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Mychonastes homosphaera (Chlorophyceae): A promising feedstock for high quality feed production in the arid environment

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ABSTRACT

Rapid development and drastic population increase in Qatar have led to increasing awareness about food security. Microalgae are considered one of the most promising feedstocks owing to their ability to produce nutrients, including lipids, carbohydrates, and proteins, in addition to antioxidants, vitamins, and minerals. In this study, 30 isolates of local freshwater microalgae were screened first based on their growth rate to select the most suitable strains for feed production. Based on the normality test, 15 fast-growing microalgae isolates were selected and subjected to further investigation of their metabolites content. The hierarchical Cluster Analysis conducted on lipids, proteins and carbohydrates contents subdivided these strains selected into 4 clusters, among them, the cluster one was grouping three *Chlorella* and two *Mychonastes* isolates with high nutritional values due to their high amounts of lipid and protein. The survey of metabolite production was performed every three days during growth in a volume of 3 L. We observed that the maximum amount of proteins and lipids was produced at day 6 and 14, respectively. The assessment of the Trolox equivalent antioxidant capacity of the top 5 strains proved that *Mychonastes homosphaera* isolate QUCCCM70 showed the highest antioxidant capacity. Moreover, results revealed the presence of essential amino acids and omega3 fatty acids. The screening evidenced a *Mychonastes homosphaera* strain QUCCCM70 with high nutritional value that can be considered as a promising alternative to produce a well-balanced animal feed supplement for a high quality of poultry and livestock products.

1. Introduction

Recently, microalgae-based feed supplement has emerged in the food industry [1]. Microalgae are composed of 11–71% proteins, 6–28% lipids, and 4–37% carbohydrates [2] In addition, microalgae produce multiple pigments, which are widely used as natural food colorants [3]. As such, the addition of microalgal biomass to food products results in a well-balanced nutritional biochemical composition with increased nutritional value [4,5]. Microalgae are considered as a very promising source of proteins that can improve the quality of food products due to the presence of essential amino acids [6]. The protein content is closely related to the nature of the microalgae and cultivation conditions. High protein content has also been reported for *Chlorella vulgaris* [7]. Batista et al., [7] demonstrated a correlation between pigmentation and protein production. These researchers reported that protein production was influenced by the type of pigmentation produced by the microalgae.

In addition to the polar lipids used for the membrane structure, microalgae can produce neutral lipids, especially triglycerides. These lipids are produced during the stationary phase and then accumulate in the vacuoles as storage [8]. Fatty acids are considered one of the most nutritionally valuable products to be obtained from algae. Furthermore, some of the polyunsaturated fatty acids (PUFAs) are considered essential, since they are crucial for physiological and health functions but cannot be synthesised by humans [9]. The microalgae PUFAs include omega 3, 6, 7, and 9 fatty acids. The most important PUFAs are eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), which are also alternatives to fish oil [7,10,11].

Carbohydrates are one of the major components of microalgae that have been well studied. Indeed, they represent 10–23% of the dry biomass [12–14]. Batista et al., [7] recorded higher concentrations of carbohydrates, reaching 34% for *H. pluvialis*, which is comparable to

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that of the algae commercialized by the American micro-algae producing Company "Cyanotech". Microalgae polysaccharides are mainly composed of glucose (21–87%) [15].

In the Algal Technologies Program, Centre for Sustainable Development, Qatar University, a Culture Collection of Cyanobacteria and Microalgae (QUCCCM) has been built and maintained in liquid nitrogen [16,17]. In the current study, an investigation of the nutritional potential of 30 fresh QUCCCM isolates was performed to select the most suitable strains that can be used as feedstock for producing poultry and livestock feed supplement with high quality. For this purpose, screening of the selected microalgae for growth rate and metabolites (proteins, lipids, and carbohydrates) was performed. Fatty acid methyl ester (FAME) and amino acid profiling were also carried out for the selected strains.

2. Material and methods

2.1. Algae cultivation and growth rate analysis

Thirty freshwater microalgal strains locally isolated from Qatar and belonging to QUCCCM [17], were screened for growth rate and metabolite production (Table 1). The microalgae were cultivated using BG11 growth medium [18]. The strains, maintained in an illuminated growth chamber (Sanyo Japan), were cultivated in 10 mL growth medium and incubated for 7 days in an illuminated shaker (Innova 44R, New Brunswick Scientific, USA) under an agitation of 150 rpm, an illumination of 100 μmol s^{-1} m^{-1} with a light-dark cycle of 12:12 h and a temperature of 30 °C, corresponding to the annual average temperature in Qatar.

Table 1
Growth rate analysis of QUCCCM isolates.
Growth rate was determined from culture of 1 L volume using vertical tubular photobioreactors under controlled condition. The microalgae isolates were identified as previously described by Saadaoui et al., [17]

Strain	Molecular taxonomic identification	μ (day^{-1})	Doubling time (day)
QUCCCM2	*Chlamydomonas* sp.	0.17 ± 0.04	4.07
QUCCCM3	*Chlorella* sp.	0.30 ± 0.07	2.23
QUCCCM4	*Chlorella* sp.	0.51 ± 0.08	0.95
QUCCCM5	*Chlorella* sp.	0.36 ± 0.02	1.92
QUCCCM6	*Mychonastes* sp.	0.43 ± 0.01	1.61
QUCCCM9	*Mychonastes* sp.	0.15 ± 0.01	4.62
QUCCCM10	*Chlorella* sp.	0.2 ± 0	3.46
QUCCCM13	*Chlorella* sp.	0.39 ± 0.04	1.77
QUCCCM27	*Chlorococcum* sp.	0.33 ± 0.11	1.47
QUCCCM28	*Neochloris* sp.	0.30 ± 0.12	1.77
QUCCCM32	*Chlorella* sp.	0.5 ± 0.06	1.38
QUCCCM37	*Desmodesmus* sp.	0.3 ± 0.05	2.31
QUCCCM38	*Chlorella* sp.	0.42 ± 0.06	1.65
QUCCCM40	*Scnenedesmus* sp.	0.28 ± 0.04	2.47
QUCCCM41	*Chlorophyta*	0.19 ± 0.06	3.64
QUCCCM43	*Mychonastes* sp.	0.45 ± 0.17	1.54
QUCCCM62	*Chlorella* sp.	0.64 ± 0	1.08
QUCCCM63	*Scenedesmus* sp.	0.13 ± 0.09	5.33
QUCCCM65	*Desmodesmus* sp.	0.35 ± 0	1.98
QUCCCM66	*Dictyosphaerium* sp.	0.3 ± 0.04	2.31
QUCCCM68	*Coelastrella* sp.	0.12 ± 0	5.77
QUCCCM70	*Mychonastes homosphaera*	0.6 ± 0.27	1.15
QUCCCM72	*Chlamydomonas* sp.	0.89 ± 0.27	0.77
QUCCCM73	*Chlorophyta*	0.34 ± 0.05	2.03
QUCCCM74	*Chlorophyta*	0.14 ± 0.08	4.95
QUCCCM75	*Protosiphon* sp.	0.17 ± 0.03	4.07
QUCCCM118	ND	0.23 ± 0.03	3.03
QUCCCM119	ND	0.28 ± 0.01	3.01
QUCCCM120	*Scenedesmus* sp.	0.7 ± 0.01	0.99
QUCCCM122	ND	0.65 ± 0.01	1.066
QUCCCM123	ND	0.104 ± 0.01	6.6

