioxide from the atmosphere, and produce a greater amount of biomass per hectare compared to vascular plants. In applied botany, the term microalgae usually include microalgae in the narrow sense and photosynthetic cyanobacteria (i.e., blue-green algae), formerly known as Cyanophyceae. However, in terms of biomass, both are considered sources of energy, fuel, food, and other commercially interesting products (García et al., 2017). The bioactive compounds found in the microalgae biomass of *spirulina*, such as protein, polyunsaturated fatty acids, carotenoids, vitamins, and minerals, play important roles in functional foods (e.g., dairy products, confectionery, pasta, oil derivatives, or dietary supplements) or animal feed (for livestock, poultry, shellfish, and fish), with positive effects on human health. These effects include antioxidant, antimicrobial, and antiviral properties, as well as the prevention of stomach ulcers, constipation, anemia, diabetes, and hypertension. Microalgae are also used as a coloring agent or supplement in the food and feed industries (Tavakoli et al., 2019). Food provides the body with many essential nutrients necessary for growth, biological functions, and maintaining overall health. Since our bodies cannot produce some nutrients, they must be obtained from our diet. On the other hand, some diseases can be linked to imbalances in the human diet, resulting from the consumption of unhealthy or insufficient food components.

The body's ability to absorb it (Roughan, 1989). The chemical composition of *spirulina* varies depending on the algae source, cultivation conditions, and production season. It contains 55-70% protein, 15-25% carbohydrates, 6-8% fat, 7-13% minerals, 3-7% moisture, and 8-10% dietary fiber on a dry weight basis (Jung et al., 2019). Amino acids are the basic building blocks of proteins, serving as the building blocks for many coenzymes, hormones, and nucleic acids. They play numerous functional and structural roles in the body (Aljobair et al., 2021). Spirulina proteins are considered complete in quality, with the essential amino acids constituting 47% of the total protein weight. These essential amino acids are leucine, methionine, tryptophan, phenylalanine, lysine, thionine, isoleucine, and valine, at a ratio of 55%, 10%, and 20%, respectively. 14, 28, 30, 33, 36, 45) mg/g respectively. The non-essential amino acids found in spirulina are cysteine, histidine, proline, tyrosine, glycine, serine, arginine, alanine, aspartic acid, and glutamate acid at a rate of (7, 10, 27, 30, 32, 33, 44, 47, 60, 92) mg/g respectively (Falquet and Hurni, 1997; Liestianty et al., 2019). It has been found that the most abundant non-essential amino acid in dried algae is glutamic acid, followed by aspartic acid, while the most abundant essential amino acids are isoleucine and phenylalanine (Afify et al., 2017). Currently, there is increasing interest in alternative methods for disease prevention or treatment. Nutrients are increasingly being used for this purpose as an alternative therapy, since conventional treatment with synthetic drugs, despite its effectiveness, has many side effects. The term "nutraceuticals" refers to the relationship between the nutritional and pharmaceutical fields. The health benefits of functional foods are attributed to their ability to alter the composition of the intestinal microbiota and can influence the health status of patients (Catinean et al., 2018). In addition, the human effort to obtain food, which is a natural need of living beings, has been increasing for centuries, and the desire to provide more suitable and better-quality food is the main motivation for striving for more diverse products (Hajalizadeh et al. 2019; Bordbar et al. 2022). This factor is basically the main driver of human thinking and his innovations in the creation and development of agricultural, animal husbandry and food industries (Amirteymoori et al. 2021; Shahsavari et al. 2023). Following the increasing role of nutrition in the livestock economy, since the middle of the last century, identifying the value of food and feedstuffs and determining the nutritional needs of (farm) animals has attracted the attention of experts, especially in animal science, and numerous researches have been conducted during this period (Vahabzadeh et al. 2020; Shahsavari et al. 2022). Rangelands are the most important part of the renewable resources of countries, which are under great pressure due to the low cost of providing fodder from them compared to the cost of producing fodder through irrigated agriculture (Vahabzadeh et al. 2021; Mohammadabadi et al. 2023). Therefore, the aim of this study was to compare the proximate composition, GC-MS volatile profile, amino acid composition, and selected functional properties (water- and oil-holding capacities) of *S. major* and *S. platensis* collected from Basrah, Iraq. The novelty lies in providing a side-by-side characterization of locally collected biomass under the same analytical workflow, generating a baseline dataset that may support future valorization of regional *Spirulina* as a functional food ingredient.

Materials and methods

Algal samples: Two Species of algae were used in the research, namely *S. Major* and *S. paltensis*, which were collected from the banks of the Shatt al-Arab in the Karma Ali area and placed in polyethylene bags and transported to the laboratory at the College of Agriculture / University of Basrah. The samples were collected during the spring season (April/ 2023).

Preparation of algae samples: After obtaining the algae, any suspended organisms, aquatic plant remains, and other impurities were removed. The algae were then washed several times with distilled water. This process was repeated multiple times to eliminate microorganisms, following the method described by Weidman *et al*. (1964). To ensure the purity of the algae, Hellebust & Craigie (2011) method was used. This method involved microscopic examination of algal filaments after inoculation on nutrient agar in Petri dishes. The cultures were incubated at 37°C for 48 hours. The samples were then examined several times to confirm the absence of microorganisms. The algae were dried and stored in freezer containers until use.

