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Original Article

Evaluation of the effect of using soaked grated beans (SGB) as a nitrogen source and sodium bicarbonate (SB) as a carbon source on the chemical composition, fatty acid, and amino acid content of the marine microalga *Nannochloropsis oceanica*

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ABSTRACT

Microalgae culture media should be cost-effective, enable high growth, meet precise requirements, and be readily available. In this study, the effect of different levels of sodium bicarbonate (SB) and Soaked grated beans (SGB) in the growth medium on the biochemical components (protein, carbohydrate, lipid, fatty acids, and amino acids) of *Nannochloropsis oceanica* was evaluated compared to standard Gaillard F/2 medium. Treatments were performed as follows: - control (OCO): Gaillard F/2 medium, OD1: 75 % of SB+25% of SGB, OD2: 50% SB+50% of SGB and OD3: 25%SB+75%of SGB. The results obtained revealed that the biochemical components of *N. oceanica* are affected by the level of sodium bicarbonate and bean sprout infusion. The highest protein and carbohydrate contents were obtained from the OD3 treatment, the highest lipid content from the OD1 treatment and the highest EAA content (54.41%) were obtained using OD3 medium compared to the control group (100% F/2). The highest biomass productivity was obtained from OD3 treatment followed by OD2 and the highest total saturated fatty acids (TSFA) content of *N. oceanica* was recorded by OD2 medium.

Keywords: Amino acids, Fatty acids, *N. oceanica*, Proximate composition

1. INTRODUCTION

Microalgae are a valuable source of bioactive substances and a treasure trove of resources. Their high capacity to produce various valuable products such as lipids, proteins, and sugars has been highlighted (Capek *et al*., 2023). Due to their high growth rates, these organisms can be easily cultured in closed bioreactor systems to obtain a high percentage of biomass (Vona, *et al*.,2018; Serive *et al.*,2012).

Since microalgae cultivation does not compete with terrestrial plant cultivation due to the use of different resources, the cultivation and utilization of microalgae biomass represents an excellent opportunity to develop alternative compounds for various bioapplications. Since 2014, the focus of algae research has value gradually shifted toward high-bioproducts and environmental applications (Rashid *et al*., 2018).

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Algae are currently being developed as a new source of polyunsaturated fatty acids (PUFAs) such as linolenic, Eicosapentaenoic, docosapentaenoic and Docosahexaenoic acids (Chen *et al*., 2021; Cui *et al*., 2021).

Algal Docosahexaenoic acids (DHA) has already been commercialized. Although the currently available deep-sea oil PUFAs are derived from fish, they are mainly derived from the oil accumulated by algae ingested by fish (Ebm *et al*., 2021).

This study aimed to measure the effect of using soaked grated beans and sodium bicarbonate on the chemical composition of the marine microalgae *Nannochloropsis oceanica*.

2.MATERIALS AND METHODS

2.1. Microalgal strains

Nannochloropsis oceanica strain was from an algae unit of the marine hatchery at Kilo 21 Alexandria – Egypt. *N. oceanica* was kept at the Institute of Oceanography and Fisheries (NIOF), Egypt, and cultured under controlled conditions of temperature (22± 2C˚), and salinity (35 ± 2 ppt). F/2 medium (Guillard and Rhyter, 1962), with continuous ventilation and 8:16 h of light, was used in three replicates. Dry weight (CDW) and chemical composition of algal cells (10 days after culturing) were determined. And dry weight (CDW) was determined, according to (Abomohra, *et al.*, 2013)

2.2. Experimental design

The F/2 medium contained (mgL^{-1}) $NaNO_3$,75; $NaH_2PO_4.H_2O$, 5; Na_2 EDTA.H_2O, 4.16; $FeC1_3.6H_2O$, 3.15;$CuSO_4.5H_2O$,0.01;$ZnSO_4.7H_2O$,0.022;COC12.6H 20,0.01;$MnC1_2.4H_2O$,0.18;$Na_2MoO_4.2H_2O$,0.006;Vita min B12, 0.0005; Vitamin B1, 0.1; and Bi-tin, 0.0005 (Guillard and Rhyter, 1962).

