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# STUDY OF THE EFFECT OF PHYCOERYTHRIN AND PHYCOCYANIN ON THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF LOW-FAT MILK

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**ABSTRACT**

In recent years, the replacement of synthetic preservatives with natural ones has gained attention due to concerns about their potential harms and the beneficial biological properties of natural preservatives. This study aimed to investigate the effects of adding natural pigments, phycocyanin (PC) and phycoerythrin (PE), extracted from the cyanobacteria *Desmonostoc ablorizicum* and *Neowestiellopsis persica* A1387, on the physicochemical properties, colorimetry, antioxidant activity, and antimicrobial activity of samples. All tests were conducted on eight treatments: T1, T2, T3, and T4 with varying concentrations of PE (0.1%, 0.5%, 1%, and 2%) and T5, T6, T7, and T8 with varying concentrations of PC (0.1%, 0.5%, 1%, and 2%) over 28 days at different time intervals. Spectrophotometric results indicated that the highest concentration and purity of PE were achieved after 72 hours ($p<0.05$). Statistical analyses showed that, compared to treatments containing PC, those with PE exhibited significantly higher acidity and pH values ($p<0.05$). However, treatments and storage duration had no significant effect on Brix, fat, or protein content ($p<0.05$). Antioxidant activity, evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) And Ferric Reducing Antioxidant Power (FRAP) methods, revealed that samples with higher PE concentrations exhibited 1.27 and 1.20 times greater antioxidant activity compared to those with PC, respectively. Additionally, colorimetric analysis showed that adding PC and PE significantly reduced the brightness index ($L^*$) while increasing the $a^*$ and $b^*$ indices of milk samples ($p<0.05$). The total color change index ($\Delta E$) also indicated a significant increase in overall color variation in milk samples with added PC and PE ($p<0.05$). Microbial counts, including total microorganisms, Enterobacteriaceae, and Yeast showed the highest growth in control and T1 treatments and the lowest in treatments containing PC and PE ($p<0.05$). Sensory evaluation of overall acceptability demonstrated that adding PC and PE significantly improved the sensory acceptance of milk samples ($p<0.05$). Overall, this study suggests that PE and PC, due to their numerous properties such as antioxidant and antimicrobial activities, can enhance the functional and safety characteristics of low-fat milk, demonstrating high potential for use in the dairy industry as safe and effective natural additives.

**Keywords:** *Neowestiellopsis persica A1387, Desmonostoc ablorizicum, Antioxidant Activity, antimicrobial activity, Cyanobacteria*

## INTRODUCTION

From a nutritional perspective, milk is considered one of the most suitable and, according to many nutrition experts, the most complete food found in nature. It is perhaps the only food capable of solely providing all the essential nutrients required by the body over a specific period **(Pietrzak- Fiećko & Kamelska-Sadowska, 2020)**. The color of milk varies from bluish-white to golden yellow, depending on factors such as the animal's breed, diet, fat content, and total solids **(Arslan *et al*., 2021)**. According to the European Food Safety Authority (EFSA), color is a key sensory attribute influencing food choice and can significantly condition consumer purchasing behavior. Amid the growing trend toward healthy diets and lifestyles, consumers have shifted their preference from synthetic additives to natural ones. Natural pigments are derived from plants, fruits, and animals, then extracted and purified. Consumers are skeptical about synthetic colors and prefer natural food pigments due to their potential benefits **(García *et al*., 2021)**.

Natural pigments exhibit beneficial biological activities, such as antioxidant and anticancer properties **(Freitas *et al*., 2021)**. Unlike their synthetic counterparts, natural pigments pose no toxic or adverse effects on the human body and even serve as nutritional enhancers with diverse biological activities. Although some plant-based pigments are commercially available, microalgal pigments have been found to exhibit significantly higher activity **(Patel *et al*., 2022)**.

Cyanobacteria are sustainably cultivated in an environmentally friendly manner and can be renewably produced on an industrial scale without limitations from seasonal, climatic, or environmental conditions. Their high pigment content, rapid growth, ability to thrive under stress, and lack of need for arable land make them a critical resource, positioning them as one of the most promising and competitive sources of natural pigments **(Gong & Bassi, 2016)**.

Cyanobacteria are rich in phycobiliproteins (PBPs), a family of water-soluble, colored biliproteins found in cyanobacteria, rhodophytes, cryptomonads, and cyanelles **(Sui, 2021)**. Phycobiliproteins exhibit numerous biological activities, including antioxidant, antitumor, neuroprotective, and hepatoprotective properties, and can be developed as photosensitizers for tumor treatment **(Tounsi *et al*., 2023)**. Four types of phycobiliproteins are found in cyanobacteria: PC, allophycocyanin (APC), PE, and phycoerythrocyanin **(Portmann *et al*., 2008)**.

Among these, PE and PC are the most well-known. PE, with its red or pinkish-red hue, is accepted as an edible colorant in foods such as confectionery, syrups, dairy products, baked goods, gelatin desserts, dry foods, fermented milk products, ice cream, and milkshakes. Additionally, PE's yellow fluorescence properties make it suitable for decorating cakes, soft drinks, and alcoholic beverages, enhancing their visual appeal and consumer attractiveness **(Karmakar *et al*., 2021)**.

PE is widely used in pharmaceuticals as an antioxidant and antimicrobial agent, and its therapeutic value, particularly its anticancer activity, has been recognized for years **(Nowruzi *et al*., 2020)**. PC, with its blue color, can be used as an edible colorant in chewing gum, candies, ice cream, jelly, dairy products, and soft drinks. For instance, yogurt fortified with PC demonstrated positive effects on color stability during storage **(Mohammadi-Gouraji *et al*., 2019)**.

PC is also considered a potent *in vivo* antioxidant with radical-scavenging properties and can function as a functional food with anti-inflammatory, anticancer, hepatoprotective, and neuroprotective effects in experimental studies **(Sun *et al*., 2023)**.

Given that no comprehensive research has yet explored the high potential of phycobiliproteins extracted from the cyanobacteria *Desmonostoc ablorizicum* and *Neowestiellopsis persica A1387* in low-fat milk as natural edible pigments to enhance antimicrobial and antioxidant activities, the findings of this study could pave the way for introducing these cyanobacteria-derived natural edible pigments for use in the dairy industry.