All measurements were recorded in triplicate (n = 3).
ND, Not determined.

The cultures were scaled up to a volume 100 mL then incubated for 7 days under the previously cited condition prior to being used to inoculate 1 L of cultivation volume using vertical tubular photobioreactors. These cultures were incubated for 12 days under a light intensity of 400 μmol s^{-1} m^{-1} with a light-dark cycle of 12:12 h and air bubbling. The strain cultivation was performed in duplicate with tow flasks per run, resulting in four OD_{750nm} values per time interval for each strain. An aliquot of 2 mL was collected daily for optical density (OD) measurements at 750 nm in order to determine the growth rates [19].

Growth rate μ = lnX2 - lnX1/t2-t1, where X1 and X2 are ODs at times t1 and t2.

The doubling time was calculated using the following equation: dt = 0.6931/μ.

Based on this screen, biomass of the 15 fast-growing microalgae strains were selected and subjected to an assessment of their protein and lipid contents. Then, the top five strains were subjected to a survey of their metabolites production in order to determine the production peak time of proteins and lipids. For this purpose, these microalgae were scaled up from 10 mLto 3 L as described previously. These cultures were performed under a photon flux density of 100 μmol photons m^{-2} s^{-1} and a 12:12 h dark: light cycle and air bubbling using a 3 L vertical tubular photobioreactor. Next, 100 mL aliquots of the culture were collected at days 3, 6, 9, 11, and 14 then centrifuged. Biomass collected was freeze-dried prior to being subjected for extraction of total proteins and total lipids.

2.2. Total protein extraction and determination

Microalgal biomass collected after 12 days of cultivation at scale of 1 L and form different time intervals of cultivation at 3 L volume was subjected to protein extraction using a Plant Total Protein Extraction Kit (Sigma # PE0230-1KT, USA). The concentration of the proteins was determined using Bradford reagent and synergy H4 hybrid multi-mode microplate reader (Bio-Tek # H4MLFPTAD, USA). Bovine Serum Albumin was used for the standard [20].

2.3. Total carbohydrate extraction and quantification

The total carbohydrates was extracted as it was described by Saadaoui et al., [20]. Dry biomass of known weight was treated with glacial acetic acid for 30 min in a water bath at 80 °C. This mixture was further treated with acetone, vortexed at a high speed, and centrifuged for 10 min. The supernatant was discarded carefully without disturbing the pellet. The left-over biomass was treated with 4 M HCl and boiled in a water bath for 2 h. An equal volume of water was added to the acid mixture. After centrifugation of the above mixture, the supernatant was mixed with phenol and sulfuric acid, and then boiled for 20 min. The carbohydrate concentration was determined via spectrophotometry at 490 nm using glucose standards.

2.4. Total lipid extraction and quantification

Total lipids were extracted from freeze-dried algal biomass using the method of Folch, Lees & Stanley [21] with some modifications as described by Saadaoui et al., [17]. A gravimetrical determination of the total lipid was performed, and the lipid content (%) and lipid productivity were determined using the following equations as described by Arora et al., [22].

Lipid content (%) = Total lipids (g)/Dry biomass (g) × 100

Lipid Productivity (mg L^{-1} day^{-1})

: (Lipid content/100)∗Biomass productivity

2.5. Polyunsaturated fatty acids extraction and profiling

The extraction was performed using a one-step trans-esterification method as described by Saadaoui et al., [17], and the analysis was conducted using a gas chromatography–flame ionization detector. The selected strain was cultured for 14 days in BG11 growth medium. Next, 10 mg of dried biomass was placed into a 20 mL crimp cap vial. Then, 4 mL of a solution consisting of 95% sulfuric acid and methanol solution (H_2SO_4: CH_3OH, 1:10) was added to the biomass. After 10 min of sonication, the extract was placed in an oven at 80 °C for 2 h. This mixture was then transferred into a centrifuge tube containing 1 mL of distilled water and 3 mL of hexane: chloroform (4:1) mixture. The tube was mixed and then centrifuged at 5000 rpm for 5 min. Finally, the top layer with the FAME was collected and filtered into a 2 mL vial for GC-FID analysis.

2.6. Amino acid profiling

Amino acids were quantified using pre-column derivatization with *O*-phtalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) after methods described by Blankenship et al. [23]. Pre-weighed algal biomass (approx. 2 mg) was hydrolysed in 100 µL 6 N HCl at 120 °C for 24 h [24]. Analysis was performed in replicate, dried, and resuspended in 100 µL 0.1 N HCl. A reaction blank (no biomass) and a known protein standard (bovine serum albuminc; Sigma# 1076192) was performed. The HPLC system (Agilent 1260) included a programmable autosampler for fully automated sample handling, derivatization, and sample injection (30 µL). Amino acid derivatives were separated by reverse-phase high-performance liquid chromatography (RP-HPLC) using a 5-µm Hypersil amino acid-octadecyl silane column (AA-ODS; 2.1 × 200 mm) using the solvent system and gradient described by Zheng et al. [25]. Amino acids derivatives were detected using a variable wavelength UV detector and an in-line fluorescence detector. Sample was quantified against a 5-point calibration curve from dilutions prepared from a standardized mixture of L-amino acids (Sigma# P0834). Sixteen amino acids were reported; due to deamination, asparagine and glutamine were reported with aspartate and glutamate, resp., as ASX and GLX. Note that tryptophan cannot be not determined by this method. The system operations and data analysis were performed on Chemstation. The assay was capable of detecting amino acid derivatives between 1 and 100 nmol

