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FULL LENGTH ARTICLE

Application of Plackett–Burman design for the high production of some valuable metabolites in marine alga *Nannochloropsis oculata*

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Artemia franciscana

Abstract Microalgae have efficient nutritional influence to obtain high survival growth and quality of fish larvae and to promote the growth of brine shrimp. In this work the Plackett–Burman statistical design was applied to specify which nutrient factor(s) optimize the nutritional contents [protein, carbohydrate, β-carotene, ascorbic acid and free radical scavenging activity (DPPH)] in the marine alga *Nannochloropsis oculata* used in aquaculture to maximize marine hatchery production. *N. oculata* was cultured on F/2 medium (as control) to reach its maximum growth. The obtained results showed that the maximum growth, chlorophyll-*a*,*b* and carotenoid contents were attained after 10 days. The contents of all studied parameters in *N. oculata* grown on the optimized medium after10 days increased significantly ($P \leqslant 0.1$) than those on control with low concentration of PO_4 ($2.5\ g\ l^{-1}$) and with high concentration of NO_3 ($112.5\ g\ l^{-1}$) except for cell numbers and DPPH. Significant increases in the protein, carbohydrate, ascorbic acid, β-carotene and DPPH in *Artemia franciscana* enriched with *N. oculata* cultured on the newly optimized medium were observed.

Introduction

Microalgae play an essential role in the nutrition of some marine animals and consequently in aquaculture (Khairy and El-Sayed, 2012). However, these algae must possess a number of key attributes to be useful aquaculture species including: size for ingestion and rapid growth rates, stable in culture to any fluctuations in light, temperature and nutrients as may occur in hatchery systems, and nutrient composition including an absence of toxins that might be transferred up the food chain (Guedes and Malcata, 2012).

The nutritional value of the microalgal diet is related to its ability to supply essential micro and macronutrients, becoming efficient as marine animal nutrients. These nutrients include protein, carbohydrates, vitamins and lipids. Protein content

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is a major factor determining the nutritional value of microalgae (Reitan et al., 1997; El-Sayed and Khairy, 2013). Microalgae represent a valuable source of nearly all essential vitamins, however, most animals can synthesize vitamins C and more specifically ascorbic acid from glucuronic acid, fish and crustaceans lack the enzyme gulonolactone oxidase necessary for the last step in this biosynthesis (Dabrowski, 1990). In addition, some microalgae are considered as rich sources of natural antioxidants (Huang and Wang, 2004). Also, carotenoids have antioxidative properties (Fang et al., 2002; Ahmed et al., 2014). Carotenoids have been used as feed for aquaculture, they are important antioxidants for human health and they are involved in other biological functions (Stahl and Sies, 2012). The β-carotene is an important carotenoid that may assist in improving color of salmon fish, and crustaceans (Laurent et al., 2005).

Nutritional requirement of marine animals from microalgae can be manipulated by the conventional or statistical methods. Conventional method involves changing one independent variable at a time, while keeping others at affixed level. This method is often used to screen suitable carbon and nitrogen sources (Bajaj et al., 2009). Plackett–Burman design is a well established and widely used statistical technique for selecting the most effective components with high significance levels for further optimization, ignoring interactions among variables (Plackett and Burman, 1946). The Plackett–Burman design was favorably used by many researchers (e.g. Haque et al., 2012; Amit, 2013) to optimize the chemically definite medium and to screen which medium components give a maximum growth. Reddy et al. (2012) studied the effect of pH, light intensity, temperature and constituents of the medium on growth of *Anabaena ambigua* using Plackett–Burman design to enhance the biomass.

The present work aims to develop an optimized medium by using Plackett–Burman design to increase the nutritive value (cell number, contents of protein, carbohydrate, and some antioxidants (β-carotene, ascorbic acid and DPPH)) of the marine microalga *Nannochloropsis oculata*, which is commonly used in aquaculture. The study also monitors the growth and metabolic compositions of the brine shrimp *Artemia franciscana* enriched with *N. oculata* grown on the optimized medium.