Identification and classification of isolated algae: Identification was based on morphological characteristics using classical taxonomic keys. Molecular identification was not performed in the present study. The isolated and purified cyanobacterial species identified, described, and classified during the current study were based on the following taxonomic sources: Desikachary (1959), Abed *et al*. (2003a, 2003b), Charpy *et al*. (2012), and Yu *et al*. (2015), as well as the updated 2017 Algaebase website (www.algaebase.org).

Preliminary phytochemical screening-detection of amino acids and peptides: Amino acids and peptides were detected using the Harborne method (1988), where 1 cm^3 of 1% Nihydrine reagent was added to 1 cm^3 of the extract, and the mixture was heated in a water bath for 10 minutes. The formation of a purple color was observed, indicating the presence of peptides and amino acids.

Carbohydrate detection: Molish's reagent was used to detect carbohydrates. α-Naphthol alcohol was added to 1 cm^3 of the extract, and a few drops of concentrated sulfuric acid were added. The appearance of a purple ring indicates a positive reaction (Harborne, 1988).

Protein detection: Biurete's reagent was used, and the appearance of a purple color indicates a positive reaction (Harborne, 1988).

Phenol detection: 2 cm^3 of the extract was moistened with filter paper, and several drops of a 1% ferrous chloride ($FeCl_2$) solution were added. The mixture was then exposed to ammonia vapor. The appearance of a bluish-green color indicates a positive reaction (Harborne, 1988).

Flavonoid detection: Was added 1 cm^3 of 0.5 N alcoholic potassium hydroxide to 1 cm^3 of the extract. The formation of a yellow precipitate indicates a positive reaction (Moufid and Eddouks, 2012; Wahnou et al., 2024).

Glycoside detection-Before glycosidic hydrolysis: Was mixed 1 cm^3 of the extract with 1 cm^3 of Benedict's reagent in a test tube. Then heat the tube in a water bath for 10 minutes. The formation of a red or brown precipitate indicates the presence of reducing sugars (Moufid and Eddouks, 2012; Wahnou et al., 2024).

After glycosidic hydrolysis: Drops of concentrated hydrochloric acid was added to 5 cm^3 of the extract. Then heat in a water bath at 40°C for 25 minutes to prevent glycosidic bond rupture.

Adjust the pH by adding 2M sodium hydroxide, then add an equal volume of Benedict's reagent and heat the solution for 10 minutes. The formation of a red or brown precipitate indicates the presence of glycosides (Moufid and Eddouks, 2012; Wahnou et al., 2024).

The chemical composition of algae: Moisture estimation: Moisture was estimated at 105°C for 3 hours. Fat was measured using a Soxhlet apparatus with petroleum ether as a solvent. Ash was measured using a muffle furnace at 550°C according to the method described in AOAC (2000). Protein was estimated by measuring the total nitrogen using the semi-microkjeldal method and then multiplying the result by the protein factor of 6.25 to calculate the protein percentage, as described in Pico (2020). The carbohydrate percentage was estimated by the difference between the components mentioned above, as described in Pico (2020).

Ethanolic extract: The 90% ethanolic extract was prepared according to the method (Michael et al., 2018), which involved mixing 20 g of powdered *S. Major* and *S. paltensis* algae with 100 mL of 90% ethyl alcohol in a 250 mL volumetric flask. The mixture was then stirred for 24 hours at laboratory temperature, allowed to cool, and filtered through Whattman No. 1 filter paper. The solution was then concentrated using a rotary evaporator at 50°C, yielding a viscous, dark green substance. This solution was stored in a refrigerator until use.

Identification of the active compounds of the alcoholic extract using gas chromatography-mass spectrometry: The biologically active compounds in the alcoholic extract isolated from algae were identified using Gas Chromatography-Mass Spectrometry (GC-mass spectrometry) (Agilent 7890B GC with 5977A MSD). The following conditions were used for isolation and identification: Column type: 5% phenylmethylsiloxane; Pressure: 100 kPa; Inert gas flow rate: 1 mL/min (helium); Column furnace temperature: 70°C, then increased to 280°C at a rate of 10°C/min; Injection temperature: 280°C; Solvent cutoff time: 4 minutes; Injection pattern: Pulsed splits; Molecular weight range: 40-600 m/z; Test time: 20 minutes; Injection volume: 1 µL. After obtaining the mass spectra for each compound, the results were processed using software. GCMS Solution and Definition of Separated Peaks Based on a Database.

Protein extraction: The method of Kandasamy et al. (2012) was followed for protein extraction from algae. Five grams of dried algae were added to 5% (w/v) deionized distilled water with continuous stirring for 12 hours at 4°C. Centrifugation was then performed (10,000 x g, 20 minutes, 4°C). The supernatant was collected, and the precipitate was treated with 0.5% (v/v) β-mercaptoethanol to achieve a pH of 12 using 1M NaOH. This was left to stand for 2 hours at room temperature, after which centrifugation was performed again. The supernatant was then collected along with the supernatant from the first cycle, and the pH was adjusted to 7 using 1M HCl. Finally, solid ammonium sulfate was added to the supernatant until it was saturated. 85%) and the solution was kept for one hour at room temperature, after which it was centrifuged (10,000 x g, 20 minutes, 4 degrees Celsius), and the precipitate was taken and washed using deionized water with dialysis bags (2 kDa) to get rid of the salts, and then it was collected and dried.

