# Innovative applications of *Spirulina platensis* L. and phycocyanin in food products: effects on the quality of sponge cakes

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The physicochemical properties of muffins incorporating different concentrations of *Spirulina platensis* L. (1 %, 3 %, and 6 %) were evaluated and compared to control muffins. The addition of *Spirulina platensis* increased the specific gravity and volume of the muffins, with the highest volume observed in muffins containing 1 % *Spirulina*. The pH of the muffins remained neutral across all samples, while the water absorption capacity decreased as the *Spirulina* concentration increased. Ash content showed a marked increase, particularly with higher *Spirulina* concentrations, indicating a boost in the muffins' mineral content. Sensory analysis revealed enhanced color, aroma, and flavor with 6 % *Spirulina*, though muffins with 1 % and 3 % *Spirulina* exhibited higher scores for shape and pore uniformity. Similarly, the incorporation of phycocyanin led to a decrease in relative mass, water absorption, and volume, while improving the color, aroma, and overall sensory attributes compared to the control. Color analysis using the CIELab system showed no significant difference in lightness between the experimental and control samples, though the red component (a\*) was significantly reduced in *Spirulina* and phycocyanin muffins. The findings suggest that both *Spirulina platensis* L. and phycocyanin significantly enhance the nutritional and sensory properties of muffins, providing a functional and aesthetically appealing food product.

Keywords: physicochemical properties, cakes, Spirulina platensis L., phycocyanin, foods.

## **INTRODUCTION**

Microalgae are natural sources of important bioactive compounds such as vitamins, essential amino acids, polyunsaturated fatty acids, minerals, carotenoids, enzymes, and fibers. They represent a valuable source of nearly all essential vitamins (A, B1, B2, B6, B12, C, E, niacin-B3, biotin, folic acid, and pantothenic acid-B5) [1]. Food products often incorporate them due to their rich chemical composition and nutritional value. Recognized as "superfoods," they have the potential to become valuable raw materials, improving both the nutritional and functional qualities of food [2, 3]. They are high in protein content with a balanced amino acid profile [4]. Certain types of microalgae have higher protein content compared to traditional animal or plant sources. For example, the protein content in Spirulina platensis L. is 65 % - higher than

in skimmed dry milk (36 %), soy flour (37 %), chicken (24 %), fish (24 %), beef (22 %), and peanuts (26 %) [5]. Proteins build every cell, tissue, and organ in the human body and are involved in forming structural components such as the musculoskeletal system, enzymes, hormones, hemoglobin, actin, myosin, blood serum, and blood cells [6]. Proteins from microalgae are valuable for supporting human health, especially for vegans or vegetarians, as well as individuals on dietary regimens where maintaining a balance of essential amino acids is challenging.

Some researchers [7] say that *Spirulina platensis* L. makes superoxide dismutase and catalase work better, stops lipid peroxidation and DNA damage, and turns on antioxidant enzymes in cells. It is rich in phycobiliproteins, known for their hepatoprotective, anti-inflammatory, immunomodulatory,

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anticancer, and antioxidant properties [1].

The goal of this study is to develop recipe ideas and technical methods for making muffins with *Spirulina platensis* L. and phycocyanin and to investigate their physical, chemical, sensory, and color properties.

#### MATERIALS AND METHODS

#### Materials

To produce sponge cake batter, the following materials were used, sourced from major retail chains in Bulgaria: wheat flour "Extra" (type 500), produced by "Sofia MEL" JSC, Sofia; chicken eggs; sugar (crystal, refined); and deionized water.

The used *Spirulina platensis* L. was grown in a bioreactor in Varvara, Bulgaria. Nikolova *et al.* [8] did a full study of its chemical makeup and properties. Phycocyanin was also used, extracted using a green method described in Nikolova *et al.* [9].

The following recipe composition formulations were made: (1) a control batter for muffins; (2) mixtures that replaced some of the wheat flour with 1 %, 3 %, and 6 % *Spirulina platensis* L. flour and phycocyanin, in that order. Table 1 provides the precise recipe compositions of the control and newly developed formulations.

We will use the following designations in the upcoming discussions: Variant 1-1 % *Spirulina platensis* L. (V1), Variant 2-3 % *Spirulina platensis* L. (V2), Variant 3-6 % *Spirulina platensis* L. (V3), Variant 4-1 % phycocyanin (V4), Variant 5-3 % phycocyanin (V5), Variant 6-6 % phycocyanin (V6).