Materials and methods

Alga cultivation and growth measurement

N. oculata was obtained from the National Institute of Oceanography and Fisheries, Alexandria, Egypt. It was cultured in filtered sterilized seawater enriched F/2 medium (Guillard and Ryther, 1962). Algal inoculum size used for inoculating the flasks was 15×10^4 cell ml^{-1} for each experiment. The culture flasks were incubated under continuous fluorescent light of 80 μmol m^{-2} s^{-1} and temperature at 26 ± 1 °C. To estimate the period of the maximum growth of *N. oculata*, cell number, and photosynthetic pigments (chlorophyll-*a*,*b* and carotenoids) were measured. Cell number was counted using hemocytometer. Contents of photosynthetic pigments were determined spectrophotometrically following the method of Mckinney (1941).

Applying Plackett–Burman experimental design

The Plackett and Burman statistical design (1946) was developed for 7 variables (elements) over 8 runs. Each independent variable was investigated at a high (+) and a low (−) level. The low level (−) was taken as a half concentration in the given media (F/2), while the high level (+) means that the element is present by 150% (i.e. one and half of the variable concentration in the standard F/2 medium).

Table 1 illustrates an array for $n = 8$ trials that well test 7 independent variables ($NaNO_3$, NaH_2PO_4, Na-EDTA, $FeCl_3$, $CuSO_4{\cdot}5H_2O + ZnSO_4{\cdot}7H_2O$, $CoCl_2{\cdot}6H_2O + MnCl_2{\cdot}4H_2O$ and $Na_2MoO_4{\cdot}2H_2O$), while vitamin concentration was constant. The main effect of each variable was determined with the following equation:

$$E\,\mathrm{xi} = \left(\sum \mathrm{Mi}+ - \mathrm{Mi}-\right)/N$$

where E xi is the main variable effect, and Mi+ and Mi− are the results in trials. The independent variable (xi) was present in the high and low concentrations, respectively, and N is the number of trials divided by 2. The main effect with a positive sign indicates that high concentration of this variable is near to optimum and the negative sign indicates that low concentration of this variable is nearer to optimum. Standard

Table 1 Plackett and Burman experimental design with low and high levels of selected seven elements.

Trials ($n = 8$)	Variables (factors)						
	$NaNO_3$	NaH_2PO_4	Na-EDTA	$FeCl_3$	$CuSO_4{\cdot}5H_2O + ZnSO_4{\cdot}7H_2O$	$CoCl_2{\cdot}6H_2O + MnCl_2{\cdot}4H_2O$	$Na_2MoO_4{\cdot}2H_2O$
	g L^{-1} stock solution				g 100 ml^{-1} stock solution		
+level	112.5	7.5	6.54	4.73	1.5 + 3.3	1.5 + 2.7	0.945
−level	37.5	2.5	2.18	1.58	0.5 + 1.1	0.5 + 0.9	0.315
1	+	+	+	−	+	−	−
2	+	+	−	+	−	−	+
3	+	−	+	−	−	+	+
4	−	+	−	−	+	+	+
5	+	−	−	+	+	+	−
6	−	−	+	+	+	−	+
7	−	+	+	+	−	+	−
8	−	−	−	−	−	−	−

t-values for sample assuming unequal variances are obtained from statistical methods (Cochran and Snedecor, 1989) by using Microsoft Excel for the determination of the variable significance.

Brine shrimp (A. franciscana) culture

A. franciscana was produced by hatching *Artemia* cysts through decapsulation. They were incubated in seawater to hatch as described by Lavens and Sorgeloos (1996). The produced nauplii were harvested after 24 h, and then washed with filtered seawater, and after 6 h from hatching time, they were enriched with *N. oculata* grown on F/2 (control) and optimized medium for 24 h. The enriched *Artemia* was harvested by plankton net (100 μm), and its nutritional value was evaluated in terms of contents of total protein, carbohydrate, ascorbic acid, β-carotene and DPPH free radical scavenging activity.