Amino acid identification: Amino acids were determined according to the method (Moran-Palacio et al., 2014). This was done by taking 3 g of the sample and adding 25 ml of 6 M hydrochloric acid at 150°C for 3 hours. The sample was then dried using a rotary evaporator, and 5 ml of 0.2% sodium citrate at a pH of 2.2 was added. The sample was then filtered using a plastic

filter with 0.45 µm openings. One ml of the extracted sample was then added to 200 µL of 5% orthophthalaldehyde (OPA), and the sample was shaken for 2 minutes. Finally, 100 µL of the resulting mixture was injected into an amino acid analyzer. The mobile phase was acetonitrile:methanol:formic acid (60:20:20), and the dimensions of the injection column were 150 x 4.6 mm.

Water holding capacity (WHC): The water-holding capacity (WHC) of algal proteins was estimated using the method of Du *et al*. (2018). This involved mixing 0.5 g of the sample with 10 mL of distilled water (M1) in a test tube of known weight. The sample was thoroughly mixed for 2 minutes, left to stand for 80 minutes, and then separated by centrifugation at 3000 rpm. The weight of the water filtrate after sample absorption (M2) was determined, and the tube was weighed along with the sample.

$$\text{Water Holding Capacity (WHC) (g/g)} = (M1 - M2) / W \times 100$$

Where, WHC = Water Holding Capacity, M1 = Weight of 10 g of water, M2 = Weight of remaining water filtrate (g), and W = Weight of the initial sample.

Oil holding capacity (OHC): The ability of algal proteins to bind lipids was estimated using the method of Du *et al*. (2018) By mixing 0.5 g (w) of the sample with 5 g of vegetable corn oil (W1) in a test tube of known weight and mixing the sample for 2 minutes, then separating it by centrifugation at 4000 rpm for 30 minutes, the free oil (W2) was determined. The oil holding capacity (OHC) was calculated using the following equation:

$$\text{Oil Holding Capacity (OHC) (g/g)} = (W1 - W2) / W \times 100$$

Where, OHC = Oil Holding Capacity, W1 = Weight of Oil Sample, W2 = Weight of Free Oil, and W = Weight of Sample.

Design and statistical analysis: The results were statistically analyzed using SPSS software and a completely randomized design (CRD). The studied factors were tested using the least significant difference (LSD) test at a probability level of 0.05.

Results and discussion

Qualitative analysis of the isolated algae: The results of the qualitative analysis showed that the two isolated algae, *S. Major* and *S. paltensis*, contained peptides, proteins, carbohydrates, phenols, and glycosides before and after analysis, as shown in Table 1.

Chemical composition: The results in Figure 1 show the percentage of the chemical composition of the algae S. Major and S. paltensis under study on a dry weight basis. There were significant differences ($P < 0.05$) between the samples, with protein percentages reaching 55.6% and 53.11%, respectively. These results are close to those obtained by Farg *et al*. (2021) when they estimated the protein percentage of the extracted alga *Spirulina platensis*, which reached 53.78%. This was within the range mentioned by Usharani *et al*. (2013), who indicated that the protein content was 55-70% on a dry weight basis. The protein content varies with the seasons and harvest time, as well as environmental factors such as light, temperature, and salinity. These have an effect on the protein content, as the protein content is relatively low in the summer compared to the winter, in which the protein content is high, as energy consumption increases

with rising temperatures due to algal respiration (Ratana-Arpom and Chirapart, 2006). The percentage of moisture was approximately 5.54% and 4.66%, with significant differences ($P < 0.05$). These results were consistent with those obtained by Sotiroudis and Sotiroudis (2013), who found the moisture content to be 4-9%, and Salah *et al*. (2025), who stated that the moisture content was 2.5-6%. The studying the chemical composition of spirulina, has shown that the moisture content was 4.74% (g/100g sample on a dry weight basis). There were also significant differences ($P < 0.05$).

Table 1. Qualitative phytochemical screening of bioactive compounds in *Spirulina major* and *Spirulina platensis*

Chemical groups	*Spirulina major*	*Spirulina platensis*
Peptides	+	+
Proteins	+	+
Carbohydrates	+	+
Phenols	+	+
Flavonoids	+	+
Glycosides (before hydrolysis)	−	−
Glycosides (after hydrolysis)	−	−

The percentage of fat in the algae was 5.09% and 5.93%, and these results were consistent with those obtained by Farg *et al*. (2021), who found that the percentage of fat was approximately 5.92% on a dry weight basis. The percentage of ash was found to be 9.98% and 8.12%, showing significant differences ($P < 0.05$). These results are consistent with those of Farg *et al*. (2021), whose ash percentage was 9.67%, and also with those of EL-Moataaz *et al*. (2019), whose ash percentage was 10.05%. The high ash percentage is attributed to the presence of inorganic compounds, particularly mineral elements, as well as variations in genera and species, geographical location, and local environmental conditions. Meanwhile, the percentage of carbohydrates was 23.97% and 28.18%, respectively. These results fall within the range covered by several studies, including those by Jung *et al*. (2019) and Hosseini *et al*. (2013). Sotiroudis and Sotiroudis (2013), who showed that the percentage of carbohydrates in spirulina algae ranges between 15-25% approximately, and that its chemical composition varies according to the conditions of cultivation and the production season.

