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EFFECT OF H_2O_2 OXIDATION STRESS ON CAROTENOID PRODUCTION IN *CHLAMYDOMONAS REINHARDTII* AND ITS ANTIOXIDANT ACTIVITY

Çiğdem Yüksel[1], Saliha Şahin[1,*], Turgay Çakmak[2]
[1]Department of Chemistry, Faculty of Science and Arts, University of Bursa Uludag, Bursa, Turkey
[2]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Istanbul Medeniyet University, Istanbul, Turkey

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ABSTRACT
The effect of H_2O_2 oxidation stress on carotenoid production in *C. reinhardtii* and its antioxidant properties were investigated in this study. For this purpose, the amount of carotenoids determined by HPLC-DAD, total of phenolic contents and antioxidant capacities determined by Folin and CHROMAC methods respectively, in different oxidative stress conditions were studied. When the concentration of H_2O_2 was increased during the incubation period, total of phenolic content and antioxidant capacity value were decreased. In the same incubation period, HPLC-PDA results showed highest amounts of carotenoids in microalgae exposed to 1 μM H_2O_2 oxidative stress and it was thought that toxic dose might be in 20 μM oxidation media for microalgae. Thus, when the oxidative stress conditions were changed, the amounts of carotenoids and the structure of carotenoids could be changed. This study is important that the relationship between carotenoid and the power of oxidation stress in microalgae system.
Keywords: Antioxidant, *Chlamydomonas reinhardtii*, ultrasonic-assisted extraction, HPLC, carotenoid, oxidative stress.

H_2O_2 OKSİDASYON STRESİNİN *CHLAMYDOMONAS REINHARDTİİ* MİKROALGİNİN KAROTENOİD ÜRETİMİ VE ANTİOKSİDAN AKTİVİTESİ ÜZERİNE ETKİSİ

ÖZ
Bu çalışmada *C. reinhardtii* mikroalginin karotenoid üretimi ve antioksidan özellikleri üzerine H_2O_2 molekülünün oksidasyon etkisi incelenmiştir. Bu amaçla karotenoid miktarları HPLC-DAD cihazı ile, toplam fenolik madde ve antioksidan kapasite miktarları sırasıyla Folin ve CHROMAC yöntemleri ile tayin edilmiştir. Oksidatif stress süresi arttıkça karotenoid miktarlarında da artma görülmüştür. İnkübasyon süresi boyunca H_2O_2 konsantrasyonu arttırıldığında, toplam fenolik madde ve antioksidan kapasite değeri azalmıştır. Aynı inkübasyon süresi boyunca HPLC-DAD sonuçlarına göre, 1 μM H_2O_2 oksidatif strese maruz kalan mikroalglerde en yüksek miktarda karotenoid

* *Corresponding author* / Yazışmalardan sorumlu yazar:
✉: salihabilgi@uludag.edu.tr ✆: (+90) 224 294 17 24 🖷: (+90) 224 2941899

Çiğdem Yüksel; ORCID no: 0000-0002-7923-9764
Saliha Şahin; ORCID no: 0000-0003-2887-5688
Turgay Çakmak; ORCID no: 0000-0002-4953-8384

sentezlendiği ve mikroalgler için toksik dozun 20 μM oksidasyon ortamında olabileceği anlaşılmıştır. Böylece, oksidatif stres koşulları değiştiğinde, karotenoidlerin miktarları ve karotenoidlerin yapısı değiştirilebilir. Bu çalışma, mikroalg sisteminde, karotenoid sentezi ile oksidasyon stresi arasındaki ilişkinin açıklanması açısından önemlidir.
Anahtar kelimeler: Antioksidan, *Chlamydomonas reinhardtii,* ultrasonik destekli ekstraksiyon, HPLC, karotenoid, oksidatif stres

INTRODUCTION

Microalgae are unicellular microscopic and photosynthetic microorganisms. Due to their photosynthetic properties, they can take H_2O and CO_2 into their structures and transform them into organic compounds in different forms with the help of sunlight (Guedes et al., 2011; Olasehinde et al., 2017). These microorganisms can be found everywhere in the aquatic media (Minhas et al., 2016). Microalgae are known to contain bioactive components such as protein, fat, carbohydrates, amino acids and carotenoids. Microalgae show that antioxidant properties because of they contain carotenoids (Abd El Baky et al., 2013). The most important microalgae are *Chlorella, Chlamydomonas, Dunaliella, Muriellopsis* and *Haematococcus* spp (Erdoğan et al., 2015).