Determination of the biochemical constituents in N. oculata and A. franciscana

Content of total protein was measured using the method of Bradford (1976), whereas total carbohydrate was quantitatively determined by the method of phenol–sulfuric acid as described by Kochert (1973). β-Carotene and ascorbic acid in both alga and *Artemia* were extracted and estimated quantitatively following Herrero-Martinez et al. (2006) and Oser (1979), respectively. Free radical scavenging activity (DPPH) of the algal and *Artemia* extracts (concentrations of 25, 50 and 100 μg ml^{-1}) was evaluated spectrophotometrically at 517 nm against the absorbance of the indicator 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (20 mg L^{-1}) as described by Lee et al. (2002). The percentage of DPPH–decolorization was calculated using the following equation:

$$\text{Free radical scavenging } \% = 1 - (\text{Ac} - \text{As})/\text{Ac} \times 100$$

where Ac = absorbance of control and As = absorbance of sample.

Statistical analysis

Results were presented as mean ± SD (standard deviation) for three replicates. The statistical analyses were carried out using SAS program (1989–1996) version 6.12. Data obtained were analyzed statistically to determine the degree of significance between treatments using one way analysis of variance (ANOVA) at $P \leqslant 0.05$ and 0.01.

Results

Algal growth in F/2 medium

Figs. 1 and 2 indicate that the cell number and contents of chlorophyll-*a*, *b* and carotenoids of *N. oculata* grown in F/2 medium increased gradually from the day zero to the 10th day (stationary phase) and then decreased gradually till the14th day (death phase).

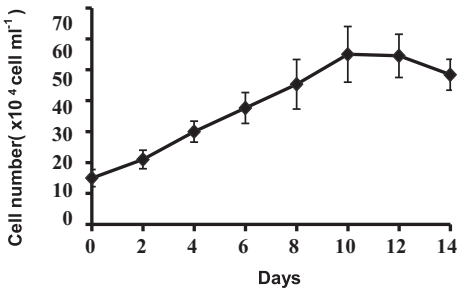

Figure 1 Mean cell number (× 10^4 cell ml^{-1}) of *Nannochloropsis oculata* grown in F/2 medium during the incubation period. Each value is the mean of three readings ± standard deviation.

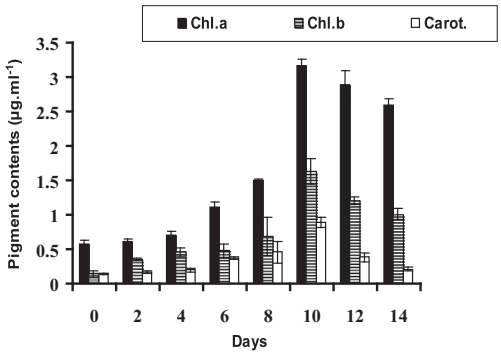

Figure 2 Chlorophyll-*a* and *b* and carotenoid contents (μg ml^{-1}) in *Nannochloropsis oculata* grown in F/2 medium during the incubation period. Each value is the mean of three readings ± standard deviation.

Nutritional factors optimizing algal growth based on Plackett–Burman experimental design

Applying Plackett–Burman experimental design showed that the maximum cell number of *N. oculata* was obtained on trial 5 (+ve concentration of NO_3, Fe, Cu + Zn and Co + Mn, while −ve concentration of PO_4, EDTA and MoO_4) (Table 2). There were three variables of the trace elements (Cu + Zn, Co + Mn and MoO_4) that caused +ve main effects, while the other variables caused negative ones (Fig. 3A). Results in Fig. 3A show that only the effects of PO_4 and Co + Mn are significant at $P \leqslant 0.05$. In trial 5, *N. oculata* yielded the highest protein content, where PO_4, Fe and Cu + Zn have a −ve main effect on the protein content (Fig. 3B), while the other variables have a +ve main effect. Statistical analysis for significance of the seven tested variables estimated that NO_3 was the significant variable at $P \leqslant 0.05$. Concerning carbohydrate contents in *N. oculata*, the maximum production was 0.12 mg ml^{-1} which was represented also in trial 5 (Table 2). According to the levels of variables, it is observed from Fig. 3C that PO_4, and EDTA caused a negative main effect on the carbohydrate contents, while the other variables have a positive main effect, and NO_3 was the significant variable on carbohydrate contents at $P \leqslant 0.05$.