#### Preparation technology of muffins

Table 1 displays the muffin recipe composition. Egg whites and two-thirds of the sugar were mixed using a mixer for 5 min at a medium speed of 3000 rad/s<sup>-1</sup>. The remaining sugar was mixed with the egg yolks. The flour and *Spirulina platensis* L. were mixed for 5 min. The powdered *spirulina* was added at 1 %, 3 %, and 6 % levels as a flour substitute in

the muffin recipe. Phycocyanin (1 %, 3 %, and 6 %) was included as a substitute for egg yolk. The ingredients were homogenized for 5 min (t = 20 °C). The muffins were baked for 20 min at 180 °C in an oven with upper and lower heating. After cooling to room temperature, they were stored in plastic bags.

#### Methods for evaluating muffins

*pH measurement*. A digital pH meter (HANNA-SN0111053Nq, electrode: HL1230B (Czech Republic) with a range of pH 0.00 to 14.00 and an accuracy of 0.01 pH was used.

Researchers analyzed *total moisture content* using standard methods [10-12].

*Weight measurement.* Samples of muffins with *Spirulina platensis* L. and phycocyanin were cooled for 2 h, then weighed on an electronic scale.

*Water absorption capacity.* Measured by determining the swelling of biscuits per BDS 15221-81 [13].

*Specific gravity for cake batter.* Calculated by dividing the cake batter weight in a standard cup by the weight of an equal volume of distilled water [14].

*Volume:* Measured with a volumeter using small, uniform grains (in cm<sup>3</sup>) per AACC method [14].

*Ash content*. The total ash content of the muffins (%) was determined by incineration in a muffle furnace (Germany) at 700°C until a constant weight was achieved [11]. The results represent the arithmetic mean of three parallel samples.

To assess the structural characteristics of the muffins, photographs of cross-sections were taken, and color parameters were measured.

The color of the muffin crust was evaluated using a high-quality technical colorimeter, PCE-CSM 5, PCE Instruments UK Ltd (Southampton, UK). The measurements were recorded in the CIELab system [15]. The parameters a and b, as well as the lightness (L\*), were determined during the color measurement process.

**Table 1.** Recipe composition of muffins (control) and with *Spirulina platensis* L. (1, 3 and 6 %) and phycocyanin (1... 3 and 6 %)

	Control	Spirulina platensis L.		Phycocyanin			
Ingredients (%)		1 (%)	3 (%)	6 (%)	1 (%)	3 (%)	6 (%)
Egg yolk	13.35	13.35	13.35	13.35	13.21	12.95	12.55
Egg white	29.88	29.88	29.88	29.88	29.88	29.88	29.88
Sugar	25.90	25.90	25.90	25.90	25.90	25.90	25.90
Wheat flour	30.88	30.57	29.95	29.02	30.88	30.88	30.88
Spirulina platensis L.	0.00	0.31	0.93	1.85	0.00	0.00	0.00
Phycocyanin	0.00	0.00	0.00	0.00	0.13	0.40	0.80

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Sensory evaluation: quantitative descriptive test for sensory profiling was applied to assess the sensory attributes. Sensory parameters (shape, color, aroma, size, sweetness, residual taste, aftertaste, and crumb softness) of the muffins were evaluated six hours after baking, in accordance with BDS EN ISO [16, 17].

Statistical analysis: All parameters examined were assessed following three repetitions. Data from all studies were analyzed to get the mean and standard deviation (SD). The statistical analyses were conducted using IBM SPSS software at a significance level of p < 0.01.

The sensory analysis data of sponge cake blots were analyzed using Kendall's concordance method to evaluate the agreement in the ranks of trained tasters about the tested samples [18].

The physicochemical parameters of muffins without (Control) and with Spirulina platensis L. (1, 3 and 6 %) were determined. The relative mass of muffins with 1 %, 3 %, and 6 % Spirulina platensis L. showed higher values than the control (0.72  $\pm$ 0.03). Increasing the amount of Spirulina platensis L. from 3 % to 6 % did not significantly affect the relative mass. The control muffin had a volume of  $207.00 \pm 6.71$  cm<sup>3</sup>, close to that of muffins with 6 % Spirulina platensis L. (208.33  $\pm$  2.89 cm<sup>3</sup>). Muffins with 1% Spirulina platensis L. exhibited the most significant volume ( $216.67 \pm 5.77$  cm<sup>3</sup>), followed by those with 3 % Spirulina platensis L.  $(215.00 \pm 5.00)$ cm<sup>3</sup>). It can be concluded that incorporating Spirulina platensis L. in concentrations of 1 % to 3 % increased both the relative mass of the batter and the volume (cm<sup>3</sup>) of the muffins (Table 2).

