OPTIMIZATION OF EXTRACTION PARAMETERS FOR FUCOXANTHIN, GALLIC ACID AND RUTIN FROM *NITZSCHIA THERMALIS*

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Abstract. Recently, microalgae have become important in their health, and cosmetic applications since they are viewed as new sources of carotenoids. Fucoxanthin is also a type of carotenoid. The anti-diabetic, anti-obesity, anti-cancer, and antioxidant properties of fucoxanthin have been widely reported. Since these valuable properties, they also represent a valuable resource of nutraceuticals for functional food applications. This study aims to determine the amount of fucoxanthin, gallic acid, and rutin in *Nitzschia thermalis* obtained from the Ege University Microalgae Culture Collection. The extraction parameters have been optimized using response surface methodology. The extraction temperature (25, 35, and 45°C), the extraction time (10, 20, and 30 min) and the biomass/solvent ratio (0.005, 0.001, and 0.015 g ml^{-1}) have been assessed as response variables in the Box – Behnken design. The amount of fucoxanthin was determined by the C_{30} column at 450 nm, while both the amount of gallic acid and rutin were separated in the C_{18} column at 275 nm by HPLC-DAD. In the present study, the optimum extraction conditions providing the maximum amount of fucoxantin, gallic acid, and rutin were selected by applying the "desirability" function approach in response surface methodology. Finally, the temperature has been determined to be 27.30°C, the extraction time 10 minutes, and the biomass ratio 0.05 g ml^{-1}. Under these conditions, the optimum fucoxanthin level has been determined as 5.8702 mg g^{-1}, the gallic acid level as 0.0140 mg g^{-1}, and the rutin level as 0.0496 mg g^{-1}. The findings are in good agreement with international published values for fucoxanthin content. In addition, response surface methodology was shown to be an effective technique for optimising extraction conditions for maximum fucoxanthin yield. In conclusion, these findings may be applied in the development of extraction methodologies for value added microalgea products as well as can serve as a reference for the extraction of fucoxanthin having high gallic acid and rutin from other brown microalgae, and therefore it could potentially be applied in both pharmaceutical and food industries.

Keywords: fucoxanthin, gallic acid, rutin, *Nitzschia thermalis*, response surface methodology.

DOI: http://dx.doi.org/10.15673/fst.v13i1.1342

Introduction. Formulation of the problem

Microalgea are strong candidate in the biofuel industry due to their high growth rates, high diversity and nutrient use efficiency. Nitzschia sp., one of microalgea strain, have important characteristics for large-scale cultivation, fast growth rates and high lipid production for biofuel production [1]. In addition to biofuel production, microalgea are good source of fucoxantin estimated to account for more than 10% of the total production of carotenoids in nature. Fucoxanthin has already been used to enrich some food products such as pasta, biscuits and dips. Moreover, fucoxanthin supplements are generally recognised as safe by the European Food Safety Authority, Japan's Food for Specified Health Uses, and the US Food and Drug Administration [2]. Fucoxanthin has also been studied clinically against many diseases [3-5]. Since its efficacy and potential in terms of health applications, it was necessary to optimize the extraction conditions to obtain the maximum amount of fucoxanthin.

Analysis of recent research and publications

Carotenoids have a tetraterpene (C_{40}) backbone and are classified as belonging to terpenoid pigments [6]. They have been usually known for their colours (brilliant yellow, orange, and red) and used mostly in food and cosmetics.

They are found in plants, in many algae, bacteria, and fungi. Their most important properties are light harvesting and photoprotection in photosynthetic organisms [7].

There are two main classes of molecules: carotenes, and xanthophylls. The only difference between carotenes (alpha-carotene, beta-carotene and lycopene) and xanthophylls (lutein, zeaxanthin, neoxanthin, violaxanthin, flavoxanthin, and fucoxanthin) is that xanthophylls include oxygen-binding hydrocarbon chain [8], and the presence of oxygen in the hydroxyl and epoxide groups of xanthophylls makes them more polar than carotenes [9].

Fucoxanthin is abundantly found in brown seaweeds (macroalgae) and contributes to more than 10% of the estimated total production of carotenoids in nature [10]. However, microalgae, especially diatoms, produce much

more fucoxanthin [11]. Moreover, they exhibit lipophilicity and antioxidant activity. All-trans fucoxanthin is the major isomer of fucoxanthin found naturally, especially [4,7]. The fucoxanthin exhibits anticarcinogenic [2,3,13], antiobesity [14], antidiabetic [9], antioxidant [10,12], and anti-inflammatory properties [17].

The term "optimization" has been used to mean attaining the maximum benefit from the performance of a system, a process, or a product. In traditional optimization, the effect of one factor on the response is monitored; other factors are kept at a constant level. However, the disadvantage of this traditional optimization is ignoring the interaction effects among the factors. To solve this problem, multivariate statistic techniques have been carried out. Response surface methodology (RSM) is among the multivariate techniques used to optimize extraction [14, 15].

The fucoxanthin was extracted from brown seaweeds and marine diatoms including *Undaria pinnatifida* [20] *Hijkia fusiformis* [21], *Sargassum* sp. [22], *Laminaria japonica* [23], *Padina tetrastomatica* [24], *Phaeodactylum tricornutum* [25], *Cyclotella cryptica* [26], *Nitzschia laevis*, *Odontella aurita* [11].