## MATERIALS AND METHODS

### Preparation and Cultivation of Cyanobacteria *Desmonostoc ablorizicum* and *Neowestiellopsis persica A1387*

The cyanobacteria *Desmonostoc ablorizicum* and *Neowestiellopsis persica A1387*

were isolated and cultured from the Cyanobacteria Culture Collection (CCC) of the Alborz Herbarium at the Islamic Azad University, Science and Research Branch. Purification and axenic cultivation were performed in 400 mL of liquid Z8 medium in a growth chamber at a temperature of 28 ± 2°C under continuous fluorescent illumination with an intensity of 300 µEinstein $m^{-2}$ $s^{-1}$ for 10 to 15 days (Figure 1) **(Maynes *et al*., 2006)**.

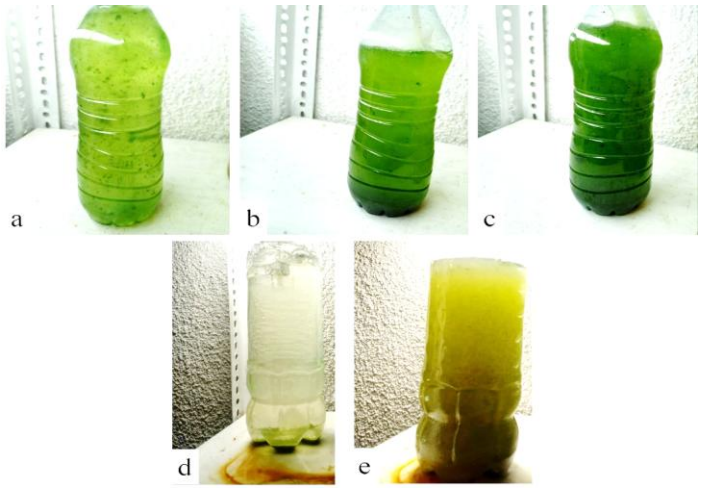



**Figure 1** Cultivation stages of the *cyanobacterium Desmonostoc ablorizicum* on day 1 (a), day 7 (b), day 14 (c), and cultivation stages of the *cyanobacterium Neowestiellopsis persica A1387* on day 1 (d), day 14 (e).

**Isolation and Extraction of Phycobiliproteins**

Two grams of freeze-dried biomass from each cyanobacterial strain were suspended in 500 mL of sodium phosphate buffer (0.1 M, pH 7.2). Phycobiliproteins were extracted by repeated freeze- thaw cycles, involving freezing at -20°C and thawing at room temperature in darkness. The resulting mixture was centrifuged at 10,000 × g for 30 minutes at 5°C, and the phycobiliprotein content in the supernatant was collected, dried, and then freeze-dried. The freeze-dried samples were stored at -20°C (Figure 2) **(Mishra *et al*., 2010)**.

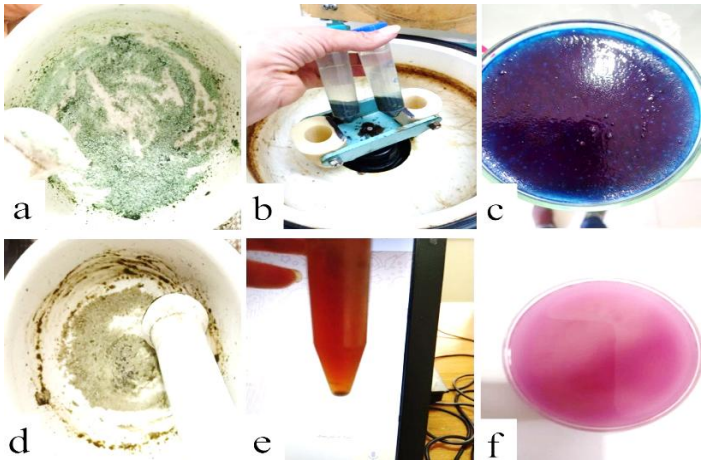



**Figure 2** Stages of isolation of PC and PE. a: Grinding and crushing of cyanobacteria. b: Mixing sodium phosphate buffer with cyanobacterial biomass. c: Extracted PC. d: Grinding and crushing of cyanobacteria. e: Mixing sodium phosphate buffer with cyanobacterial biomass. f: Extracted PE.

**Determination of Stability, Concentration, and Purity of PC and PE**

The stability, concentration, and purity of the PC and PE were calculated over 72 hours according to the following formulas (Antelo *et al*., 2010):

PC $(mgmL^{-1}) = [A_{620} - 0.474(A_{652})]/\,5.34$
APC $(mgmL^{-1}) = [A_{652} - 0.208(A_{620})]/\,5.09$
PE $(mgmL^{-1}) = [A_{568} - 2.41(\text{pc}) - 0.849(\text{APC})]/\,9.62$

To calculate the purity of PE and PC, the formulas OD $A_{562}$/OD $A_{280}$ and OD $A_{620}$/OD $A_{652}$ were used, respectively.

**Preparation of Low-Fat Milk Containing PC and PE**

Low-fat milk was commercially sourced. To ensure the absence of contamination, the samples were heat-treated at 72°C for 15 seconds. After cooling to 42°C, different concentrations of PC and PE (0%, 0.1%, 0.5%, 1%, and 2%) were added (Figure 3) **(Galetović*et al*., 2020)**.



**Figure 3** Preparation of low-fat milk treatments with concentrations of 0.1%, 0.5%, 1%, and 2% PC (blue color) and PE (pink color), and without PC or PE as the control.

**Tests on Low-Fat Milk Containing PC and PE**

**Acidity Measurement**

Acidity was measured using a modified method **(Tomovska *et al*., 2016)**. A 10 mL sample was placed in a beaker, and 0.5 mL of phenolphthalein indicator was added. The sample was titrated with 0.1 N sodium hydroxide until a stable pink color appeared (lasting for 5 seconds). Acidity, expressed as lactic acid, was calculated using the following formula:

$$\text{Acidity (\%)} = \frac{N \times 0.009 \times 100}{v}$$

Where:

- V: Sample volume
- N: Volume of sodium hydroxide consumed (mL)

**pH Measurement**

A portion of the milk samples containing PC and PE was poured into a beaker, and the electrode of a pH meter (calibrated with buffers 4 and 7) was fully immersed in the sample. After the reading stabilized, the pH was measured and recorded at 20°C **(Kandeel *et al*., 2019)**.