The maximum β-carotene content production in *N. oculata* at day 10 was 10.25 mg 100 g^{-1} represented in trial 5 (Table 2). The result represented in Fig. 3D cleared that the NO_3, Cu

Table 2 Cell number and the nutritional compositions of *Nannochloropsis oculata* after applying the Plackett–Burman experimental design.

Trials ($n = 8$)	Element (factor)							Cell number ($\times 10^4$ cell ml^{-1})	Protein mg ml^{-1}	Carbohydrates mg ml^{-1}	β-Carotene mg 100 g^{-1}	Ascorbic acid mg g^{-1}	DPPH		
	NO_3	PO_4	EDTA	Fe	Cu + Zn	Co + Mn	MoO_4						100 µl	50 µl	25 µl
1	+	+	+	−	+	−	−	68.4 ± 4.7	0.08 ± 0.005	0.06 ± 0.004	2.93 ± 0.1	2.62 ± 0.07	47.09 ± 2.7	33.78 ± 3.5	24.45 ± 4.2
2	+	+	−	+	−	−	+	62.7 ± 8.7	0.09 ± 0.005	0.09 ± 0.006	4.75 ± 0.3	3.81 ± 0.04	49.81 ± 2.2	35.19 ± 3.2	27.68 ± 3.5
3	+	−	+	−	−	+	+	134.1 ± 5.5	0.092 ± 0.004	0.10 ± 0.005	5.77 ± 0.3	3.8 ± 0.02	52.01 ± 1.4	37.52 ± 2.5	30.4 ± 4.6
4	−	+	−	+	−	+	+	127.4 ± 3.7	0.075 ± 0.004	0.08 ± 0.005	6.75 ± 0.2	3.18 ± 0.02	55.37 ± 3.5	41.27 ± 3.3	33.25 ± 4.4
5	+	−	−	+	+	+	−	137.3 ± 7.5	0.098 ± 0.005	0.12 ± 0.01	10.25 ± 0.1	3.54 ± 0.04	59.38 ± 3.1	45.28 ± 4.4	36.74 ± 4
6	−	−	+	+	+	−	+	80 ± 13.5	0.058 ± 0.004	0.07 ± 0.004	4.06 ± 0.05	4.48 ± 0.03	62.87 ± 3	47 ± 6	39.46 ± 5.7
7	−	+	+	+	−	+	−	77.2 ± 12	0.052 ± 0.002	0.06 ± 0.002	2.54 ± 0.06	3.06 ± 0.04	65.20 ± 3.1	49.68 ± 4.2	42.17 ± 5
8	−	−	−	−	−	−	−	119.2 ± 8.1	0.075 ± 0.004	0.05 ± 0.004	6.87 ± 0.07	1.25 ± 0.05	68.56 ± 1.7	52.13 ± 5.6	45.67 ± 4.8

+ Zn and Co + Mn have +ve main effects on β-carotene content, while the other variables showed a negative one, and only EDTA was the significant variable affecting β-carotene content at $P \leqslant 0.05$. Ascorbic acid in *N. oculata* cultured in trial 6 gave the maximum amount of ascorbic acid (Table 2), where Fe, EDTA, MoO_4 and Cu + Zn were present in +ve concentrations, while other elements were present in −ve concentrations. In the range of the examined data represented in Fig. 3E, the PO_4 have the −ve main effect, while the other variables showed +ve main effects on the ascorbic acid content. Statistical analysis showed that MoO_4 was the significant variable at $P \leqslant 0.05$.

The algal extracts have the highest DPPH scavenging capacity (Table 2), with increasing concentration of the extract (from 25 to 100 µg ml^{-1}) in trial 8, where all variables were present in −ve concentrations. Fe and Co + Mn have the positive main effects, while the other variables showed negative main effects on antioxidant activity of *N. oculata* (Fig. 3F). Statistical analysis showed that NO_3 was the significant variable affecting the DPPH scavenging capacity in *N. oculata* at $P \leqslant 0.05$.