Based on our knowledge, fucoxanthin from brown microalgae, such as *Nitzschia thermalis*, have not yet been investigated.

Objectives of the study:

– to maximize the extraction of fucoxanthin from *N. thermalis*,
– to reach the maximum gallic acid and rutin concentration as the highest phenolic compounds found in *N. thermalis.*

Research materials and methods

Materials and Chemicals. Triethylamine (CAS Number 121-44-8), all-trans fucoxanthin (CAS Number 3351-86-8), all-trans neoxanthin (CAS Number 14660-91-4), pyrogallol (CAS Number 87-66-1), and calcium carbonate (CAS Number 471-34-1) were all purchased from Sigma-Aldrich. All the solvents used in this study were of LC-grade and supplied from Merck.

Cultivation of N. thermalis. *N. thermalis* was obtained from the Ege University Microalgae Culture Collection, Ege-MACC (coded 56) with National Center for Biotechnology Information (NCBI) accession number JQ886458. It was isolated from Sigacik-Teos/Turkey. *N. thermalis* was cultivated in the F2 medium at 22±2°C, at the light intensity 30 µE $m^{-2}s^{-1}$ during 14 days. The cell growth (cells ml^{-1}) was determined daily by a *Neubauer* counting chamber using bright field microscopy (Olympus CH40). The specific growth rate (µ) of the cells was calculated from the exponential phase, as:

$$\mu = \frac{In\,N2 - In\,N1}{d(t2 - t1)} \quad (1)$$

where N_2 is the final cell concentration, N_1 is the initial cell concentration, and dt is the time required for the increase in concentration from N_1 to N_2.

Doubling time (DT) was also calculated as: (according to Demirel et al. 2015)

$$DT = \frac{In\,2}{\mu},. \quad (2)$$

The dry weight diatom biomass was measured by filtering a 5 mL culture sample through pre-weighed MN GF-3 filter paper (Macherely-Nagel, Germany) and washed with distilled water, then the cell mass was dried at 65°C for 12 h.

To identify *N. thermalis*, an optical image was obtained by using a trinocular light microscope (Olympus BX53), and a SEM image was provided using Philips XL-30S FEG.

Preparation of biomass. *N. thermalis* was harvested by filtering with a 47 mm cellulose acetate membrane filter (Sartorius, 0.45 µm) and washed with deionized water in order to remove the salts that can come from the culture medium. Then, the cells were collected and lyophilized for 24 hours. The frozen samples were then grounded in a mortar to obtain fine powder and stored at -20°C until the extraction.

Optimization of the extraction process parameters. The extraction procedures described by Erdoğan et al., 2016 [27] were chosen as a starting point to optimize the procedure of extraction from *N. thermalis.* The biomass/solvent ratio (0.005, 0.01 and 0.015 g mL^{-1}, B), the extraction temperature (25, 35, and 45 C, C), and the extraction time (10, 20, and 30 min, T) were chosen as the design variables depending on the literature survey and preliminary experiments. The explored experimental domain was to be fixed by taking into account industrial practice. The calculated amount of fucoxanthin (FX), gallic acid (GA) and rutin (R) in *N. thermalis* were assessed as the response variables when a Box – Behnken design was used. By this design, seventeen randomized samples were prepared in two steps and treated for extraction in the methanol/acetone (70:30%). After $CaCO_3$ was added, the biomass was extracted with tetrahydrofuran-dimethylformamite containing 0.010% (w/v) pyrogallol according to the design plan. Then, the mixture was placed in an ultrasonic bath according to the duration mentioned in the design plan. The residue was extracted three times, and the supernatant was collected. Then, the supernatant was filtered with a nylon filter having the pore size 0.2 µm before HPLC analysis.

The effects of the design variables on the response variables were evaluated using an analysis of variance (ANOVA) with a significance level of 0.05. Moreover, the effects of the design variables, their interactions, and results from the design of the response variables were used to visualize the formation of the response surfaces.

HPLC analysis of fucoxanthin. In this study, an Agilent Technologies (USA) HPLC instrument was used. It consisted of a 1200 Quaternary pump, a 1200 Diode array detector, and a YMC-Carotenoid column (WATERS, C_{30}, 250 x 4.6 mm, 5µm). The solvent system consisted of 70% of methanol and 30% of acetonitrile, both including 0.01% Triethylamine as the modifier. The simple 15 minutes-isocratic elution was applied to

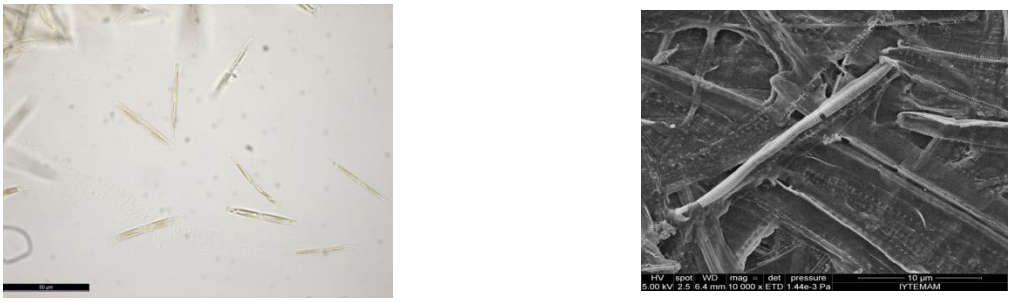
separate fucoxanthin. The wavelengths used to determine fucoxanthin was 450 nm. The injection volume was 50 µL with a constant flow rate of 1.0 mL/min. The full spectrum was recorded from 300 nm to 600 nm.