Identification of bioactive compounds by GC-MS: The results in Figures 2 and 3 illustrate the active compounds in the algae identified by gas chromatography-mass spectrometry (GC-MS). The peaks shown in the figures, which correspond to each volatile active compound. Figure 2 shows the appearance of 39 volatile compound peaks in the alga *S. Major*, the sequence of which is shown in Table 2. The highest peak, number 32, was represented by the compound Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, followed by peak 13, represented by the compound Hexadecanoic acid, methyl ester. Then peak 14, represented by the compound n-Hexadecanoic acid, and then peak 8, represented by the compound Phytol. Other compounds that appeared included Heptadecane, Oxiraneoctanoic acid, 3-octyl-, methyl ester, and 9,12-

Octadecadienoic acid (Z,Z)-, methyl ester. 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester is shown by peaks 5, 16 and 35 respectively, and other compounds within different ratios.

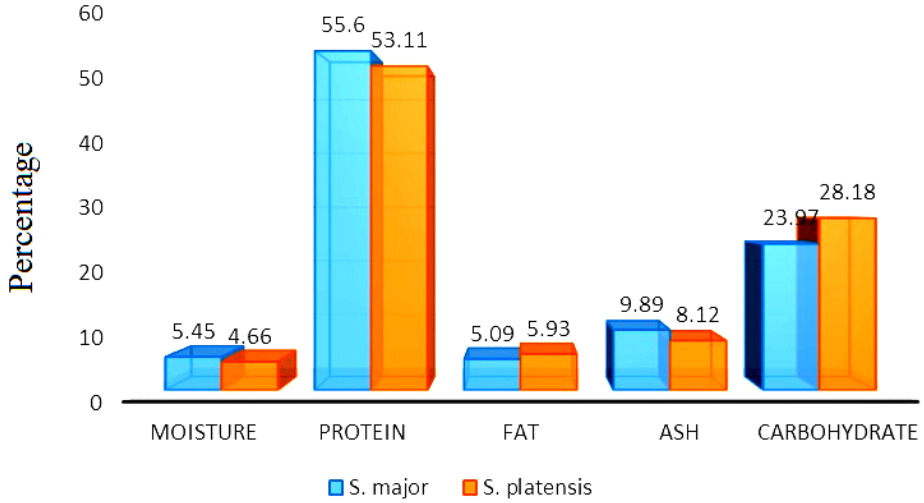

Figure 1. Chemical composition of the algae *S. major* and *S. platensis*

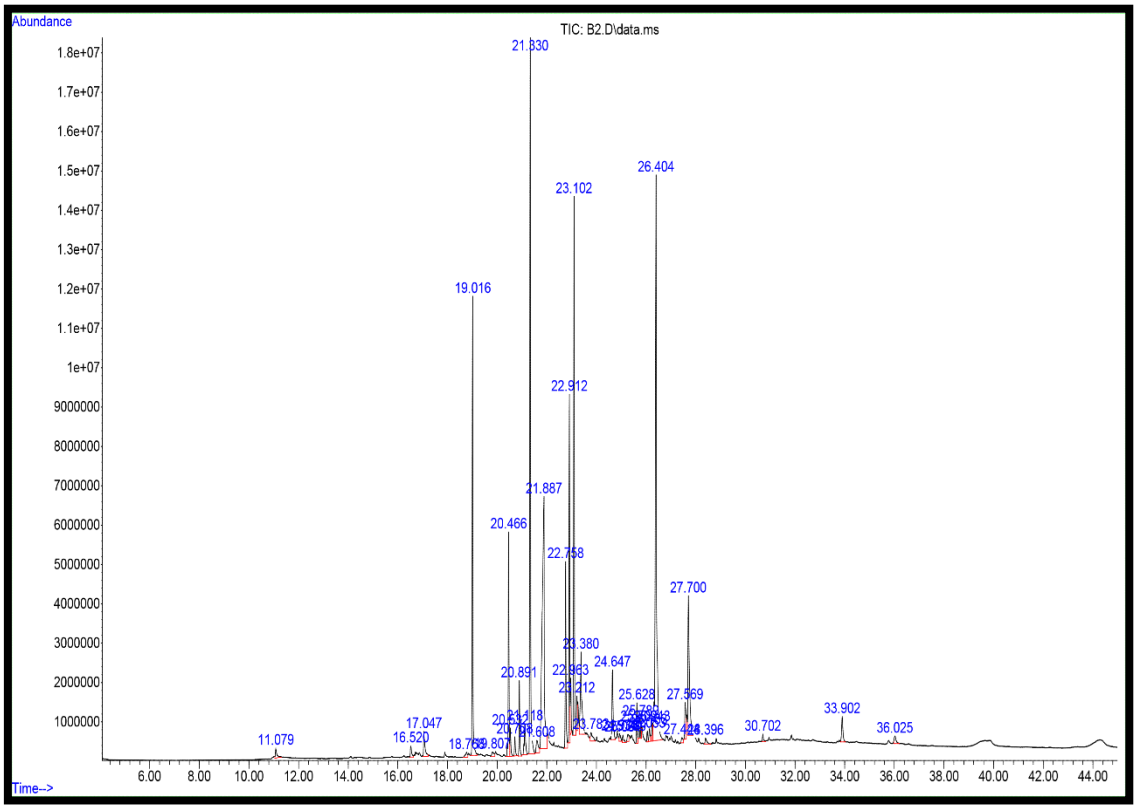

Figure 2. Profile of the bioactive compounds isolated by GC-MS from the alga *Spirulina major*

Table 2. Retention time (RT) and relative peak area of bioactive compounds identified by GC–MS in *Spirulina major*