Chlamydomonas reinhardtii belongs to the *Chlorophyta* group, also called green algae. It is an organism with an oval cell shape, usually 10 μm in size and 3 μm in width. It can live in many different environments such as fresh water, moist soil, sea and snow. Because of the ability of photosynthetic properties and the presence of whips, they are regarded as the predecessors of both plants and animals (Erdoğan et al., 2015; Rochaix 1995; Çakmak et al., 2015).

Carotenoids are natural compounds that can be synthesized in all photosynthetic organisms and also synthesized by some non-photosynthetic bacteria, yeasts and fungi (Rao et al., 2007; Moran et al., 2010; Cazzonelli 2011). The antioxidant properties of carotenoids due to increasing disease (Alzheimer, cancer, cardiovascular disease, heart diseases, etc.) in recent years have become the focus of many studies. There are more than 600 carotenoids in the sources and many researchers have identified carotenoids using fruits, vegetables and microorganisms (algae, bacteria, fungi). The most common carotenoids are phytophthora, lycopene, γ-carotene, β-carotene, α-carotene, ξ-carotene, β-cryptoxanthin, α-cryptoxanthin, zeaxanthin, lutein, violaxanthin and astaxanthin in vegetables and fruits. Some diseases are tried to be prevented by taking carotenoids through food or medicines (Olasehinde et al., 2017). For this reason, the assignment of carotenoids becomes important.

Free radicals are formed in an unstable structure due to chemical reactions or external factors in our body. In order to become stable, these molecules attack cell components such as lipids, proteins and DNA and cause damage. Antioxidant defense mechanisms exist in organisms to control the formation of free radicals and to prevent harmful effects of molecules. Antioxidants are molecules that prevent chain reactions that can cause many diseases by neutralizing free radicals that damage the cells. In some cases, however, the antioxidant defense mechanism of the body doesn't completely prevent the effect of free radicals, and the condition called oxidative stress occurs. For this reason, external antioxidant supplementation is required (El-Bahr 2013).

Recent studies have examined the effects of oxidative stress-inducing factors such as light, pH, and nutrient stress on carotenoids in order to increase antioxidant content in microalgae. *Phaeodactylum tricornutum, Tetraselmis suecica* and *Chlorella vulgaris* were selected as target microalgae and antioxidant activity, total phenolic content, amount of carotenoid, vitamins E and C were compared in the presence of nitrogen and phosphorus to optimize antioxidant production in microalgae (Goiris et al., 2015). Compared to the control group for all three target microalgae, antioxidant activity, total phenol content, and total carotenoid content were reduced in microalgae exposed to nitrogen stress (Goiris et al., 2015). There are several research about regulation of carotenoid biosynthesis and

accumulation that conducted on a limited number of microalgal species such as β-carotene accumulation in the *Dunaliella salina.* (Nikulina et al., 2016). There are few studies about carotenoid biosynthesis and accumulation of *C. reinhardtii* in the literature (Nikulina et al., 2016).

In this study, carotenoid biosynthesis and accumulation of *C.reinhardtii* were investigated under H_2O_2 oxidation with different concentrations and time. The carotenoids of *C.reinhardtii* microalgae produced under different oxidative stress environments were analyzed by HPLC-DAD. Total phenolic content and antioxidant capacity of *C.reinhardtii* were determined by Folin-Ciocalteu method and CHROMAC method, respectively under oxidative stress.

MATERIAL AND METHODS

Chemicals

Ethanol, acetone, dichloromethane, ter-butyl methyl ether and methanol were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteau reagent, trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid], gallic acid, neoxanthin, beta-carotene and alpha-carotene were purchased from Sigma Aldrich (St Louis, USA). Lutein and beta cryptoxanthin were purchased from Extrasynthese (France), astaxanthin and zeaxanthin were purchased from Dr.Ehrenstorfer GmbH (UK). The other chemicals used were of analytical grade.

Isolation, identification, and cultivation

Chlamydomonas reinhardtii sp TMCC8 was isolated from Lake Nemrut, located in the Nemrut stratovolcano in Bitlis Province, Turkey (N 42°15'25"- 42°24' 48" and E 38° 31'11"-38°36'12"). The strain was first morphologically identified via microscopic observation according to Kessler (1976). The species was also characterized via sequencing of the 18S rRNA gene. Accordingly, the genomic DNA fragment was amplified by PCR, sequenced, and analyzed following Hoham et al. (2002). Sequence comparison of the 18S rRNA genes was performed using the NCBI databases with BLASTN search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and BioEdit-graphical biological sequence editor v7.0.9 and submitted to NCBI gene bank with an accession number, KP259886. For experiments, C. reinhardtii was cultured in 500 ml of TAP medium (Rippka et al., 1979, Çakmak et al., 2015) in 1 liter Erlenmeyer flasks under continuous light (150 PAR) on an orbital shaker at 120 rpm and 25°C. The authors adhered and confirmed that ethical statement as noted on the journal's author guidelines page. The Directive 2010/63/EU standard was followed for this study.

