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Determination of Biological Activities and Color Kinetics of *Spirulina platensis* During Convective Air Drying

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Abstract: *Spirulina platensis (S.platensis*), procaryotic microalgae, is favored as a functional food owing to the high protein content, fatty acid, phenolics, vitamins, and minerals preferred by consumers. Producers prefer to sell it in dry form because of the shipping and shelf-life advantages. This study intends to describe the effect of convective drying on biological activities and color changes of *S.platensis.* Firstly, mass production of *S. platensis* was accomplished in flat-panel bioreactor, and harvested cells were dried by a convective hot air dryer.

Convective drying treatments were carried out at 40, 45, and 50 °C. On the contrary of the total phenolic content values, an increase in the temperature from 45ºC to 50 ºC was more effective than the increase from 40 ºC to 45 ºC on the antioxidant capacity of the samples. Activation energy values were calculated for the antioxidant capacity and total phenolic content were about 24.46 kJmol^{-1} and 24.94 kJmol^{-1}, respectively. The color values (*L, a, b*) were measured, and the total color difference (ΔE) was calculated as 3.019, 2.98, and 2.94 at 40, 45, and 50°C, respectively. ΔE characterized by a zero-order model and the activation energy (Ea) for change in ΔE was calculated as 50.15 kJ.mol^{-1}. The results indicated that the most suitable temperature most suitable temperature for convective drying for *S.platensis* would be nearly 50°C.

Key words: Antioxidant capacity, convective drying, drying and color kinetics, *Spirulina platensis*, total phenolic content.

Konvektif Kurutma Süresince *Spirulina platensis*'in Renk Kinetiği ve Biyolojik Aktivitelerinin Belirlenmesi

Öz: Prokaryotik bir mikroalg olan *Spirulina platensis (S.platensis)*, yüksek protein içeriği, yağ asidi, fenolikler, vitaminler ve mineraller nedeniyle tüketiciler tarafından fonksiyonel gıda olarak tercih edilmektedir. Üreticiler ise nakliye ve raf ömrü avantajları nedeniyle *S.platensis'in* kurutularak satışa sunulmasını arzu etmektedir. Bu çalışma ile kurutma metotlarından biri olan konvektif kurutmanın *S.platensis*'in biyolojik aktivitesi ve renk değişikliği üzerindeki etkisi incelenmiştir. Öncelikle panel biyoreaktörde *S.platensis*'in üretimi gerçekleştirilmiştir ve hasat edilen aynı biyokütleye sahip hücreler konvektif sıcak hava kurutucusu ile kurutulmuştur.

Konvektif kurutma işlemleri 40, 45 ve 50 °C' de gerçekleştirilmiştir. Sıcaklığın 45 ºC' den 50 ºC' ye kadar olan artışı antioksidan kapasitesi üzerinde etkili olurken 40 ºC' den 45 ºC' ye yükseltilmesi toplam fenolik içerik değerleri üzerinde etkili olmuştur. Antioksidan kapasite ve toplam fenolik içerik için hesaplanan aktivasyon enerjisi değerleri sırasıyla 24.46 kJmol^{-1} ve 24.94 kJmol^{-1} olarak hesaplanmıştır. Renk değerleri (L, a, b) ölçülmüş ve 40, 45 ve 50 °C' deki toplam renk farkı (ΔE) sırasıyla 3.019, 2.98 ve 2.94 olarak hesaplanmıştır. ΔE sıfır dereceli bir model ile karakterize edilmiştir ve ΔE' deki değişim için aktivasyon enerjisi (Ea) 50.15 kJ.mol^{-1} olarak hesaplanmıştır. Sonuçlar en etkin kurutma sıcaklığının 50 °C olduğunu göstermiştir.

Anahtar Kelimeler: Antioksidan kapasitesi, konvektif kurutma, kurutma ve renk kinetiği, *Spirulina platensis*, toplam fenol içeriği.