#### **RESULTS AND DISCUSSION**

Table 2. Physicochemical parameters of muffins without and with Spirulina platensis L. (1, 3 and 6 %)

	Amount added of Spirulina platensis					
Physical parameters	Control	1 % Spirulina	3 % Spirulina	6 % Spirulina		
		<i>platensis</i> L.	<i>platensis</i> L.	<i>platensis</i> L .		
Specific gravity for cake batter	$0.72 \pm 0.03^{b}$	$0.82{\pm}0.02^{a}$	$0.86{\pm}0.04^{a}$	$0.86{\pm}0.04^{a}$		
Volume (cm <sup>3</sup> )	207.00±6.71 <sup>b</sup>	216.67±5.77 <sup>a</sup>	215.00±5.00 <sup>a</sup>	208.33±2.89 <sup>b</sup>		
Water absorption capacity (%)	332.50±7.72 <sup>a</sup>	262.00±6.24 <sup>b</sup>	261.67±4.047 <sup>b</sup>	267.67±6.51b		
pH	$8.00{\pm}0.06^{a}$	$7.10{\pm}0.02^{b}$	$7.17 \pm 0.10^{b}$	7.10±0.03 <sup>b</sup>		
Ash content	$0.57 \pm 0.04^{d}$	0.73±0.02°	$0.94{\pm}0.06^{b}$	$1.57{\pm}0.04^{a}$		
Moisture (%)	28.49±0.36 <sup>a</sup>	$27.82 \pm 0.37^{b}$	27.70±0.54 <sup>b.c</sup>	27.16±0.17°		

Means in a row with a common superscript letter (a-c) differ (p < 0.05) as analyzed by Duncan test.

Table 3. Physicochemical parameters of muffins without and with phycocyanin (1, 3, 6 %)

Physical parameters	Amount added of phycocyanin						
Thysical parameters	Control	1 % phycocyanin	3 %phycocyanin	6 %phycocyanin			
Specific gravity for cake batter	0.72±0.03ª	$0.68{\pm}0.03^{a.b}$	$0.63{\pm}0.03^{b}$	0.56±0.03°			
Volume (cm <sup>3</sup> )	207.00±6.71ª	193.33±2.89 <sup>b</sup>	178.33±7.64°	171.67±7.64°			
Water absorption capacity (%)	332.50±7.72ª	309.00±7.21 <sup>b</sup>	291.00±5.00°	273.33±2.89 <sup>d</sup>			
pН	$8.00{\pm}0.06^{a}$	7.71±0.20 <sup> a.b</sup>	7.32±0.26 <sup>b</sup>	7.30±0.28 <sup>b</sup>			
Ash content	$0.57{\pm}0.04^{a}$	0.56±0.04 ª	0.60±0.05 ª	0.62±0.02 <sup>a</sup>			
Moisture (%)	28.49±0.36 <sup>b</sup>	28.67±0.52 <sup>b</sup>	27.70±0.54 <sup>b.c</sup>	29.23±0.10 <sup>a</sup>			

Means in a row with a common superscript letter (a-d) differ (p < 0.05) as analyzed by Duncan test.

The pH of the new products tended toward alkaline (slightly above 7.00), which is considered balanced according to literature data [19, 20].

The newly developed muffins' water absorption capacity (%) decreased by 21-20 % compared to the control ( $332.50 \pm 7.72$ ) % as the *Spirulina platensis* L. concentration increased from 1 % to 6 %. This indicates that water absorption capacity decreases proportionally with the increasing concentration of *Spirulina platensis* L.

Adding 1% Spirulina platensis L. increased the ash content by 1.3 times, while 3 % Spirulina platensis L. resulted in a 1.6-fold increase, and 6% Spirulina platensis L. led to a 2.8-fold increase compared to the control ( $0.57 \pm 0.04$ ). Thus, adding Spirulina platensis L. significantly enhanced the mineral content of the muffins, contributing to their nutritional and biological value. The addition of Spirulina platensis L. (1 %, 3 %, and 6 %) did not significantly affect the moisture content (%). The

addition of phycocyanin reduced the relative mass of the batter in the newly developed muffins by 23 %. A similar trend, although less pronounced (17 %), was observed for the volume (cm<sup>3</sup>).

Phycocyanin addition visibly influenced the phycocyanin products' pH. Increasing the concentration from 3 % to 6 % had no significant effect on this parameter. It is important to note that the newly developed products retained a neutral pH, similar to the control, which is considered balanced according to literature data. When 1 %, 3 %, and 6 % phycocyanin were added, the ability to absorb water dropped by about half at each concentration. Muffins with 6 % phycocyanin showed a 14 % increase in ash content compared to the control (Table 3).