2.7. Trolox equivalent antioxidant activity (TEAC) assay

The antioxidant assay consists of the formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) to produce the radical cation ABTS+, which is a soluble chromogen that is green in colour and can be determined spectrophotometrically at 405 nm [26]. The TEAC assay was performed using an antioxidant assay kit (CS0790, Sigma, USA). The decrease in absorbance due to the compounds being tested, measured after 20 min of incubation at room temperature and in the dark, reflected the ABTS+ radical-scavenging capacity [27]. Trolox and ascorbic acid were used as positive controls [28]. All measurements were performed in triplicate ($n = 3$).

2.8. Statistical analysis

The results from the experiments were expressed as mean of two independent parallel experiments and represented as means ± standard deviation (SD). Statistical analysis was carried out by using IBM SPSS software (Version 26). Normality of variable distribution was checked by the Kolmogorov-Smirnov test to aid in sample screening [29]. Two-step cluster analysis was performed to group samples based on their level of lipids, carbohydrates and proteins. Such analysis will allow us to select the best microalgae isolate showing high nutritional balance [30]. Later, the optical density, metabolites content and antioxidant activity were tested statistically using one-way analysis of variance (ANOVA) and post-hoc Turkey's honestly significant difference (HSD) test. The significant level was set at $P < .05$.

3. Results

3.1. Growth rate analysis of the local microalgae isolates

Thirty local microalgae strains isolated from the Qatar desert environment, which represented the major genera of the QUCCCM [17], were selected randomly for growth rate and metabolite composition in order to select the most suitable strain for feed-supplement production (Table 1). Growth characteristics are present in the Table 1. The five fastest strains are as follows: *Mychonastes homosphaera* (*M. homosphaera*) QUCCCM70, *Chlorella* sp. QUCCCM62, a *Chlorophyte* QUCCCM122, *Scenedesmus* sp., QUCCCM120 and *Chlamydomonas* sp. QUCCCM72. These strains had growth rates between 0.6 and 0.89 day^{-1}. Normality test proved that growth rate followed normal distribution with significance for Kolmogorov-Simirnov test higher than 0.05 ($\alpha = 0.2$). Hence, the strains presenting a growth rate above the average ($\mu = 0.326\ day^{-1}$) were selected for further investigation of their metabolites content. Accordingly, 15 microalgae isolates were selected such as *Chlorella* sp. QUCCCM4, *Chlorella* sp. QUCCCM5, *Mychonastes* sp. QUCCCM6, *Chlorella* sp. QUCCCM13, *Chlorococcum* sp. QUCCCM27, *Chlorella sp.* QUCCCM32, *Chlorella* sp. QUCCCM38, *Mychonastes* sp. QUCCCM43, *Chlorella* sp. QUCCCM62, *Desmodesmus* sp. QUCCCM65, *M. homosphaera* QUCCCM70, *Chlamydomonas* sp. QUCCCM72, *Chlorophyta* QUCCCM73, *Scenedesmus* sp. QUCCCM120, and QUCCCM122.

3.2. Metabolic characterization of the selected microalgae isolates

The 15 fastest growing strains were selected for further investigation of their potential to produce primary metabolites such as lipids, proteins, and carbohydrates. The protein content ranged between 23 and 41% g dry $weight^{-1}$. The highest protein content was observed with the *Chlorella* sp. isolates: *Chlorella* sp. strains QUCCCM4, QUCCCM13 with ~41% g dry $weight^{-1}$, followed by *Mychonastes* sp. QUCCCM6 and *Chlorella* sp. QUCCCM38 (Fig. 1).

The highest lipid content was obtained for *Mychonastes homosphaera* QUCCCM70 (40.7% g dry $weight^{-1}$) (Fig. 1). Nine other strains of microalgae present relatively high lipid content ranging between 30.8 and 37% g dry $weight^{-1}$. These strains correspond successively to *Scenedesmus* sp. QUCCCM120 (33.1% g dry $weight^{-1}$); *Chlamydomonas* sp. QUCCCM72 (36.5% g dry $weight^{-1}$); *Chlorella* sp. strains such as QUCCCM4, QUCCCM13, and QUCCCM38 with a lipid content of ~37% g dry $weight^{-1}$ and QUCCCM122 (37% g dry $weight^{-1}$). The comparative analysis of the carbohydrate content showed that it varies from 12 to 30% g dry $weight^{-1}$. The highest content was observed with the *Chlorococcum* sp. strain QUCCCM27 (30 ± 0.009%).

3.3. Metabolites-based clustering analysis

First, hierarchical clustering analysis (HCA) was performed to determine the metabolomic proximity between strains and select the strain showing the highest metabolite content. ANOVA test showed significant difference between analysed samples with ($p < 0.05$) Then, K means cluster analysis was performed to distribute the microalgae isolates into the four clusters. The averages of metabolites content of all clusters are present in the Table 2. A dendrogram with 2 main branches was obtained (Fig. 2A). One branch for only *Chlorococcum sp.* QUCCCM27 presenting a particular metabolites profile with broadly similar protein, carbohydrate, and lipid content. The other branch groups three clusters among them appears the cluster I, composed of *Chlorella sp* QUCCCM4, *Mychonastes sp.* QUCCCM6, *Chlorella sp.*