1.Introduction

S.platensis which is the most commonly cultured, filamentous, spiral-shaped procaryotic organism of the class *Cyanophyceae* (Blue-green algae), is widely used in medicine, cosmetics, as well as human and animal food in various industrial fields. Due to its high protein value and other nutrient content, it is grown

phototrophically in open pools on the industrial scale (Kargin Yilmaz and Duru, 2011).

In food industry, *Spirulina* is considerably important because of its high nutritional value in the production of certain chemicals and animal feed. Moreover, it is a rich source of antioxidants, amino acids, vitamins, and minerals (Koru and Cirik, 2002).

Spirulina is a low-fat and low-calorie, cholesterol-free protein source containing all essential amino acids and consists of 60-70% protein in its dry weight. This ratio is much higher than many protein sources (Gutiérrez-Salmeán *et al*. 2015). Besides, the organism is a source of some micro and macro minerals such as iron, selenium, magnesium, calcium and contains high provitamin A, Vitamin E, thiamine, cobalamin, biotin, and inositol (Kay and Barton, 1991; Belay *et al*. 1996; Belay, 1997). Moreover, it also contains more than 2000 enzymes, many essential fatty acids i.e. gamma-linolenic acid, linoleic acid, and arachidonic acid which have recently come to the fore, as well as growth factors, nucleic acids, and high biologically essential amino acids (Belay *et al*. 1996; Cohen, 1997).

The cell wall of *S.platensis* consists of mucopolysaccharides and does not contain cellulose in its structure. This structural feature facilitates the digestion and absorption of *S.platensis*, which is important for older people and those with intestinal absorption disorders. *S.platensis* also contains some antioxidant pigments that help in synthesizing a large number of enzymes necessary for human metabolism. One of these pigments is Phycocyanin, which is one of the most important pigments containing iron in the structure of *S.platensis*. Another significant pigment is chlorophyll, an herbal pigment with strong cleansing and detoxifying effects. The most known pigment of *S.platensis* is beta-carotene. Aside from beta-carotene, *S.platensis* contains at least 10 kinds of carotenoids with opulent antioxidant properties. These carotenes and xanthophylls are active in different parts of the body together with the coactivation of other essential vitamins and minerals (Babadzhanov et al. 2004). In studies conducted, it has been reported that the sulfolipids and polysaccharides in *S.platensis* are active in cancer treatment, their regular intake accelerates antiviral processes, supports the immune system, reduces kidney toxicity and the severity of radiation-induced diseases and injuries (Iijima et al. 1983; Zhang et al. 2001). In addition, the United Nations and the World Health Organization reported that *S.platensis* is a natural and safe food for children and adults (Michaelsen et al. 2009).

Microalgae are the primary biological CO_2 / O_2 converters of aquatic systems, the most important biomass producer, and the most important prospective source of biotechnology (Borowitzka, 1992; Tsoglin and Gabel, 2000; Pulz, 2001). Thus, *they* are one of the most important types of algae cultivated today. Among all algal species, *S.platensis* and *Spirulina maxima* are the species that have commercial value and are undergoing extensive research (Richmond, 2004).

Considering the market, *S.platensis* is sold in powder form due to both ease of storage and extended shelf life. Among drying methods, spray-dryer is preferred for large-scale productions, sun drying for small-scale applications, and convective drying for laboratory-scale ones. As a result of sun drying, the traditional method, the nutritional value of microalgae may decrease, and drying cannot be carried out in a sterile manner. In addition, there is an inability to realize a uniform drying at all points for large amounts of microalgae (Brennan and Owende, 2010; Chen et al. 2015).