H_2O_2 oxidation of C. *reinhardtii*

In order to count the algae at different times, an amount of 4-day microalgae culture was allowed to incubate in the control medium. After sowing in the sterile chamber, the newly cultivated samples were cultured in a shaking incubator at 120°C at 23° C under continuous light at 100 μmol photon m^{-2} s^{-1} intensity. The growth of microalgae grown in 250 mL erlene containing 100 mL liquid growth culture was divided into four groups with three replicates when they reached the absorbance value of 1.0 (λ=680 nm). In addition to the control group, the cultures were treated with H_2O_2 at a concentration of 1 mM, 5 mM and 20 mM and were harvested at 2, 8, 24 and 72 h after application. The concentration of H_2O_2 in sample was determined according to the literature (Çakmak et al., 2015).

Ultrasonic-assisted extraction

C. reinhardtii samples were lyophilized for an hour (freezing at -86°C, followed by drying at 0,1 mbar). Then the lyophilized samples were extracted with ethanol (2 mL) and mixed by vortex. The samples were placed in ultrasonic cleaning bath at 40°C for 2 hours. Ultrasonic-assisted extraction was performed in a temperature-controlled ultrasonic cleaner bath (40 kHz and 200 W) and also the temperature was monitored using a thermometer. Then, the extract was filtered and the filtrate was used for spectroscopic and chromatographic analysis.

Folin-Ciocalteu method

Determination of total phenolic content using the Folin–Ciocalteu method was performed

according to literatures (Güçlü et al., 2006; Şahin et al., 2013). 0.2 mL of sample was taken, 1.8 mL of distilled water and 2.5 mL of Lowry C solution (50 mL Lowry A +1 mL Lowry B) were added in a test tube. After vortexed samples, 0.25 mL of Folin-Ciocalteu reagent was added a tube and the blue color was allowed to stabilize under darkness for about 30 min. The absorbance was measured at 750 nm by UV-VIS spectrophotometry (Varian Cary-50 UV/vis, in Melbourne, Australia) equipped with 10 mm quartz cuvettes. The results were expressed as mg gallic acid equivalent (GAE) of per gram of sample.

CHROMAC method

Antioxidant capacities of microalgae samples under oxidative stress were determined using the CHROMAC method was performed according to literatures (Işık et al., 2013; Nasir et al., 2017). 0.2 mL of sample and 0.3 mL of distilled water were added in a test tube. After vortexed samples, 3.5 mL of freshly prepared pH 2.8 phosphate buffer and 0.5 mL of $K_2Cr_2O_7$ (50 mg L^{-1}) were added in a tube. After incubation for 1 min, 0.5 mL of 1,5-diphenylcarbazide (3.4×10^{-4}mol L^{-1}) was added and stirred. The test tube was waited in the dark for 50 min to complete the reaction. The absorbance was measured at 540 nm by UV-VIS spectrophotometry. The results were expressed as mg Trolox equivalent (TE) of per gram of sample.

HPLC- PDA analysis

The carotenoids were analyzed by high performance liquid chromatography. An Agilent 1200 HPLC- PDA system (Waldbronn, Germany), consisting of a vacuum degasser, binary pump, autosampler, and a diode aray dedector, was used. Carotenoid separation was carried out on an YMC C30 carotenoid column (4.6 x 250 mm, 5 μm id.) from Waters (USA). Mobile phase consists of methanol: water (95:5, % v/v, consists of % 0.05 triethylamine in water, solvent A) and ter-butyl methyl ether (solvent B). Gradient conditions were as follows: 0-15 min 5% B (95% A), 15-20 min 20% B (80% A), 20-30 min 30% B (70% A), 30-40 min 40% B (60% A), 40-45 min 75% B (25% A), total run time is 45 min. The flow rate was 1 mL/min, injection volume was 20 μL and the column temperature set to 25 °C. The eluent was detected at 450 nm using a UV-visible detector.

Statistical analysis

Statistical analysis was performed with Minitab 17.0 for Windows (Minitan Inc., State College PA). ANOVA was used for data comparing at 5% significance level. Principal component analysis (PCA) was used for visualizing the effect of experiments (Total phenolic content, antioxidant capacity, carotenoid amount). The data was standardized for PCA analysis. With eigenvalues of 73.55 (PC1) , 24.19 (PC2) and 3.98 (PC3) that are estimated from the eigenvectors of the matrix represent 70.05, 23.04 and 3.80% of the total variance.