**Measurement of Soluble Solids (Brix)**

A few drops of milk containing PC and PE were placed on the prism of a refractometer. After eliminating light scattering, the concentration of soluble solids was measured in Brix at 20°C and reported **(Todaro *et al*., 2024)**.

**Fat Content Measurement**

The fat content of milk samples containing PC and PE was determined using the Gerber method. For this, 10 mL of concentrated sulfuric acid was transferred to a Gerber tube. Milk samples were slowly added to form a layer over the acid. After a 3-second pause, 1 mL of isoamyl alcohol was added. The Gerber tube was sealed, its contents thoroughly mixed, and centrifuged at 30 × g for 4 minutes. After cooling, the fat content was read from the Gerber tube and reported **(Kleyn *et al*., 2001)**.

**Soluble Protein Measurement**

Ten boiling stones, 15 g of potassium sulfate, and 1 mL of copper sulfate solution were placed in a digestion flask. Then, 1 g of the sample and 25 mL of sulfuric acid were added, and the contents were mixed. The flask was heated at a low temperature to prevent foaming until white fumes appeared from the flask's opening, and heating continued for 20 minutes. The temperature was then increased to half its maximum setting, and digestion continued for another 15 minutes. Finally, the temperature was raised to its maximum, and digestion proceeded for 1.5 to 2 hours until a clear blue-green solution was obtained. The solution was cooled to room temperature under a laboratory hood, and the contents were titrated with a standard hydrochloric acid solution until the first stable pink color appeared. The nitrogen content was calculated using the following formula and reported **(Urbat *et al*., 2019)**:

$$W_n = \frac{14007 \times (Vs - Vb) \times Mt}{m}$$

Where:

$W_n$: Nitrogen content of the sample (% w.w)
$V_S$: Volume of standard hydrochloric acid consumed for the sample
$V_b$: Volume of hydrochloric acid consumed for the blank
$m_t$: Molarity of hydrochloric acid m: Sample mass (g)

**Antioxidant Activity Evaluation**

**FRAP Antioxidant Activity Assay**

The FRAP (Ferric Reducing Antioxidant Power) assay measures antioxidant potential by reducing ferric iron ($Fe^{3+}$) to ferrous iron ($Fe^{2+}$) through antioxidants in the samples. Results were reported as ferrous sulfate equivalents capable of reducing iron ions. A calibration curve was prepared using various concentrations of ferrous sulfate (125 to 1000 µg. mL). Then, 1.5 mL of prepared FRAP reagent was added to test tubes and incubated at 37°C for 5 minutes. Subsequently, 50 µL of each concentration was added to the respective tubes and incubated at 37°C for 10 minutes. The resulting color intensity was measured at 593 nm against a blank. Based on the standard concentrations (ferrous sulfate), absorbance at 593 nm was measured, and a calibration curve was plotted **(Torruco-Uco *et al*., 2009)**.

**DPPH Antioxidant Activity Assay**

This method is based on scavenging free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH), with the color change from purple to colorless measured by absorbance at 520 nm using a spectrophotometer. For this, 1 mL of samples was mixed with 1 mL of 0.002% DPPH in methanol. After incubation in the dark for 30 minutes at room temperature, absorbance was measured at 517 nm. Ascorbic acid was used as a positive control, and antioxidant activity was calculated using the following formula, with results expressed in µg. mL **(Torruco-Uco *et al*., 2009)**:

I% = (A control - A sample) / A control × 100

**Color Index Measurement**

Colorimetric evaluation was performed using the HunterLab system, measuring the color indices L* (brightness), a* (redness-blueness), and b* (yellowness-greenness). The overall color change was calculated and reported using the following formula (Galetović *et al*., 2020):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

**Total Microbial Count**

One milliliter of each prepared sample was transferred to a sterile Petri dish, and 12–15 mL of Plate Count Agar (PCA) medium was added. The plates containing the sample and medium were carefully mixed by rotary motion, then placed on a flat, cool surface to solidify. The plates were incubated upside down at 30°C for 72 hours. After incubation, the number of colonies grown on the plates was reported as colony-forming units per gram (cfu/g) (Yang & Wei, 2021).

**Enterobacteriaceae Count**

One milliliter of each prepared sample was transferred to a sterile Petri dish, and 12–15 mL of Violet Red Bile Glucose (VRBG) agar medium was added. The plates containing the sample and medium were carefully mixed by rotary motion, then placed on a flat, cool surface to solidify. The plates were incubated upside down at 37°C for 24 hours. After incubation, the number of colonies grown on the plates was reported as cfu/g **(ISO 21528-2:2017)**.

**Mold and Yeast Count**

A modified method was used for this test (Talukder *et al*., 2019). One milliliter of each sample was transferred to a sterile Petri dish, and 12–15 mL of Chloramphenicol Agar medium was added. The plates were placed on a flat, cool surface to solidify. The plates were incubated upside down at 25°C for 5 days. After incubation, the number of colonies grown on the plates was reported as cfu/g.

**Sensory Evaluation (Hedonic Test)**

Sensory evaluation was conducted by a trained panel of 51 semi-skilled assessors. The evaluated factors included appearance, texture and form, aroma and taste, color and appearance, and overall acceptability, assessed using a 5-point hedonic scale (1 = least desirable, 5 = most desirable) **(Pertuzatti *et al*., 2015)**.

**Statistical Analysis**

The results obtained from the experiments were expressed as mean ± standard deviation based on three replicates. Colony-forming units (CFUs) in all experiments were converted to logarithmic values before statistical analysis. Experimental data were analyzed using one-way analysis of variance (ANOVA), and significant differences between mean values (in cases where the overall effect of treatments was significant) were determined using Duncan's multiple range test. Statistical analyses were performed using SPSS software version 26.

**RESULTS**

**Spectrophotometric Results**

The spectrophotometric analysis of PE and PC extracted from *Desmonostoc ablorizicum* and *Neowestiellopsis persica* showed maximum absorbance at λmax 567 nm with an absorbance of 1.83 for PE and λmax 620 nm with an absorbance of 0.653 for PC, respectively. According to the results, PE exhibited the highest absorbance.

**Stability, Purity, and Concentration of PE and PC**

The results indicated a significant decrease in the purity and concentration of PC and PE over 72 hours of storage ($p<0.05$) (Table 1). The highest stability in terms of purity and concentration over 72 hours was observed for PE ($p<0.05$).