Peak	RT (min)	Area (%)	Identified compound
1	11.079	0.3632	Cyclohexanol, 2,6-dimethyl-
2	16.520	0.3495	trans-β-Ionone
3	17.047	0.8493	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl
4	18.768	0.2251	3-Heptadecene (Z)
5	19.016	8.2140	Heptadecane
6	19.807	0.2310	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one
7	20.466	4.0439	Neophytadiene
8	20.532	0.5671	2-Hexadecene, 3,7,11,15-tetramethyl
9	20.708	0.4084	Neophytadiene
10	20.891	1.3690	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans
11	21.118	1.0298	9-Hexadecenoic acid, methyl ester (Z)
12	21.330	13.8936	Hexadecanoic acid, methyl ester
13	21.608	0.6802	Palmitoleic acid
14	21.887	12.7741	n-Hexadecanoic acid
15	22.758	3.6400	γ-Linolenic acid, methyl ester
16	22.912	5.8904	9,12-Octadecadienoic acid (Z,Z), methyl ester
17	22.963	0.9236	11-Octadecenoic acid, methyl ester
18	23.102	9.6565	Phytol
19	23.212	0.7005	Methyl stearate
20	23.380	3.0574	9,12-Octadecadienoic acid (Z,Z)
21	23.783	0.4566	Tetradecanamide
22	24.647	1.8688	Oxiraneoctanoic acid, 3-octyl-, methyl ester (trans)
23	24.933	0.2337	Methyl 18-methylnonadecanoate
24	25.064	0.2328	(Z,Z)-4,16-Octadecadien-1-ol acetate
25	25.269	0.2595	Cyclopentadecanone, 2-hydroxy
26	25.628	0.7663	Octadecanoic acid, 9,10-dihydroxy-, methyl ester
27	25.709	0.3207	2-Methyl-(Z,Z)-3,13-octadecadienol
28	25.789	0.3644	2-Methyl-(Z,Z)-3,13-octadecadienol
29	26.053	0.2461	Cyclododecyne
30	26.133	0.3627	1H-Indole, 6-methyl
31	26.243	0.3345	cis,cis-7,10-Hexadecadienal
32	26.404	17.7512	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
33	27.444	0.2134	(Z,Z)-6,9-Pentadecadien-1-ol
34	27.569	1.1362	1,8,11,14-Heptadecatetraene (Z,Z,Z)
35	27.700	4.9780	9,12-Octadecadienoic acid (Z,Z), 2-hydroxy-1-(hydroxymethyl)ethyl ester
36	28.396	0.2573	9,17-Octadecadienal (Z)
37	30.702	0.1835	dl-α-Tocopherol
38	33.902	0.8248	Phytyl palmitate
39	36.025	0.3429	1H-Purine-2,6-dione, 7-ethyl-3,7-dihydro-8-(isothiocyanatomethyl)-1,3-dimethyl

The results in Figure 3 show the presence of 39 volatile compounds in *S. paltensis* alga, the sequence of which is shown in Table 3. The highest percentage was for peak 34, represented by the compound Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, followed by peak 13, represented by the compound Hexadecanoic acid, methyl ester. Then peak 15, represented by the compound n-Hexadecanoic acid, then peak 19, represented by the compound Phytol. Peak 6, represented by the compound Heptad cane, and peak 17, represented by the compound 9,12-Octadecadienoic acid (Z,Z)-, methyl ester. Other peaks that appeared included: 36, 16, 8, and 21, which represented the compounds 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester. gamma-Linolenic acid, methyl ester, Neophytadiene and 9,12-Octadecadienoic acid (Z,Z)- respectively. In addition, there are other compounds that appeared in different proportions depending on the time of their appearance in the same alga.

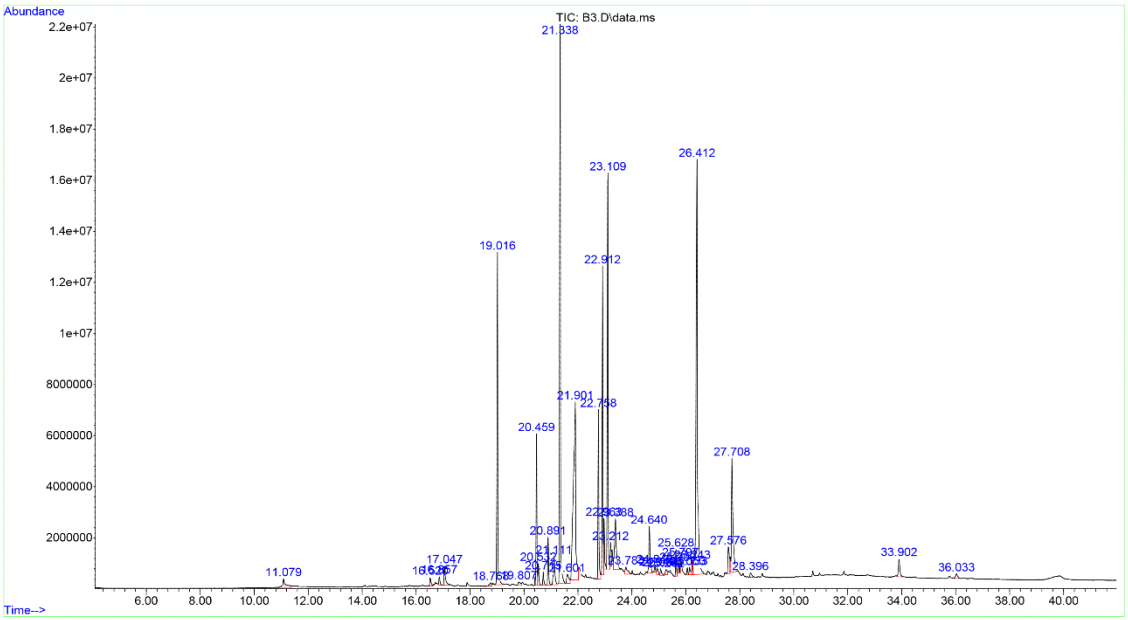

Figure 3. Profile of the bioactive compounds isolated by GC-MS from the alga *Spirulina platensis*

