



## Determination of the Phycocyanin, Protein Content and Sensory Properties of Muffins Containing Spirulina Powder or Fresh Spirulina

### Taze Spirulina veya Spirulina Tozu İçeren Muffinlerin Fikosiyanin, Protein İçeriği ve Duyusal Özelliklerinin Belirlenmesi

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#### Abstract

**Objective:** The aim of this study was to evaluate the nutritional and sensory qualities of muffins containing fresh Spirulina (*Arthrospira platensis*) or Spirulina powder (dried Spirulina).

**Materials and Methods:** Spirulina forms of fresh or dried was added to muffins at 3 different levels (4%, 6% and 8%). Spirulina-free muffins was prepared as a control group. Groups were evaluated by sensory analysis in terms of appearance, texture, taste, odor, color and general acceptability. Protein and phycocyanin analyzes were performed in muffins.

**Results and Conclusion:** In groups containing 6% and 8% fresh Spirulina were found to contain higher phycocyanin than the all groups containing dried Spirulina ( $p > 0,05$ ). In the group containing 8% fresh Spirulina, the purity of phycocyanin was determined at food grade ( $A_{620} / A_{280}$ ). The group containing 8% dried Spirulina was found to be the group with the lowest scores in terms of odor and color ( $p < 0,05$ ). A higher sensory score was detected in the groups with 6% and 8% fresh Spirulina compared to the group containing 8% dried Spirulina ( $p < 0,05$ ).

**Keywords:** Spirulina, Muffin, Phycocyanin, Protein, Sensory Properties

#### Öz

**Amaç:** Çalışmada, taze Spirulina (*Arthrospira platensis*) veya Spirulina unu (kurutulmuş Spirulina) içeren muffinlerin protein ve fikosiyanin içeriği ile duyusal özellikleri değerlendirilmiştir.

**Materyal ve Yöntem:** Spirulina'nın taze veya kurutulmuş formları 3 farklı seviyede (%4, %6 ve %8) muffin karışımlarına eklenmiştir. Kontrol grubuna Spirulina katılmamıştır. Gruplar görünüm, doku, tat, koku, renk ve genel kabul edilebilirlik açısından duyusal analizlerle değerlendirilmiş olup muffinlerin protein ve fikosiyanin içeriği analiz edilmiştir.

**Bulgular ve Sonuç:** %6 ve %8 taze Spirulina içeren gruplarda, diğer gruplardan daha yüksek fikosiyanin içeriği tespit edilmiştir ( $p > 0,05$ ). %8 taze Spirulina içeren grubun fikosiyanin saflığı gıda derecesi ( $A_{620} / A_{280}$ ) olarak ölçülmüştür. %8 kurutulmuş Spirulina içeren grup, koku ve renk açısından en düşük puan alan grup olarak bulunmuştur ( $p < 0,05$ ). %6 ve %8 taze Spirulina içeren grupların, %8 kurutulmuş Spirulina içeren gruba kıyasla duyusal puanlamada daha yüksek skor elde ettiği tespit edilmiştir ( $p < 0,05$ ).

**Anahtar Sözcükler:** Spirulina, Çörek, Fikosiyanin, Protein, Duyusal Özellikler

#### 1.Introduction

It is thought that bakery products, including pastries, are a carbohydrate-rich energy source that plays an important role in human biology and disease development. Functional carbohydrate foods are considered as an alternative to the protection of health, since a significant part of the daily energy demand is provided from carbohydrates. Bioactive

components in foods refer to compounds derived from animal and plant sources that have a regulatory function beyond adequate nutrition in the human system (Hayes and Tiwari 2015). Functionally, the use of bioactive components in carbohydrate foods is not sufficiently common. Functional foods are a food or food ingredient with good health benefits that should be preferred during the day's energy needs.