**Table 1** Mean results of purity and concentration indices for PE extracted from *Desmonostoc ablorizicum* and PC extracted from *Neowestiellopsis persica* over 72 hours of storage.

| | **0 hours** | **24 hours** | **48 hours** | **72 hours** |
|---|---|---|---|---|
| **PC purity** | 0.173±0.00$^{a}$ | 0.172 ± 0.00$^{b}$ | 0.171±0.00$^{c}$ | **0.170±0.00$^{d}$** |
| **PE purity** | 1.75±0.02$^{a}$ | 1.680±0.00$^{b}$ | 1.67 ± 0.00$^{b}$ | **1.65± 0.00$^{c}$** |
| | **0 Hours** | **24 hours** | **48 hours** | **72 hours** |
| **PE purity** | 100.00±00.00$^{a}$ | 99.55± 0.10$^{b}$ | 98.76±0.22$^{c}$ | **98.26±0.27$^{d}$** |
| **PC purity** | 100.00±00.00$^{a}$ | 96.21±0.51$^{b}$ | 95.04±0.73$^{c}$ | **93.00±0.30$^{d}$** |

Different lowercase letters indicate statistical differences within rows ($p0<05$).

**Physicochemical Results of Low-Fat Milk Containing PC and PE**

**Acidity and pH Results**

According to the results, treatments and storage duration had a significant effect on the acidity of milk samples containing PC and PE ($p<0.05$). On day zero, no statistically significant difference was observed between the control sample and samples containing PE and PC ($p>0.05$). From day four of storage, statistically significant differences were observed between treatments ($p<0.05$). The addition of PC and PE significantly increased the acidity of milk samples compared to the control ($p<0.05$). Among the treatments containing PC and PE, those with PC exhibited higher acidity levels ($p<0.05$) (Table 2).
According to the results, treatments and storage duration had a significant effect on the pH of milk samples containing PC and PE ($p<0.05$). On day zero, no statistically significant difference was observed between the control sample and samples containing PE and PC ($p>0.05$). From day four of storage, statistically significant differences were observed between treatments ($p<0.05$). The addition of PC and PE significantly reduced the pH of milk samples compared to the control ($p<0.05$). Among the treatments containing PC and PE, those with PE exhibited higher pH levels ($p<0.05$) (Table 2).

**Table 2** Mean acidity and pH results of milk containing PC and PE over 28 days of storage

| | **Acidity Results** | | | | |
|---|---|---|---|---|---|
| Treatments | 0 Day | 4 Day | 7 Day | 14 Day | 28Day |
| Co | 0.11 ±0.00$^{Ea}$ | 0.141±0.00$^{Da}$ | 0.171±0.00$^{Ca}$ | 0.203±0.00$^{Bb}$ | 0.243±0.00$^{Aa}$ |
| T1 | 0.11 ±0.00$^{Eb}$ | 0.137±0.00$^{Db}$ | 0.164±0.00$^{Cc}$ | 0.20±0.00$^{Bb}$ | 0.239±0.00$^{Ab}$ |
| T2 | 0.11 ±0.00$^{Ea}$ | 0.133±0.00$^{Dcd}$ | 0.160±0.00$^{Cd}$ | 0.196±0.00$^{Bc}$ | 0.233±0.00$^{Ac}$ |
| T3 | 0.11 ±0.00$^{Ea}$ | 0.128±0.00$^{De}$ | 0.156±0.00$^{Ce}$ | 0.187±0.00$^{Bd}$ | 0.229±0.00$^{Ad}$ |
| T4 | 0.11 ±0.00$^{Ea}$ | 0.123±0.00$^{Df}$ | 0.152±0.00$^{Cf}$ | 0.182±0.00$^{Be}$ | 0.223±0.00$^{Ae}$ |
| T5 | 0.11 ±0.00$^{Ea}$ | 0.138±0.00$^{Db}$ | 0.1685±0.00$^{Ca}$ | 0.202±0.00$^{Bab}$ | 0.240±0.00$^{Aab}$ |
| T6 | 0.11 ±0.00$^{Ea}$ | 0.134±0.00$^{Dc}$ | 0.1681±0.00$^{Cab}$ | 0.201±0.00$^{Bab}$ | 0.237±0.00$^{Ab}$ |
| T7 | 0.11 ±0.00$^{Ea}$ | 0.132±0.00$^{Dd}$ | 0.165±0.00$^{Cbc}$ | 0.196±0.00$^{Bc}$ | 0.233±0.00$^{Ac}$ |
| T8 | 0.11 ±0.00$^{Ea}$ | 0.127±0.00$^{De}$ | 0.160±0.00$^{Cd}$ | 0. 189±0.00$^{Bd}$ | 0.230±0.00$^{Ad}$ |

| pH Results | | | | | |
|---|---|---|---|---|---|
| **Treatments** | **0 Day time** | **4 Day** | **7 Day** | **14 Day** | **28Day** |
| **Co** | 6.70±0.01 $^{Aa}$ | 6.39±0.01 $^{Bf}$ | 5.91±0.01 $^{Cg}$ | 5.38±0.01 $^{De}$ | **4.65 ± 0.01 $^{Ee}$** |
| **T1** | 6.70±0.01 $^{Aa}$ | 6.41±0.01 $^{Be}$ | 6.02 ± 0.01 $^{Cf}$ | 5.40±0.02 $^{Dd}$ | **4.66 ± 0.02$^{Ee}$** |
| **T2** | 6.71±0.01 $^{Aa}$ | 6.45±0.01 $^{Bd}$ | 6.18 ± 0.01 $^{Cc}$ | 5.44±0.01 $^{Dc}$ | **4.73 ± 0.01$^{Ee}$** |
| **T3** | 6.71±0.01 $^{Aa}$ | 6.48 ± 0.01 $^{Bc}$ | 6.20 ± 0.01 $^{Cb}$ | 5.49 ± 0.01 $^{Db}$ | **4.77 ± 0.01$^{Eb}$** |
| **T4** | 6.71±0.00 $^{Aa}$ | 6.56±0.01 $^{Ba}$ | 6.26 ± 0.02 $^{Ca}$ | 5.56 ± 0.10 $^{Da}$ | **4.83 ± 0.01 $^{Ea}$** |
| **T5** | 6.71±0.02 $^{Aa}$ | 6.39±0.01 $^{Bf}$ | 6.01 ± 0.02 $^{Cf}$ | 5.37±0.01 $^{De}$ | **4.65 ± 0.01$^{Ee}$** |
| **T6** | 6.71±0.01 $^{Aa}$ | 6.41±0.01 $^{Be}$ | 6.07 ± 0.02 $^{Ce}$ | 5.40±0.01 $^{Dd}$ | **4.70 ± 0.01$^{Ea}$** |
| **T7** | 6.70±0.02 $^{Aa}$ | 6.45±0.02 $^{Bd}$ | 6.12 ± 0.01 $^{Cd}$ | 5.44±0.02 $^{Dc}$ | **4.72 ± 0.01$^{Ecd}$** |
| **T8** | **6.71±0.01 $^{Aa}$** | **6.53±0.01 $^{Bb}$** | **6.20 ±0.01 $^{Cbc}$** | **5.48±0.01 $^{Db}$** | **4.77±0.01 $^{Eb}$** |