Newly formulated culturing media based on the Plackett–Burman design

Compositions of the newly formulated media are shown in Table 3. The media components showed that, NO_3 was present at low concentration (−ve sign) giving higher cell number and DPPH free radical scavenging activity, while the higher production of the other metabolites was present with high NO_3 concentration (+ve sign). On the other hand, low PO_4 concentration (−ve sign) gives higher production of all studied parameters in *N. oculata*.

Culturing N. oculata in the optimized medium

As shown in Table 4 there was a significant increase in the cell numbers of *N. oculata* on the new optimized medium as compared with F/2 medium after 10 days culturing, and these increases were about 4 folds ($P \leqslant 0.05$). The percentage of increase of total protein in *N. oculata* cultured on optimized medium was about 48.9% as compared with F/2 medium (control). ANOVA test showed a significant increase in protein content at $P \leqslant 0.1$ (Table 4).

Carbohydrate contents in *N. oculata* cultured on the optimized medium for 10 days were higher about 110% than that cultured on the F/2 medium (Table 4). From the statistical analysis, it was obvious that, there was a highly significant increase in carbohydrate content at $P \leqslant 0.05$.

Ascorbic acid content increased about 28.3% in *N. oculata* cultured on the optimized medium than that cultured on F/2 medium (Table 4). One way analysis of variance estimated a high significant increase in ascorbic acid in *N. oculata* at ($P \leqslant 0.01$). β-Carotene content increased about 26.2% in *N. oculata* cultured on the optimized medium than that on F/2 medium. The suggested statistical model (ANOVA) revealed that, there was a highly significant increase in β-carotene content at $P \leqslant 0.05$ (Table 4).

DPPH scavenging activity of *N. oculata* cultured on the optimized medium increased by 53.5%, 26.1% and 18.4% at the three different concentrations, respectively as compared