RESULTS AND DISCUSSION

Validation of analytical method

The linearity of the HPLC- PDA method was investigated for the determination of carotenoids at a concentration of 0.25-10 mg/L. Regression coefficients for each of the calibration plots were obtained using different carotenoid compounds. As shown in Table 1, the limits of detection (LOD, 3 s/m) and limits of quantification (LOQ, 10 s/m) of some carotenoids (lutein, β-carotene, α-carotene, zeaxanthin, neoxanthin) in *C.reinhardtii* microalgae were calculated. The peak purity values, capacity (k') and selectivity factors (α) of the carotenoids were also calculated. Based on the obtained values, it has been shown that carotenoids can be distinguished by HPLC- PDA method successfully.

Identification of carotenoids under oxidative stress

This study was taken up to understand the influence of H_2O_2 oxidation stress on carotenogenesis. After the growth phase of *C. reinhardtii,* different concentration levels (1, 5 and 20μM) of H_2O_2 were studied. The *C. reinhardtii* cultures exposed to H_2O_2 for 72 h and accumulated higher carotenoid production as compared to control within 24 h (Figs. 1-5). However, the decrease on carotenoid production was observed at 72 h. When trials were conducted using different concentration of H_2O_2, cell rupturing and damage was observed at higher

concentration (>20μM). Growing cells were found to be more sensitive to higher concentration of H_2O_2 compared to control group. H_2O_2, which is non-toxic cellular metabolite, can act as a signaling molecule that mediates responses to various stimuli and stresses, such as the regulation of the antioxidant defense system (Mittler 2002; Neill et al., 2002; Apel et al., 2004; Mittler et al., 2004). Carotenoids are natural pigments synthesized from plants as well as from algae and microorganisms. Neoxanthin, lutein, zeaxanthin, α-carotene and β-carotene were determined in *C. reinhardtii* under H_2O_2 oxidative stress. The significant differences that detected in amount of carotenoids among the samples depending on the concentration of H_2O_2 and oxidation time (p<0.05). The amount of carotenoid was decreased in control group except zeaxanthin and α-carotene. At the beginning, the amounts of carotenoids have similar decrease behavior at the same concentration of H_2O_2 and then increased gradually after 24 h. The amounts of synthesized carotenoid in the live culture medium under oxidation condition are sensitive to some factors such as concentration of oxidant in the environment, enzyme concentration, etc (Çakmak et al., 2015).

Table 1. Analytical parameters for analysis of carotenoids by HPLC- PDA

Carotenoids	Concentration (mg/L)	y=mx+b	R^2	LOD (mg/L)	LOQ (mg/L)	Peak purity (%)	Capacity factor (k')	Selectivity factor (α)
Lutein	0.25-10	y = 71.224x - 1.9159	0.998	0.02	0.06	99.88	5.04	1.19
β-carotene	0.25-10	y = 154.46x - 19.5	0.998	0.06	0.21	99.99	10.56	1.04
α-carotene	0.25-10	y = 118.09x –17.225	0.999	0.02	0.06	98.52	9.53	1.11
Zeaxanthin	0.25-10	y = 265.7x-23.055	0.998	0.04	0.12	99.50	6.00	1.06
Neoxanthin	0.25-10	y = 35.158x+2.4447	0.997	0.01	0.02	96.89	3.15	1.15

Carotenoids, which are specific for each microalgae, can be synthesized using necessary enzymes. According to the biosynthesis diagram of microalgae, α-carotene can be converted to lutein, β-carotene can be converted to zeaxanthin and neoxantin from these carotenoids (Cordero et al., 2011; Butnariu 2016). In this study, the amounts of lutein and neoxanthin were found to be high in HPLC analysis when oxidative stress was applied. Because it was thought that α-carotene converted to lutein and β-carotene converted to neoxantin after oxidative stress according to the biosynthesis diagram of microalgae.

Neoxanthin has shown an increase of 2 and 3-fold than control group under 1 μM H_2O_2 oxidation stress at first 24 h (Fig. 1). And also, the amount of nexoanthin was approximately 1.5-fold higher than control group under 5 and 20 μM H_2O_2 oxidation stress. Because of oxidation of carotenoid and damaging of algae cell, the amount of neoxanthin was decreased during 72 h.