Co: Control sample (low-fat milk). T1: Control + 0.1% PE. T2: Control + 0.5% PE. T3: Control + 1% PE. T4: Control + 2% PE. T5: Control + 0.1% PC. T6: Control + 0.5% PC. T7: Control + 1% PC. T8: Control + 2% PC.

**Different lowercase and uppercase letters indicate statistically significant differences within rows and columns, respectively ($p<0.05$).

**Brix Results**

According to the results, treatments and storage duration had no significant effect on the Brix value (10.00 ± 0.001) %of milk samples containing PC and PE ($p<0.05$).

**Fat Content Results**

According to the results, treatments and storage duration had no significant effect on the fat content (0.60 ± 0.001) % of milk samples containing PC and PE ($p<0.05$).

**Protein Content Results**

According to the results, treatments and storage duration had a significant effect on the protein content of milk samples containing PC and PE ($p<0.05$). The addition of PC and PE significantly increased the protein content of milk samples ($p<0.05$). This increase in protein content was dependent on the percentage of PC and PE added ($p<0.05$). When comparing samples with the same percentages of PC and PE, no statistically significant difference in protein content was observed ($p<0.05$) (Table 3).

**Antioxidant Activity Results**

According to the results, different treatments and storage duration had a significant effect on the DPPH antioxidant activity of milk samples containing PC and PE ($p<0.05$). The lowest antioxidant activity was observed in the control sample ($p<0.05$). The addition of PC and PE significantly increased the antioxidant activity of milk samples ($p<0.05$). This increase in antioxidant activity was dependent on the percentage of PC and PE used ($p<0.05$). When comparing PC and PE, the highest antioxidant activity was observed in samples containing high concentrations of PE, which was 1.27 times greater than that of samples with high concentrations of PC.However, a decrease in the antioxidant activity of milk samples was observed over time across all examined groups ($p<0.05$) (Table 4).

**Table 3** Mean protein content results (expressed as %) of milk containing PC and PE.

| O Day | Treatments |
|---|---|
| **Co** | 15.6±0.08$^{e}$ |
| **T1** | 16.33±0.04$^{d}$ |
| **T2** | 17.60±0.08$^{c}$ |
| **T3** | 19.73 ±0.09$^{b}$ |
| **T4** | 25.33 ±0.24$^{a}$ |
| **T5** | 16.36 ±0.04$^{d}$ |
| **T6** | 17.56 ±0.12$^{c}$ |
| **T7** | 19.76 ±0.04$^{b}$ |
| **T8** | 25.40 ±0.05$^{a}$ |

*Different lowercase letters indicate statistically significant differences within columns ($p<0.05$).

*Co: Control sample (low-fat milk)/ T1: Control + 0.1% PE/ T2: Control + 0.5% PE/ T3: Control + 1% PE/ T4: Control + 2% PE/ T5: Control + 0.1% PC/ T6: Control + 0.5% PC/T7: Control + 1% PC/ T8: Control + 2% PC.