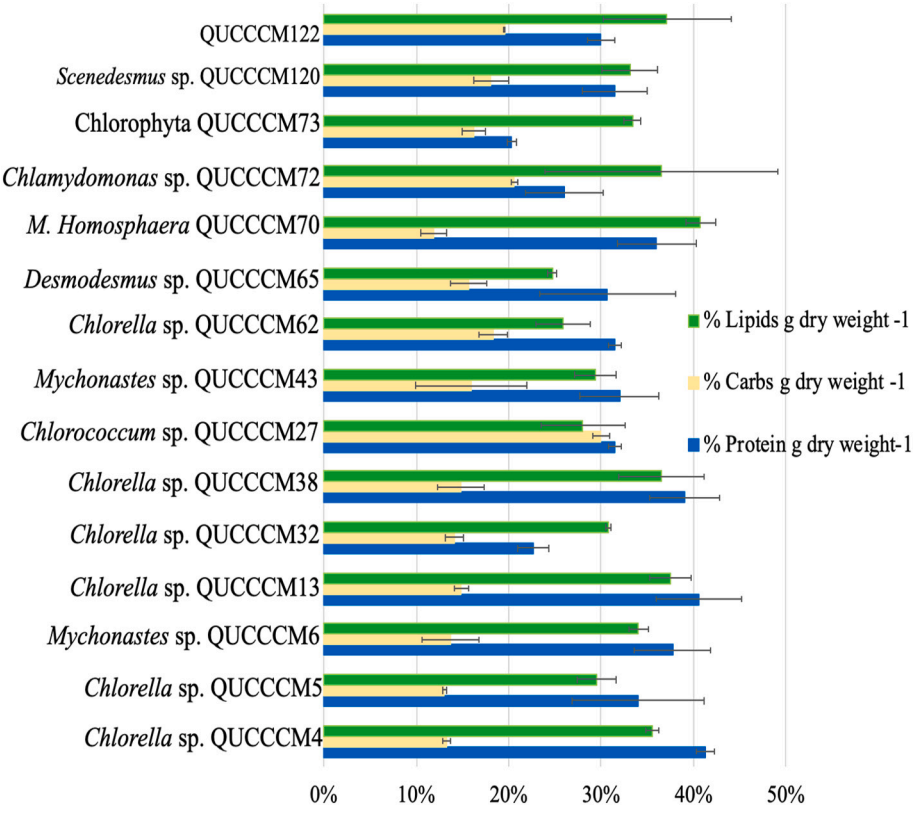

Fig. 1. Metabolite content of the 15 fast growing microalgae strains. Results are presented in terms of % metabolites g dry weight $^{-1}$. All measurements were recorded in triplicate ($n = 3$).

Table 2
Means of lipids, proteins and carbohydrates content of the four clusters generated by K means cluster analysis. of the microalgae isolates. K-means cluster analysis was performed to distribute the microalgae isolates into the four clusters.

Metabolites content	Cluster I	Cluster II	Cluster III	Cluster IV
Total lipids	36.86	27.38	28.00	34.17
Total protein	38.92	32.17	32.00	26.26
Total carbohydrates	13.7	15.76	30.00	17.80

QUCCCM13, *Chlorella sp.* QUCCCM38 and *M. homosphaera.* QUCCCM70. These strains present high nutritional values due to their high amounts of lipid and protein contents (Fig. 2B). These strains were subjected to survey of lipids and proteins content during the growth in order to identify the peak time of their production. This will ultimately help in harvesting biomass enriched with proteins and lipids to be used for feed production.

3.4. Determination of the optimal metabolite production time for the selected microalgae isolates

To identify the most suitable time for harvesting the algal culture to collect biomass enriched with metabolites of interest, a survey of lipid and protein contents during their growth was conducted for the 5 microalgal strains showing fast growth and high metabolite content. These algae were in cluster one showing higher protein and lipid content.

Growth analysis demonstrated that *M. homosphaera* QUCCCM70 and *Chlorella* sp. QUCCCM13 presented a considerable biomass productivity of 20 ± 0.4 mg L^{-1} day $^{-1}$ and 27 ± 0.5 mg L^{-1} day $^{-1}$, respectively. However, the remaining strains *Chlorella* sp. QUCCCM4, *Mychonastes* sp. QUCCCM6, and *Chlorella* sp. QUCCCM38 maintained relative fast growth. These five strains were considered as scalable and thus they were surveyed for their protein and lipid production capability during growth in a volume of 3 L.

The protein accumulation increased from day3 to day6 for the five selected isolates. However, three different patterns were observed from day6 to day14. Indeed, *Chlorella* sp. strains QUCCCM4, QUCCCM38 and *Mychonastes* sp. QUCCCM6 and showed a gradual decrease of their protein content, while *Chlorella* sp. QUCCCM13 maintained a stable protein content up to day11 and *M. homosphaera* QUCCCM70 showed increase up to day9 after which it decreased (Fig. 3). *M. homosphaera* QUCCCM70 showed the highest protein content (39.1% g dry weight^{-1}) and a protein productivity of 5.34 ± 0.4 mg L^{-1} day $^{-1}$ after 6 to 9 days of cultivation (end of exponential phase), followed by QUCCCM38 with a protein content of 38.75% g dry weight^{-1} and a protein productivity of 6.81 ± 0.53 mg L^{-1} day $^{-1}$ after 6 days of cultivation (exponential phase).

The lipid content of all the strains increased during the cultivation time, and the maximum was observed at day14 for the five strains investigated (Fig. 4). The highest amount measured was for Chlorella sp. QUCCCM13 reaching 56.21% g dry weight^{-1} with a lipid productivity of 18.67 ± 0.53 mg L^{-1} day $^{-1}$, followed by QUCCCM4, 6, and 70 showing similar lipid amount between 48.2% - 49.32% and a lipid productivity between 9.6 ± 1.6 and 11.19 ± 2.6 mg L^{-1} day $^{-1}$. No significant difference was seen between the five strains ($\alpha > 0.05$). Therefore, further investigation of the antioxidant capacity was performed to select the most promising for feed production.

