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# Effects of Different Levels of Urea as Nitrogen Source on Chemical Composition of Marine Microalgae *Nannochloropsis oculata*

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**Abstract**: Microalgae breeding media must be cost-effective, enable high growth, meet exact requirements and be readily available. The effect of different levels of urea [25, 50, 75, and 100%] in the growth medium on the biochemical constituents (protein, carbohydrates, lipids, fatty acids, and amino acids) of the *Nannochloropsis oculata,* was assessed compared to the F/2 Guillard standard medium. The obtained results revealed that the chemical constituents of *N. oculata* were influenced by the different levels of urea. The highest total protein was obtained by A4 medium (100% urea) (26.44%) and A3 medium (75% urea) (25.84%). The maximum percentage of essential amino acids (EAA) (51.54%) was obtained by using the A4 medium (100% urea) as compared to the control (100% F/2). The highest total lipid content was achieved by using the A1 medium (25% urea) producing (17.33 %) and A4 medium (100% urea) (16.98%). Accordingly, the highest total saturated fatty acids percentage (TSFA) of *N. oculata* was recorded by the A3 medium. In conclusion, the addition of urea is an excellent policy to increase chemical composition and lipid accumulation. The present study recommended taming results for aquaculture feeding through using the proposed A1 medium as a lipid promoter or A4 medium as a protein promoter.

**Keywords:** Amino Acids, Fatty Acids, *Nannochloropsis oculata*, Proximate Composition

## INTRODUCTION

Algae are a diverse group of eukaryotic organisms with important roles in marine, freshwater, and even terrestrial ecosystems. For instance, 30–50% of the planetary net photosynthetic productivity (the difference between autotrophic gross photosynthesis and respiration) is of marine origin and dependent on phytoplankton biomass (Boyce et al., 2010; Field et al., 1998). Unicellular microalgae are capable of harnessing sunlight and $CO_2$ to produce energy-rich chemical compounds, such as lipids and carbohydrates, which can be converted into fuels (Hu et al., 2008; Rodolfi et al., 2009; Wijffels & Barbosa, 2010). Marine phytoplankton is often categorized into groups based on taxonomic traits, its abundance role in biogeochemical fluxes, and/or primary production. While diatoms are considered the principal group contributing to primary production and carbon export in coastal areas, dinoflagellates are important contributors to biomass in stratified or silica-limited areas, and microalgae are the dominant group in the marine continental shelf and oceanic waters (da Silva et al., 2009). Microalgae strains are fast-growing microorganisms with very high growth rates under optimal culture conditions. It is rapidly growing, along with great chemical diversity, it opens up applications in many fields, such as aquaculture, biotechnology, and food science (Spolaore et al., 2006; Templeton & Laurens, 2015). Applications involving microalgae are expected to increase and diversify as a result of the ongoing search for more productive systems to supply

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the community with food, feed stock, and high-value biochemical products (Lee Chang et al., 2013; Zeng et al., 2011) Particularly in food-stuffs, microalgae are beneficial in improving the nutritional content of traditional foods, and thus, have a positive effect on human health due to their proper chemical composition.On the other hand, biological issues include the domestication of promising strains (Lim et al., 2012) and successful uses of mechanisms stimulating microalgae to grow and produce target substances (Kaye et al., 2015).

All possible applications of microalgae are directly associated with high growth and a favourable chemical profile of the species (Borges-Campos et al., 2010). Fluctuations in the chemical profile of microalgae in cultures are a key issue in their study and applications (Lourenco et al., 2002). The chemical content of microalgae can vary with culture age and with changes in culture conditions (Carvalho et al., 2009). *Nannochloropsis* is considered the main algal species cultured in marine hatcheries and plays an important role in aquaculture development (Bondioli et al., 2012). The effect of variation of culture parameters on many microalgae species has been studied in order to better understand their physiology, as well as to answer specific and relevant questions for mass culture (Grobbelaar, 2014). In the industrial production scale in marine hatcheries, it is very important to optimize a suitable nutrient culture media for culturing this species (Ashour & Kamel, 2017). The microalgae nutrient media should be easy to prepare, economical, achieve high growth, and satisfy all the microalgae quality and quantity. Although the F/2 Guillard medium is considered the most commonly used medium in the culturing of *Nannochloropsis* in marine hatcheries, it has some disadvantages, such as being difficult to prepare and set up for outdoor mass culture and being expensive (Ashour & Kamel, 2017). The objective of this study is to determine the effect of adding a percentage of $CH_4N_2O$ on the chemical composition, fatty acid, and amino acid of *Nannochloropsis oculata.*

## MATERIALS AND METHODS

**Microalgal strains and culture condition:** The *Nannochloropsis oculata* strain was from an algae unit of the marine hatchery at the kilo 21 Alexandria - Egypt.