**Table 4** Mean DPPH and FRAP antioxidant activity results of milk containing PC and PE over 28 days of storage.

| | **DPPH Antioxidant Activity Results (**IC50 = mg. mL) | | | | |
|---|---|---|---|---|---|
| **Treatments** | **0 Day time** | **4 Day** | **7 Day** | **14 Day** | **28Day** |
| **Co** | 5.84±0.08 $^{Dd}$ | 7.08±0.07 $^{CDa}$ | 8.77 ± 0.08 $^{Ca}$ | 12.55 ± 2.17 $^{Ca}$ | 17.27 ± 0.92 $^{Aa}$ |
| **T1** | 5.72±0.08 $^{Eb}$ | 6.75±0.12 $^{Db}$ | 8.45 ± 0.10 $^{Cb}$ | 9.40 ±0.16 $^{Bbc}$ | 14.78 ± 0.38$^{Ac}$ |
| **T2** | 5.12±0.04 $^{Dd}$ | 6.05 ± 0.06 $^{Cc}$ | 7.26 ± 0.15 $^{Bda}$ | 7.31 ± 0.65 $^{Bde}$ | 12.03 ± 0.42$^{Ad}$ |
| **T3** | 4.66±0.03 $^{Cf}$ | 5.01 ± 0.25 $^{Cd}$ | 6.07 ± 0.04 $^{Bf}$ | 6.25 ± 0.49 $^{Bef}$ | 9.13 ± 0.22$^{Af}$ |
| **T4** | 3.75±0.03 $^{Eh}$ | 4.33 ± 0.03 $^{De}$ | 4.77 ± 0.03 $^{Ch}$ | 5.26 ± 0.10 $^{Bf}$ | 6.31 ± 0.04 $^{Ah}$ |
| **T5** | 5.86±0.05 $^{Ea}$ | 6.89 ± 0.07 $^{Dab}$ | 8.43 ± 0.06 $^{Cb}$ | 9.98 ± 0.23 $^{Bb}$ | 16.10 ± 0.35 $^{Ab}$ |
| **T6** | 5.32±0.09 $^{Eg}$ | 6.65 ±0.30 $^{Db}$ | 7.77 ± 0.08 $^{Cc}$ | 8.62 ± 0.11 $^{Bbcd}$ | 12.55 ± 0.21 $^{Ad}$ |
| **T7** | 5.02±0.03 $^{Ea}$ | 5.86 ± 0.09 $^{Dc}$ | 6.80 ± 0.02 $^{Cg}$ | 7.62 ± 0.13 $^{Bcde}$ | 10.80 ± 0.38$^{Ae}$ |
| **T8** | 4.31±0.03 $^{Ea}$ | 4.84± 0.05 $^{Dd}$ | 5.53 ±0.08 $^{Cg}$ | 6.13 ± 0.02 $^{Bef}$ | 8.05±0.18 $^{Ag}$ |
| **Treatments** | **0 Day time** | **4 Day** | **7 Day** | **14 Day** | **28Day** |
| | **FRAP Antioxidant Activity Results (ppm)** | | | | |
| **Co** | 102.80 ± 0.38 $^{Ah}$ | 92.91 ± 0.38 $^{Bf}$ | 66.56 ± 0.50 $^{Cg}$ | 51.60 ± 0.50 $^{Df}$ | 45.36 ± 0.25 $^{Eg}$ |
| **T1** | 104.05 ± 0.38 $^{Ag}$ | 94.07 ± 0.52 $^{Be}$ | 67.31 ± 0.75$^{Cg}$ | 56.00 ± 0.52 $^{Dd}$ | 48.85 ± 0.90$^{Ee}$ |
| **T2** | 107.62 ± 0.29 $^{Ae}$ | 96.48 ± 0.50 $^{Bc}$ | 72.04 ± 0.05$^{Cd}$ | 58.66 ± 0.38 $^{Dc}$ | 51.93± 0.00$^{Ec}$ |
| **T3** | 110.86 ± 0.38 $^{Ac}$ | 99.98 ± 0.50 $^{Bb}$ | 77.86 ± 0.14$^{Cb}$ | 61.82 ± 0.25 $^{Db}$ | 55.84 ± 0.25 $^{Eb}$ |
| **T4** | 114.36 ± 0.14 $^{Aa}$ | 104.30 ± 0.14 $^{Ba}$ | 84.68 ± 0.29 $^{Ca}$ | 67.81 ± 0.25 $^{Da}$ | 60.57 ± 0.50 $^{Ea}$ |
| **T5** | 103.55 ± 0.38 $^{Ag}$ | 91.33 ± 0.38 $^{Bg}$ | 69.97 ± 0.38 $^{Cf}$ | 52.76 ± 0.29$^{De}$ | 45.20 ± 0.52 $^{Eg}$ |
| **T6** | 106.46 ± 0.25 $^{Af}$ | 95.49 ± 0.43 $^{Bd}$ | 70.80 ± 0.75$^{Ce}$ | 55.59 ± 0.50 $^{Dd}$ | 46.53 ± 0.38 $^{Ef}$ |
| **T7** | 108.62 ± 0.38 $^{Ad}$ | 95.57 ± 0.38 $^{Bd}$ | 74.29 ± 0.25 $^{Cc}$ | 58.50 ± 0.63 $^{Dc}$ | 48.19 ± 0.38 $^{Ee}$ |
| **T8** | 111.95 ± 0.25 $^{Ab}$ | 94.41 ± 0.63 $^{Be}$ | 77.61 ± 0.38 $^{Cb}$ | 61.24 ± 0.52 $^{Db}$ | 50.18 ± 0.38 $^{Ed}$ |

*Different lowercase and uppercase letters indicate statistically significant differences within rows and columns, respectively ($p<0.05$).

*Co: Control sample (low-fat milk)/ T1: Control + 0.1% PE/ T2: Control + 0.5% PE. T3: Control + 1% PE/ T4: Control + 2% PE/ T5: Control + 0.1% PC/T6: Control + 0.5% PC/ T7: Control + 1% PC/ T8: Control + 2% PC.

According to the results, different treatments and storage duration had a significant effect on the FRAP antioxidant activity of milk samples containing PC and PE ($p<0.05$). The lowest antioxidant activity was observed in the control sample ($p<0.05$). The addition of PC and PE significantly increased the FRAP antioxidant activity of milk samples ($p<0.05$). This increase in FRAP antioxidant activity was dependent on the percentage of PC and PE used ($p<0.05$). When comparing PC and PE, the highest antioxidant activity was observed in samples containing high concentrations of PE, which was 1.20 times greater than that of samples with high concentrations of PC. However, a decrease in FRAP antioxidant activity of milk samples was observed over time across all examined groups ($p<0.05$) (Table 4).

**Color Index Results**

According to the results, different treatments and storage duration had a significant effect on the brightness index (L*) of milk samples containing PC and PE ($p<0.05$). The highest brightness index was observed in the control sample ($p<0.05$). The addition of PC and PE significantly reduced the brightness index of milk samples ($p<0.05$). This reduction in the brightness index was dependent on the percentage of PC and PE used ($p<0.05$). However, a significant decrease in the brightness index of milk samples was observed over time across all examined groups ($p<0.05$). No significant difference was found in the brightness index (L*) between milk samples containing PE and PC (Table 5).

According to the results, different treatments and storage duration had a significant effect on the redness index (a*) of milk samples containing PC and PE ($p<0.05$). The lowest redness index was observed in the control sample ($p<0.05$). The addition of PC and PE significantly increased the redness index of milk samples ($p<0.05$). When comparing PC and PE, treatments containing PE significantly increased the redness of milk samples ($p<0.05$). This increase in the redness index was dependent on the percentage of PC and PE used ($p<0.05$) (Table 5).

According to the results, different treatments and storage duration had a significant effect on the yellowness index (b*) of milk samples containing PC and PE ($p<0.05$). The lowest yellowness index was observed in the control sample ($p<0.05$). The addition of PC and PE significantly increased the yellowness index of milk samples ($p<0.05$). When comparing PC and PE, treatments containing PE significantly increased the yellowness of milk samples ($p<0.05$). This increase in the yellowness index was dependent on the percentage of PC and PE used ($p<0.05$) (Table 5).

According to the results, different treatments and storage duration had a significant effect on the total color change index (ΔE) of milk samples containing PC and PE ($p<0.05$). As expected, no color changes were observed on the first day ($p>0.05$). The addition of PC and PE significantly increased the total color change index of milk samples ($p<0.05$). This increase in the total color change index was dependent on the percentage of PC and PE used ($p<0.05$). No significant difference was found in the total color change index (ΔE) between milk samples containing PE and PC (Table 5).