A color analysis of the muffin surface and a sensory evaluation of their consumer qualities were conducted. Figure 1 illustrates the morphological features of the muffins' surface and interior compared to the control.



**Fig. 1**. Photographs of top surfaces and cross-sections of a muffin without *Spirulina platensis* L., with *Spirulina platensis* L, and with phycocyanin.

A quantitative descriptive sensory profiling test (Meilgaard, 1991) was used to determine the sensory attributes (shape, color, aroma, pore size and uniformity, sweet taste, residual taste (aftertaste)) of the newly developed muffins.

To fully describe the product characteristics, specific indicators and quantitative scales were developed to measure their intensity. The intensity results were statistically analyzed to determine significant differences among the evaluated muffins by a selected panel of ten trained sensory assessors, ensuring the accuracy of the evaluation [21]. The crumb softness of the muffins was also determined six hours after baking, Muffin samples were prepared one hour before evaluation. The crumb layers from each type were cut into  $1.5 \times 1.5 \times 1.5$  cm samples, which were stored in coded containers covered with aluminum foil. The sensory assessors evaluated the coded samples in random order, presented simultaneously.

As shown in Figure 2, for the shape parameter, muffins with 1 % and 3 % *Spirulina platensis* L. achieved the highest values, equal to those of the control. The intensity of color and aroma perception was highest in muffins with 6 % *Spirulina platensis* L. Uniform pore size was observed in samples with 1 % and 3 % *Spirulina platensis* L., while those with 6 % were similar to the control.

A stronger residual taste than that of the control was noted in all muffins with *Spirulina platensis L.*, with intensity increasing with higher concentrations, though less noticeable at lower levels. The tasters did not detect a difference in the softness of the developed muffins, but the values were lower than those of the control.



**Fig. 2.** Sensory profile of muffins without (Control) and with *Spirulina platensis* L. (1, 3 and 6 %): V1 - 1 % *Spirulina platensis* L. V2 - 3 % *Spirulina platensis* L. V3 - 6 % *Spirulina platensis* L.

The 1 %, 3 %, and 6 % phycocyanin muffins showed highly pronounced sensory attributes of shape, color, aroma, sweet taste, and softness (Figure 3). The intensity of perception of color, smell, residual taste, and softness was higher than that of the control.

For all concentrations of phycocyanin (1 % to 6 %), the values for color and aroma were distinctly different from those of the control. A concentration of 1 % phycocyanin did not affect the size of the muffins, whereas muffins with 3 % and 6 % of phycocyanin had similar and higher size values. Adding 3 % and 6 % of phycocyanin resulted in a more pronounced increase in muffin size.



**Fig. 3.** Sensory profile of muffins without (Control) and with phycocyanin (1, 3 and 6 %): V4 - 1 % phycocyanin V5 - 3 % phycocyanin V6 - 6 % phycocyanin.



**Fig. 4.** Color parameters of muffin crusts with phycocyanin (1, 3, and 6 %), *Spirulina platensis* L. (1, 3, and 6 %), and control.

The results for the color parameters in the CIELab colorimetric system for the muffin crusts with phycocyanin and *Spirulina platensis* L. are presented in Figure 4.

It was found that the L\* parameter which measures how light the muffin crust is, was the same for samples containing 1 %, 3 %, and 6 % of *Spirulina platensis* L. and 1 %, 3 %, and 6 % of phycocyanin as it was for the control sample. This shows that adding *Spirulina platensis* L. and phycocyanin at the chosen concentrations doesn't have a big effect on how light the crust is of the new products.

The a\* values, reflecting the contribution of the red component to the crust's color, showed a significant decrease compared to the control sample. For samples with 1 % concentrations of *Spirulina platensis* L. and phycocyanin, the a\* values were nearly five times lower than those of the control. We observed a similar trend for the other two concentrations, particularly in samples containing

phycocyanin, where the effect was more pronounced.

The b\* values, which represent the yellow component in the color of the crust, showed no significant differences among the muffin samples with *Spirulina platensis* L., added at the selected concentrations, did not alter the color of the crust.

# CONCLUSION

✓ Increased nutritional value: The incorporation of Spirulina platensis L. into muffins significantly enhanced their mineral content, particularly with higher concentrations of Spirulina (3 % and 6 %), which resulted in increased ash content. This suggests an improvement in the nutritional value of the muffins, providing additional health benefits.