The experiment was conducted in the marine hatchery of the National Institute of Floating Seas and Fisheries Alexandria (NIOF), Egypt. *N. oculata* were kept and cultured under controlled conditions of temperature (22± 2C°), salinity (35 ± 2 ppt), and illumination (750-3000 Lux /24 h.) using F/2 Guillard standard medium (Guillard & Ryther, 1962), with continuous aeration.

**Experiment design:** The experiment was conducted in plastic bottles of 1.5 liters filled with 1 liter of sterile saline water (35 ± 2 ppt). Comparing to F/2 standard media as a control treatment (CO), four treatments (A1, A2, A3, and A4) were conducted with different levels of urea as a replacement of nitrogen source, as presented in Table 1. To prepare a stock solution of urea ($CH_4N_2O$), 4 g was dissolved in 250 ml of distilled water and used in proportions as shown in Table(1) with 24 hours lighting for eight days and then harvested using NaOH.

**Table (1):** Present of media F/2 and urea.

| | CO | A1 | A2 | A3 | A4 |
|---|---|---|---|---|---|
| F/2 | 100 | 0.75 | 0.50 | 0.25 | 0 |
| urea | 0 | 0.25 | 0.50 | 0.75 | 100 |

**Biochemical Analysis**

**Dry matter:** Approximately 2 g of sample was added into a pre-weighed porcelain crucible and oven-dried at 60°C for 24 h. It was then cooled in a desiccator and then weighed to a constant weight. The moisture content and dry matter (DM) content was calculated as follows: DM (%) = 100 – Moisture (%)

**Crude ash:** After moisture measurement, the crucible was incinerated at 500°C for 2 h. Once cooled in a desiccator, the crucible was re-weighed.

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**Crud proteins:** Extraction and determination of total protein were conducted according to the methods of (Hatree, 1972; Rausch, 1981), respectively.

**Crude fiber:** Crude fiber was determined according to the method (Chemists & Horwitz, 1995).

**Crude carbohydrates:** Extraction and determination of total carbohydrates were conducted according to (Dubois et al., 1956; Myklestad & Haug, 1972), respectively.

**Total lipid fatty acids profile:** Total lipid and fatty acids were extracted as described by (Bligh & Dyer, 1959; Folch et al., 1957). Preparation of fatty acids methyl ester from total lipids was performed according to the procedure of (Radwan, 1978).

All analyses for the identification of fatty acid fractions were performed on GS-MS model HP (Hewlett Packard) 7890 GC equipped with a flame ionization detector. GC Conditions: Device Model: HP (Hewlett Packard) 6890GC, Column: HP-INNOWax (Polyethylene glycol), 60m, 0.25mm ID, 0.2µm film thickness. Detector: FID (Flame Ionization Detector). Detector temperature: 250°C. Injector temperature: 220°C, injection volume 3µl, split ratio 50:1.

**Amino acids determination:** Amino acids of *N. oculata* were analyzed by hydrolysis in 6N HCL for 22hrs at 110°C; after hydrolysis, the acid was evaporated in a vacuum oven. The residue of the algal sample was dissolved in 1 ml of sample dilution (diluting buffer) (0.2M, pH 2.2) to complete the sample dissolving. An automatic amino acid analyzer was used for amino acid determination (Dionex ICS3000) (Block, 1948).

**Statistical analysis:** Data were presented (n=3) as mean± standard deviation (SD). Statistical analysis was performed using analysis of the one-way (ANOVA), followed by Duncan's test was used to test for statistically significant differences between all treatments at p <0.05 SPSS (2007).