Table (1): Design of the experiment used to grow *Nannochloropsis oceanica*

Treatments	OCO	OD1	OD2	OD3
control F/2	100	------	------	------
Sodium bicarbonate (SB)	---	0.75	0.50	0.25
Soaked grated beans (SGB	-----	0.25	0.50	0.75

Control (OCO), Treatment1(OD1), Treatment2(OD2), Treatment3 (OD3)

2.3. Culture conditions

Liter plastic containers filled with sterile seawater (35 ± 2) ppt were used. 5 grams of grated beans were taken and soaked in 50 ml of sterile water for 30 minutes, placed on the stove for 15 minutes, and left to cool**.** 1.5 The solution was filtered with filter paper and used at the levels as shown in (Table 1).

2.4. Estimation of the biochemical constituents of N. oceanica

The total protein and carbohydrate content were estimated by (Lowry et al., 1951) using Bovine Serum Albumin (BSA) as a standard. (Dubois et al., 1956) and quantification of total carbohydrate "phenol-sulfuric acid" using d-glucose µg/ml as a standard.

Biomass productivity (mg L^{-1}/ day) = (CDWL – CDWE) x (tL- tE)-1 With CDWE representing the CDW (mg/l) on the days of the early exponential phase (tE) and CDWL on the days of the late exponential phase (tL). (Abomohra, *et al*., 2016).

2.5.Total lipid content and fatty acids profile

Total lipids and fatty acids were extracted as described by (Folch *et al*. 1957; Bligh and Dyer, 1959). The fatty acids were determined using methyl ester from total fat according to a procedure (Radwan, 1978).

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

2.6. Amino acids determination

Amino acids of *N. oceanica* 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 (DionexICS3000) (Block, 1948).

2.7. Statistical analysis

the Statistical Package for the Social Sciences SPSS version 16 was used to Statistical analysis. One-way ANOVA was conducted to match the mean differences. Differences between means were set as significant at $P<0.05$ with least significant difference (LSD) for multiple ranges of post hoc comparisons.

3. RESULTS

Biochemical components change under different ratio of carbon source and nitrogen source Protein, carbohydrate and lipid are the main components of microalgal cells and are estimated to account for about 60%-85% of microalgal cells (Marcu, 2012). Except for stress conditions, autotrophic, heterotrophic and mixotrophic culture conditions have little effect on these components, which is an inherent physiological feature of microalgae (Marcu and Georgakakis, 2011).

Nannochloropsis oceanica was cultured at different concentrations as shown in Table (1) in the early stationary stage, from which samples were harvested for chemical composition analysis after the late stationary stage (10 days).

Cells dry weight and chemical composition were examined. The presented results indicated that there was no significant difference in dry weight (CDW) between the media containing different levels of mixture and control. Samples for analysis were harvested from *Nannochloropsis oceanica* after the stationary phase with different concentrations of sodium bicarbonate carbon source (10 days). Nitrogen source (soaked bean sprouts), where chemical analysis was performed and furthermore statistics were performed using general linear univariate model analysis (ANOVA). Differences between means were set as significant at $P< 0.05$ with least significant difference (LSD) for multiple ranges of post hoc comparisons used to resolve differences between means of replicates using SPSS (2007).
The presented results indicated that there was no significant difference in cell dry weight (CDW) between media containing different levels of SB, SGB and control. The data obtained (Table 2) showed significant differences in the biochemical composition of *N. oceanica* among the different treatments. The highest percentages of total protein and carbohydrates of dry weight (115.20% and 490.21%, respectively) were achieved by OD3 medium compared to controls and other treatments. The highest total lipid content (485.19%) was displayed by OD1 medium compared to controls and other treatments.