Due to the short drying time, spray dryer can dry even temperature-sensitive products easily, and without damage. For obtaining both tablets or powdered form of microalgae, spray dryer is preferred today. It provides a controlled drying process in a short time at high temperature. Although convective drying is not suitable for large-scale drying, it is the most controlled and sterile drying type among all drying methods. It is possible to adjust conditions such as temperature, air speed, and the entire harvest dries evenly. The aim of this study is describing the effect of convective drying on the biological

activities and color changes of *S.platensis.*

2.Materials and Method

2.1.Materials

S.platensis was from the collection of the Department of Bioengineering at Ege University. Flat-panel bioreactor (3L) made by glass, and air feed controlled by a flowmeter.

2.2.Methods

Concentrated Zarrouk's medium was used as the culture media for production of *S.platensis* (Becker, 1995). After all ingredients were dissolved in 1L distilled water, the concentrated medium was autoclaved at 120 °C for 15 min.

2.2.1.Production of Spirulina platensis

Fresh medium was inoculated with the culture at 10% (v/v) in 250 mL Erlenmeyer flasks for 15 days. Sterilized Zarrouk's medium transferred to a flat-panel bioreactor (3L) by using sterile tubes (Figure 1a). The light source was 40W daylight fluorescent lamp. The air was supplied by an air pump. The growth was monitored by optical density and the dilution amount was controlled to have OD between 0.200 and 0.260 at 560 nm. For determining the changes in optical density of bioreactor medium, samples were taken once a day. The cells were separated from the media by Whatman No:1 filter.

2.2.2.Convective drying

Throughout convective drying, the first step was washing the strains by dH_2O to remove the media remaining. Washed strains were spread on the filter paper as 5 g and dried at 40, 45, and 50 °C air temperatures for 160 min. The dryer consists of a centrifugal blower for supplying desired air velocity, an electric heater for heating up the drying chamber, and an electronic proportional controller (ENDA, EUC442, Istanbul, Turkey). The air rate was kept constant at 0.2 m/s in the whole process. Samples were taken for every temperature at specific intervals and weighed. Drying process continued until the samples reached the constant weigh. The final moisture content of the sample was determined following each drying experiment. After measuring the sample weighs, all samples were kept at -20°C. All of the experiments were triplicated at each air temperature parameter and the average values were used.

The oven method was carried out for determining the moisture content (kg water/kg dry matter) of the samples throughout convective drying. The drying curves were obtained by generating plots of instant moisture contents of the samples (on dry basis) versus drying time.

2.2.3.Dry weights of the strains

Harvested cell debris was spread on a sterile filter paper and air-dried for 24h at 105°C and kept in the hot air oven to a constant weight. Then the samples were placed in desiccators until cold and weighed. The difference in weight gave as dry weight. The moisture ratio of the samples during drying was calculated by using Equation 1.

$$MR = \frac{M - M_e}{M_i - M_e} \quad (1)$$

where MR is the moisture ratio, M is the moisture content (kg water/kg dry matter) at any specific time, M_i is the initial moisture content, and M_e is the equilibrium moisture content.

2.2.4.Color measurements

The color measurements of *S.platensis* samples was carried out before and throughout the drying process by a Hunter Lab MiniScan XE colorimeter (Hunter Associates Laboratory, Reston, VA). Color values were measured three times and expressed in Hunter Color Scale (L, a, b). These parameters were converted to numerical values of the total color difference (ΔE) calculated by Equation 2.

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (2)$$

where Δa, Δb, and ΔL are the differences between the values of the coordinates a, b, and L of fresh and dried samples, respectively (Costa *et al*. 2016).

2.2.5. 2,2, diphenyl 1-picryl hydrazyl (DPPH) radical scavenging assay

Samples (200 µL) were diluted in to the different concentrations with ddH_2O for the 2,2,

diphenyl 1-picryl hydrazyl (DPPH) assay. First 600 µL DPPH solution (0.2 mM) mixed with the samples and then vortexed and incubated for 30 minutes at 25°C . The samples were measured at 570 nm.