## RESULTS

The effect of different experimented media on the biochemical composition of *N.oculata* was presented in Table2. The total protein, lipid, and fiber content were significantly (*p<0.05*) higher in all treatments comparing to control, while the carbohydrates were significantly higher in control. In addition, the highest protein and lipid were in treatment A4 (26.44%) and A2 (17.330%) respectively, while the lowest fiber (6.18%) and carbohydrate (19.74%) were in treatment A1 and A4 respectively.

**Table (2):** The effect of different levels of urea on the chemical composition of marine microalgae *Nannocloropsis oceanica*

| | Dry matter | Protein | Lipid | Carbohydrate | Fiber | Ash |
|---|---|---|---|---|---|---|
| CO | $10.27 \pm 0.01^{d}$ | $22.28 \pm 0.01^{e}$ | $14.03 \pm 0.01^{e}$ | $26.44 \pm 0.01^{a}$ | $6.18 \pm 0.01^{e}$ | $20.74 \pm 0.01^{b}$ |
| A1 | $10.68 \pm 0.01^{b}$ | $24.12 \pm 0.00^{d}$ | $17.33 \pm 0.01^{a}$ | $22.29 \pm 0.01^{b}$ | $6.35 \pm 0.01^{c}$ | $19.18 \pm 0.01^{e}$ |
| A2 | $10.92 \pm 0.01^{a}$ | $25.50 \pm 0.01^{c}$ | $16.46 \pm 0.01^{c}$ | $21.17 \pm 0.01^{c}$ | $6.37 \pm 0.01^{b}$ | $19.52 \pm 0.01^{d}$ |
| A3 | $10.22 \pm 0.00^{e}$ | $25.84 \pm 0.01^{b}$ | $15.46 \pm 0.01^{d}$ | $20.49 \pm 0.01^{d}$ | $6.65 \pm 0.01^{a}$ | $21.29 \pm 0.01^{a}$ |
| A4 | $10.34 \pm 0.01^{c}$ | $26.44 \pm 0.01^{a}$ | $16.98 \pm 0.01^{b}$ | $19.74 \pm 0.01^{e}$ | $6.20 \pm 0.01^{d}$ | $20.24 \pm 0.01^{c}$ |

Mean different between CO, A1, A2, A3, A4, significant at P<0.05.

**Fatty acid profile:** The highest total fatty acid profile (TFA, µg/100g/DW) of *N. oculata* was obtained by CO (252.38), followed by A4 (248.26), and A3 (247.83), as presented in Table 3.The highest total saturated fatty acids (TSFA) was obtained by A2 (33.18%), followed by A3 (33.12%), A4 (32.69%), and CO (32.37%), while the lowest TSFA were obtained by A1 (32.24%). The highest Palmitic acid C16:0 percent was obtained in CO (22.13%) followed by A2 (21.89%), A1 A3 (21.78%), and A4 (21.36%). Myristic acid C14:0 percentages were high in A3 (4.19%), A2A4 (4.17%), CO (3.64%) and A1 (3.29%).

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Stearic acid C18:0 percentages of CO, A1, A2, A3, and A4 were 3.29 %, 3.70 %, 3.68 %, 3.59 % and 3.72 %, respectively, as shown in Table 3. On the other hand, the lowest unsaturated fatty acids (UFA) were obtained by CO (51.34%), presenting monounsaturated fatty acids (MUFA) of 24.87%, and polyunsaturated fatty acids (PUFA) of 26.47%. The highest UFA was obtained by A1 (55.39%), and consisted of MUFA of 30.25% and PUFA of 25.09.

In addition, CO obtained the highest HUFA (∑U-3 and ∑U-6), (26.47%), whereas the highest MUFA was obtained by A1 (30.25%), followed by A3 (29.17%), A4 (28.28%), A2 (27.81%), and CO (24.87%), respectively. The lowest n-3 (HUFA) was achieved by A1 (10.96%), and the highest was obtained by CO (13.71%), followed by A3 (11.40%), A4 (11.32%) and A1 (11.13%). Table 3 showed that the highest ΣU-3/ΣU-6 ratio was recorded at CO (1.07), followed by A4 (0.88), A2 (0.85), A3 (0.83), and the lowest ratio was obtained by A1 (0.79). As well as the highest Docosahexaenoic acid (DHA) was achieved by (8.17%), A3 (7.64%), A4 (7.58%), and A1 (7.39%), while the lowest was achieved by A2 (7.21%), as shown in Table 3.