3.5. Evaluation of the antioxidant capacity of the selected microalgae isolates

The highest and lowest TEAC radical scavenging activities in this study was detected in strain *M. homosphaera* QUCCCM70 (127.73 µmol TE g dry weight^{-1}) and *Chlorella sp.* QUCCCM13 (62 µmol TE g dry weight^{-1}), respectively (Fig. 5). Significant difference between the

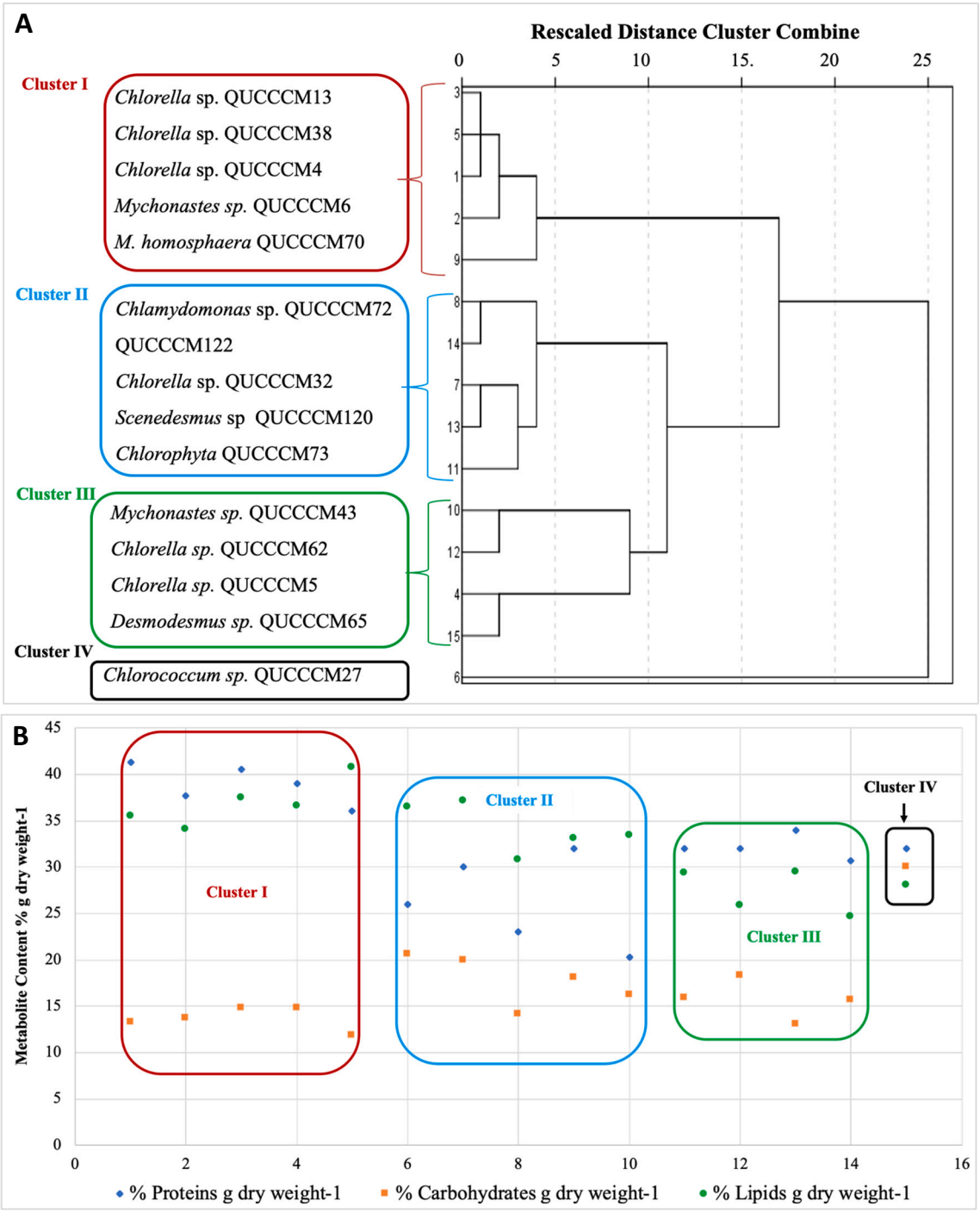

Fig. 2. Hierarchical Cluster analysis of the selected microalgae isolates **2A**: Dendrogram representing the outcome of a Hierarchical Cluster Analysis conducted on metabolites content grouping three variables such as lipid, protein and carbohydrates of 15 differential microalgae isolates grown under standard cultivation condition. **2B:** Cluster plot based on the Hierarchical Cluster Analysis.

antioxidant activities of the five strains was observed ($\alpha < 0.5$). Subsequently, Post-hoc multiple comparisons were carried out to compare their TEAC. This test proved that *Chlorella* sp. QUCCCM4, *Chlorella* sp. QUCCCM13, and *Mychonastes* sp. QUCCCM6 are similar ($\alpha > 0.05$). However, *Chlorella* sp. QUCCCM38 and *M. homosphaera* QUCCCM70 are different than the other strains (mean higher than 111 µmol TE g dry weight^{-1}) with the latter exhibiting higher value ($\alpha = 0.011$).

Among the microalgae strains investigated, the strain *M. homosphaera*. QUCCCM70 is a very good source of both proteins and lipids. Indeed, it produced the highest amount of proteins from day 6 to day 9 and high amounts of lipids at day 14. In addition, this strain had considerable antioxidant capacity, and a high growth rate. Accordingly, *M. homosphaera* QUCCCM70 can be considered a very promising alternative for feed supplement production. The harvest of *M. homosphaera* QUCCCM70 at different time intervals of 6 and 14 days from the same batch is recommended to collect nutritionally balanced biomass enriched with proteins and lipids, respectively.