Lutein, which is one of the oxygenated derivatives of carotenes, has the highest amount (5.75 mg/g sample under 1 μM H_2O_2 oxidation stress) in *C. reinhardtii* under H_2O_2 oxidative stress (Fig. 2). The amount of lutein that found in human tissues was decreased in that order: 2 h> 24 h> 8 h> 72 h. It was seen that the amount of carotenoid depends on the several factors such as H_2O_2 concentration, time, temperature and also presence of other antioxidants. And also, these factors can attract the redox properties of carotenoids.

Zeaxanthin has the more ability to scavenge reactive oxygen species than lutein (Woodall et al., 1997). It has shown an increase 4 and 5-fold than control group under 1 μM H_2O_2 oxidation stress at first 24 h (Fig. 3). Its reactivity to reactive oxygen species is higher than that of lutein in the Fenton based system (Siems et al., 2005). Because of this reason the increase at the amount of zeaxanthin is higher than that of lutein within 24 h under 1 μM H_2O_2 oxidation stress.

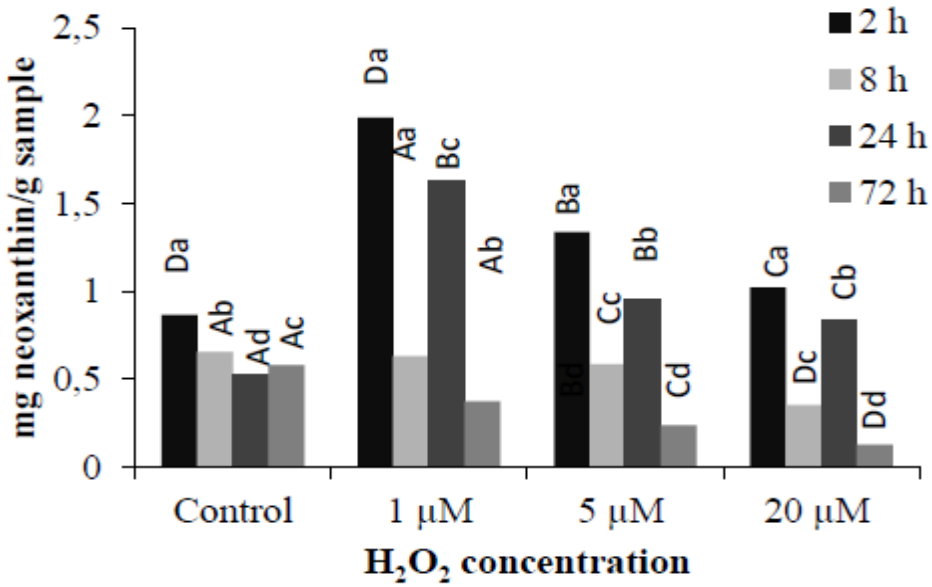

Fig. 1. Effect of H_2O_2 on the amount of neoxanthin in *C. reinhardtii*. Different uppercase letters (A-D) indicate significant differences between applied H_2O_2 concentrations at same time (p<0.05), different lowercase letters (a-d) indicate significant differences between times at the same H_2O_2 concentration (p<0.05).