3.1. Biomass productivity and lipid productivity

The obtained data (Table 2) showed significant variations in the biomass productivity of *N. oceanica* between was exhibited byOD3 and OD2 medium relative to the control and other treatments.

Table (2): The Average biochemical composition (in % dry basis) mg/g DW of *N. oceanica* at different levels of sodium bicarbonate (SB) and Soaked grated beans (SGB) medium harvested after 10 days incubation period.

Items	CDW (g L^{-1})	Protein (%CDW)	Lipid (%CDW)	Carbohydrate (%CDW)	Biomass productivity (mg/l. day^{-1})
OCO	10.285 ±0.005^{d}	16.280± 0.010d	3.665±0. 005^{d}	18.370±0.010^{d}	67.78±0.02^{d}
OD1	10.655 ±0.005^{b}	18.335± 0.005c	19.485± 0.005^{a}	21.070±0.010^{b}	87.65±0.02^{c}
OD2	10.415 ±0.005^{c}	19.080± 0.010b	18.165± 0.005^{b}	20.125±0.005^{c}	94.06±0.02^{b}
OD3	10.100 ±0.010^{a}	20.115± 0.005^{a}	15.850± 0.010^{c}	21.490±0.010^{a}	98.15±0.02^{a}

3.2. Fatty acid profiles

The fatty acid profile of *N. oceanica* is presented in Table 3. The data revealed no change in the fatty acid profile among the different treatments. In contrast, there was a significant change in the content of each individual fatty acid among the different treatments.
The most abundant saturated fatty acid was palmitic acid (C16:0), which recorded its highest value (26.140%) with OD2 medium than the other media.

In addition, oleic acid (C18:1) was the most prevalent monounsaturated fatty acid in all treatments, however, its value decreased to reach the minimum (14.130%) with OCO medium. Palmitoleic acid (C16:1) showed the highest value (6.120%) with OD3 medium, while it recorded the lowest value with F/2 medium. Eicosapentaenoic acid (EPA) was the second polyunsaturated fatty acid, as its highest percentage value (7.530%) was recorded with OD3 medium. Similarly, docosahexaenoic acid (DHA) was the third polyunsaturated fatty acid, as its highest value (11.660%) was recorded with the same medium. Moreover, linoleic acid (C18:2) was the most common polyunsaturated fatty acid with all treatments, as the data revealed that the highest value of this fatty acid (15.940%) was recorded with OD2 medium compared to control medium (10.350%).

Table (3): Total fatty acids profiles and their individual (%) of *N. oceanica* at different levels of sodium bicarbonate (SB) and Soaked grated beans (SGB) medium harvested after 10 days incubation period.