Edible algae are currently a source of bioactive compounds in Europe and America, due to their richness in nutrients, pigments, and antioxidants. These bioactive compounds are considered secondary metabolites of algae (Yang *et al*. 2023). Volatile organic compounds consist of several major groups: amino acids, phenolic compounds, terpenes and terpenoids, alkaloids, fatty acids, and polysaccharides (Gil *et al*. 2020). These compounds play a crucial role in improving human health, such as reducing the risk of cardiovascular disease and exhibiting anti-inflammatory and neuroprotective properties (Munekata *et al*. 2022). These findings are consistent with those of Yamamoto *et al*. (2014), who identified 41 major volatile compounds, classified as alkanes, alkenes, ketones, aldehydes, sulfur compounds, alcohols, and esters, in Japanese green algae. Organic compounds refer to a wide range of biochemicals extracted from algae, such as amino acids, phenols, terpenes, terpenoids, alkaloids, fatty acids, and polysaccharides (Vo *et al*. 2024).

Table 3. Retention time (RT) and relative peak area of bioactive compounds identified by GC–MS in *Spirulina platensis*

Peak	RT (min)	Area (%)	Identified compound
1	11.079	0.7796	Cyclohexanol, 2,6-dimethyl-
2	16.520	0.3417	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)
3	16.857	0.2986	2,4-Di-tert-butylphenol
4	17.047	0.8281	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl (R)
5	18.768	0.2041	3-Heptadecene (Z)
6	19.016	7.9976	Heptadecane
7	19.807	0.2449	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one
8	20.459	3.3516	Neophytadiene
9	20.532	0.5886	2-Hexadecene, 3,7,11,15-tetramethyl
10	20.715	0.3790	Neophytadiene
11	20.891	1.2251	Cyclohexane, 1-methyl-4-(1-methylethenyl), trans
12	21.111	1.2165	9-Hexadecenoic acid, methyl ester (Z)
13	21.338	14.3725	Hexadecanoic acid, methyl ester
14	21.601	0.6142	Palmitoleic acid
15	21.901	12.3016	n-Hexadecanoic acid
16	22.758	4.1594	γ-Linolenic acid, methyl ester
17	22.912	6.9708	9,12-Octadecadienoic acid (Z,Z), methyl ester
18	22.963	1.1564	11-Octadecenoic acid, methyl ester
19	23.109	9.4867	Phytol
20	23.212	0.6390	Methyl stearate
21	23.388	2.7027	9,12-Octadecadienoic acid (Z,Z)
22	23.783	0.4974	9-Octadecenamide (Z)
23	24.640	1.6925	9-Octadecenoic acid (Z), methyl ester
24	24.845	0.1845	9,12-Tetradecadien-1-ol acetate (Z,E)
25	24.940	0.2521	12-Methyl-(E,E)-2,13-octadecadien-1-ol
26	25.064	0.2587	9,12-Octadecadienoic acid (Z,Z)
27	25.262	0.2546	9,17-Octadecadienal (Z)
28	25.628	0.6606	Octadecanoic acid, 9,10-dihydroxy-, methyl ester
29	25.709	0.2933	Methyl 2-octylcyclopropene-1-octanoate
30	25.797	0.3166	12-Methyl-(E,E)-2,13-octadecadien-1-ol
31	26.053	0.1990	Cyclododecyne
32	26.133	0.2570	1H-Indole, 1-methyl
33	26.243	0.3842	cis,cis-7,10-Hexadecadienal
34	26.412	17.3224	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
35	27.576	1.1697	Ethyl 6,9,12-hexadecatrienoate
36	27.708	5.0150	9,12-Octadecadienoic acid (Z,Z), 2-hydroxy-1-(hydroxymethyl)ethyl ester
37	28.396	0.2736	7-Pentadecyne
38	33.902	0.8198	Phytyl dodecanoate
39	36.033	0.2903	1,2-Bis(trimethylsilyl)benzene

Amino acids: The results of the amino acid analysis of the algae *S. Major* and *S. paltensis* using the amino acid analyzer showed the presence of 13 amino acids: aspartic acid, glutamic

acid, asparagine, histidine, serine, arginine, methionine, alanine, valine, proline, phenylalanine, leucine, and tyrosine. These amino acids exhibited different retention times and varying concentrations, as illustrated in Figure 4, which shows the retention times of the amino acids, including both essential and non-essential amino acids present in the two algae.