5 mg of all-trans fucoxanthin as the stock solution and 5 mg of all-trans-neoxanthin as the internal standard were prepared in chloroform (50 mL) separately. To the internal standard (2 mg L^{-1}), the calibration standards (0.010–5 mg L^{-1}) were added.

HPLC analysis of gallic acid and rutin. To determine gallic acid and rutin, the same instrument combination was used, however, a C_{18} column was preferred to these compounds due to the nature of the compounds separated. To separate gallic acid and rutin, as the mobile phases, water-acetic acid (99:1, v/v, mobile phase A) and water-acetonitrile-acetic acid (67:32:1, v/v/v, mobile phase B) were chosen, in the gradient mode (0–10 min: 90% A+10% B, 10–16 min: 80 % A+20% B, 16–20 min: 60% A+40% B, 20–25 min: 50% A+ 50% B, 25–27 min: 60% A+40% B, 27–35 min: 90% A+ 10% B). The injection volume was 10 µL with a constant flow rate of 1.0 mL/min^{-1}. The wavelength used to determine gallic acid and rutin was 275 nm. Gallic acid and rutin were identified by comparison of their retention time with that of pure standards [28].

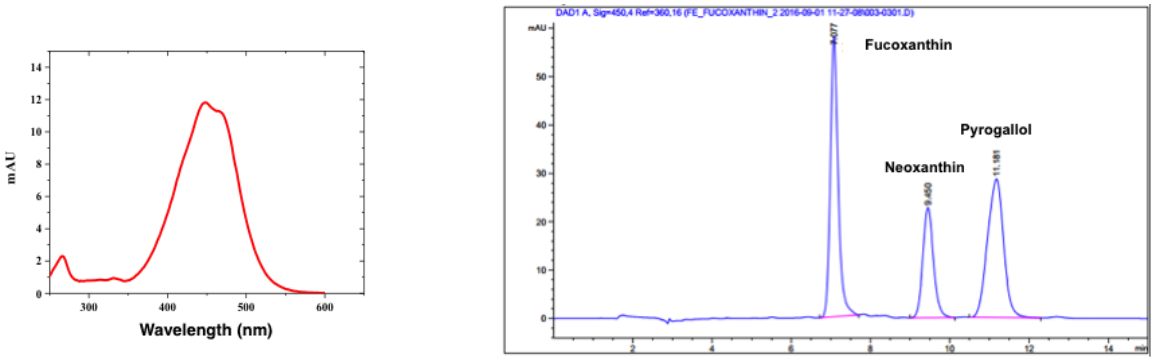
Results of the research and their discussion

As presented in Fig. 1, *N. thermalis* was identified using an optical image and a SEM image. Fig. 2a illustrates the full spectrum of fucoxanthin recorded from 300 nm to 600 nm and Fig. 2b illustrates the separation of fucoxanthin in an overlay chromatogram in fucoxanthin standard peak; neoxanthin and pyrogallol, respectively.



A **B**

Fig. 1. Optical image (A) and electromicrosopy image (B) of *N. thermalis*



A **B**

Fig. 2. (A) The full spectrum of fucoxanthin
(B) The separation of fucoxanthin, neoxanthin (internal standard), and pyrogallol

The effect of extraction parameters. Response surface methodology was selected as the statistical method since it has been successfully applied in our laboratory for the optimization of extraction from a variety of food samples [29]. The specific growth rate of (µ) *Nitzschia thermalis* has been found to be 0.1147 day^{-1}, and doubling time (DT) to be 6.0424 day. The biomass reached the productivity 0.2929±0.0016 mg $L^{-1}day^{-1}$ in 14 days. Freeze-dried biomass of *N. thermalis* was extracted, with the biomass/solvent ratio, extraction temperature, and extraction time, by using response surface methodology (RSM), which consisted in an arranged experimental design based on a Box – Behnken design to detect the amounts of FX, GA, and R as presented in Table 1.

The extraction temperature affected the extraction rate due to increased kinetic energy and penetration between solvent and biomass, but the higher temperatures can cause the degradation of some bioactive components (Fig. 3). The extraction can be controlled by diffusion and for that reason, the extraction was directly related with the extraction time and stopped when it reached the equilibrium [30]. Fig. 3 shows the effect of extraction time on the amount of FX, GA, and R. When the biomass/solvent ratio was increased, the diffusivity of the solvent into the cell and the extractability might be

easier. However, the amount of solvent is important for absorption of cavitation energy. The excess of solvent absorbs the cavitation energy, while a smaller amount of solvent is not enough to extract biomolecules [31]. As shown in Fig. 3, the effect of the biomass/solvent ratio on the amounts of FX, GA, and R was changeable and depended on the other parameters and interactions.