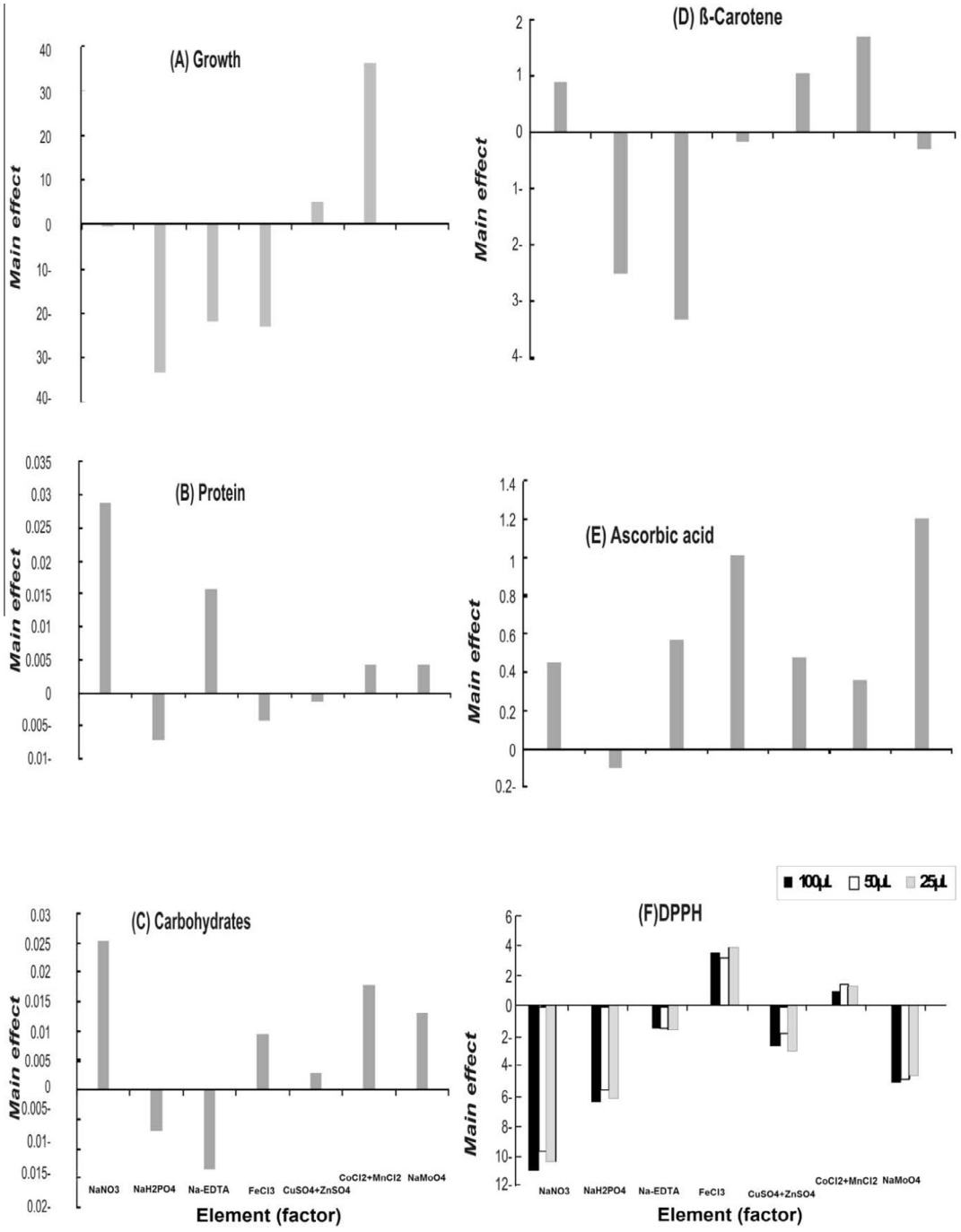

Figure 3 The main effects of the different variables affecting the growth and different metabolites in *Nannochloropsis oculata*.

with F/2 medium (Table 4), and these increases were highly significant at $P \leqslant 0.05$.

Nutritional value of A. franciscana

The data recorded in Table 5 reveal that the optimized parameters (protein, carbohydrate, ascorbic acid and β-carotene) in *A. franciscana* enriched with *N. oculata* cultured on the optimized medium were increased by 65%, 28%, 40.5% and 14.1%, respectively as compared with the *A. franciscana* fed on alga cultured on the F/2 medium. Statistical analysis cleared that there were highly significant increases of protein and β-carotene in *A. franciscana* fed on *N. oculata* cultured on the newly optimized medium at $P \leqslant 0.001$ while carbohydrates and ascorbic acid increased significantly at $P \leqslant 0.01$. On the other hand, there was a significant increase ($P \leqslant 0.001$) in DPPH scavenging activity in *A. franciscana* enriched with *N. oculata* cultured on the newly optimized medium than that fed on alga grown on F/2 medium (Table 5).

Table 3 Constituents of the newly formulated media for optimization of the cell number, protein, carbohydrate, ascorbic acid, and β-carotene and DPPH free radical scavenging activity in *Nannochloropsis oculata*.

Parameter	Variable (factors)							
	NO_3	PO_4	EDTA	Fe	Cu + Zn	Co + Mn	MoO_4	Vitamins ml L^{-1}
	g L^{-1} stock solution				g 100 ml^{-1} stock solution			
+level	112.5	7.5	6.54	4.73	1.5 + 3.3	1.5 + 2.7	0.945	0.5
−level	37.5	2.5	2.18	1.58	0.5 + 1.1	0.5 + 0.9	0.315	0.5
F/2 medium	75	5	4.36	3.15	1 + 2.2	1 + 1.8	0.63	0.5
Cell number	−	−	−	−	+	+	+	0.5
Protein	+	−	+	−	−	+	+	0.5
Carbohydrate	+	−	−	+	+	+	+	0.5
Ascorbic acid	+	−	+	+	+	+	+	0.5
β-Carotene	+	−	−	−	+	+	−	0.5
DPPH	−	−	−	+	−	+	−	0.5

Table 4 Cell number, total protein, carbohydrate, ascorbic acid, and β-carotene and DPPH free radical scavenging activity in *Nannochloropsis oculata* grown on F/2 medium as compared with optimized medium after 10 days culturing.