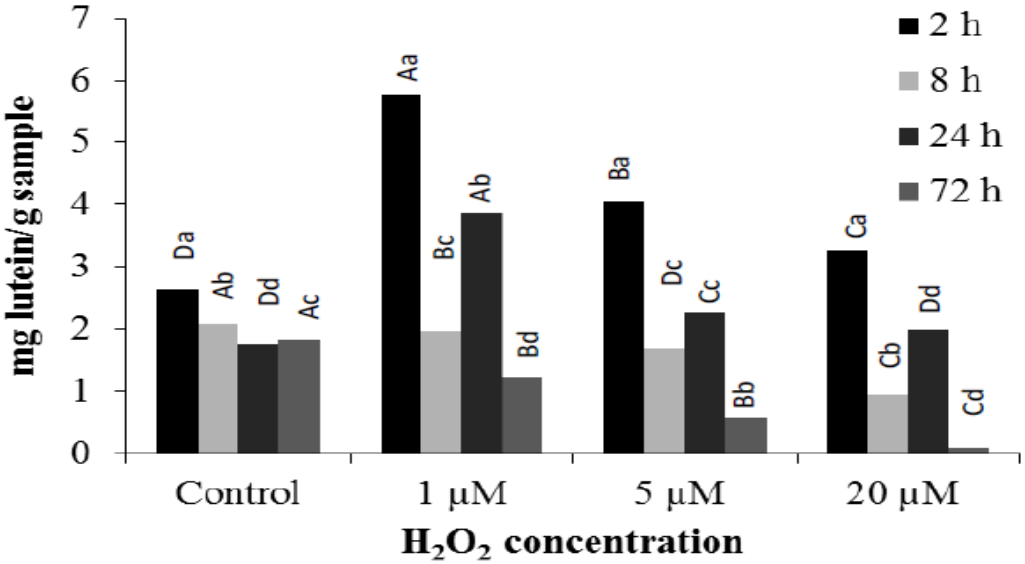

Fig. 2. Effect of H_2O_2 on the amount of lutein in *C. reinhardtii*. Different uppercase letters (A-D) indicate significant differences between applied H_2O_2 concentrations at same time (p<0.05), different lowercase letters (a-d) indicate significant differences between times at the same H_2O_2 concentration (p<0.05).

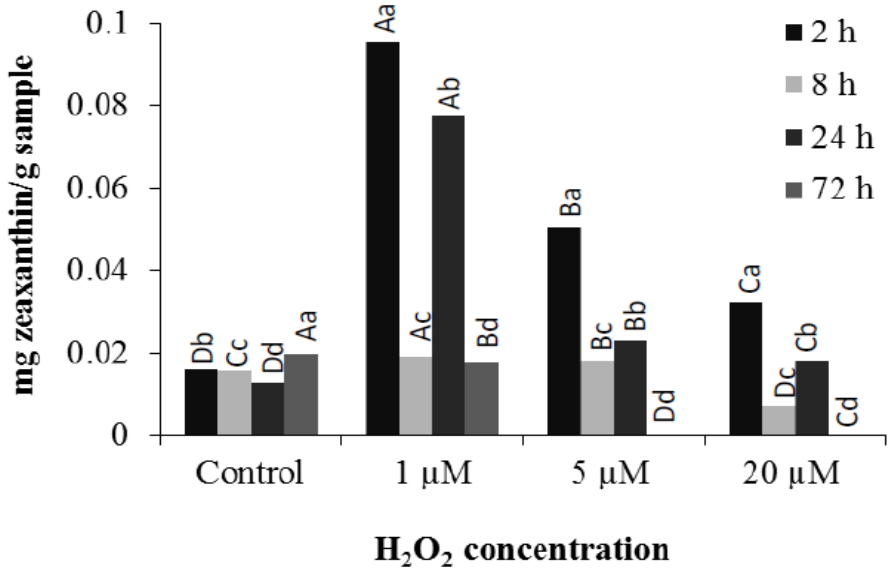

Fig. 3. Effect of H_2O_2 on the amount of zeaxanthin in *C. reinhardtii*. Different uppercase letters (A-D) indicate significant differences between applied H_2O_2 concentrations at same time (p<0.05), different lowercase letters (a-d) indicate significant differences between times at the same H_2O_2 concentration (p<0.05).

β-carotene and α-carotene, that are pro-vitamin A derivatives, have the same behavior under oxidative stress. There was no α-carotene detection under 5 µM H_2O_2 oxidation stress after 72 h in Fig. 4. When comparing with β-carotene, 0.06 mg β-carotene was quantified by HPLC-PDA in Fig. 5. As a result, the quenching activity of α-carotene is bigger than that of β-carotene. The amounts of these carotenoids were decreased in that order: 2 h> 24 h> 8 h> 72 h without control group.

While little is known about carotenogenosis pathways in algae, some suggestion pathways are presented based on carotenoid structures (Takaichi, 2011). Carotenoids protect organisms from photodegradation by damping the photodegradation created by singlet oxygen and triplet chlorophyll (Cl) species that can participate in redox reactions. Carotenoids help keep the membrane structure stable (Lohr et al., 2005). In case of formation of damaging reactive oxygen types, it is seen that some unstable and photoreactive species are formed during Cl biosynthesis. Therefore, carotenoid and Cl biosynthesis occur in chloroplasts and the formation of all enzymes in vascular plants is encoded by nuclear genes and synthesized in cytoplasm cells. A sudden increase in total carotenoid levels is observed in the medium of over pressure on the phytoene syntase genes (Lohr et al., 2005). In this study, the amount of carotenoids changed depending on the H_2O_2 concentration in the environment. In *C. reinhardtii* microalgae, α and β carotene are formed from lycopene carotenoid as a result of catalysis of the enzymes lycopene ε-cyclase (LCYe) and lycopene β-cyclase (LCYb). Lutein and zeaxanthin are then hydroxylated in α and β carotene. The enzymes involved here are P450 hydroxylases (P450b-CHY for α and β carotene, P450e-CHY for α-carotene). Neoxanthin can then be formed from zeaxanthin. This reaction can also be catalyzed by neoxanthin synthase (NSY) enzyme (Cordero et al., 2011). However, it is not known exactly how these enzymes are affected in oxidative stress environment and how they affect carotenoid biosynthesis in the literature. Under oxidative stress the carotenoids and their oxidation products have not studied yet in biological

samples such microalgae. The knowledge in similar study seems to be important to put forth the relationship between the amount of carotenoid and the power of oxidation stress. *C. reinhardtii* has been selected as the most valuable microalgae for production of carotenoids as a source of natural pigment when grown under defined conditions.