**Table (3):** Fatty acids profiles (% total fatty acid) TFA (µg/100g/DW) of selected *N.oculata*.

| Fatty acid | CO | A1 | A2 | A3 | A4 |
|---|---|---|---|---|---|
| TFA (µg/100g/DW | 252.38 | 245.78 | 244.78 | 247.83 | 248.26 |
| C14:0 ( Myristic acid) | 3.61±0.04[c] | 3.28±0.01[d] | 4.16±0.01[b] | 4.18±0.01[a] | 4.16±0.01[bb] |
| C15:0 (Pentadecylic acid ) | 0.57±0.01[d] | 0.64±0.02[b] | 0.64±0.01[bb] | 0.68±0.01[a] | 0.63±0.01[c] |
| C16:0 ( Palmitic acid ) | 22.12±0.01[a] | 21.77±0.01[c] | 21.88±0.01[b] | 21.77±0.02[cc] | 21.35±0.01[d] |
| C17:0 ( Margaric acid) | 0.23±0.01[d] | 0.43±0.01[b] | 0.38±0.01[c] | 0.45±0.01[a] | 0.38±0.01[cc] |
| C18:0 ( Stearic acid ) | 3.28±0.02[e] | 3.60±0.01[c] | 3.68±0.01[b] | 3.58±0.01[d] | 3.71±0.02[a] |
| C21:0 ( Heneicosanoic acid ) | 0.92±0.01[a] | 0.85±0.01[d] | 0.91±0.02[b] | 0.86±0.01[c] | 0.83±0.01[e] |
| C24:0 ( Lignoceric acid ) | 1.56±0.01[a] | 1.51±0.02[c] | 1.47±0.01[d] | 1.53±0.02[b] | 1.56±0.01[aa] |
| ∑Saturated (SFA) | 32.29 | 32.08 | 33.12 | 33.05 | 32.62 |
| C14:1 ( Myristoleic acid ) | 0.13±0.01[c] | 0.14±0.01[b] | 0.16±0.02[a] | 0.12±0.01[d] | 0.12±0.01[dd] |
| C15:1 (cis-10-pentadecenoic acid) | 0.06±0.01[c] | 0.07±0.01[b] | 0.09±0.01[a] | 0.06±0.01[cc] | 0.06±0.01[cd] |
| C16:1 (Palitoleic acid) | 4.77±0.02[e] | 5.16±0.01[b] | 5.09±0.01[d] | 5.14±0.01[c] | 5.18±0.01[a] |
| C17:1(cis-10-Heptadecenoic acid) | 0.40±0.01[e] | 0.42±0.01[d] | 0.46±0.01[a] | 0.43±0.01[c] | 0.45±0.01[b] |
| C20:1 (Paullinic acid ) | 2.92±0.01[a] | 1.47±0.02[c] | 1.52±0.01[b] | 1.41±0.02[e] | 1.44±0.01[d] |
| C18:1n9 (Oleic acid) | 15.86±0.01[e] | 22.54±0.01[a] | 20.04±0.01[d] | 21.53±0.01[c] | 20.55±0.01[b] |
| C22:1 (Erucic acid methyl ) | 0.68±0.01[a] | 0.40±0.01[c] | 0.38±0.01 | 0.42±0.01[b] | 0.40±0.01[cc] |
| ∑Monosaturated (MUFA) | 24.82 | 30.20 | 27.74 | 29.11 | 28.20 |
| C18:2n6 (Linoleic acid) | 11.68±0.01[e] | 13.40±0.01[b] | 12.35±0.01[c] | 13.51±0.02[a] | 12.26±0.01[d] |
| C18:3n6 ( y-Linoleic acid) | 0.15±0.01[c] | 0.22±0.01[a] | 0.20±0.01[b] | 0.22±0.01[aa] | 0.20±0.01[bb] |
| C18:3n3 (α- Linoleic acid) | 1.42±0.01[a] | 0.86±0.01[d] | 0.90±0.01[c] | 0.83±0.02[e] | 0.91±0.02[b] |
| C20:2n6 (Eicosadienoic acid) | 0.90±0.01[a] | 0.37±0.01[b] | 0.32±0.01[e] | 0.36±0.01[c] | 0.34±0.02[d] |
| C20:5n-3 (Ecosapentaenoic acid) | 4.12±0.01[a] | 2.86±0.01[c] | 2.83±0.01[d] | 2.91±0.02[b] | 2.81±0.02[e] |
| C22:6n-3 (Docosahexaenoic acid) | 8.16±0.01[a] | 7.38±0.01[d] | 7.20±0.01[e] | 7.63±0.01[b] | 7.57±0.01[c] |
| ∑Polyunsaturated (PUFA) | 26.45 | 25.09 | 23.80 | 25.46 | 24.09 |
| ∑Usaturated | 51.27 | 55.29 | 51.54 | 54.57 | 52.29 |
| Sat./Monosat. | 1.30 | 1.06 | 1.19 | 1.14 | 1.16 |
| Sat./Polsat. | 1.22 | 1.28 | 1.39 | 1.30 | 1.35 |
| Sat./Unsat. | 0.63 | 0.58 | 0.64 | 0.61 | 1.35 |
| ∑U-3 | 13.70 | 11.10 | 10.93 | 11.37 | 11.29 |
| ∑U-6 | 12.76 | 13.99 | 12.87 | 14.09 | 12.80 |
| ∑U-3/∑U-6 | 1.07 | 0.79 | 0.85 | 0.81 | 0.88 |
| EPA/DHA | 0.51 | 0.39 | 0.39 | 0.38 | 0.37 |
| DHA/EPA | 1.98 | 2.58 | 2.54 | 2.62 | 2.69 |