**Table 5** Mean Color Index Results of milk containing PC and PE over 28 days of storage.

| | **Mean brightness index (L*)** | | | | |
|---|---|---|---|---|---|
| Treatments | 0 Day | 4 Day | 7 Day | 14 Day | 28Day |
| Co | 81.84 ± 0.11 $^{Aa}$ | 81.68 ± 0.11 $^{Aa}$ | 79.54 ± 0.18 $^{Ba}$ | 78.75 ± 0.25 $^{Ca}$ | 78.78 ± 0.04$^{Ca}$ |
| T1 | 68.38 ± 0.10 $^{Ae}$ | 67.31 ± 0.13$^{Be}$ | 66.57 ± 0.08 $^{Ce}$ | 63.75 ± 0.35 $^{Def}$ | 61.01 ± 0.21$^{Ec}$ |
| T2 | 68.40 ± 0.20 $^{Ae}$ | 66.58 ± 0.09$^{Ba}$ | 66.13 ± 0.17 $^{Cf}$ | 63.95 ± 0.15 $^{De}$ | 61.30 ± 0.15$^{Ec}$ |
| T3 | 68.37 ± 0.10 $^{Ae}$ | 66.52 ± 0.15 $^{B}$ | 66.70 ± 0.15 $^{Be}$ | 64.31 ± 0.38 $^{C}$ | 61.68 ± 0.17$^{Dc}$ |
| T4 | 64.15 ± 0.15 $^{Ag}$ | 64.45 ± 0.08 $^{Aa}$ | 63.51 ± 0.12 $^{Bh}$ | 63.41 ± 0.57 $^{Bf}$ | 62.03 ± 0.02$^{Cc}$ |
| T5 | 79.85 ± 0.01 $^{Ab}$ | 78.15 ± 0.08 $^{Ab}$ | 78.40 ± 0.07 $^{Ab}$ | 78.79 ± 0.10 $^{Aa}$ | 71.55 ± 0.11 $^{Ab}$ |
| T6 | 78.38 ± 0.28 $^{Ac}$ | 76.50 ± 0.08 $^{Bc}$ | 75.92 ± 0.04 $^{Cg}$ | 75.03 ± 0.00 $^{Db}$ | 72.52 ± 0.49 $^{Eb}$ |
| T7 | 71.74 ± 0.46 $^{Ad}$ | 70.63 ± 0.13 $^{Bd}$ | 69.21 ± 0.04 $^{Cd}$ | 68.55 ± 0.05 $^{Dc}$ | 67.25 ± 0.07 $^{Ebc}$ |
| T8 | 66.29 ± 0.15 $^{Af}$ | 66.59 ± 0.08 $^{Aa}$ | 65.65 ± 0.57 $^{Ba}$ | 65.55 ± 0.57 $^{Bd}$ | 64.17 ± 0.02 $^{Cc}$ |
| | **Index (a*)** | | | | |
| Treatments | 0 Day | 4 Day | 7 Day | 14 Day | 28Day |
| Co | 4.29 ± 0.08 $^{Dh}$ | 4.13 ± 0.08 $^{Di}$ | 4.78 ± 0.08 $^{Ch}$ | 5.24 ± 0.14$^{Bh}$ | 7.35 ± 0.11 $^{Ad}$ |
| T1 | 22.65 ± 0.28 $^{Bc}$ | 23.03 ± 0.22 $^{ABc}$ | 23.21±0.10 $^{Ac}$ | 23.31±0.09$^{Ac}$ | 23.26 ± 0.51$^{Ab}$ |
| T2 | 22.15 ± 0.15 $^{Cd}$ | 22.71 ± 0.07 $^{Bd}$ | 22.77±0.08$^{Bd}$ | 22.92 ±0.14 $^{ABd}$ | 23.04 ± 0.10 $^{Ab}$ |
| T3 | 23.34 ± 0.46 $^{Bb}$ | 23.35 ± 0.07 $^{Bb}$ | 23.56 ±0.18$^{Abb}$ | 23.81 ± 0.25 $^{ABb}$ | 23.92 ± 0.22 $^{Aab}$ |
| T4 | 24.15 ± 0.09 $^{ca}$ | 24.35 ± 0.01 $^{Ca}$ | 25.44 ± 0.06 $^{Ba}$ | 25.82 ±0.04 $^{Aa}$ | 25.80 ± 0.43 $^{Aa}$ |
| T5 | 4.48 ± 0.04 $^{Bh}$ | 4.58 ± 0.04 $^{Bh}$ | 8.62 ± 0.05 $^{ABh}$ | 4.80 ±0.04$^{ABh}$ | 8.37 ± 4.31 $^{Ad}$ |
| T6 | 10.60 ± 0.42 $^{Cg}$ | 11.14 ± 0.10 $^{Bg}$ | 11.51 ± 0.07 $^{ABg}$ | 11.36±0.18$^{Bg}$ | 11.85 ± 0.10 $^{Ac}$ |
| T7 | 12.78 ± 0.15 $^{Cf}$ | 13.01 ± 0.27 $^{BCf}$ | 13.30 ± 0.17$^{Abcf}$ | 13.68 ± 0.03$^{Af}$ | 13.55 ± 0.65$^{ABc}$ |
| T8 | 13.79 ± 0.51 $^{Ae}$ | 13.24 ± 0.16 $^{Be}$ | 3.88±0.06$^{Ae}$ | 13.96± 0.04$^{Ae}$ | 14.28 ± 0.19 $^{Ac}$ |
| | **Index (b*)** | | | | |
| Treatments | 0 Day | 4 Day | 7 Day | 14 Day | 28Day |
| Co | 7.32 ± 0.14 $^{Di}$ | 7.16 ± 0.14 $^{Di}$ | 8.19 ± 0.34 $^{Ch}$ | 9.46 ± 0.30 $^{Ba}$ | 12.91 ± 0.11 $^{Ad}$ |
| T1 | 10.31 ± 0.07 $^{Cg}$ | 10.46 ± 0.24 $^{Cg}$ | 10.62 ± 0.17 $^{BCg}$ | 10.84 ± 0.08 $^{Ba}$ | 11.53 ± 0.21$^{Ad}$ |
| T2 | 25.37 ± 0.10 $^{Dc}$ | 25.63 ± 0.13 $^{Cc}$ | 25.78 ± 0.06 $^{Cc}$ | 26.06 ± 0.02 $^{Ba}$ | 26.39 ± 0.16 $^{Ab}$ |
| T3 | 27.60 ± 0.20 $^{Ab}$ | 28.09 ± 0.17 $^{Ab}$ | 28.43 ± 0.44 $^{Ab}$ | 28.51 ± 0.35 $^{Aa}$ | 28.46 ± 0.15 $^{Aab}$ |
| T4 | 29.08 ± 0.02 $^{Ea}$ | 29.64 ± 0.03 $^{Da}$ | 30.43 ± 0.13 $^{Ca}$ | 30.60 ± 0.09 $^{Ba}$ | 30.84 ± 0.12 $^{Aa}$ |
| T5 | 7.66 ± 0.03 $^{Bh}$ | 8.36 ± 0.13 $^{ABh}$ | 8.62 ± 0.05 $^{ABh}$ | 8.81 ± 0.08 $^{ABa}$ | 12.47 ± 0.14 $^{Ad}$ |
| T6 | 12.46 ± 0.03$^{Df}$ | 12.79 ± 0.17 $^{Df}$ | 13.56 ± 0.35 $^{Cf}$ | 14.60 ± 0.14 $^{Ba}$ | 16.47 ± 0.08 $^{Ac}$ |
| T7 | 17.37 ± 0.20 $^{Ce}$ | 17.51 ± 0.07 $^{Ce}$ | 17.64 ± 0.08 $^{Ce}$ | 18.01 ± 0.10 $^{Ba}$ | 18.37 ± 0.24 $^{Ac}$ |
| T8 | 18.22 ± 0.02 $^{Dd}$ | 18.36 ± 0.03 $^{Dd}$ | 18.57 ± 0.13 $^{Cd}$ | 18.91 ± 0.08 $^{Ba}$ | 19.13 ± 0.07 $^{Ac}$ |
| | **Total Color Change Index (ΔE)** | | | | |
| Treatments | 0 Day | 4 Day | 7 Day | 14 Day | 28Day |
| Co | 0.0 ± 0.00 $^{Da}$ | 0.28 ± 0.00 $^{Dd}$ | 0.85 ± 0.13 $^{cf}$ | 1.08 ± 0.16 $^{Bg}$ | 2.40 ± 0.15 $^{Ag}$ |
| T1 | 0.0 ± 0.00 $^{Ea}$ | 0.69 ± 0.05 $^{Dc}$ | 1.94 ± 0.17 $^{Cde}$ | 2.41 ± 0.27 $^{Be}$ | 3.23 ± 0.04 $^{Ae}$ |
| T2 | 0.0 ± 0.00 $^{Ea}$ | 1.17 ± 0.47 $^{Db}$ | 1.99 ± 0.44 $^{Cde}$ | 3.89 ± 0.36 $^{Bcd}$ | 4.70 ± 0.41 $^{Ac}$ |
| T3 | 0.0 ± 0.00 $^{Ea}$ | 1.93 ± 0.13 $^{Da}$ | 2.54 ± 0.34 $^{Cbc}$ | 4.07 ± 0.24 $^{Bbc}$ | 6.77 ± 0.02 $^{Ab}$ |
| T4 | 0.0 ± 0.00 Ea | 1.98 ± 0.27 Da | 2.60 ± 0.08 $^{Cbc}$ | 4.23 ± 0.29 $^{Babc}$ | 7.23 ± 0.19$^{Aab}$ |
| T5 | 0.0 ± 0.00 $^{Da}$ | 0.74 ± 0.39 Cc | 1.77 ± 0.05 $^{Be}$ | 1.80 ± 0.47 $^{Bf}$ | 4.04 ± 0.17 $^{Af}$ |
| T6 | 0.0 ± 0.00 $^{Ea}$ | 1.18 ± 0.03 $^{Db}$ | 1.98 ± 0.13 $^{Cde}$ | 3.39 ± 0.41 $^{Bd}$ | 7.07 ± 0.13 $^{Aab}$ |
| T7 | 0.0 ± 0.00 $^{Ea}$ | 1.84 ± 0.05 $^{Da}$ | 2.39 ± 0.06 $^{Ccd}$ | 4.57 ± 0.31 $^{Bab}$ | 7.23 ± 0.55$^{Aab}$ |
| T8 | 0.0 ± 0.00 $^{Ea}$ | 2.02 ± 0.11 $^{Da}$ | 2.89 ± 0.35 $^{Ca}$ | 4.71 ± 0.25 $^{Ba}$ | 7.49 ± 0.29 $^{Aa}$ |