Fatty acid	Medium			
	OCO	**OD1**	**OD2**	**OD3**
C14:0 (Myristic acid)	3.520±0.01^{b}	4.780±0.01^{a}	4.520±0.01^{b}	3.880±0.01^{c}
C15:0 (Pentadecylic)	0.550±0.01^{d}	0.820±0.01^{c}	0. 870±0.01^{a}	0.823±0.01^{b}
C16:0 (Palmitic)	20.280±0.01^{d}	25.330±0.01^{b}	26.140±0.01^{a}	24.770±0.01^{c}
C17:0 (Margaric)	0.320±0.01^{d}	0.530±0.01^{b}	0.620±0.01^{a}	0.520±0.01^{c}
C18:0 (Stearic)	3.650±0.01^{d}	4.170±0.01^{b}	4.130±0.01^{c}	4.660±0.01^{a}
C21:0 (Heneicosylic)	0.680±0.01^{c}	1.170±0.01^{c}	1.360±0.01^{b}	1.370±0.01^{a}
C24:0 (Lignoceric)	1.420±0.01^{d}	1.880±0.01^{b}	1.770±0.01^{c}	1.930±0.01^{a}
∑Saturated (SFA)	30.42±0.01^{c}	38.68±0.11^{a}	39.41±0.01^{a}	37.95±0.21^{b}
C14:1 (Myristoleic acid)	0.134±0.01^{a}	0.131±0.01^{c}	0.130±0.01^{d}	0.132±0.01^{b}
C15:1 (cis-10-pentadecenoic)	0.074±0.01^{a}	0.072±0.01^{b}	0.070±0.01^{d}	0.071±0.01^{c}
C16:1 (Palitoleic)	4.380±0.01^{d}	5.680±0.01^{c}	5.770±0.01^{b}	6.120±0.01^{a}
C17:1 (Heptadecenoic)	0.470±0.01^{d}	0.540±0.01^{c}	0.580±0.01^{a}	0.570±0.01^{b}
C20:1 (Paullinic)	2.150±0.01^{a}	1.360±0.01^{d}	1.580±0.01^{b}	1.470±0.01^{c}
C18:1n9 (Oleic)	14.130±0.01^{d}	19.320±0.01^{a}	18.680±0.01^{b}	18.670±0.01^{c}
C22:1 (Erucic)	0.550±0.01^{d}	0.680±0.01^{b}	0.670±0.01^{c}	0.730±0.01^{a}
∑Monosaturated (MUFA)	21.89±0.01^{b}	27.78±0.01^{a}	27.48±0.01^{a}	27.76±0.01^{a}
C18:2n6 (Linoleic)	10.350±0.01^{c}	15.260±0.01^{b}	15.940±0.01^{a}	15.780±0.01^{b}
C18:3n6 (y-Linoleic)	0.220±0.01^{d}	0.260±0.01^{c}	0.320±0.01^{a}	0.270±0.01^{b}
C18:3n3 (α- Linoleic)	1.320±0.01^{d}	2.273±0.01^{a}	1.770±0.01^{c}	1.830±0.01^{b}
C20:2n6 (Eicosadienoic)	0.760±0.01^{a}	0.630±0.01^{c}	0.720±0.01^{b}	0.580±0.01^{d}
C20:5n3 (Ecosapentaenoic)	6.680±0.01^{d}	7.250±0.01^{c}	7.480±0.01^{b}	7.530±0.01^{a}
C22:6n3(Docosahexaenoic)	7.660±0.01^{d}	10.440±0.01^{c}	10.680±0.01^{b}	11.660±0.01^{a}
∑Polyunsaturated (PUFA)	26.99	36.11	36.91	37.65
∑Usaturated	48.88	63.89	64.39	65.41
Sat./Monosat.	1.39	1.39	1.43	1.37
Sat./Polsat.	1.13	1.07	1.07	1.01
Sat./Unsat.	0.62	0.61	0.61	0.58
∑U-3	15.66	19.96	19.93	21.02
∑U-6	11.33	16.15	16.98	16.63
∑U-3/∑U-6	1.38	1.24	1.17	1.26
EPA/DHA	0.87	0.69	0.70	0.65
DHA/EPA	1.15	1.44	1.43	1.55

Low percentage values of linolenic acid (C18:3) were detected with control medium and OCO medium. However, the highest value of linolenic acid was achieved by OD1 medium (2.273%). The results revealed that the highest percentage of total saturated fatty acids (TSFA) (39.41%) was achieved by OD2 medium, which was higher than the percentage of TSFA (30.42%) recorded by control medium (CO) (100% F/2). The present study showed that the highest percentage of total unsaturated fatty acids (USFA) (65.41%) was detected by OD3 medium, where this percentage mainly consisted of 76.27% MUFA and 65.37% PUFA. On the other hand, the highest ratio (0.62) between SFA/USFA was achieved by control medium OCO, in addition, the highest ratios between n-3/n-6 and DHA/EPA were shown by OCO 1.38% and 1.55% were shown by OD3 medium, (Table 3).