Spirulina is a micro-algae with functional food properties. Dairy products (Güroy et al. 2016, Güroy and Keskin 2016), bakery products (Abd El Baky et al. 2015) and the use of functional foods in the baby food market are becoming widespread (Bigliardi and Galati 2013). Diplock et al. (1999) have been reported that functional foods can be used to modulate physiological systems to improve the physiological functions positively with anti-carcinogenic antimutagenic antioxidative effects that may reduce the risk of exposure to a disease or increase the welfare of the body's health. Memije-Lazaroa et al. (2018) which states that it is necessary to develop new therapeutic strategies to reduce the causes of cardiovascular complications associated with chronic kidney disease, has used nutraceuticals such as *Arthrospira maxima* (Spirulina) and C-phycoerythrin. They found that *A. maxima* and C-phycoerythrin reduce the causes of hypertension, left ventricular hypertrophy, renal dysfunction and oxidative stress associated with chronic kidney disease in the kidney and heart. Results suggests that *A. maxima* or C-phycoerythrin can be used to prevent the development of chronic kidney disease associated cardiovascular complications and to delay chronic kidney disease.

In general, microalgae can produce a great variety of secondary metabolites, which do not occur in other organisms. Spirulina is one of the fast-growing microalgae species that can produce many bioactive compounds, especially phycocyanin (Güroy et al. 2017) most commonly used in industrial areas.

*Arthrospira platensis* (Spirulina) used in this study was produced in Schlösser (1994) nutrient conditions and in 635 L volume raceway type algal tanks under laboratory conditions at 30°C temperature. The aim of this study was to determine the optimum use of Spirulina powder and fresh Spirulina in muffins.

## 2. Materials and Methods

### 2.1. *Spirulina (Arthrospira) platensis* Culture

The *Spirulina (Arthrospira) platensis* was cultured in Algae Culture Unit of Yalova University. The starter cultures were prepared by inoculating *Spirulina platensis* in Schlösser (1994) medium at 30°C and illumination of 2500 lux (16 h). The *Arthrospira (Spirulina) platensis* was cultivated 625 L of Schlösser's medium. Cultural homogenization was achieved using air pumps with an air flow rate of 30 L/h.

### 2.2. Fresh Spirulina biomass and Spirulina powder

Fresh Spirulina Preparation: Microalgae culture (*Arthrospira platensis*) was collected by passing through 80 micron fabric, washed with tap water and then filtered to harvest a dark green slurry. The dark green-colored paste biomass that filtered from the water was called "fresh Spirulina" (Figure 1).



**Figure 1.** Fresh Spirulina  
Spirulina Powder Preparation: The freshly harvested Spirulina was first dried with freeze drier for 22 hours at -60°C. It was then ground to obtain "Spirulina powder" (Figure 2).



**Figure 2.** Spirulina powder

### 2.3. Formulation of Muffins

The experimental muffins formula is shown in Table 1. Butter, sugar and eggs were mixed using a mixer for 2 min at medium speed. Then, all ingredients were added and blended 1 minute. Muffin dough was prepared using a mixer and special muffin dough bowl. Fresh or powder Spirulina was added at ratios

4‰, 6‰ and 8‰ by weight of the muffin dough mixtures (Figure 3). Muffin were immediately baked for 35 min at 170°C in an oven, cooled, and then removed from baking bowl. They were further cooled to room temperature and stored in plastic bags until used.

**Table 1.** Ingredients of muffins prepared using Spirulina

Ingredients (g)	Control	Fresh Spirulina biomass			Spirulina powder		
		4 ‰	6 ‰	8 ‰	4 ‰	6 ‰	8 ‰
Egg	15	16,7	17,02	17,59	17,28	17,52	17,86
Sugar	26,6	27,9	28,4	28,37	28,8	29,21	29,76
Milk	39,9	41,9	42,5	42,56	43,2	43,81	44,65
Butter	31,9	33,5	34,1	34,05	34,56	35,05	35,72
Baking powder	1,33	1,4	1,42	1,41	1,44	1,46	1,48
Vanillin	1,33	1,4	1,42	1,41	1,44	1,46	1,48
Wheat Flour	47,9	50,3	51,1	51,07	51,84	52,58	53,58
Spirulina biomass	0	0,63	0,95	1,32	0	0	0
Spirulina powder	0	0	0	0	0,64	0,98	1,34

Dough weight respectively; 149,9 g, 157,2 g, 159,7 g, 165,1 g, 162,1 g, 164,4 g, and 167,5 g