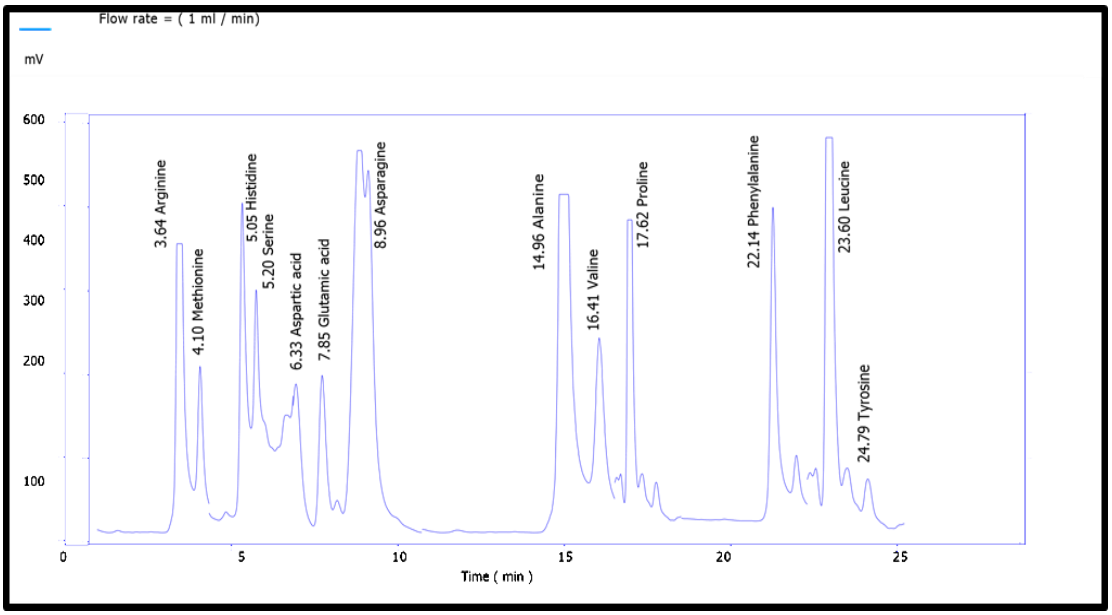

Figure 4. Amino acid retention times of *S. major* and *S. platensis*

The results of the amino acid analysis in the *S. Major* sample showed that the highest concentration of amino acids was serine, with a retention time of 5.20 minutes and a concentration of 52.1 µg/gm. This was followed by glutamic acid, with a retention time of 7.85 minutes and a concentration of 32.8 µg/gm, then methionine, with a retention time of 4.10 minutes and a concentration of 30.1 µg/gm. Arginine followed with a concentration of 18.5 µg/gm, and then aspartic acid and tyrosine with concentrations of 16.4 µg/gm and 16.3 µg/gm, respectively. The lowest concentration of amino acids was alanine, at 6.4 µg/gm, as shown in Table 4. While the results in *S. paltensis* showed the appearance of 13 amino acids, the highest amount of amino acids was in the amino acid Serine with a retention time of 5.20 minutes and a concentration of 62.4/ug gm, followed by Glutamic acid with a retention time of 7.85 minutes and a concentration of 62.1/ug gm, followed by Methionine with a retention time of 4.10 minutes and a concentration of 52.1/ug gm, then Histidine with a concentration of 45.9/ug gm, and then the acid Arginine with a concentration of 33.6/ug gm. The lowest concentration of the amino acid was found in asparagine, at 22.8 µg/gm. We also observe a higher percentage of amino acids in the *Spirulina platensis* algae sample compared to *Spirulina major*, as shown in Table (4). The study conducted by El-Moataz *et al*. (2019) on *Spirulina platensis* algae showed that this alga contains a wide range of amino acids. These included non-essential amino acids such as arginine, glutamic acid, aspartic acid, alanine, cysteine, tyrosine, serine, glycine, and proline. In addition, the algae contained essential amino acids that the body cannot synthesize, such as leucine, phenylalanine, lysine, valine, isoleucine, threonine, histidine, and methionine. These essential amino acids are typically obtained through plant and animal diets.

Table 4. Amino acid composition of *Spirulina major* and *Spirulina platensis*

No.	Amino acid	Retention time (min)	*Spirulina major* (µg/g)	*Spirulina platensis* (µg/g)
1	Arginine	3.64	18.5	33.6
2	Methionine	4.10	30.1	52.1
3	Histidine	5.05	20.9	45.9
4	Serine	5.20	52.1	62.4
5	Aspartic acid	6.33	16.4	30.5
6	Glutamic acid	7.85	32.8	62.1
7	Asparagine	8.96	9.4	22.8
8	Alanine	14.96	6.4	24.7
9	Valine	16.41	10.1	33.0
10	Proline	17.62	9.5	23.6
11	Phenylalanine	22.14	9.7	24.8
12	Leucine	23.60	11.4	29.8
13	Tyrosine	24.79	16.3	33.5

Functional properties-Water and oil absorption capacity: The results in Table 5 showed the water carrying capacity of the algal proteins of *S. major* and *S. paltensis*, as well as the oil absorption capacity, with significant differences ($P < 0.05$) for the two algae. The water carrying capacity was good, reaching 410 g/g in *Spirulina major*, while its oil absorption was 380 g/g. In *Spirulina paltensis*, the water absorption capacity was 400 g/g and the oil absorption was 350 g/g.