The 2,2, diphenyl 1-picryl hydrazyl (DPPH) radical scavenging activities of samples were calculated by following Equation 3.

$$DPPH(\%)=[1–(A_s/A_b)]\times1000 \quad (3)$$

where A_s is the absorbance of sample and A_b is the absorbance of blank (Aytekin *et al*. 2011).

2.2.6.Total reducing power (FRAP) assay

Samples were mixed with 1 mL sodium phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%, 1 mL) was added to initiate the reaction. The mixtures were incubated at 50°C for 20-min. The reaction was stopped by adding 1 ml of trichloroacetic acid (10%). After centrifuged the sample, supernatant was collected and diluted with deionized water (1:1, v/v), 0.2 mL of ferric chloride (0.1%) was added to the mixture and incubated for a 5-min at the room temperature, samples were measured at 700 nm against the blank. A higher absorbance indicates a higher reducing power (Rice-Evans *et al*. 1997).

2.2.7.Total phenolic content

The total phenolic content was measured by the Folin–Ciocalteu assay. Firstly, 100µL sample added to 200µL Folin–Ciocalteu solution (diluted with ddH2O 1:1, v/v) and were mixed by 800µL sodium carbonate (75 g L^{-1}). The mixture was incubated at room temperature for 2 h. After the incubation, the absorbance of the samples was measured at 765 nm. Gallic acid was the standard, and the results were expressed as milligram gallic acid equivalents (GAE) per liter (Üstün-Aytekin *et al*. 2020).

2.2.8.Determination of protein amount

The bicinchoninic acid assay (BCA) was used to calculation of the protein concentration. The manufacturer's protocol was followed, Protein Assay Reagent Kit (Pierce; Thermo Scientific, USA).

2.2.9.Calculation of kinetic parameters for DPPH, total phenol and color

The biological activities of *S.platensis* were measured for specific intervals (every 10 min at 50˚C, 15 min. at 45˚C and 10 min. at 50˚C) at each drying temperature. The kinetics of DPPH, total phenol, and color degradation in dried *S.platensis* followed a first-order reaction (Equation 4).

$$lnC = lnC_0\text{-k.t} \quad (4)$$

where C is the concentration at time t, C_0 is the initial concentration of DPPH, FRAP, total phenol, and color value, and k is the reaction rate constant. Temperature dependence of the reaction rate constants followed the Arrhenius equation (Equation 5)

$$k = k_0.\exp\left(-\frac{E_a}{RT}\right) \quad (5)$$

where k is the reaction rate constant, k_0 is the preexponential constant , Ea is the activation energy (kJ/mol), R is the universal gas constant (kJ/mol.K) and T is the absolute temperature (K).

Temperature coefficient (Q_{10}) is the criterion indicating the effect of raising the temperature by 10˚C on the rate of reaction, and it was calculated by Equation 6.

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2-T_1}} \quad (6)$$

where k_1 and k_2 are reaction rate constants at temperatures T_1 and T_2.

2.2.10.Statistical Analysis

The experimental results are expressed as mean value ± standard deviation of triplicate measurements.

3.Results and Discussion

3.1.The growth of *Spirulina platensis*

S.platensis cells were grown in the selective media for 15 days at specific conditions to obtain maximum biomass amount. The growth curve was occurred by measuring optical density of the cells. Production has been ended, and the cells were harvested at OD 1.250 at the end of the production period (Figure 1b and 1c).