**Figure 3.** Dough mixture of different group muffins

### 2.4. Protein and Phycocyanin Analysis

Protein was analyzed by Kjeldahl method. Phycocyanin analysis were performed by spectrophotometrically methods. The percentage of crude phycocyanin was calculated according to the formula stated in Equation 1 (Boussiba and Richmond 1979, Oliveira et al. 2009). The method followed is that to extract blue supernatant; dry weight of sample of all experiment groups was calculated after drying in the oven at 80°C for 6h. To determine the percentage of crude phycocyanin, 40 mg of each group was weighed, 10 mL of phosphate buffer

(100 mM) added and stirred until complete dissolution. The samples were stored in refrigerator at 4°C overnight. The samples were subsequently mixed in centrifuge at 10°C, at 3500 rpm for 5 min. After centrifuge blue supernatant was reserved for spectrophotometric analysis. The analysis procedure was conducted in triplicate. After centrifuge, blue supernatant was separated from residue. The absorbance value of blue supernatant was read in a spectrophotometer at 620 nm using phosphate buffer as blank. Phycocyanin was calculated according to the following Equation 1.

$$\text{Equation 1: \% Crude C- Phycocyanin (C-PC)} = \frac{[A(620) \times 10 \times 100]}{3,39 \times \text{sample (mg)} \times (\% \text{ dry weight})}$$



$A(620)$  is the absorbance of sample supernatant at 620 nm, 10 is the dilution volume, 100 is the representative of 100%, and 3,39 is the extinction of coefficient for phycocyanin at 620 nm.

The purity of C-phycocyanin is determined by the equality of  $A_{620} / A_{280}$  (Antelo et al. 2010). When  $A_{620} / A_{280}$  ratio is  $\geq 0,7$  and above, the degree of purity of phycocyanin is expressed in the food grade as a natural blue color additive in foods. When the  $A_{620} / A_{280}$  is between 0,7 and 3,9 the phycocyanin purity is considered to be of the reactive grade.  $A_{620} / A_{280}$  is considered to be an analytical grade

when 4 and above. In this study, the degree of purity of the phycocyanin was calculated according to the Equality 2 according to  $A_{620} / A_{280}$  absorbance ratio using the spectrophotometry-based method (Antelo et al. 2010). The C-phycocyanin purity ratio is considered as the food grade when  $A_{620} / A_{280}$  is  $\geq 0,7$ , and as the reagent grade when  $A_{620} / A_{280}$  is between 0,7 and 3,9; and as analytical grade when  $A_{620} / A_{280}$  is  $\geq 4,0$ . C-phycocyanin purity ratio was calculated using the spectrophotometry-based method on the absorbance ratio  $A_{620} / A_{280}$ . Calculations of purity ratio is given below;

$$\text{Equation 2: Purity ratio} = \frac{A(620)}{A(280)}$$

Phycocyanin concentration refers to the amount of the phycocyanin in blue supernatant. The C-phycocyanin concentration in mg/mL was calculated from the optical densities at

652 and 615 nm by spectrophotometry-based method (Bennett and Bogorad 1973). Calculations of phycocyanin concentration is presented Equation 3.

$$\text{Equation 3: C-PC (mg/mL)} = \frac{[A(615) - 0.474 * A(652)]}{5.34}$$

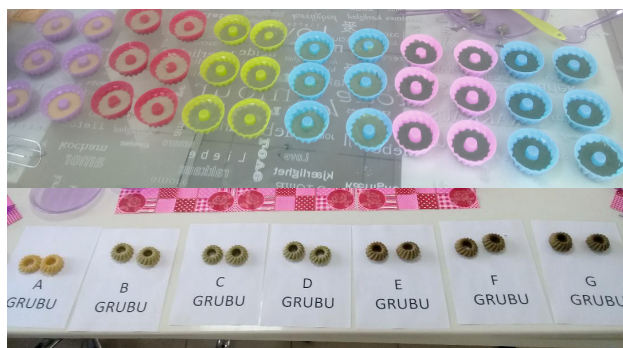
Yield refers to the amount of the phycocyanin in cakes containing Spirulina. The Phycocyanin yield was calculated using the method described by

(Silveira et al. 2007). Calculations of phycocyanin yield is presented Equation 4.

$$\text{Equation 4: Yield of phycocyanin (mg/g)} = \frac{[C-PC (mg/mL) * \text{Solvent volume (mL)}]}{\text{dry biomass (g)}}$$

## 2.5. Sensory Analysis

The sensory evaluation was performed by a panel of 10 people trained in the Department of Food Processing at Yalova University. We evaluated muffins the 6-point hedonic scale. 5 represented the highest degree and represented at least 1, color, taste, texture, appearance, texture and overall acceptability. For sensory analysis, muffins from different groups are presented in Figure 4.