Table 5. Water- and oil-holding capacities of proteins extracted from *Spirulina major* and *Spirulina platensis*

Property	*Spirulina platensis* (g/g)	*Spirulina major* (g/g)
Water-holding capacity	400	410
Oil-holding capacity	350	380

The ability of proteins to bind to lipids depends on the number and type of hydrophobic bonds in their structure, the presence of amino acids (which may be responsible for their tendency to absorb lipids), and the presence of numerous nonpolar side chains capable of binding to lipid hydrocarbon chains, leading to increased lipid binding capacity (Mirhosseini and Amid, 2013; Noorlaila et al., 2015). The water-holding capacity, or oil-absorbing capacity, of proteins significantly impacts food properties such as texture, mouthfeel, and flavor retention in a wide range of foods, including soups, desserts, jellies, sauces, mayonnaise, baked goods, sausage rolls, meat analogs, and fried products (Day et al., 2022; Ma et al., 2022). Meanwhile, the ability of proteins to absorb oil refers to their capacity to trap and retain oil or lipids within their networks, pores, and capillaries. Water retention capacity is supported by the interaction of the protein's charged, polar, hydrophilic amino acid groups with water. Oil absorption capacity, on the other hand, is facilitated by the binding of hydrophobic or nonpolar amino acid side chains to the

aliphatic chains of oils. These amino acid groups or side chains are typically present in the protein in its native state. (Lima et al., 2023; Yousefi and Abbasi, 2022).

Conclusion: Overall, the results of this study showed that both *Spirulina major* and *Spirulina platensis* algae species have significant nutritional and biological value. The high protein content, the presence of diverse bioactive compounds identified by GC-MS, and the presence of 13 different amino acids indicate the high potential of these algae as a food source and functional compounds. In both species, the amino acid serine was the most abundant, and compounds such as hexadecanoic acid derivatives were dominant in their chemical composition. Also, the functional properties showed that S. major has a higher water retention and oil absorption capacity than S. platensis. Therefore, these algae can be used as a valuable source for the development of functional foods and dietary supplements.

Author contributions

L. S. M. contributed to developing the research methodology, conducting physicochemical tests, analyzing the chemical composition, and determining the amino acids of the algae. M. S. H. contributed to data analysis in the GC-Mas assay, to the estimation of the functional properties of the algal proteins and the statistical analysis of the research. L. S. M., M. S. H., and M. A. W. contributed to drafting, reviewing, discussing, and approving the final version of the manuscript.

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Data availability

The data contributing to the findings of this study are available from the investigating researcher upon request.

Ethical considerations

No human or animal participants were used in this study. All laboratory procedures were performed in accordance with standard procedures.

Conflict of interest

The researchers declare no conflict of interest regarding the publication of this paper.

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مطالعه اسیدهای آمینه و ویژگی‌های عملکردی دو نوع جلبک (*Arthrospira platensis* و *Arthrospira* sp بومی)

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چکیده

هدف: جلبک‌ها توانایی رشد سریع دارند، از انرژی نور و دی‌اکسید کربن موجود در اتمسفر استفاده می‌کنند و در مقایسه با گیاهان آوندی مقدار بیشتری زیست‌توده در هر هکتار تولید می‌کنند. ترکیبات زیست‌فعال موجود در جلبک‌ها دارای خواص آنتی‌اکسیدانی، ضدمیکروبی و ضدویروسی هستند و همچنین در پیشگیری از زخم معده، یبوست، کم‌خونی، دیابت و فشار خون بالا نقش دارند. هدف این مطالعه مقایسه ترکیب تقریبی، پروفایل ترکیبات فرّار با استفاده از GC-MS، ترکیب اسیدهای آمینه و برخی ویژگی‌های عملکردی منتخب (ظرفیت نگهداری آب و روغن) در دو جلبک *Spirulina major* و *Spirulina platensis* جمع‌آوری‌شده از بصره، عراق بود.

مواد و روش‌ها: این مطالعه بر روی دو نوع جلبک، *Spirulina platensis* و *Spirulina major*، انجام شد که از منابع آبی منطقه کرمه‌علی در استان بصره در جنوب عراق جمع‌آوری شدند. آنالیزهای اولیه عصاره‌های جلبکی شامل اندازه‌گیری پروتئین‌ها، کربوهیدرات‌ها، ترکیبات فنولی، فلاونوئیدها و گلیکوزیدها و همچنین بررسی ترکیب شیمیایی آن‌ها انجام گرفت.

نتایج: میزان پروتئین در *S. major* برابر با ۵۵/۶٪ و در *S. platensis* برابر با ۵۳/۱۱٪ بود. ترکیبات زیست‌فعال با استفاده از کروماتوگرافی گازی–طیف‌سنجی جرمی (GC-MS) تعیین شدند و در مجموع ۳۹ ترکیب زیست‌فعال در هر دو جلبک شناسایی شد. بیشترین غلظت در *Spirulina major* مربوط به پیک ۳۲ بود که با ترکیب Hexadecanoic acid, 2-hydroxy-1-

(hydroxymethyl)ethyl ester مشخص شد و پس از آن پیک ۱۳ مربوط به Hexadecanoic acid, methyl ester قرار داشت. در *Spirulina platensis* نیز بیشترین غلظت مربوط به پیک ۳۴ با همان ترکیب Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester و سپس پیک ۱۳ مربوط به Hexadecanoic acid, methyl ester بود. پروفایل اسیدهای آمینه نیز بررسی شد و در هر دو گونه ۱۳ اسید آمینه شناسایی گردید. سرین فراوان‌ترین اسید آمینه بود که به‌ترتیب به مقادیر ۵۲/۱ و ۶۲/۴ میکروگرم بر گرم در *S. major* و *S. platensis* رسید.

نتیجه‌گیری: از نظر ویژگی‌های عملکردی مانند ظرفیت نگهداری آب و اتصال به لیپیدها، *S. major* نسبت به *S. platensis* برتری نشان داد.

کلمات کلیدی: اسیدهای آمینه، ترکیبات زیست‌فعال، جلبک‌ها، ویژگی‌های عملکردی، GC-MS

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