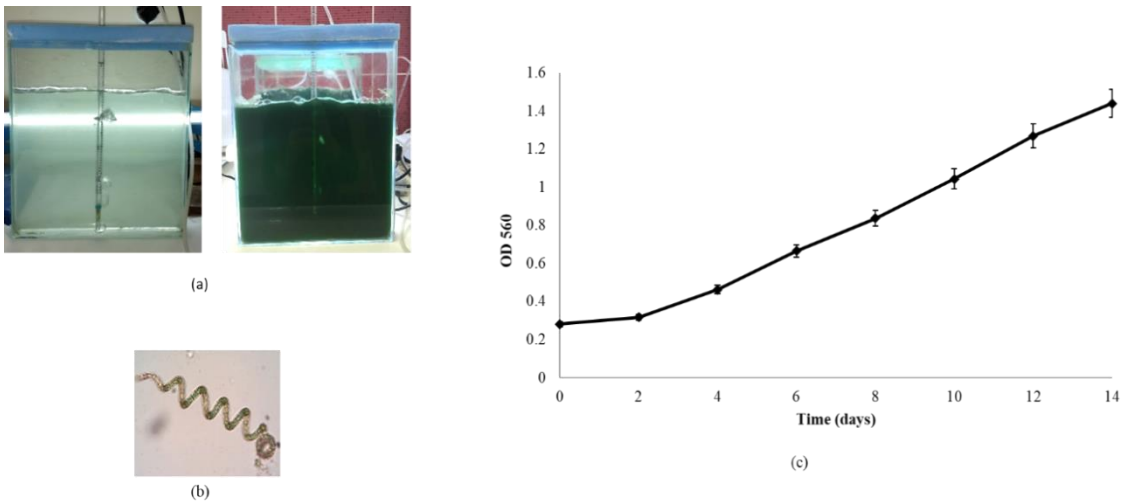

Figure 1. Flat-panel bioreactor system **(a)** *Spirulina platensis* cells under the binoculer microscope (Olympus (CX21, 40 X) **(b)** The growth curve of *Spirulina platensis* **(c)**

Şekil 1. *Düz panel biyoreaktör sistemi* ***(a)*** *Spirulina platensis hücrelerinin binoküler mikroskop görüntüsü (Olympus (CX21, 40X)* ***(b)*** *Spirulina platensis'in büyüme eğrisi* ***(c)***

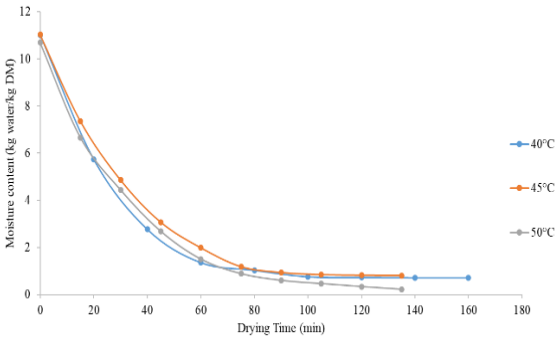

Figure 2. Moisture content variation of *Spirulina platensis* with time at different temperatures

Şekil 2. *Spirulina platensis'in kurutulmasında farklı sıcaklıklardaki nem içeriği değişimi*

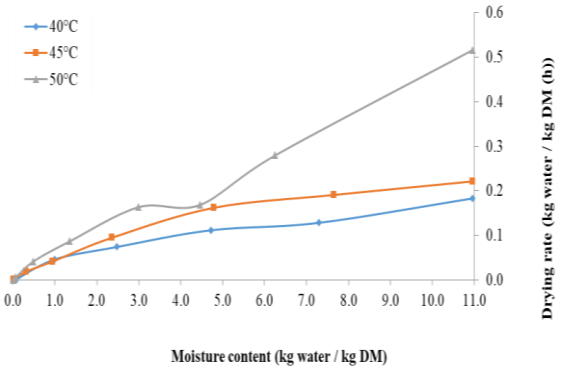

Figure 3. Drying rate versus moisture content of *Spirulina platensis* at different temperatures

Şekil 3. *Spirulina platensis'in farklı sıcaklıklardaki kuruma hızının nem içeriğine göre değişimi*

The drying rates-moisture content graph was shown in Figure 3, mostly decreasing rate drying period was observed at all drying air temperatures. Unlike other temperatures (40 and 45°C), a constant rate drying period was clearly observed at 50°C. Targeted drying time was sustained by elevated drying air temperature that increased drying rate.