Algae	*N. oculata*	
Media	F/2 medium	Optimized medium
Cell number ($\times 10^4$ cell ml^{-1})	55 ± 9.0	205** ± 15
Total protein mg ml^{-1}	0.45 ± 0.1	0.67 ± 0.1*
Carbohydrate mg ml^{-1}	0.08 ± 0.004	0.17 ± 0.05**
Ascorbic acid mg g^{-1}	9.2 ± 0.4	11.8 ± 0.3*
β-Carotene mg 100 g^{-1}	11.17 ± 0.5	14.1 ± 0.2**
DPPH (25 µl) (contain 50 µg of extract)	31.81 ± 1.5	48.74 ± 2.1**
DPPH (50 µl) (contain 100 µg of extract)	50.63 ± 1.1	63.85 ± 1.8**
DPPH (100 µl) (contain 200 µg of extract)	65.74 ± 0.9	77.83 ± 1.2**

Each value is the mean of three readings ± standard deviation.
* Significant at $P \leqslant 0.01$ using one way analysis of variance.
** Highly significant at $P \leqslant 0.05$ using one way analysis of variance (ANOVA).

Table 5 Comparison between protein, carbohydrate, ascorbic acid and β-carotene contents in *Artemia franciscana* fed on *Nannochloropsis oculata* grown on the F/2 and optimized media.

Parameter	*A. franciscana* fed on *N. oculata* grown on F/2 medium	*A. franciscana* fed on *N. oculata* grown on optimized medium
Total protein mg ml^{-1}	39.3 ± 2.4	64.85 ± 5.7*
Carbohydrate mg ml^{-1}	83.2 ± 10.2	106.6 ± 5.8**
Ascorbic acid mg g^{-1}	261.6 ± 10	367.6 ± 14**
β-Carotene mg 100 g^{-1}	12.44 ± 0.4	14.2 ± 0.4*
DPPH (25 µl)	36.54 ± 2.0	52.84 ± 2.1*
DPPH (50 µl)	55.43 ± 1.3	69.35 ± 1.8*
DPPH (100 µl)	70 ± 1.50	77.8 ± 0.2*

Each value is the mean of three readings ± standard deviation.
* Highly significant at $P \leqslant 0.001$ using one way analysis of variance (ANOVA).
** Highly significant at $P \leqslant 0.01$ using one way analysis of variance (ANOVA).

Discussion

Many reports have proved the applicability of Plackett–Burman design in the optimization of media components for various culture activities (Haque et al., 2012). Applying the Plackett–Burman design estimates that variables caused the growth optimization in the tested *N. oculata* is not the same. The results showed that, NO_3 and PO_4 were the most important factors affecting the *N. oculata* growth and its studied parameters. The growth of *N. oculata* on the optimized medium significantly increased by 4 folds than on the control F/2 medium.

The protein content increased by 48.9% in *N. oculata* cultured in the optimized medium as compared with F/2 medium. This increase was the result of increasing the concentration of NO_3 by 1.5 times than F/2 (112.5 g l^{-1}). So nitrogen sufficient condition induced a marked increase in total soluble protein contents of *N. oculata*. Zaki and Saad (2010) concluded that, nitrate concentration at 1.5 times that of the basal medium was highly significant for optimization of the growth of *Chlorella salina* up to two times. Phosphorus is an important component required for normal growth and development of algal cells (Hu, 2004). In *N. oculata* decreases in phosphorus concentration (2.5 g l^{-1}) increase the protein content. These results agree with those of Kilham et al. (1997).

Carbohydrate synthesis in *N. oculata* increased significantly with culturing in the optimized medium, than when cultured on the F/2 medium. This increase was the result of increase in NO_3 and decrease in PO_4 concentrations. This result was in conformity with the result of Kilham et al. (1997) who reported that phosphorus starvation causes increases of the relative carbohydrate content in algal cells. Also, Thomas et al. (1984) reported an increase in the carbohydrate content of *Phaeodactylum tricornutum* when cultured in N sufficient medium. In accordance, Zaki and Saad (2010) reported that the content of carbohydrate in *Nannochloropsis salina*

increased by 98% as compared to the basal medium at 1.5 times of NO_3 that of the basal medium.