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**Amino acids analysis**: Amino acid profiles of different experimented media were presented in Table 4. The present study revealed that there is no change in the amino acid profile between the different media. In contrast, there is a clear variation in the content of each individual amino acid between the different treatments. The results showed that *N. oculata* recorded the highest percentage of essential amino acids EAA (51.54%) by A4 medium (100% urea), while the lowest value was achieved by CO medium (100% F/2). The results showed that the highest four EAA in the A1 medium were arginine (5.67%), leucine (7.92%), phenylalanine (5.64%), and threonine (5.30%), as presented in Table 4. The most abundant four NEAA in the CO medium were glutamine (11.89%), aspartate (9.70%), proline (7.90%), and alanine (6.60%), as presented in Table 4.

**Table (4):** Amino acids profiles (%) in *N.oculata* of different level urea and F/2 media.

| Amino acid | CO | A1 | A2 | A3 | A4 |
|---|---|---|---|---|---|
| Essential amino acids ( EAA) | | | | | |
| Arginine | 5.09±0.01[e] | 5.63±0.01[d] | 5.21±0.02[c] | 5.30±0.01[b] | 5.67±0.01[a] |
| Histidine (HIS) | 1.69±0.01[e] | 2.27±0.01[d] | 2.73±0.02[c] | 2.88±0.02[b] | 2.92±0.01[a] |
| Isoleucine (ILE) | 5.09±0.01[c] | 4.49±0.01[e] | 4.66±0.01[d] | 4.42±0.01[b] | 5.05±0.02[a] |
| Leucine (LEU) | 8.19±0.01[a] | 6.51±0.02[e] | 6.66±0.02[d] | 6.64±0.01[b] | 7.92±0.02[c] |
| Lysine (LYS) | 4.09±0.01[e] | 4.70±0.01[d] | 5.12±0.01[c] | 4.35±0.02[b] | 4.51±0.02[a] |
| Methionine (MET) | 2.49±0.01[e] | 3.44±0.02[d] | 3.83±0.02[c] | 4.80±0.01[b] | 4.92±0.01[a] |
| Phenylalanine (PHE) | 5.69±0.01[a] | 5.47±0.01[e] | 4.62±0.01[d] | 5.36±0.02[c] | 5.46±0.01[b] |
| Threonine (THR) | 4.59±0.01[c] | 4.06±0.01[e] | 4.43±0.01[d] | 5.26±0.02[b] | 5.30±0.01[a] |
| Tryptophan (TRP) | 2.49±0.01[e] | 3.40±0.01[d] | 4.25±0.01[c] | 4.32±0.01[b] | 4.43±0.01[a] |
| Valine (VAL) | 5.09±0.01[a] | 4.69±0.01[e] | 5.18±0.01[d] | 5.25±0.01[c] | 5.36±0.02[b] |
| *Total EAA* | 44.50 | 44.66 | 46.69 | 48.06 | 51.54 |
| Alanine (ALA) | 6.60±0.02[a] | 5.76±0.02[b] | 5.39±0.02[d] | 5.36±0.02[e] | 5.41±0.02[c] |
| Aspartate (ASP) | 9.70±0.01[a] | 9.61±0.01[b] | 9.43±0.01[c] | 7.57±0.01[d] | 6.63±0.01[e] |
| Cystine (C-C) | 3.64±0.01[e] | 4.41±0.01[d] | 5.01±0.01[c] | 5.62±0.01[a] | 5.52±0.01[b] |
| Glutamate (GLU) | 11.89±0.02[c] | 11.63±0.02[d] | 12.27±0.02[b] | 12.54±0.02[a] | 10.71±0.02[e] |
| Glycine (GLY) | 5.27±0.01[c] | 5.31±0.01[b] | 5.42±0.01[a] | 4.72±0.01[d] | 4.75±0.01[e] |
| Proline (PRO) | 7.90±0.01[a] | 7.57±0.01[b] | 7.34±0.01[c] | 6.58±0.01[e] | 7.13±0.01[d] |
| Serine (SER) | 5.20±0.01[b] | 5.63±0.01[a] | 4.29±0.01[d] | 4.68±0.01[c] | 4.24±0.01[e] |
| Tyrosine (TYR) | 5.30±0.01[b] | 5.42±0.01[a] | 4.16±0.01[d] | 4.93±0.01[c] | 4.07±0.01[e] |
| *Total NEAA* | 55.5 | 55.34 | 53.31 | 51.94 | 48.46 |
| *EAA/NEAA* | 0.80 | 0.81 | 0.88 | 0.93 | 1.07 |
| *NEAA/EAA* | 1.25 | 1.24 | 1.14 | 1.08 | 0.94 |