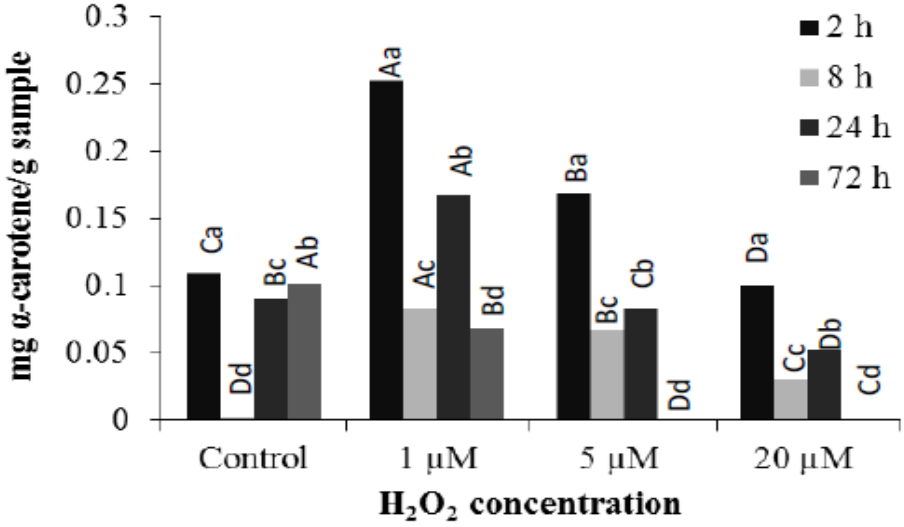

Fig. 4. Effect of H_2O_2 on the amount of α-carotene in *C. reinhardtii*. Different uppercase letters (A-D) indicate significant differences between applied H_2O_2 concentrations at same time (p<0.05), different lowercase letters (a-d) indicate significant differences between times at the same H_2O_2 concentration (p<0.05).

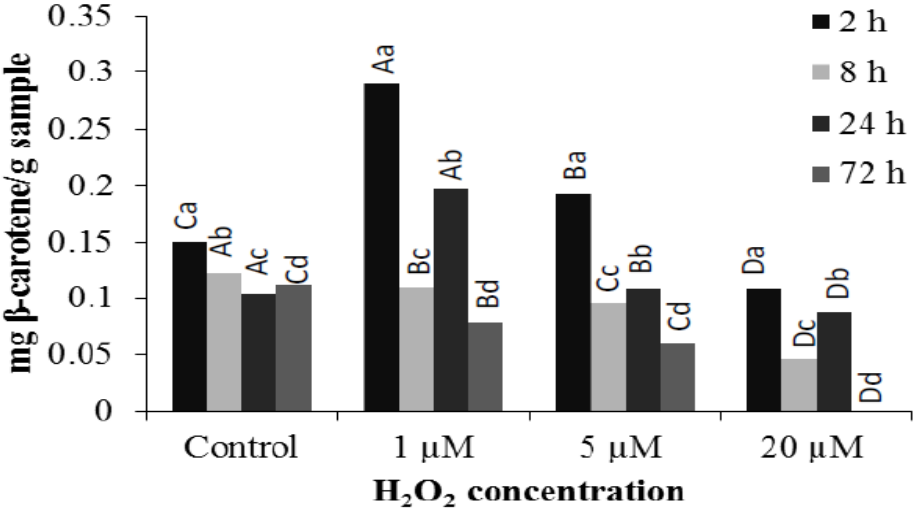

Fig. 5. Effect of H_2O_2 on the amount of β-carotene in *C. reinhardtii*. Different uppercase letters (A-D) indicate significant differences between applied H_2O_2 concentrations at same time (p<0.05), different lowercase letters (a-d) indicate significant differences between times at the same H_2O_2 concentration (p<0.05).