Table 1 – The amounts of FX, GA, and R of *N. thermalis* extracts at different extraction conditions

No	C (°C)	T (min)	B (g ml^{-1})	FX (mg g^{-1})	GA (mg g^{-1})	R (mg g^{-1})
1	25	20	0.15	5.8031	0.0035	0.0118
2	25	30	0.10	5.7948	0.0051	0.0209
3	25	20	0.05	5.8424	0.0104	0.0556
4	25	10	0.10	5.8702	0.0052	0.0209
5	35	20	0.10	4.9820	0.0058	0.0216
6	35	30	0.05	5.5920	0.0153	0.0386
7	35	20	0.10	5.2572	0.0074	0.0167
8	35	20	0.10	5.2610	0.0077	0.0205
9	35	10	0.15	5.0837	0.0051	0.0086
10	35	20	0.10	4.9134	0.0054	0.0207
11	35	20	0.10	5.0498	0.0058	0.0201
12	35	30	0.15	5.4301	0.0052	0.0096
13	35	10	0.05	5.4171	0.0173	0.0405
14	45	20	0.05	5.5996	0.0186	0.0271
15	45	10	0.10	5.0687	0.0027	0.0117
16	45	30	0.10	5.6267	0.0050	0.0122
17	45	20	0.15	5.4648	0.0037	0.0065

Optimization of the parameters of extraction from N. Thermalis. The amounts of FX, GA, and R were fitted into a quadratic model (Equations 1–3, respectively), which was the most compatible with the experimental data.

$$FX=12.82–0.21C–0.09T16.73B+ 1.58x10^{-3}CT–0.05CB+0.09TB+3.97x10^{-3}C^2+ 1.00x10^{-3}T^2+75.09B^2 \quad (1)$$

$$GA=2.08x10^{-3}+1{,}62x10^{-3}C–2.5x10^{-4}T– 0.34B+6.10x10^{-6}CT–4.02x10^{-3}CB+9.85x10^{-4}TB– 1.81x10^{-5}C^2—1.43x10^{-6}T^2+1{,}77B^2 \quad (2)$$

$$R=0.12–9.40x10^{-4}C+6.88x10^{-4}T–1.28B+1.28x10^{-6}CT+0.01CB+1{,}50x10^{-3}TB–1.28C^2– 2.22x10^{-5}T^2+2.66B^2 \quad (3)$$

Analysis of variance partitioned the variability in FX, GA, and R yield for each of the three independent factors. The statistical significance of each effect and their interaction were determined by comparing the mean squares against an estimate of the experimental error. The calculated quadratic models of each response were statistically significant at $p < 0.05$ level whereas the lack of fit of models was statistically insignificant (Table 2). The statistical analysis indicated that the suggested model was adequate since R^2, adjusted R^2, predicted R^2, and the prediction error sum of squares (PRESS) were checked (Table 2).

Response surface plots (Fig. 3-5) were constructed according to the modelled experimental data. In each case, the effects of three variables on the amount of FX, GA, and R were presented in three-dimensional surface plots while the two other variables were kept constant at zero level. The ANOVA results in Table 2 and the counterplot in Fig. 3a make it clear that the C in the assessed range 25–45°C and T in the assessed range 10–30 min had a significant effect on the amount of FX. The analysis of the results has shown that B in the studied range 0.05–0.15 (g mL^{-1}) does not effect on the amount of FX. As shown in Fig. 3, the counter line belonging to B is almost straight. The amount of GA, another response found in this optimization process, is also an important compound. According to Table 2 and Figure 4, the amount of GA is significantly influenced by B, while the amount of R was changed significantly by C and B (Figure 5). B was found as insignificant in the amount of FX, but it was affected significantly by the amounts of GA and R.

The desired goals for each variable and response were chosen and different weights were assigned to each goal to adjust the shape of its particular desirability function. The software generated optimum conditions of independent variables have been found as 0.05 g ml^{-1}, 27.30°C, and 10 min for B, C, and T, respectively, and the maximum desirability has been found to be 0.868.

Five validation experiments at the optimum conditions have been performed to confirm the experimental data and model predicted data. The amounts of FX, GA, and R were significantly ($p<0.05$) different from the predicted values determined by Design-Expert version 10.0 software as shown in Table 3.

The amount of FX. The fucoxanthin has been preferred due to its pigmentation properties for food and cosmetic application [9] and known to be one of the most common carotenoids in brown algae [32]. The extraction of fucoxanthin from various brown algae depended on both algae types and extraction techniques [18,29-31]. Roh et al. (2008) extracted fucoxanthin from *Undaria pinnatifida* using freeze-drying as pretreatment in supercritical fluid extraction and obtained 7.53 mg g^{-1} of dry matter, while the

amount of fucoxanthin extracted from *Undaria pinnatifida* was found as 0.99 mg g^{-1} of dry matter [34].