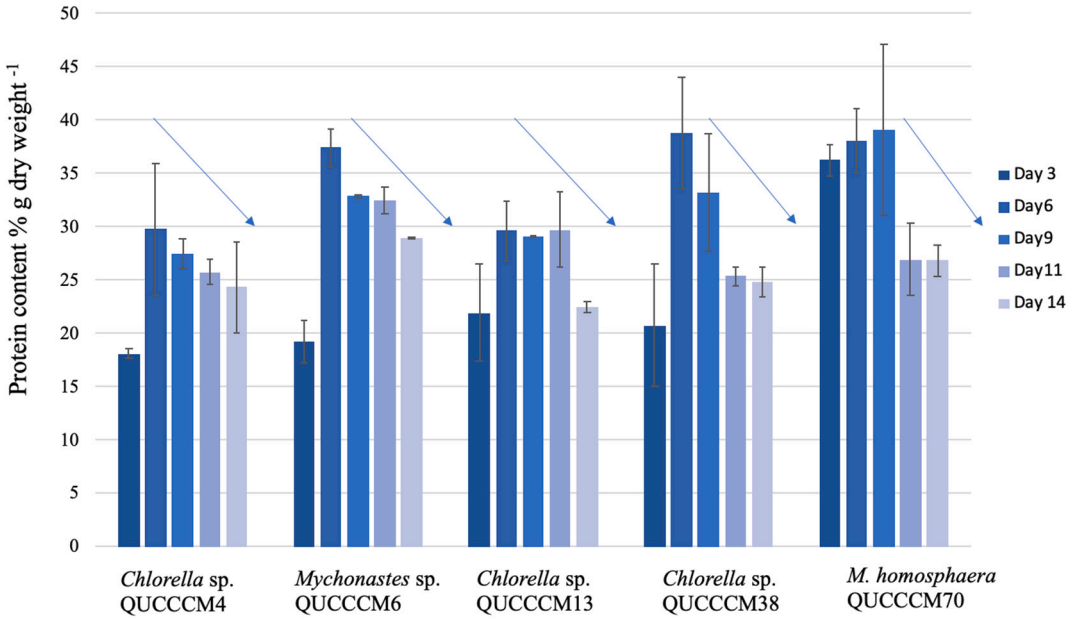

Fig. 3. Survey of the Protein production during the microalgae growth. The culture was performed using 3 L vertical tubular photobioreactors. The arrows indicate the trend observed during the culture time. All measurements were recorded in triplicate ($n = 3$).

3.6. Amino acids and fatty acids profiling of *M. homosphaera* QUCCCM70

The amino acid profile of *M. homosphaera* QUCCCM70 revealed the presence of 9 essential amino acids (tryptophan (Trp), isoleucine (Ile), leucine (Leu), valine (Val), methionine (Met), threonine (Thr), histidine (His), phenylalanine (Phe), and lysine (Lys)) with different frequencies (Fig. 6). Additionally, the FAME profiling evidenced the presence of essential fatty acids such as omega 3, 6, 7, and 9 (Table 3).

4. Discussion

The screening of freshwater microalgae isolates revealed large variations in all screening criteria. Among the 30 freshwater microalgae strains subjected to the comparative growth rate analysis, 15 fast-growing microalgae isolates with appropriate metabolite contents were selected. Among them, *Scenedesmus* (0.7 day^{-1}) presented a growth rate significantly higher than the average described for *Scenedesmus* isolates (0.2 day^{-1}) [31].

As per our findings, the strains classified as protein-rich belong to three main genera of microalgae: *Chlorella* sp., *Mychonastes* sp., and *Scenedesmus* sp. The strains *Chlorella* sp. QUCCCM4, QUCCCM13, QUCCCM38, and QUCCCM62, which are locally isolated *Chlorella* sp., were considered as protein-rich isolates since they exhibited protein contents 31–42% *w*/w higher than that previously described by Guccione et al., [32]. Accordingly, these isolates were considered as suitable candidates for the production of protein-rich feed supplement.

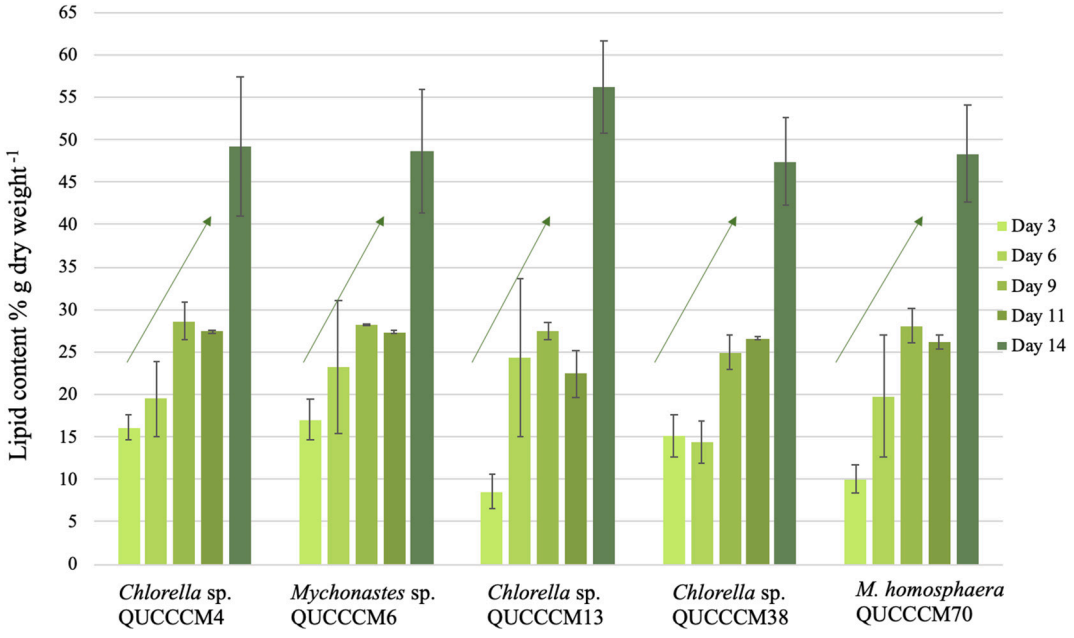

Fig. 4. Survey of the Lipid production during the microalgae growth. The cultures were performed using 3 L vertical tubular photobioreactors. The arrows indicate the trend observed during the culture time. All measurements were recorded in triplicate (n = 3).