✓ *Physicochemical properties*: The addition of *Spirulina platensis* L. led to a higher relative mass and increased volume, especially at the 1 % and 3 % concentrations. However, increasing the *Spirulina* content beyond 3 % did not significantly affect these parameters. The pH of the muffins remained balanced, slightly alkaline, regardless of *Spirulina* concentration. Water absorption capacity decreased with increasing *Spirulina* content, indicating its impact on dough consistency.

✓ Sensory qualities: Sensory evaluation revealed that muffins with 1 % and 3 % Spirulina platensis L. were preferred for their shape, color, and uniformity. Muffins with 6% Spirulina exhibited a stronger aroma and flavor, although the aftertaste increased with higher concentrations. The sensory attributes of muffins with Spirulina showed an overall enhancement in aroma, color, and taste, with lower concentrations being preferable for texture and shape.

✓ *Effect of phycocyanin*: The addition of phycocyanin reduced the relative mass and volume of muffins, particularly at 3 % and 6 %. Despite this, phycocyanin significantly improved color and aroma, contributing positively to the sensory appeal of the muffins. Phycocyanin also caused a decrease in water absorption and a slight increase in ash content, though its effect was less pronounced than that of *Spirulina*.

✓ Color analysis: Both Spirulina platensis L. and phycocyanin influenced the color of the muffin crust, with reductions in the red component ( $a^*$ ) being particularly notable in phycocyanincontaining muffins. However, these additions did not significantly affect the lightness (L\*) of the crust, indicating minimal impact on the overall appearance. P. Mustakova et al.: Innovative applications of Spirulina platensis L. and phycocyanin in food products...

✓ Functional ingredient potential: Both Spirulina platensis L. and phycocyanin proved to be valuable functional ingredients in muffin production, enhancing the product's nutritional profile and sensory properties. Their incorporation into bakery products can contribute to the development of healthier, more appealing food options, with positive implications for product innovation in the bakery industry.

## REFERENCES

- 1. E.W. Becker, Microalgae in human and animal nutrition, in: A. Richmond (ed.), Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Second edn., 2013, p. 312.
- 2. A. Marzec, P. Kramarczuk, H. Kowalska, J. Kowalska, *Applied Sciences*, **13** (13), 632 (2023).
- 3. A. Barka, C. Blecker, *Biotechnol. Agron. Soc. Environ.*, **20**, 427 (2016).
- 4. Y. Wang, S.M. Tibbetts, P.J. McGinn, *Foods*, **10**, (12), 3002 (2021).
- 5. K. Moorhead, B. Capelli, G. R. Cysewski, Spirulina Nature's Superfood, Cyanotech Corporation, 3rd edn., 2012.
- 6. **B.** Popov, Hygiene, nutrition and occupational diseases, 2018. ISBN 978-954-9977-62-2.
- P. Grover, A. Bhatnagar, N. Kumari, A.N. Bhatt, D.K. Nishad, J. Purkayastha, *Saudi Journal of Biological Sciences*, 28, 3, 853 (2021).
- Kr. Nikolova, G. Gentscheva, D. Gyurova, V. Pavlova, I. Dincheva, M. Velikova, A. Gerasimova, L. Makedonski, G. Gergov, *Life*, **14(2)**, 174 (2024).

- 9. BDS 3412:1979. Bread and bread products. Regulation for taking samples and testing methods (1979).
- BDS 5313-1985. Confectionery products. Physical and chemical tests. Methods for determination of dry material, moisture, ash and ash alkalinity (2015). American Association of Cereal Chemists Approved Methods. Stati Uniti: The Committee, (2000) Method 44-15.02 Moisture—Air-Oven Methods.
- 11. BDS 15221-1981. Confectionery. Method for determination the swelling of biscuits (1981).
- American Association of Cereal Chemists Approved Methods (10-05. 01 and 10-95): Guidelines for Measurement of Volume by Rapeseed Displacement; American Association of Cereal Chemists: St. Paul, MN, USA, (1998)
- 13. International Commission on Illumination, Vienna, Austria, 2 (1978).
- 14. BDS EN ISO 8586:2023. Sensory analysis Selection and training of sensory assessors (2023).
- 15. BDS EN ISO 13299:2016. Sensory analysis -Methodology - General guidance for establishing a sensory profile (2016).
- 16. F.S. Daniel, Wood, John Wiley & Sons, Hoboken, NJ, USA, **20**, 1980.
- 17. AOAC. Official methods of analysis, 25th end. (2010).
- American Association of Cereal Chemists Approved Methods. Stati Uniti: The Committee, (2000) Method 02-31.01.
- 19. M.R. Baeva, I.N. Panchev, V.V. Terzieva, *Food/Nahrung*, **44** (4), 242 (2000).