## DISCUSSION

The production cost of microalgae utilized as live food in marine hatcheries is nearly 30% of the total cost of the fish larva production (Borowitzka, 1997). However, if stable and economical microalgae production can be developed, the production cost of marine fish larvae will decrease. The present study showed that additions of urea to replace F/2 medium, to reduce the cost production of marine fish larvae production enhance the medium culture of *N. oculata* (the most important species used in marine hatcheries). Using *N. oculata* grown on urea achieved biochemical composition (such as lipid, protein, fiber, and carbohydrates), and the amino acids from the fatty acids are close to those cultured on F/2 Guillard medium.

Although F/2 Guillard medium has been widely used in the cultivation of microalgae for more than fifty years, nowadays, due to different applications of microalgae in biotechnology fields, F/2 Guillard medium has some disadvantages, such as its price and materials used for installation. Additions of urea in all replacement levels could increase protein while carbohydrate contents were decreased in *N. oc-*

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*ulata.* Simultaneously to the increase of protein quantity, carbohydrate amount tended to decrease in the microalgal species studied (Otero & Fábregas, 1997). High microalgae protein content can be explained by the intake of nitrogen internally from urea media.

The results of this study indicated that some urea formulas obtained significant (P <0.05) biochemical composition higher than F/2, to optimize the production of *N. oculata* for aquaculture purposes in marine hatcheries. A possible explanation for the protein content in microalgae is that it results from nitrogen consumption, which is assumed to have arisen due to the abundant absorption of nitrates. Protein in all ratios was close to the result (Lourenço et al., 1998; Millán-Oropeza et al., 2015; Paes et al., 2016).

The accumulation of high concentrations of carbohydrates may contribute to enlarge cells. (Dean et al., 2010; Lourenço & Vieira, 2004; Millán-Oropeza et al., 2015; Paes et al., 2016; Traller & Hildebrand, 2013).

As for lipid concentrations, these results are similar to those found by (Li et al., 2008; Machado & Lourenço, 2008; Millán-Oropeza et al., 2015; Paes et al., 2016). Fiber was higher than CO and was, therefore, higher than what was found by (Kalpa W et.al., 2015).

**Fatty acids:** Several pieces of research concluded the presence of UFAs in algae lipids; they have been considered as wellsprings of PUFAs for the aquaculture industry, as stated by (Patil et al., 2005).