Table 2 – ANOVA results for the amounts of FX, GA, and R of *N. thermalis* extracts in terms of "Extraction Temperature," "Extraction time," and "Biomass/solvent ratio" and the corresponding interactions

Source	FX (mg g^{-1})		GA (mg g^{-1})		R (mg g^{-1})	
	Sum of Squares	p-value Prob> F	Sum of Squares	p-value Prob> F	Sum of Squares	p-value Prob> F
Model	1.51	0.0045	$3.59x10^{-4}$	0.0014	$2.64x10^{-3}$	<0.0001
C	0.30	0.0054	$4.09x10^{-6}$	0.2880	$3.35x10^{-4}$	0.0002
T	0.13	0.0371	$1.77x10^{-8}$	0.9419	$1.81x10^{-8}$	0.9603
B	0.06	0.1304	$2.44x10^{-4}$	<0.0001	$1.96x10^{-3}$	<0.0001
CT	0.10	0.0556	$1.49x10^{-6}$	0.5105	$6.60x10^{-8}$	0.9243
CB	$2.28x10^{-3}$	0.7397	$1.61x10^{-5}$	0.0566	$1.35x10^{-4}$	0.0030
TB	$7.35x10^{-3}$	0.5546	$9.71x10^{-7}$	0.5929	$2.26x10^{-6}$	0.5824
C^2	0.66	0.0006	$1.37x10^{-5}$	0.0732	$6.86x10^{-6}$	0.3485
T^2	0.04	0.1797	$8.63x10^{-8}$	08721	$2.08x10^{-5}$	0.1241
B^2	0.15	0.0270	$8.25x10^{-5}$	0.0013	$1.86x10^{-4}$	0.0012
Residual	0.13		$2.17x10^{-5}$		$4.76x10^{-5}$	
Lack of Fit	0.03	0.7495	$1.71x10^{-5}$	0.0780	$3.33x10^{-5}$	0.1516
Pure Error	0.10		$4.59x10^{-6}$		$1.43x10^{-5}$	
Total	1.65		$3.81x10^{-4}$		$2.69x10^{-3}$	
R-Squared	0.92		0.9431		0.9823	
Adjusted R^2	0.81		0.8700		0.9595	
Predicted R^2	0.59		0.2641		0.7936	
Adeq Precision	7.91		11.156		22.628	
Std. Dev.	0.14		$1.76x10^{-3}$		$2.61x10^{-3}$	
Mean	5.42		$7.61x10^{-3}$		0.021	
C.V. %	2.55		23.13		12.19	
PRESS	0.67		$2.8x10^{-4}$		$5.55x10^{-4}$	

Table 3 – The results of statistical analysis to verify the optimization

Responce	Predicted value	Experimental value	Mean standard error	Difference	p value
FX (mg/g)	5.8702	6.4100	0.563	0.5398	0.00
GA	0.0140	0.069	0.491	0.055	0.00
R	0.0496	0.1076	0.495	0.058	0.066

The amount of GA and R. GA and R as phenolic compounds have been analyzed by the extraction method optimized using RSM. The amount of these phenolic compounds have been compared with the published data including those on other brown algae and food sources. However, it has been understood that different brown algae have various phenolic compounds and their amounts are different. This situation can be explained by many factors such as the algae species, the geographical origin, the cultivation area, the extraction conditions and types [28]. In this study, gallic acid was chosen as a representative of phenolic acids determining the food colour, flavour, quality, and health effects [36], while rutin was chosen as a representative of flavonoids associated with reducing the risks of the chronic diseases [37]. Machu et al., in 2015 [28], detected the amount of gallic acid in different brown algae. The gallic acid amount of *Eisenia bicyclis* and *H. fusiformis* were determined as 2.80 and 14.10 μg g^{-1} in a sample, respectively. The studies of brown algae are generally about the detection of total phenolic content, and their antioxidant activities using different antioxidant activity methods [34,35]. Yumiko et al., in 2003 [40], investigated the content of flavonoids in some red, brown, and green algae from Japan. The amount of rutin of *U. pinnatifida*, *Ecklonia cava*, and *Ishige okamurae* were found to be 457.00, 2730.00, and 996.00 μg g^{-1} of dry matter. In this study, the amount of rutin has been found to be 49.60 μg g^{-1} in a sample.

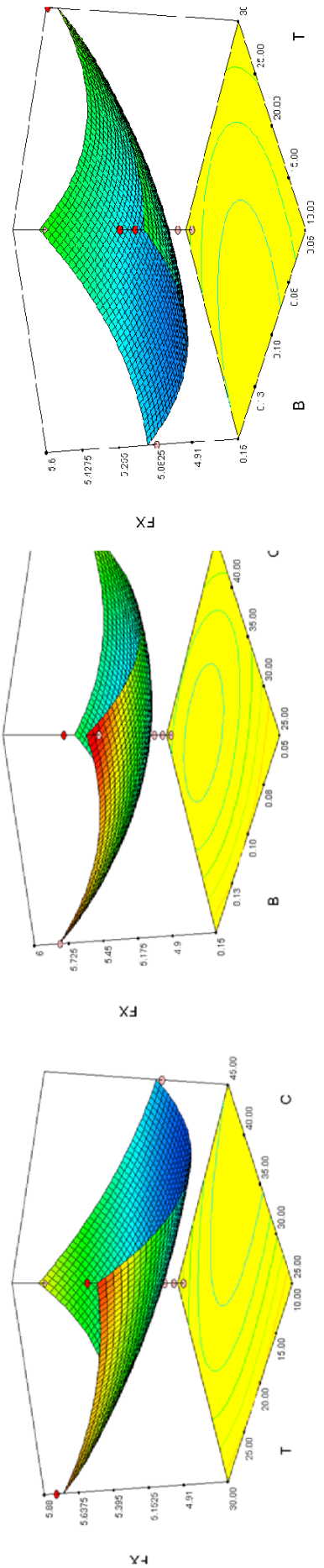


Fig. 3. Calculated effect of independent variables: C (°C), T (min), B (g mL^{-1}) on the amount of FX (g mL^{-1}).