**Figure 4.** Muffins from different groups for sensory analysis

## 2.6. Statistical analysis

Statistical analysis was performed with SPSS software (version 20.0, SPSS Inc. Chicago, IL, USA). Experimental groups were compared using ANOVA followed by Tukey post hoc test ( $p < 0,05$ ).

## 3. Results and Discussion

### 3.1. Sensory Analysis

In the sensory analysis, there were no significant differences between the groups in terms of appearance, texture, taste and general acceptability parameters (Table 2). Depend on the scores, 8% fresh Spirulina groups were the highest with regard to appearance, texture and general acceptability parameters ( $p > 0,05$ ). Even though there were no significant differences between the groups, 8% fresh biomass group had the highest score in terms of general acceptability. Although there was no statistically significant difference in taste parameters with other groups, in 4 % Spirulina powder group had the highest score ( $p > 0,05$ ).

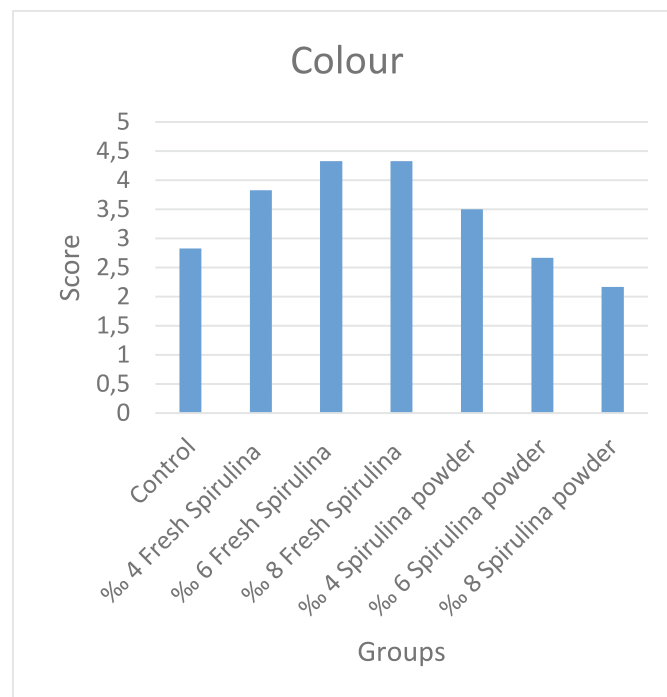


**Table 2.** Sensory analysis

Parameters	Control	4‰ biomass	6‰ biomass	8‰ biomass	4 ‰ powder	6‰ powder	8‰ powder
Appearance	2,83±1,47	3,00±1,26	3,50±0,84	4,17±0,75	4,00±1,26	2,67±1,97	3,00±1,90
Texture	3,50±1,38	3,67±1,21	3,50±1,05	4,33±0,82	4,00±0,89	2,83±1,72	3,00±1,55
Taste	3,17±1,17	3,50±1,38	3,50±1,52	3,50±1,38	3,83±1,17	3,50±1,05	3,17±1,17
Odour	4,00±1,55 <sup>ab</sup>	4,67±0,52 <sup>b</sup>	3,83±1,17 <sup>ab</sup>	4,17±0,75 <sup>ab</sup>	4,00±1,26 <sup>ab</sup>	3,33±1,63 <sup>ab</sup>	2,83±1,83 <sup>a</sup>
Colour	2,83±1,47 <sup>ab</sup>	3,83±1,33 <sup>ab</sup>	4,33±1,03 <sup>b</sup>	4,33±0,82 <sup>b</sup>	3,50±1,76 <sup>ab</sup>	2,67±1,97 <sup>ab</sup>	2,17±1,83 <sup>a</sup>
Acceptability	2,67±1,37	3,33±1,37	3,17±1,33	3,83±1,17	3,50±1,22	3,50±1,38	3,17±1,17