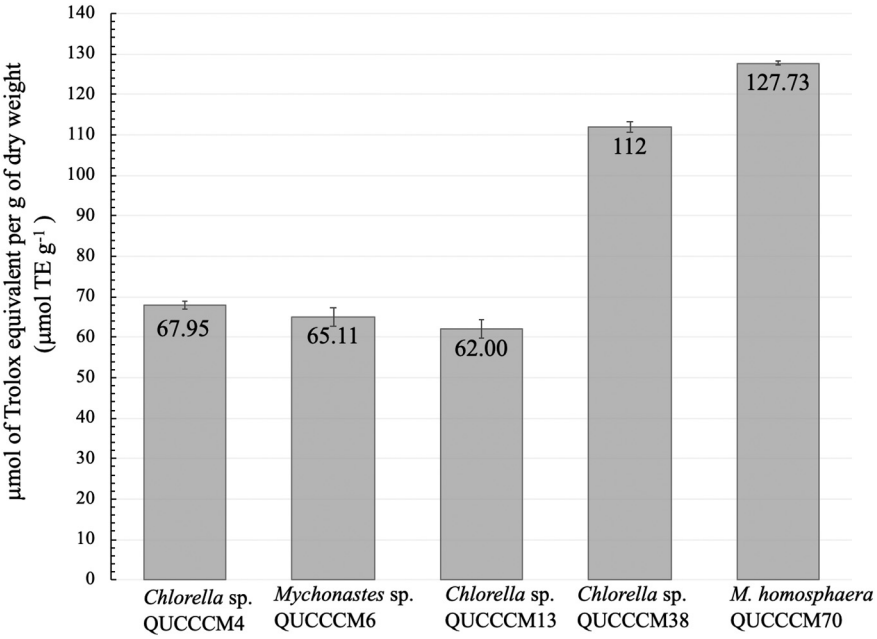

Fig. 5. Assessment of the Trolox Equivalent Antioxidant Capacity (TEAC) of the selected strains. TEAC was determined for the methanolic extract of the selected strains harvested after 14 days of cultivation. Values were recorded in triplicate ($n = 3$).

Additionally, *M. homosphaera*. QUCCCM70 showed a rich protein content of 38%, higher than that of the *Mychonastes timauensis*, as reported by Duong et al., [1].

Moreover, *M. homosphaera* was observed to have the highest amount of lipids of up to 40% in the scale of 1 L volume and this amount increased after cultivation using 3 L vertical tubular bioreactor to reach 48.32% (Figs. 1 and 4, respectively). This was in accordance with a screening program carried out by Yuan et al., [33] and demonstrated the presence of a lipid-rich *Mychonastes afer* isolate that was considered as a potential new feedstock for biodiesel production.

The isolate with the second highest lipid content was found in the *Chlorella* species. The values were consistent with that published previously [34,35], but were higher than that previously reported for the same species by Lim et al., [36]. Regarding *Scenedesmus* sp. isolate QUCCCM120, results by Xin, Hong-ying & Yu-ping [37] showed that at 30 °C, the lipid content per microalgal biomass was only 22% (w/w), whereas it was 33% in our study. *Chlamydomonas*, which is currently the best model organism for microalgal lipid research, had a lipid content of 36.5%. This amount was 1.5-fold higher than the normal amounts found by Sajjadi et al., [38] in other *Chlamydomonas* isolates that were screened. The results obtained from our study highlighted the existence of local potential strains with high nutritional value.

Since the metabolites production depends on the state of the cells [39], we conducted a survey for the peak lipids and protein production during the microalgae growth phase to determine the best time for harvesting. For that reason, we scaled up the culture from 1 L to 3 L using a vertical tubular photobioreactor to be able to collect culture samples suitable for metabolite extraction and quantification. However, we noticed a decrease in the growth rate of the microalgae isolates compared to the corresponding growth rates in a volume of 1 L. This might have been due to the decrease of light distributed in the photobioreactor since the 3 L vertical tubular photobioreactor has a thicker membrane, longer diameter, and higher depth [40,41]. In addition, a reduction in light intensity inside the photobioreactor is considered as a stress condition that can also affect metabolite production [42].

We noticed a significant increase in lipid content with the microalgae growth for all strains that were investigated. This result was consistent with the findings of Xia et al., [41]. Our strains showed almost the same ~2-fold increase between exponential and stationary phases; however, the final lipid content registered is much higher than

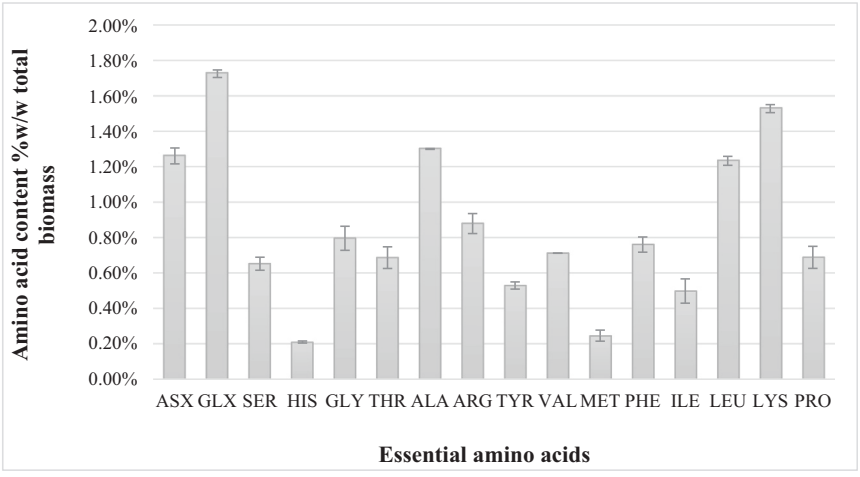

Fig. 6. Amino acid profile of *M. homosphaera* QUCCCM70 as determined by reverse-phased liquid chromatography with UV detection at 254 nm. All measurements were recorded in triplicate ($n = 3$). Asx: Asparagine/Aspartate; Glx (Glutamine/glutamate); Ser: Serine; His: Histidine; Gly: Glycine; Thr: threonine; Ala: Alanine; Arg: Arginine; Tyr: Tyrosine; Val: Valine; Met: Methionine; Phe: Phenylalanine; ILE: Isoleucine; Leu: Leucine; Lys: Lysine; Pro: Proline.

Table 3
Relative percentage of the FAME of the *M. homosphaera* QUCCCM 70. All measurements were recorded in triplicate (n = 3).

FAME	Relative percentage of the FAME (%)
Myristic acid (C14: 0)	1.37 ± 0.89
Myristoleic acid (C14: 1)	0.23 ± 0.09
Palmitic acid (C16: 0)	20.07 ± 1.38
Palmitoleic acid (C16: 1)	3.53 ± 1.75
Margaric acid (C17:00)	0.11 ± 0.014
Stearic acid (C18: 0)	1.12 ± 0.1
Elaidic acid C18: 1 (n-9) omega 9	4.76 ± 0.32
Vaccenic acid18:1(n-7) omega 7	1 ± 0.5
Linoleic acid (C18: 2n6) omega 6	13.84 ± 2.2
Gamma-linolenic acid 18:3(n-6) GLA omega 6	0.61 ± 0.48
Linolenic acid (C18: 3n6); ALA omega 3	24.4 ± 3.37
Stearidonic acid 18:4(n-3)	1.36 ± 0.19
Arachidic acid (C20: 0)	0.18 ± 0.110
22:00	0.05 ± 0.06
Erucic acid 22:1	0.12 ± 0.03
21:05	0.25 ± 0.07
24:00	0.75 ± 0.07
Docosahexaenoic acid 22:6(n-3) DHA omega 3	0.1 ± 0.04
Nervonic acid 24:1 (n9) omega 9	0.17 ± 0.04
PUFA	47.74 ± 1.3
Total	73.3 ± 2.41

the content described (34%) by Hu et al., [5]. Indeed, the lipid content of our local *Chlorella* reached 56.21% in the case of *Chlorella* sp. QUCCCM38.