3.3.Amino acid profiles

Results of amino acid profiles of different culture media of *N. oceanica* diet were presented in (Table 4). The present study revealed no change in the amino acid profile among different media. In contrast, there was a clear variation in the content of each individual amino acid among different treatments. The results showed that *N. oceanica* recorded the highest content of essential amino acids EAA (54.41%) by OD3 medium, while the lowest value was achieved by OCO medium. (Table 4). Non-essential amino acids (NEAA), where the highest content of non-essential amino acids NEAA (50.93%) was detected by OCO medium, while the lowest value of NEAA was achieved by OD3 medium.

Table (4): Amino acids profile (%) in *N. oceanica* at different levels of sodium bicarbonate (SB) and Soaked grated beans (SGB) medium harvested after 10 days incubation period.

Amino acid (AA)%	**Medium**			
	OCO	**OD1**	**OD2**	**OD3**
	Essential amino acids (*EAA*)			
Arginine	5.530±0.01^{d}	5.720±0.01^{c}	6.220±0.01^{b}	6.450±0.01^{a}
Histidine (HIS)	1.840±0.01^{d}	2.580±0.01^{c}	3.260±0.01^{b}	4.420±0.01^{a}
Isoleucine (ILE)	3.740±0.01^{d}	4.280±0.01^{c}	5.320±0.01^{b}	5.470±0.01^{a}
Leucine (LEU)	9.160±0.01^{a}	5.670±0.01^{c}	6.150±0.01^{d}	6.840±0.01^{b}
Lysine (LYS)	4.330±0.01^{d}	7.320±0.01^{b}	7.430±0.01^{a}	7.130±0.01^{c}
Methionine (MET)	4.420±0.01^{d}	4.730±0.01^{b}	4.840±0.01^{a}	4.470±0.01^{c}
Phenylalanine (PHE)	6.520±0.01^{a}	5.630±0.01^{c}	5.730±0.01^{b}	5.320±0.01^{d}
Threonine (THR)	5.640±0.01^{a}	4.530±0.01^{d}	4.680±0.01^{c}	4.770±0.01^{b}
Tryptophan (TRP)	1.960±0.01^{d}	4.220±0.01^{c}	4.320±0.01^{b}	4.370±0.01^{a}
Valine (VAL)	5.970±0.01^{a}	5.450±0.01^{b}	5.260±0.01^{c}	5.170±0.01^{d}
Total EAA	49.11	50.13	53.21	54.41
	Non-essential amino acids (*NEAA*)			
Alanine (ALA)	6.660±0.01^{a}	5.340±0.01^{d}	5.380±0.01^{c}	5.450±0.01^{b}
Aspartate (ASP)	10.130±0.01^{a}	8.370±0.01^{b}	8.320±0.01^{c}	7.630±0.01^{d}
Cystine (C-C)	4.450±0.01^{c}	4.670±0.01^{a}	4.180±0.01^{d}	4.640±0.01^{b}
Glutamine (GLU)	11.250±0.01^{a}	9.463±0.12^{b}	8.270±0.01^{c}	7.320±0.01^{d}
Glycine (GLY)	4.350±0.1^{d}	5.630±0.01^{a}	5.420±0.01^{c}	5.523±0.01^{b}
Proline (PRO)	5.250±0.01^{d}	8.240±0.01^{a}	7.320±0.01^{b}	7.220±0.01^{c}
Serine (SER)	6.320±0.01^{a}	5.660±0.01^{c}	5.680±0.01^{b}	5.430±0.01^{d}
Tyrosine (TYR)	2.520±0.01^{c}	2.420±0.01^{d}	2.530±0.01^{b}	2.640±0.01^{a}
Total NEAA	50.93	49.79	47.1	45.85

4. DISCUSSION

Studies to improve culture conditions are necessary to increase the efficiency and economic value of microalgae production in the future. New methods of extraction, production and cultivation can be established efficiently to improve productivity and reduce costs. For more than 50 years, Guillard F/2 medium has been popular in marine aquaculture for microalgae cultivation, currently, due to the various uses of microalgae in different biotechnology fields; the use of natural materials.