Significant differences were found in odor and color parameters between the groups. The highest score in terms of odor was 4‰ fresh Spirulina group ( $p<0,05$ ). The lowest score in terms of odor was 8‰ Spirulina powder group ( $p<0,05$ ). In control group were similar score in terms of odor 4‰ and 6‰ Spirulina powder groups with 6‰ and 8‰ fresh Spirulina groups. Natural or synthetic colorants are used to improve the

color quality of food products. Phycocyanin a natural blue pigment and is a functional bioactive substance that is preferred in the food industry instead of synthetic pigments (Jespersen et al. 2005). According to the results of sensory analysis, higher scores were determined in color scores 6‰ and 8‰ fresh Spirulina groups compared to 8‰ Spirulina powder group ( $p<0,05$ ) (Figure 5).

**Figure 5.** Sensory analysis results in terms of colour

Sensory results of this study have shown that the use of Spirulina biomass can be more preferable than the control in general acceptability assessments. The fresh Spirulina in muffins provided a more pronounced color and increased preference. According to these results, the Spirulina biomass-containing muffins were determined to be as palatable as control group. Saggi and Sundaravalli (2013) reported that they added 6,6 g, 100 g, and 10 g of Spirulina flour to 100 g of a cupcake mixture, and obtained positive results in sensory analysis in

different traditional products including Spirulina. Moreover, according to the results of microbial analysis, it was reported that bacteria and fungi did not occur at the 30th day of the study. Additionally, Beaulieu et al. (2015) reports that antibacterial peptides isolated from algal protein hydrolysates show inhibitory activity against *Staphylococcus aureus*. Although no shelf life study was conducted in this study, results were obtained that could shed light on different researches.

### 3.2. Protein and Phycocyanin Analysis

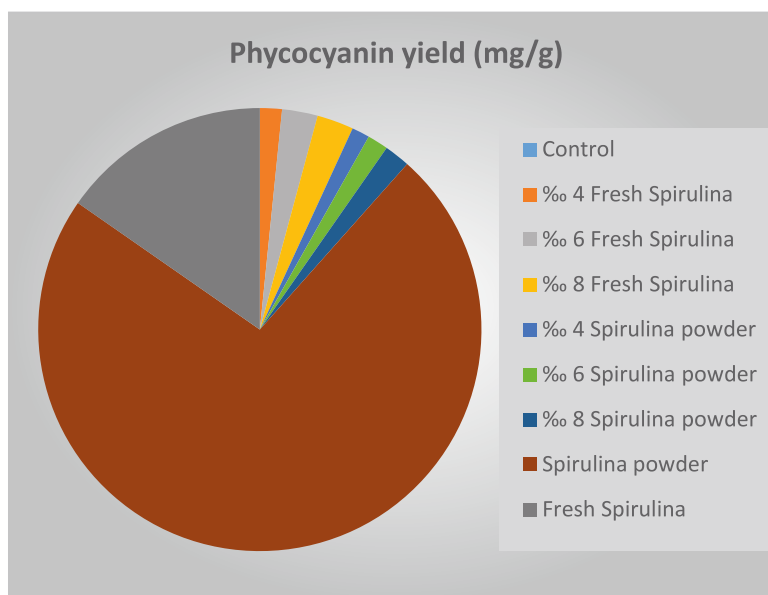
Nutritional quality were evaluated among the research groups by protein and phycocyanin analysis. Results showed that significant differences were found between the content of phycocyanin (C-PC)

and the purity ratio among the groups containing Spirulina (Table 3). The highest phycocyanin yield was 21,05% in 8‰ fresh Spirulina and the lowest phycocyanin content was 10,21% in 4‰ Spirulina powder (Figure 6).