Cultivation at a volume of 1 L as the first step of screening was performed for 9 days, thus allowing for an accurate determination of growth rate. However, to perform the survey on metabolite production, we extended the time of cultivation to 14 days corresponding to the end of stationary phase to maximize the chance of identifying peak times of both lipid and protein production. Therefore, lipids were stored at the end of the stationary phase as an energy source [5,43].

The comparative analysis of lipid content between cultures in 1 and 3 L cylindrical photobioreactors at day 9 revealed a decrease for all of the strains that were investigated, and this might be related to the reduced light quantity in the 3 L cylindrical photobioreactor. However, the lipid content after 14 days was very high compared to that in 1 L, which exceeded 47% for the five strains selected. Hence, we can consider that day 14 was the best timepoint to harvest lipid-rich biomass. In addition, these five strains can also be considered as very promising feedstock for biofuel production [5]. In addition, the lipid content observed using the 3 L vertical tubular photobioreactor was higher than that previously reported (Fig. 4). Indeed, *Chlorella* sp. QUCCCM38 had a lipid content of 56.21% g dry weight^{-1}, which was significantly higher than that of other *Chlorella* strains cultivated under normal and nitrogen depletion conditions, showing a lipid content of ~21% and 36%, respectively [44]. The strain *M. homosphaera* QUCCCM70 also presented a lipid content of 48.32%, similar to that of *Mychonaster afer* (53.9%), which has been considered as a very promising strain for biodiesel production [33].

Microalgae proteins can be considered as very good alternative for feed supplement production since it has protein levels similar to conventional sources of proteins used for feed such as soya bean [45]. As per the previously described results, 6 days of cultivation represent the peak time for protein production of the microalgae isolates. Results converges with the recent results of Blifernez-Klassen et al. [46]. The strain *M. homosphaera* QUCCCM70 presented the highest protein content observed during peak metabolite production. Such protein content of 44.5% was much higher than the protein content of the microalgae strain selected recently in Australia (33%) to produce cattle feed supplement [1]. Therefore, for an accurate screening of the microalgae isolates in terms of metabolite production, the correct time leading to the maximum metabolite production is needed to identify strains with high nutritional potential.

Several publications stated that *Mychonastes* is very suitable for biodiesel production, however we confirmed for the first time in the present research work and based on its metabolites profiling and growth characteristics that this species is also very promising for high quality poultry and livestock products. Furthermore, such strain is naturally adapted to the desert climate since its optimal temperature of *Mychonastes* is 30 °C which corresponds to the annual average temperature in Qatar. Accordingly, this local microalga strain can be considered as good alternative to supplement the conventional feed and support achieving food security in arid climate regions where all animal feeds are imported and this is associated with a high cost, huge logistic efforts to store and maintain its nutritional value.

Recently, there has been a considerable increase in the number of studies on microalgae antioxidants because of an increasing interest to identify novel natural and safe sources of antioxidants [47–49]. Subsequently, several screening programs of microalgae based on their antioxidant activities have been carried out. Compared to previously described results, the antioxidant capacity of the our local strains were higher than that by Goiris et al., [50]. These researchers described the antioxidant capacities using the TEAC assay, and reported values of 0–69 μmol Trolox equivalent g dry weight^{-1} after screening 32 microalgae isolates. Strains with high TEAC are expected to be enriched with phenolic compounds and carotenoids since they have been described as major contributors to the high antioxidant activity [50,51], and further investigation of such interesting molecules have been envisaged. Recent studies have proven that the production of these high value compounds with antioxidant potential from algae can be enhanced by various physico-chemical stresses [52–54]. As the Qatar climate poses quite a number of stress factors, such as high light intensities, temperatures, and salinities, this could explain the high antioxidant capacity of our microalgae isolates since these strains have naturally adapted to harsh environmental conditions of the Qatar desert climate.

Finally, the presence of essential fatty acids (omega 3, omega 6 and omega 9) with multiple health benefits and essential aminoacids in the *M. homoeospharea* QUCCCM70 biomass proved its high nutritional value as feedstock for animal feed production.

5. Conclusion

The overall procedure adopted ultimately led to the identification of five microalgae isolates, belonging to the genera *Chlorella* and *Mychonastes,* with high nutritional potential. Among them, a fast-growing *M. homosphaera* isolate QUCCCM70 presented considerable lipid and protein contents and has an interesting antioxidant capacity. Additionally, this strain exhibited essential amino acids and omega 3 fatty acids which increase its nutritional value. Finally, the survey of metabolites production during the growth proved that the harvest of *M. homosphaera* QUCCCM70 at different time points (days 6 and 14) led to a very well-balanced biomass suitable for the production of high-quality animal feed supplement.

CRediT authorship contribution statement

Imen Saadaoui: Conceptualization, Supervision, Writing - original draft, Project administration, Funding acquisition.**Maroua Cherif:**Investigation, Data curation, Writing - review & editing.**Rihab Rasheed:**Investigation, Writing - review & editing.**Touria Bounnit:**Investigation, Writing - review & editing.**Hareb Al Jabri:**Writing - review & editing.**Sami Sayadi:**Writing - review & editing.**Radhouane Ben Hamadou:** Conceptualization, Writing - review & editing.**Schonna R. Manning:**Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions statement

I.S. designed the study, led the task performance, analysed the data, drafted the manuscript and secured the fund, M.C. performed the experiments and participated in the reviewing the manuscript, R.R and T.B provided technical support and reviewed the manuscript. S·S and H.A.J participated in discussions and critical revision of the manuscript. RBH and S.M. discussed the results and reviewed the manuscript. All authors approved the final version before submission.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

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