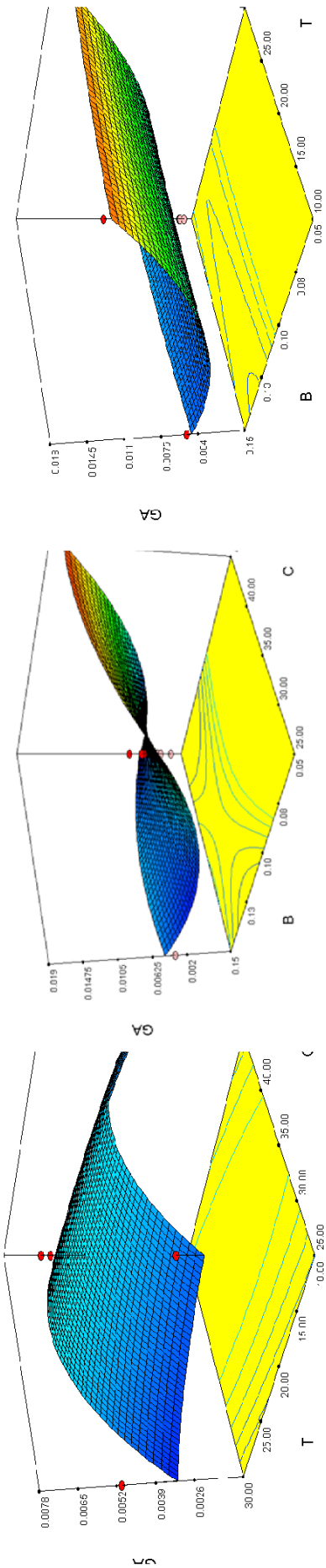


Fig. 4. Calculated effect of independent variables: C (°C), T (min), B (g mL^{-1}) on the amount of GA (g mL^{-1}).

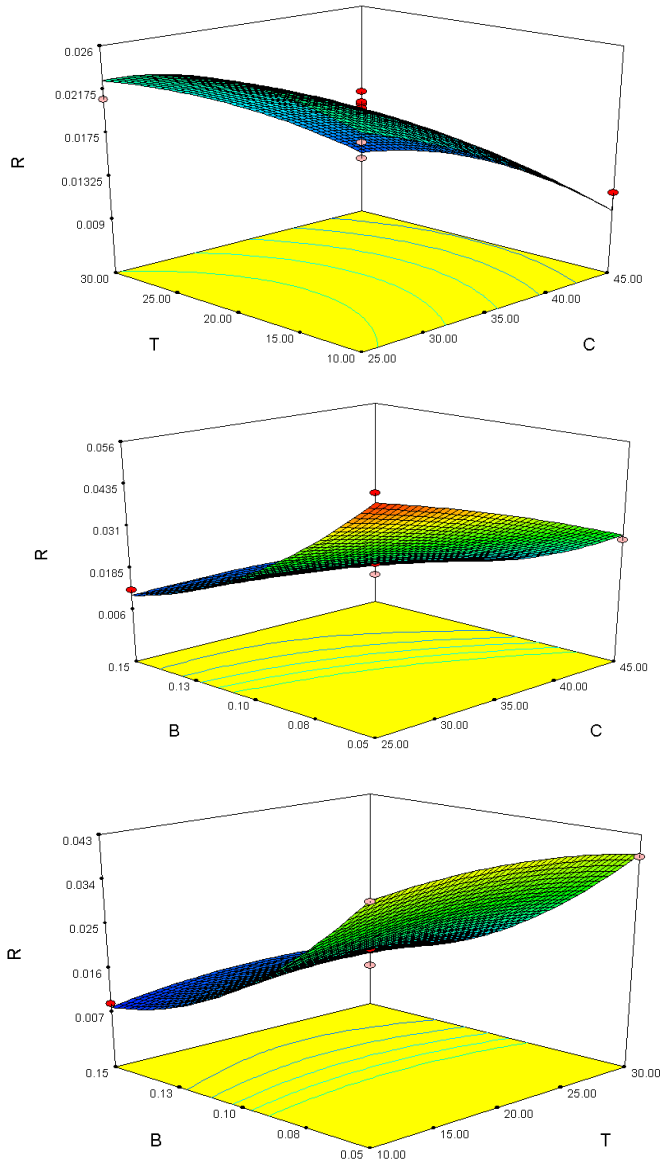


Fig. 5. Calculated effect of independent variables: C (°C), T (min), B (g mL^{-1}) on the amount of R (g mL^{-1}).

Conclusion

One of the problems that has gained importance in recent years is determining the kinds and concentrations of carotenoids contained in food products and important for human health. Several studies have been conducted on obtaining carotenoids from microalgae. Since microalgae differ from each other in their structures, the obtained carotenoids and/or amounts will be different, too. In order to follow these changes, many researchers need new methods of analysis. One of the major problems in carotenoid analysis is that there is no commercial standard for many carotenoids. Every contribution in this area is also important in the context of finding/purifying new species, finding new species in the health and food sector, and using them as analytical standards. The amount of fucoxanthin of *Nitzschia thermalis* has been found to be very high in commercial terms and it sheds light on the future studies of how to obtain commercial amounts of fucoxanthin from this microalgae. Moreover, this study has shown that the algae products have a considerable amount of phenolic compounds. The project team will continue to work in this field. The findings of the project and the fact that the production of carotenoid from microalgae on an industrial scale is important in different countries will also guide our future work.