Oleic acid was recorded in *N.oculata*, and these results are also consistent with those of his findings (Ötleş & Pire, 2001). Furthermore, (Gerasimenko et al., 2010) stated that algal lipids could be a source of polyunsaturated fatty acids (PUFAs) of ω-3 and ω-6 series. In this current study, PUFAs ω-6 have been detected with nearly high concentrations in *N.oculata*. These results were higher than the results of (Cavonius et al., 2015; Malakootian et al., 2015; Olofsson et al., 2014), who revealed that the PUFAs contents in *N.oculata* were low, which is due to the type of media used.

Typical amino acid analysis of experimented microalgae is shown in Table 4. It indicated the presence of essential amino acids in the profile of screened microalgae. Essential amino acids cannot be made by the body, as a result, they must come from food. From the nine essential amino acids, seven are found in the profile of algae: Histidine, Leucine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine.

The high protein content of various microalgae species is one of the main reasons to consider them as an unconventional source of protein (Soletto et al., 2005) which is well-illustrated by the great interest in microalgae as a single-cell protein (SCP) during the 1950s. In addition, the amino acid pattern of almost all algae compares favorably with that of other food proteins. Since the cells are capable of synthesizing all amino acids, they can provide the ones essential to humans and animals (Guil-Guerrero et al., 2004). As other bioactive compounds are synthesized by microalgae, amino acids, especially the free amino acids, vary greatly between species as well as with growth conditions and growth phase (MA, 1988). The amino acid profile of the experiment showed a good pattern including essential amino acids which cannot be produced by the human body. These are needed to be absorb by external sources. The results were higher than what was reached by (Safi et al., 2013) for the same algae with a different type and ratio of nitrogen source, and also with the results of (Safi et al., 2014).

## CONCLUSION

The study showed that the percentage of protein was higher in A4, fat percentage was higher in A1, fiber was higher in A1, and carbohydrates were higher in A3.

In summary, using urea as a source of nitrogen at different rates gives high protein and fat rati-

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os as well as the essential amino acids and fatty acids content of the microalgae.

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# تأثير مستويات مختلفة من اليوريا كونها مصدرا للنيتروجين على التركيب الكيميائي للطحالب البحرية المجهرية نانوكلوربس أوكولاتا

**على محمود أبوغرارة**

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**المستخلص:** يجب أن تكون وسائط تربية الطحالب الدقيقة فعالة من حيث التكلفة، وتتيح نموًا عاليًا، وتفي بالمتطلبات الدقيقة، وتكون متاحة بسهولة. تم تقييم تأثير المستويات المختلفة من اليوريا [25 ، 50 ، 75 و 100٪] في وسط النمو على المكونات الكيميائية الحيوية (البروتين، والكربوهيدرات، والدهون، والأحماض الدهنية، والأحماض الأمينية) في نانو كلوريس أوكولاتا مقارنةً بـ F / 2 جيلارد كوسط قياسي. أوضحت النتائج المتحصل عليها أن المكونات الكيميائية للطحلب قد تأثرت بالمستويات المختلفة لليوريا المستخدمة. تم الحصول على أعلى نسبة بروتين باستخدام بيئة الزرع A4 (100٪ يوريا) (26.44٪) و A3 وسط (75٪ يوريا) (25.84٪)، وأعلى نسبة من الأحماض الأمينية الأساسية (EAA) (51.54٪) باستخدام بيئة الزرع A4 (100٪ يوريا) بالمقارنة مع الوسط القياسي (100٪ F / 2). تم تحقيق أعلى محتوى إجمالي للدهون باستخدام بيئة الزرع A1 (25٪ يوريا) ينتج (17.33٪) وبيئة الزرع A4 (100٪ يوريا) (16.98٪). وفقًا لذلك، تم تسجيل أعلى نسبة إجمالية للأحماض الدهنية المشبعة (TSFA) للطحلب بواسطة بيئة الزرع A3. خلصت الدراسة الى ان اضافة اليوريا هي ساسة ممتازة لزيادة المكونات الكيميائية وتجمع الدهون. أوصت الدراسة الحالية بإمكانية استخدام بيئة الزرع A4 المقترح كونها محفزا للدهون، أو بيئة الزرع A1 كونها محفزا للبروتين.

**الكلمات المفتاحية:** الأحماض الأمينية، الأحماض الدهنية، الطحالب البحرية المجهرية، التركيب الكيميائي.

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