β-Carotene and ascorbic acid synthesis in *N. oculata* increased by 26.2% and 28.3%, respectively with culturing in the optimized medium, than when cultured on the F/2 medium. This increase was the result of increase in NO_3 and decrease in PO_4 concentrations.

On the contrary, Sujatha and Nagarajan (2013) demonstrated that the carotenoid production by *Spirulina platensis* content was found to be increased at low levels of nitrogen while Phadwal and Singh (2003) reported that the increase of β-carotene accumulation in *Dunaliella salina* was obtained with phosphorus starvation and this finding agreed with the present study. In contrast, Celekli et al. (2009) reported that phosphate supply increased carotenoid production of a blue green alga *S. platensis*.

The higher ascorbic acid content in *N. oculata* was obtained by algal culturing in the optimized medium at concentrations 1.5 and 0.5 times of NO_3 and PO_4, respectively. These results disagree with those obtained by El-Baz et al. (2002), as *D. salina* accumulated a large amount of ascorbic acid when grown in media containing limiting nitrogen concentration.

The optimized *N. oculata* extracts have higher DPPH scavenging capacity, and increased with increasing their concentration (from 25 to 100 μg ml^{-1}). The antioxidant activity of *N. oculata* cultured on the optimized medium was increased by 53.5%, 26.1% and 18.4%, respectively at the three different concentrations. These increase with the decreasing concentration of both NO_3 and PO_4 (0.5 times those of the F/2 medium). Radical scavenging activity may correlate to nitrogen limitation stress. Several studies improved that the increase in carotene content in the starved cells may be attributed to excessive formation of free radicals under the stress produced in order to protect the cells and to continue their growth. On the other hand, most carotenoids are efficient antioxidants, quenching singlet oxygen, and trapping peroxyl radicals (Surai et al., 2003).

Enhancement in the biochemical composition of the *A. franciscana* after 24 h of enrichment with *N. oculata* cultured on optimized medium proves the importance of the biochemical composition of the algal food in modulating the biochemical composition of the filter-feeder. Good quality microalgae constitute an excellent diet for enrichment since they provide rotifers with essential nutrients (Dhert et al., 2001). In aquaculture, the brine shrimp *Artemia* is the most popular live diet. In this connection, the nutritional adequacy of zooplankton used for feeding larvae in marine hatcheries depends on nutritional value of microalgae used to enrich brine shrimp *Artemia salina* (Zaki and Saad, 2010). The obtained results revealed that protein, carbohydrate, ascorbic acid and β-carotene in *A. franciscana* enriched with *N. oculata* cultured on the optimized medium for 24 h were increased by 65%, 28%, 40.5% and 14.1%, respectively as compared with the *A. franciscana* fed on alga cultured on control. Similar results were obtained by Reitan et al. (1997) who found that after 24 h enrichment with *Tetraselmis suecica* and *Isochrysis galbana*, protein and lipid contents increase between 50% and 70% with regard to the initial values. Higher content of vitamins (ascorbic acid and β-carotene) in *A. franciscana* enriched with *N. oculata* cultured on the newly optimized medium revealed that the content of vitamins in *A. franciscana* depends largely on its content in the algal diets. The present results are in accordance with Brown et al. (1998) who demonstrated that microalgae are the primary source of vitamins in aquatic food chains.

Conclusions

Applying the Plackett–Burman design is powerful to find variables of the growth optimization of *N. oculata*. The optimization was likely to produce high amounts of protein, carbohydrates, vitamins and antioxidant. Feeding *N. oculata* to *A. franciscana* enhanced its biochemical composition. Thus the newly formulated media were costless media for culturing *N. oculata* for live feeds, which can be easily prepared and at the same time can offer the desired growth and production of secondary metabolites in the tested alga which can be transported by feeding organisms.

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