3.2.The color kinetics of *S.platensis* drying

S.platensis is a blue-green *Cyanobacterium* and it contains chlorophyl (green), carotenoid (yellow), and phyocyanin (blue) in the cellular structure (Prasetyaningrum and Djaeni, 2012). The color of *S.platensis* might be affected by environmental conditions such as pH and processes temperature. Therefore, three different drying temperatures and their effects on the color values (*L*, *a*, *b*) of *S.platensis* were measured and given in Figure 4. *L* value represents lightness (from 0 to 100), a value indicates the redness (+*a*)/greenness (-*a*) and *b* value indicates the yellowness (+*b*)/blueness (-*b*). *L*, *a*, and *b* values often used for quality control or preparing formulation. In the study, *L* value increased from 12.39-13.8 to 22.73-24.09 with drying time at

different temperatures from 40 to 50 ºC, respectively. *L* value of foods has turned to darker as a general drying tendency because of their sugar content. However, there was no carbon molecule in the selective media for *S.platensis*, phototrophic microorganism. Kuatrakul *et al*. (2017) reported similar results for *L* value (23.22 to 36.20) in their study. The results for *a* value showed an increase in the green color from chlorophyl while the temperatures and the process time increased because of evaporation. It is not detected significant differences in the b value, because carotenoid molecules have resistant the thermal effect up to 60°C (Jaeschke et al. 2019). The reason for not detected blue color on the sample was the phycocyanin had highly unstable and decreased the amount of phycocyanin above 40°C (Sarada *et al*. 1999; Oliveira *et al*. 2008). Total color difference (ΔE) calculated as 3.019 at 40 °C, 2.98 at 45 °C and 2.94 at 50°C. It could be characterized by a zero-order model. The activation energy (Ea) for change in ΔE was calculated as 50.15 kJ.mol^{-1}. The results showed a decrease in ΔE during thermal treatment, unlike the other studies which had reported an increase in ΔE pineapple, carrot, and apple (Jokic *et al*. 2014; Demiray and Tulek, 2014).