**Table 3.** Protein and phycocyanin analysis

Groups	Purity Ratio	% C-PC	Phycocyanin concentration(mg/mL)	Phycocyanin yield (mg/g)	% Protein
% 4 Spirulina Biomass	0,2	0,61	0,05	12,62	6,76
% 6 Spirulina Biomass	0,5	0,99	0,08	20,44	7,68
% 8 Spirulina Biomass	0,7	0,99	0,09	21,05	7,73
% 4 Spirulina powder	0,3	0,47	0,04	10,21	6,74
% 6 Spirulina powder	0,4	0,60	0,05	11,96	7,14
% 8 Spirulina powder	0,3	0,71	0,06	14,74	7,55
Control	none	none	none	none	6,32
Spirulina powder*	4,1	22,82	2,35	575,12	60
Fresh Spirulina *	1,3	39,05	0,51	120,53	60

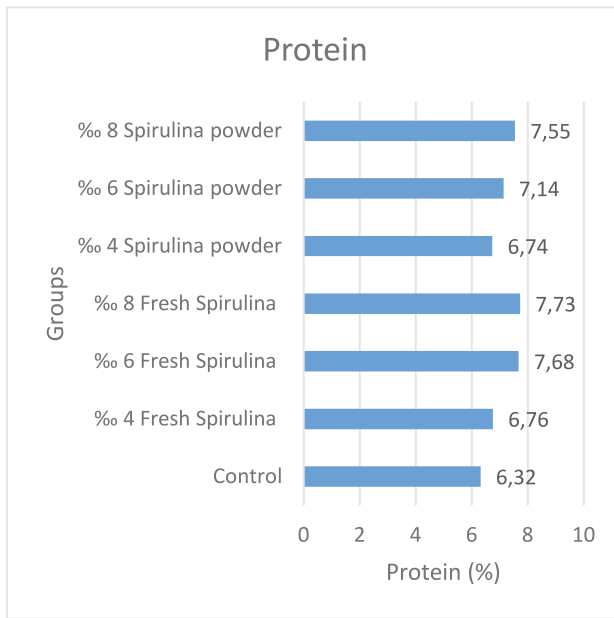
\* *Spirulina platensis* (Spirulina powder/fresh) used in this study was analyzed before adding the muffins.



**Figure 6.** Phycocyanin yield of muffin groups

Phycocyanin is a pigment sensitive to heat treatment (Güroy et al. 2017). About 20% of the dry weight of Spirulina may contain phycocyanin (Vonshak 1997). Spirulina powder used in the experiments is high in phycocyanin content and above 4 purity ratio (Table 3). In this study, the dried Spirulina group was twice exposed to heat treatment. When Spirulina is subjected to heat treatment, losses occur in the amount of phycocyanin in its composition (Figure 8). Although freeze drying preserves the properties of the components (Güroy et al. 2017), the expected results were not observed in this study. Spirulina, which was

brought into the powder form, was probably affected by external factors (such as light). Therefore, the damage of phycocyanin may have seen more. Even so, with the increase in the proportion of Spirulina in muffins, it has been determined that the content of phycocyanin is also positively affected, among the experimental groups. The use of Spirulina in fresh biomass or powder form in muffins has changed the values of phycocyanin. Spirulina powder containing groups contained lower level phycocyanin than those made with fresh biomass (Figure 6).

