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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Effects of different drying methods on C-phycocyanin content of *Spirulina platensis* powder

Farklı kurutma yöntemlerinin *Spirulina platensis* unundaki fikosiyanin içeriğine etkileri

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Abstract: The aim of this research was to determine the effects of different drying methods on C-phycocyanin extracted from *Spirulina platensis*. Two different drying techniques, including freeze-drying and oven-drying, were used in this research. 27.3% phycocyanin content was determined by freeze drying. Compared with that group, by oven drying method has been observed loss of 35.4% phycocyanin. The phycocyanin purity ratio (A_{620} / A_{280}) was found 1.26 and 1.16 by using freeze drying method and oven drying method, respectively.

Keywords: *Spirulina platensis, C-phycocyanin, drying mehod, freeze-drying*

Öz: Çalışmanın amacı, farklı kurutma yöntemlerinin *Spirulina platensis* mikroalginden elde edilen fikosiyanin üzerine etkilerinin belirlenmesidir. Çalışmada, freeze-dry tekniği ve fırın kurutma olmak üzere iki farklı yöntem denenmiştir. Freeze-dry tekniğinde fikosiyanin miktarı %27,3 olarak belirlenmiştir. Fırın kurutma yönteminde ise diğer gruba kıyasla %35,4 oranında fikosiyanin kaybı gözlenmiştir. Fikosiyanin saflık oranı (A_{620} / A_{280}), freeze-dry tekniğinde 1,26 ve fırın kurutma tekniğinde 1,16 olarak bulunmuştur.

Anahtar kelimeler: *Spirulina platensis, fikosiyanin, kurutma tekniği, freeze-drying*

INTRODUCTION

Phycocyanin is a blue pigment found in cyanobacteria (blue-green algae, procaryotic), rhodophytes (red algae, eukaryotic), and cryptomonads (Patel et al., 2005; Patil et al., 2006). *Spirulina platensis* is a source of cyanobacterial phycocyanin (C-PC). Phycocyanin constitutes approximately 20% of the dry weight of *Spirulina* (Vonshak, 1997).

In recent years, using cyanobacterial phycocyanin in industrial areas is increased. Phycocyanin is used as a natural colorant that is called as "Lina blue" in the commercial area. The purity ratio and quantity of phycocyanin may vary depending on the processing method. The purity of the phycocyanin is an important factor that determines its application area. The C-phycocyanin purity ratio is considered as the food grade when A_{620} / A_{280} is ≥ 0.7, and as the reagent grade when A_{620} / A_{280} is between 0.7 and 3.9; and as an analytical grade when A_{620} / A_{280} is ≥ 4.0 (Antelo et al., 2010; Kuddus et al., 2013). Food-grade phycocyanin is utilized safely as a dye in the food formulation without offering health risk (Kim et al., 2008). Analytical-grade phycocyanin is evaluated in medicine because it has antioxidant, anti-inflammatory, anti-cancer, and hepato-protective effects (Kuddus et al., 2013; Fernández-Rojas et al., 2014).

Phycocyanin is an expensive pigment with high value and applications in various biotechnology sectors. The price of food-grade phycocyanin is $ 500 per kg average. Used as the colorant phycocyanin is priced at $ 1-5 per gram. Reagents and analytical grade phycocyanin is priced at $ 14 to 25.000 per milligram.

The use of the C-phycocyanin is limited for its high cost. C-phycocyanin is a heat sensitive component. Traditional operating and drying methods can reduce the phycocyanin cost, with a lower purity and lower quantity. Freeze drying has been known as preserving the original material to avoid thermal damages. During freeze drying process, water is removed from the material while it is frozen via vacuum pumping (Nireesha et

al., 2013). The aim of this research is to determine the effects of different drying processes on phycocyanin and compare the phycocyanin content of the *Spirulina* powder obtained with Freeze Drying (F-D) or the Oven Drying (O-D) methods.

MATERIALS AND METHODS

Culture conditions of *Spirulina platensis*

Spirulina platensis was cultivated under the influence of seasonal temperature in greenhouse in 12 L plexiglass cylindrical algae ponds during 3 weeks. *Spirulina platensis* was grown in Zarrouk medium in repeated batches (Zarrouk, 1966).