Funding. EGE-BAP (Ege University Scientific Research Fund with the project number: 16-MÜH-025).

Conflict of interest. The authors declare that there is no conflict of interest.

References:

1. Jiang Y, Nunez M, Laverty KS, Quigg A. Coupled effect of silicate and nickel on the growth and lipid production in the diatom Nitzschia perspicua. 2014;
2. Shannon E, Abu-Ghannam N. Optimisation of fucoxanthin extraction from Irish seaweeds by response surface methodology. J Appl Phycol. 2017;29(2):1027-36. http://dx.doi.org/10.1007/s10811-016-0983-4
3. Nakazawa Y, Sashima T, Hosokawa M, Miyashita K. Comparative evaluation of growth inhibitory effect of stereoisomers of fucoxanthin in human cancer cell lines. J Funct Foods. 2009;1(1):88-97. http://dx.doi.org/10.1016/j.jff.2008.09.015
4. Jaswir I, Noviendri D, Salleh HM, Miyashita K. Fucoxanthin Extractions of Brown Seaweeds and Analysis of Their Lipid Fraction in Methanol. Food Sci Technol Res. 2012;18(2):251-7. http://japanlinkcenter.org/DN/JST.JSTAGE/fstr/ 18.251?lang=en&from= CrossRef&type=abstract
5. Beppu F, Hosokawa M, Niwano Y, Miyashita K. Effects of dietary fucoxanthin on cholesterol metabolism in diabetic / obese KK- A y mice. 2012;1-8.
6. Das SK, Hashimoto T, Shimizu K, Yoshida T, Sakai T, Sowa Y, et al. Fucoxanthin induces cell cycle arrest at G0/G1phase in human colon carcinoma cells through up-regulation of p21WAF1/Cip1. Biochim Biophys Acta - Gen Subj. 2005;1726(3):328-35.
7. Namitha KK, Negi PS. Chemistry and biotechnology of carotenoids. Crit Rev Food Sci Nutr. 2010;50(8):1040-8398.
8. Kotake-Nara E, Sugawara T, Nagao A. Antiproliferative effect of neoxanthin and fucoxanthin on cultured cells. Fish Sci. 2005;71(2):459-61.
9. Peng J, Yuan JP, Wu CF, Wang JH. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health. Mar Drugs. 2011;9(10):1806-28.
10. Hosokawa M, Kudo M, Maeda H, Kohno H, Tanaka T, Miyashita K. Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPARγ ligand, troglitazone, on colon cancer cells. Biochim Biophys Acta - Gen Subj. 2004;1675(1-3):113-9.
11. Wang S, Verma SK, Hakeem Said I, Thomsen L, Ullrich MS, Kuhnert N. Changes in the fucoxanthin production and protein profiles in Cylindrotheca closterium in response to blue light-emitting diode light. Microb Cell Fact. 2018;17(1):1-13.
12. Poojary MM, Barba FJ, Aliakbarian B, Donsì F, Pataro G, Dias DA, et al. Innovative alternative technologies to extract carotenoids from microalgae and seaweeds. Mar Drugs. 2016;14(11):1-34.
13. Kotake-Nara E, Asai A, Nagao A. Neoxanthin and fucoxanthin induce apoptosis in PC-3 human prostate cancer cells. Cancer Lett. 2005;220(1):75-84.
14. Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K. Effect of medium-chain triacylglycerols on anti-obesity effect of fucoxanthin. J Oleo Sci. 2007;56(12):615-21.
15. Sudhakar MP, Ananthalakshmi JS, Nair BB. Extraction, purification and study on antioxidant properties of fucoxanthin from brown seaweeds. J Chem Pharm Res. 2013;5(7):169-75.
16. Shannon E, Abu-Ghannam N. Optimisation of fucoxanthin extraction from Irish seaweeds by response surface methodology. J Appl Phycol. 2017;29(2):1027-36.
17. Li FL, Wang LJ, Fan Y, Parsons RL, Hu GR, Zhang PY. A rapid method for the determination of fucoxanthin in diatom. Mar Drugs. 2018;16(1):1-13.
18. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008;76(5):965-77.
19. Vera Candioti L, De Zan MM, Cámara MS, Goicoechea HC. Experimental design and multiple response optimization. Using the desirability function in analytical methods development. Talanta. 2014;124:123-38. http://dx.doi.org/10.1016/j.talanta.2014.01.034
20. Billakanti JM, Catchpole OJ, Fenton TA, Mitchell KA, Mackenzie AD. Enzyme-assisted extraction of fucoxanthin and lipids containing polyunsaturated fatty acids from Undaria pinnatifida using dimethyl ether and ethanol. Process Biochem. 2013;48(12):1999–2008. http://dx.doi.org/10.1016/j.procbio.2013.09.015
21. Yan X, Chuda Y, Suzuki M, Nagata T. Fucoxanthin as the Major Antioxidant in Hijikia fusiformis , a Common Edible Seaweed. Vol. 63, Bioscience, Biotechnology, and Biochemistry. 1999. p. 605-7. http://www.tandfonline.com/doi/full/10.1271/bbb.63.605
22. Conde E, Moure A, Domínguez H. Supercritical CO2 extraction of fatty acids, phenolics and fucoxanthin from freeze-dried Sargassum muticum. J Appl Phycol. 2015;27(2):957-64.
23. Jang EJ, Kim SC, Lee JH, Lee JR, Kim IK, Baek SY, et al. Fucoxanthin, the constituent of Laminaria japonica, triggers AMPK-mediated cytoprotection and autophagy in hepatocytes under oxidative stress. BMC Complement Altern Med. 2018;18(1):1–11.
24. Raguraman V, Stanley Abraham L, Mubarak Ali D, Narendrakumar G, Thirugnanasambandam R, Kirubagaran R, et al. Unraveling rapid extraction of fucoxanthin from Padina tetrastromatica: Purification, characterization and biomedical application. Process Biochem. 2018; https://doi.org/10.1016/j.procbio.2018.08.006
25. Kim SM, Jung YJ, Kwon ON, Cha KH, Um BH, Chung D, et al. A potential commercial source of fucoxanthin extracted from the microalga Phaeodactylum tricornutum. Appl Biochem Biotechnol. 2012;166(7):1843–55.
26. Guo B, Liu B, Yang B, Sun P, Lu X, Liu J, et al. Screening of diatom strains and characterization of Cyclotella cryptica as a potential fucoxanthin producer. Mar Drugs. 2016;14(7).
27. Erdoğan A, Demirel Z, Conk Dalay M, Eroğlu AE. Fucoxanthin Content of Cylindrotheca closterium and Its Oxidative Stress Mediated Enhancement. Turkish J Fish Aquat Sci. 2016;16:499-506.
28. Machu L, Misurcova L, Ambrozova JV, Orsavova J, Mlcek J, Sochor J, et al. Phenolic content and antioxidant capacity in algal food products. Molecules. 2015;20(1):1118–-33.
29. Ozyurt VH, Otles S. Determination of 3-nitrotyrosine in food protein suspensions. Talanta. 2017.
30. Strati IF, Oreopoulou V. Effect of extraction parameters on the carotenoid recovery from tomato waste. Int J Food Sci Technol. 2011;46(1):23-9.
31. Samavati V. Polysaccharide extraction from Abelmoschus esculentus: Optimization by response surface methodology. Carbohydr Polym. 2013;95(1):588-97. http://dx.doi.org/10.1016/j.carbpol.2013.02.041
32. Mikami K, Hosokawa M. Biosynthetic pathway and health benefits of fucoxanthin, an algae-specific xanthophyll in brown seaweeds. Int J Mol Sci. 2013;14(7):13763-81.
33. Roh MK, Uddin MS, Chun BS. Extraction of fucoxanthin and polyphenol from Undaria pinnatifida using supercritical carbon dioxide with co-solvent. Biotechnol Bioprocess Eng. 2008;13(6):724-9.
34. Kanda H, Kamo Y, Machmudah S, Wahyudiono, Goto M. Extraction of fucoxanthin from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether. Mar Drugs. 2014;12(5):2383-96.
35. Sivagnanam SP, Yin S, Choi JH, Park YB, Woo HC, Chun BS. Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO2 extraction. Mar Drugs. 2015;13(6):3422–42.
36. Craft BD, Kerrihard AL, Amarowicz R, Pegg RB. Phenol-Based Antioxidants and the In Vitro Methods Used for Their Assessment. Compr Rev Food Sci Food Saf. 2012;11(2):148-73.

37. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols : food sources and bioavailability . Am J Clin Nutr. Am J Clin Nutr. 2004;79(October 2015):727-47.
38. Dang TT, Bowyer MC, Van Altena IA, Scarlett CJ. Comparison of chemical profile and antioxidant properties of the brown algae. Int J Food Sci Technol. 2017;174–81. http://doi.wiley.com/10.1111/ijfs.13571
39. Chakraborty K, Praveen NK, Vijayan KK, Rao GS. Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to Turbinaria spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar. Asian Pac J Trop Biomed. 2013;3(1):816.
40. Yumiko YS, Ya-Pei H, Takeshi S. Distribution of flavonoids and related compounds from seaweeds in Japan. J Tokyo Univ Fish. 2003;89:16.

Отримано в редакцію 14.01.2019 Received 14.01.2019
Прийнято до друку 06.03.2019 Approved 06.03.2019

Цитування згідно ДСТУ 8302:2015
Özyurt V.H., Erdoğan A., Demirel Z., Conk Dalay M., Ötleş S. Optimization of extraction parameters for fucoxanthin, gallic acid and rutin from *Nitzschia thermalis* // Food science and technology. 2019. Vol. 13, Issue 1. P. 74-82. DOI: http://dx.doi.org/10.15673/fst.v13i1.1342

Cite as Vancuver ctyle citation
Özyurt VH, Erdoğan A, Demirel Z, Conk Dalay M, Ötleş S. Optimization of extraction parameters for fucoxanthin, gallic acid and rutin from *Nitzschia thermalis*. Food science and technology. 2019; 13(1): 74-82. DOI: http://dx.doi.org/10.15673/fst.v13i1.1342