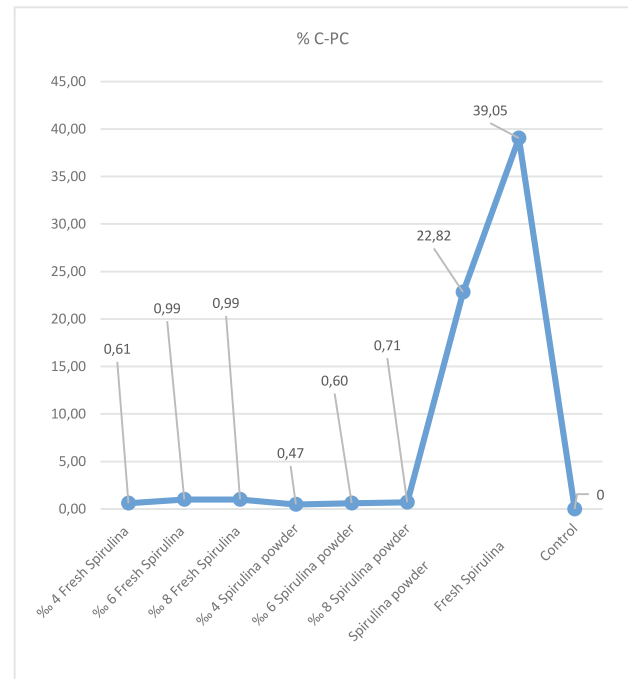


**Figure 7.** Protein analysis of muffin groups

In this trial, phycocyanin purity was positively influenced by the increase in Spirulina ratio in muffins. Phycocyanin purity degree is an important criterion for the determination of industrial use. If the purity ratio is 0,7 and above, the purity of phycocyanin at the food grade, the purity of the phycocyanin ratio between 2 and 3,9 is considered to be the reactive grade. It is accepted that it has an analytical purity degree of 4 and above (Kuddus et al. 2013). The green color in the muffins groups prepared with dried biomass was more prominent and bright. In the group containing 8‰ fresh Spirulina biomass, the purity of phycocyanin (Table 3) was determined at food grade ( $A_{620}/A_{280}$ ). However, it is possible to talk about phycocyanin in the group which contains only 8‰ fresh biomass. Although Spirulina powder provides a greener appearance in the dough mixture (Figure 3), we can understand that it is caused by chlorophyll. Although many plant sources contain chlorophyll, Spirulina is one of the rare sources of phycocyanin. According to the results of this research, the use of fresh Spirulina biomass in the production of muffin will give us the opportunity to benefit more from the phycocyanin. In this trial, it was determined that the adding fresh Spirulina biomass improved the phycocyanin quality of muffins. In a scientific research aimed at functional food development with the addition of Spirulina biomass and phycocyanin to biscuits has been reported to result in a more accentuated green color in the biscuit with an increase in the amount of biomass (3‰, 6‰ and 9‰). Foods containing bioactive substances are nutritional groups

known as functional food that have the role of improving, treating and positively promoting human health (Vulic et al. 2014). Although all groups the muffins adding Spirulina contain phycocyanin, the 6‰ and 8‰ fresh Spirulina containing groups best meet this definition.

While bakery products are a food source consumed in daily nutrition, most of them are known to be low protein. However, it is possible to produce high quality products when prepared with high value added foods such as Spirulina. With the increase in the ratio of Spirulina, protein content in all groups showed a tendency to increase. While 6,32% protein content was determined in the control group, 7,73% protein was determined in the 8% fresh Spirulina containing muffins (Figure 7).



**Figure 8.** Phycocyanin analysis in Spirulina groups

In this study, it was determined that Spirulina addition to muffin improved the protein content. Ak et al. (2016) achieved results that improved protein content (11.63%) with the addition of 10% Spirulina powder in bread. It is also reported that mold is prevented in bread stored in room conditions. To investigate new alternative protein sources that can be used for human in bakery products that it has been reported that a protein-rich substance (47% protein) of insect powder (*Acheta domestica*) is useful for protein and fiber content replace wheat meal (González et al. 2019). Shahbazizadeh et al. (2015) reported that Iranian traditional cookies have changed the dried *Spirulina platensis* biomass by 0.5%, 1% and 1.5% instead of wheat flour, and that Spirulina reinforced cookies have a favorable effect on antioxidant properties.



In addition that they found increasing of iron, protein and gamma-linolenic acid content of Spirulina adding and organoleptic evaluation made by hedonic tests, the samples containing 1% and 1.5% Spirulina powder the highest scores after the control. According to the results of this study, the positive effects of the use of Spirulina in muffins are seen. In terms of protein, phycocyanin and its overall acceptability, the author recommends using 6% and 8% fresh Spirulina in muffins.

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- ## 4. Conclusion
- Experimental groups containing Spirulina biomass gave positive results in terms of nutritional quality and preference. In fact, biomass can be said to have better protected of freshness due to less heat treatment. Among marketable Spirulina products, fresh Spirulina is recommended to be widely used. The author believes that fresh Spirulina biomass should be considered just like daily milk and Spirulina culture should be expanded.
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