Total phenolic contents and antioxidant capacity of samples under oxidative stress

Antioxidant capacities and total phenolic contents of *C. reinhardtii* samples were determined by CHROMAC and Folin-Ciocalteu methods, respectively (Table 2). The correlation coefficient between total phenolic contents and antioxidant capacities is 0.57. This indicates that the results are compatible. When the concentration H_2O_2 was increased, the antioxidant capacity and total phenolic content of extract decreased at the same time. The highest amounts were obtained from extracts that were exposed to 1 μM H_2O_2 oxidative stress for 72 hours. This shows that the carotenoids are more abundant in the environment because of cell destruction at the end of 72 hours.

Table 2. The total of phenolic content and antioxidant capacity value in microalgae samples of *Chlamydomonas reinhardtii* exposed to different oxidative stresses

Time	Samples	Antioxidant capacity (mg TE/g sample)	Total phenolic content (mg GAE/g sample)
2 h	Control	6.861 ±0.096	0.334±0.004
	1μM H_2O_2	7.550±0.400	0.456±0.005
	5μM H_2O_2	6.954±0.057	0.323±0.004
	20μM H_2O_2	5.474±0.589	0.301±0.002
8 h	Control	7.037±0.396	0.344±0.011
	1μM H_2O_2	7.569±0.020	0.411±0.010
	5μM H_2O_2	7.189±0.002	0.398±0.002
	20μM H_2O_2	7.044±0.220	0.382±0.006
24 h	Control	6.759±0.374	0.356±0.021
	1μM H_2O_2	7.073±0.244	0.408±0.010
	5μM H_2O_2	6.934±0.113	0.372±0.003
	20μM H_2O_2	6.581±0.051	0.353±0.003
72 h	Control	7.490±0.053	0.342±0.005
	1μM H_2O_2	9.174±0.162	0.469±0.016
	5μM H_2O_2	8.517±0.596	0.348±0.010
	20μM H_2O_2	7.487±0.068	0.307±0.002

Principal component analysis

Many factors are correlated with each other and their contributions to the variation have been major or minor. The two outliers (total phenolic content and antioxidant capacity) have seen in the loading plot of microalgae extract on PC1-PC2 (Fig. 6). These outliers were distinguished from other factors by significantly. The amounts of carotenoid (neoxanthin, lutein, zeaxanthin, α-carotene and β-carotene) were correlated in each other.

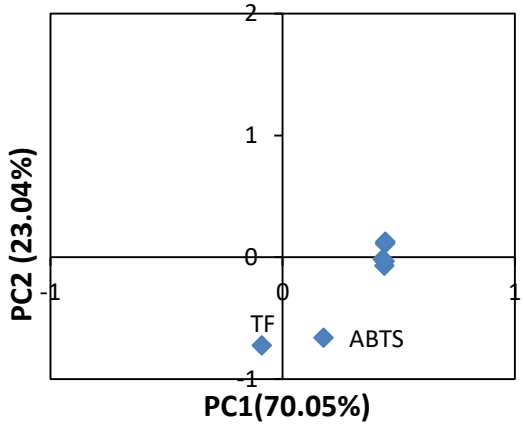

Fig. 6. The loadings plot of *Chlamydomonas reinhardtii* extracts on PC1-PC2 (TF: total phenolic content, ABTS: antioxidant capacity).

CONCLUSIONS

The effect of H_2O_2 oxidation stress on carotenoid production in *C. reinhardtii* for 72 h was investigated in this study. And after oxidative stress, total phenolic contents and antioxidant capacities of remaining samples were determined. The amounts of lutein and neoxanthin were found to be higher when determined by HPLC-PDA under oxidative stress. The five carotenoids' quantities were rapidly increased under 1 μM H_2O_2 oxidation stress. However, the highest total phenolic contents and antioxidant capacities were taken under 1 μM H_2O_2 oxidation stress for 72 h. The carotenoids are predominant antioxidants that effects on these results because of higher increase on their amount in that media. This study is important that the relationship between the amount of carotenoid and the power of oxidation stress was put forth successfully. Later, the oxidation products of these carotenoids can study to reveal anti- or pro-oxidant effects and biological potentials of carotenoids.

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CONFLICT OF INTEREST

The authors declare that there are no potential conflicts or competing of interest.

AUTHOR CONTRIBUTIONS

The conception and design of the study: Saliha Şahin, Turgay Çakmak. Acquisition of data: Çiğdem Yüksel, Saliha Şahin, Turgay Çakmak. Analysis and interpretation of data: Çiğdem Yüksel, Saliha Şahin. Drafting the article: Saliha Şahin. Revising the article critically for important intellectual content: Saliha Şahin. Final approval of the version to be submitted: Saliha Şahin

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