Each culture was inoculated with initial *Spirulina platensis* biomass concentration of 0.237 OD_{680}. The samples were taken once a day to determine the optical density at A_{680} measurements (A: Absorbance value by spectrophotometer). The temperature and pH of culture tanks were measured as daily. At the end of cultivation, *Spirulina* biomass was dried by two different drying methods after filtration from 45 µ plankton cloth.

Applications of drying methods

Harvested biomass was dried in two different techniques, including oven drying and freeze drying. In the first method, *Spirulina* biomass was spread in the oven tray as a thin layer and dried in a laboratory type oven at +80 °C during 7 hours. In the second method, *Spirulina* biomass was dried at -60 °C in freeze dryer for 22 hours. *Spirulina* powder was obtained by grinding of dried samples and stored at +4 °C until analysis. The content of C-phycocyanin was calculated from these samples and was compared among themselves.

Determination of phycocyanin content

40 mg samples were placed in a 10 mL centrifuge tube containing 100 mM phosphate buffer (10.64 g K_2HPO_4 and 5.29 g KH_2PO_4 per L, 10 mL, pH 7.0) added and stored in refrigerator overnight after vortex. The blue supernatant was separated from the cell residue after centrifugation. C-phycocyanin calculations were determined using the spectrophotometry-based methods on the absorbance ratio.

The content of C-phycocyanin was calculated according to (Setyoningrum and Azimatun Nur, 2015) and (Boussiba and Richmond, 1979). Phycocyanin has a single visible absorbance maximum between 615 and 620 nm.

Calculations:

% C-phycocyanin = (A_{620} x V x100) / [3.39 x Sample (mg) x (% dry weight)]

3.39 is extinction coefficient of CPC at 620 nm; V is total volume; 100 represents 100%.

The purity of C-PC preparations was evaluated based on the ratio between absorbencies from phycocyanobilin at 620 nm and aromatic amino acids in all proteins in the preparation at 280 nm (Antelo et al., 2010).

Purity ratio of C-phycocyanin = A_{620} / A_{280}

RESULTS

Growth of *Spirulina platensis*

The Spirulina biomass was harvested when optical density of the culture reached 0.887 (A_{680}). Wet biomass concentration was 7.32 g/L end of cultivation. Biomass yield was 0.44 g/L. It is possible to claim that it is suitable for harvesting when the yield of biomass is 0.4-0.8 g/L (Vonshak, 1997; Kargın Yılmaz and Duru, 2011).

During the Spirulina cultivation, it was observed with an increase in the optical density when the temperature rises. As shown in Figure 1, the optical density values were affected by daily temperature changes. Temperature is accepted as an important factor that influences the overall biochemical composition in Spirulina culture (Cohen, 1997; Xie et al., 2015).