Our results showed that some levels of sodium bicarbonate and Soaked grated beans achieved significantly higher biochemical components than F/2 medium (control).

The present study showed that low addition of SB and SGB to OD1, OD3 medium could improve the protein, carbohydrate, unsaturated fatty acid and EAA contents of *N. oceanica*, which may be demonstrated by increasing the concentration of inorganic dissolved carbon as an additional energy source. Similar results were found with *Chlorella pyrenoidosa* and *Scenedesmus obliquus* exposed to increased CO_2 (Yang and Gao, 2003; Srinivasan *et al*., 2018). Pancha *et al.* (2015) reported that bicarbonate addition increased the protein content of freshwater algae *Scenedesmus sp*.

Jegan *et al.* (2013) reported that the protein and carbohydrate contents of *Desmococcus sp*., *Chlorococcum sp*. and *Chlorella sp*. strains were elevated when they were grown in bicarbonate-supplied media.

The protein content of microalgae could be explained by internal nitrogen intake, possibly due to high level of nitrate intake (Table 2). The lower nitrogen level in OD1 medium than that in F/2 medium (control) caused an increase in carbohydrate content in OD1 medium due to nitrogen deficiency.

This result follows (Millán-Oropeza *et al*. 2015), who revealed that nitrogen starvation caused carbohydrate accumulation in *Chlorella sp*. In this study, replacing all nutrient salts from the culture in OD1 medium significantly decreased the protein content of *N. oceanica*. Similarly, Pancha *et al*. (2015) showed a decrease in protein content under nutrient deficiency conditions.

The present study also revealed that OCO medium significantly reduced carbohydrate content this finding is in contrast to (Pancha *et al*., 2015).

Fatty acid profiles (Table3) of *N. oceanica* grown under nitrogen source (SGB) and carbon source (SB) showed the C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:5, and C22:6. However, C16:1 was higher at OD3.

The percentage of fatty acid component was calculated (Table 3). The relative abundance of major fatty acid products varied among microalgae grown under OD2 sources. The main fatty acid composition of nitrate-grown microalgae consisted of 4.520%, 26.140%, 4.130%, 18.680%, 15.940%, 7.480%, and 10.680% of C14:0, C16:0, C18:0, C18:1, C18:2, C20:5, and C22:6. With the difference of OCO, OD1, and OD3, it is obvious that nitrogen source (SGB) and carbon source (SB) can affect the fatty acid composition of microalgae. Campos *et al.* (2014) reported the same lipid content in *N. salina* regardless of nitrogen source.

Moreover, the major fatty acids in *N. oceanica* when grown in f/2 medium were C16:0 and C16:1, which were close to those reported by (Xiao *et al.*, 2013).

One of the most prominent factors that affects the properties of biodiesel is profile of fatty acid, because the molecular features of FAMEs, including length of carbon chain and the double bond number, directly influence some characteristics of biodiesel such as the viscosity, ignition quality, oxidative constancy, and property of cold flow (Sing *et al.,* 2014; Knothe, 2009). Physicochemical conditions as well as growth phases can change the composition of fatty acid (Mata *et al*.,2010; Kim and Hur, 2013).

Serrano et al. (2014) found that oxidation stability and cold flow performance have reverse relationships to variations in composition of fatty acids. For example, the raise in unsaturated fatty acids (UFAs) would

improve the cold flow performance, while decreasing the oxidative stability. Inversely, the increase of saturated fatty acids (SFAs) could result in better oxidative stability but poor cold flow property.

CONCLUSION

From the results of this study, it was suggested that in terms of cost effectiveness and commercial availability, 75% sodium bicarbonate and 25% Soaked grated beans can be used to obtain high fat content and 25% sodium bicarbonate and 75% Soaked grated beans can be used to obtain high protein content and are the best for large-scale commercial cultivation of *N. oceanica* microalgae.

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