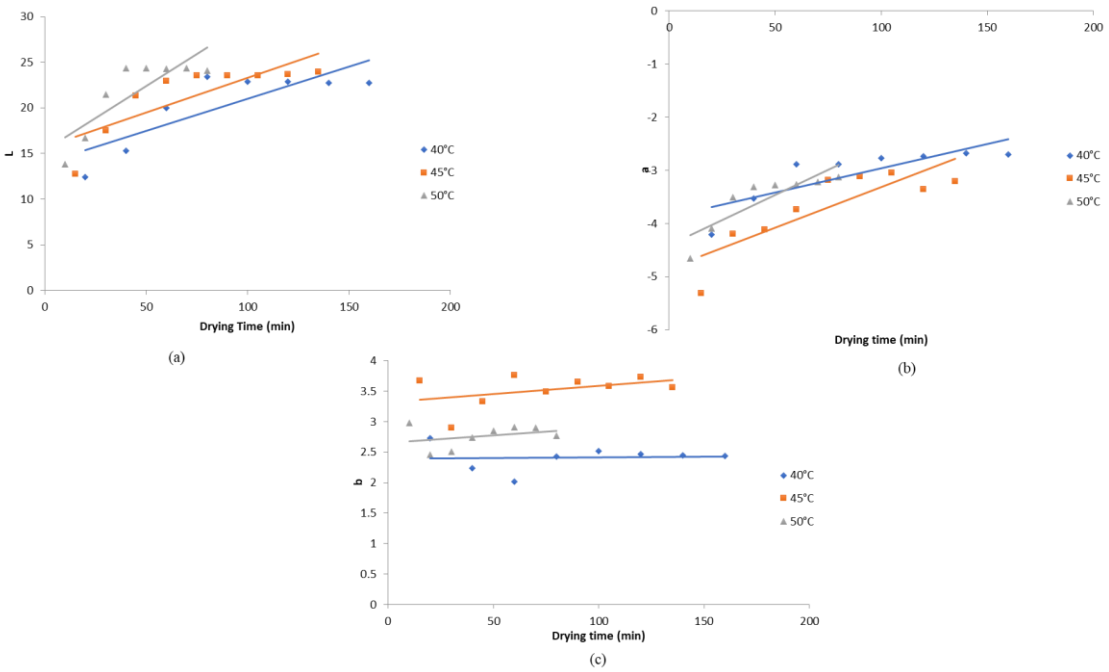

Figure 4. Kinetics of change of the *L* value (a), *a* value (b*)* and *b* value (c) as a function of drying time at different drying temperatures (Zero-order model)
Şekil 4. *Farklı kurutma sıcaklıklarında L, a, b renk değerlerinin kurutma zamanına göre değişim kinetikleri*

3.3.Kinetic parameters of *S.platensis* at different drying for DPPH, FRAP and total phenol

The antioxidant amount of the samples was determined during the drying process. The results of the present study were in accord with Zaid *et al*. (2015), and Al-Dhabi and Valan Arasu (2016), who reported that *S.platensis* has a high amount of DPPH activity. Researchers reported that the reason of the high antioxidant activity is a number of natural pigments such as chlorophyll, beta-carotene, phycoerythrin and phycocyanin (Gad *et al*. 2011; Zaid *et al*. 2015; Al-Dhabi and Valan Arasu, 2016). However, there was a slight increase in the amount of FRAP from 0.016 to 0.021 ABS at 700 nm. The kinetic parameters about DPPH and total phenol amounts were given in Table 1.

Regarding Table 1, as a result of the kinetic

models, it was determined that the kinetics of DPPH and Total phenol amounts in dried of *S.platensis* samples fit the first-order reaction model. The Ea value of the DPPH was calculated as 24.46 kJmol^{-1} and The Q_{10} values were showed that the increase of the temperature from 45ºC to 50 ºC is more effective than the increase from 40 ºC to 45 ºC. However, the effect of the temperatures on the total phenol was showed the opposite effect in the same ranges.

Table 1. The estimated kinetic parameters for DPPH and total phenolic content of *Spirulina platensis* at different drying temperatures.
Çizelge 1. *Spirulina platensis'in farklı kurutma sıcaklıklarındaki DPPH ve toplam fenolik madde içeriğine ait kinetik parametreler.*

	(ºC)	Q_{10}	k (min^{-1})	Ea (kJmol^{-1})
DPPH	40	1.263	0.6265	24.46
	45	1.417	0.7043	
	50		0.8384	
Total phenol	40	3.210	0.2912	24.94
	45	0.561	0.5218	
	50		0.3911	

There was no significant difference between the protein amounts of the *S.platensis* at the beginning of the drying processes and the end of the drying process, at 40 ºC. However, it was 1.5 fold increased at the end of the processes at 45 ºC and 50 ºC.

4.Conclusion

This study examined the influences of the convective drying method on antioxidant capacity, total phenolic content, and color changes of *S.platensis*. The results have indicated that the most intense color change was found at 40ºC. The zero-order kinetic model was applied to interpret the color change kinetics and ΔE. The activation energy for change in ΔE was figured up as 50.15 kJ.mol^{-1}. *L* value increased from 12.39-13.8 to 22.73-24.09 with drying time at different temperatures from 40 to 50 ºC. The most effective temperature increase for the antioxidant capacity was determined from 45ºC to 50 ºC. A constant rate drying period was clearly observed at 50°C. The highest Q_{10} value for total phenolic content was calculated by increasing drying temperature from 40ºC to 45 ºC. According to the results, the most suitable temperature for convective drying for *S.platensis* would be nearly 50°C.

5.Acknowledgement

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