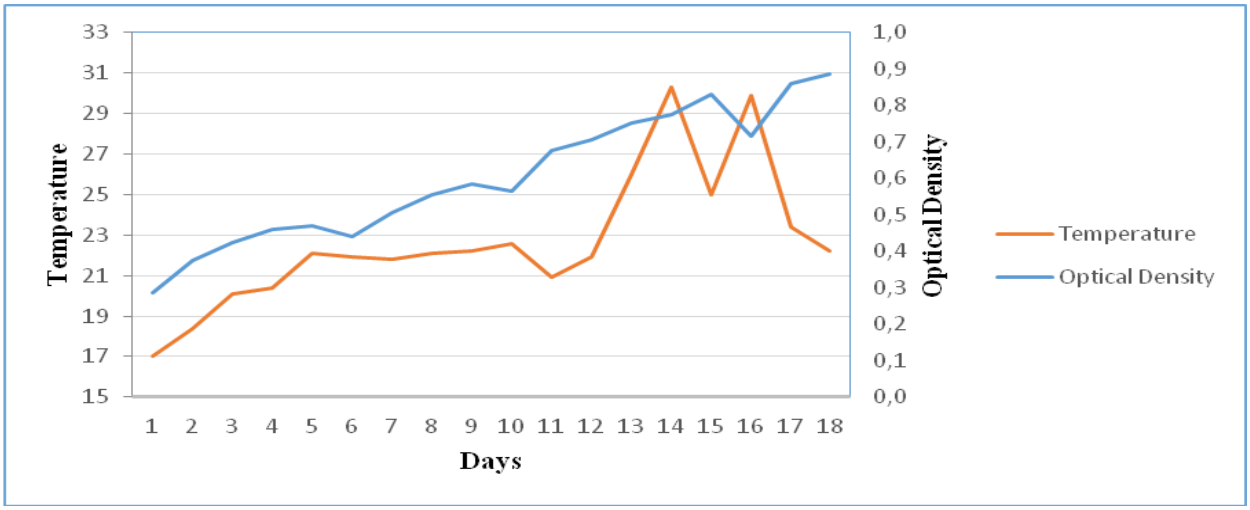

Figure 1. Optical density and temperature of the *Spirulina platensis* culture during 18 days

The effect of the drying methods on C-phycocyanin

The amount of phycocyanin obtained by freeze-drying method was considered as 100%. A significant difference observed between the two groups concludes that phycocyanin content. In the samples obtained by freeze drying method were determined 27.3% when phycocyanin content of the samples dried in the oven were 17.6%. Oven drying process has led to loss of the phycocyanin at 35% ratio (Table 1). Phycocyanin is a pigment sensitive to heat treatment. In this study, phycocyanin was determined to be sensitive to heat treatment and demonstrated negative effects of high temperature application. Sarada et al. (1999) reported that *Spirulina* contains 19% of wet biomass phycocyanin (Sarada et al., 1996). They have determined that spray drying process (150 °C) leads to the loss of the phycocyanin 45% while the loss of phycocyanin as 46% at oven dried (7 h at 60 °C). These findings were similar to O-D method data in our results. Freeze drying has been known as the best drying method to preserve the original properties of the resultant dried product because of the fact that it allows the removal of the water at low temperature, thereby avoiding thermal damage caused by the traditional drying operations. Sarada et al. (1996) have recommended the use of "freezing and thawing" as the best methods to prevent the loss of 50% phycocyanin among various drying methods. The freezing method was used in this present study as in the previous research, and our percentage on loss of phycocyanin has been lower than their data.

Phycocyanin is extremely sensitive to factors such as temperature, light, and pH. These factors can be lead to excessive loss of phycocyanin (Martelli et al., 2014). Oliveira et al. (2008) reported that high drying temperature (>60 °C) decreased the amount of the phycocyanin extractable from *Spirulina platensis*. Drying temperature of 80 °C applied in this study was the real reason for the loss of phycocyanin. The heat-sensitive phycocyanin was protected with the low temperature process in our study.

As shown in Table 1, purity ratio with freeze drying method was found to be more effective compared with the oven- drying method. By O-D and F-D method, obtained purity ratio was 1.16 and 1.26, respectively. In our study, phycocyanin purity ratio was found to be reagent grade in the both drying methods. Findings both in O-D and F-D methods were similar with Silva et al. (2009) and Antelo et al. (2010).

Table 1. Quantities of the C-phycocyanin in different drying methods

	Freeze- Dried	Oven Dried
Crude C-phycocyanin (mg) / *Spirulina* powder (100 mg)	27.3±0.08[a]	17.6±0.09[b]
Loss of Phycocyanin (%)	0	35.4
Phycocyanin purity ratio	1.26±0.11[a]	1.16±0.15[b]

Values are presented in terms of percent phycocyanin per 100 mg dry weight of *Spirulina*

The initial amount of phycocyanin was 27.3 mg for each drying methods

Spray drying, freeze drying, solar drying, and convective hot air drying methods are used to produce *Spirulina* powder. The phycocyanin is mostly extracted from *Spirulina* powder in the world for it is more practical. According to the results of our study it can be said that low-temperature applications may have positive effects on production costs of phycocyanin.

In conclusion, the freeze drying method is generally used to dry the heat-sensitive material in food and medicine industry. It is an expensive and long process of drying method. Oven drying is one of the most conventional methods known in the food industry. Unfortunately, heat sensitive ingredients in food can be damaged during the conventional drying processes. In this study, despite a rapid method of drying in the oven, it has shown that it may reduce the amount of the phycocyanin. Freeze drying is a better technology to preserve the original material and prevent thermal damage. Freeze drying should be preferred to obtain higher amount phycocyanin.

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