*Different lowercase and uppercase letters indicate statistically significant differences within rows and columns, respectively ($p<0.05$).

*Co: Control sample (low-fat milk)/ T1: Control + 0.1% PE/ T2: Control + 0.5% PE/ T3: Control + 1% PE/ T4: Control + 2% PE/ T5: Control + 0.1% PC/ T6: Control + 0.5% PC/ T7: Control + 1% PC/T8: Control + 2% PC.

**Results of microbiological analysis**

According to the results, different treatments and storage duration had a significant effect on the total microbial count in milk samples containing PC and PE ($p<0.05$). During seven days of storage, no bacterial growth was observed in any of the treatments ($p>0.05$). On the 14th day, only the control sample and T1 showed bacterial growth ($p<0.05$), while no growth was observed in the other treatments ($p>0.05$). On the 28th day, total microbial count growth was observed in all tested

groups. The highest total microbial count was observed in the control and T1 treatments ($p<0.05$). The lowest total bacterial growth was found in treatments containing PC and PE; however, no significant difference was found in the total bacterial count growth between samples containing PC and PE ($p<0.05$) (Table 6).
According to the results, different treatments and storage duration had no significant effect on the count of Enterobacteriaceae bacteria in milk samples containing PC and PE ($p>0.05$). No growth of Enterobacteriaceae bacteria was observed in any of the tested groups during 28 days ($p>0.05$).
According to the results, different treatments and storage duration had a significant effect on the mold and yeast count in milk samples containing PC and PE ($p<0.05$). During 14 days of storage, no bacterial growth was observed in any of the treatments ($p>0.05$). On the 28th day, mold and yeast growth was observed in all tested groups. The highest mold growth was observed in the control treatment, and the lowest was in the T8 treatment, with a significant difference ($p<0.05$) (Table 6).

**Table 6** Mean results of microbiological analysis (in Log CFU.ml) containing PC and PE during 28 days of storage.

| | **total microbial count (Log CFU.ml)** | | | | |
|---|---|---|---|---|---|
| Treatments | 0 Day | 4 Day | 7 Day | 14 Day | 28Day |
| Co | $0.00 \pm 0.00^{Ca}$ | $0.00 \pm 0.00^{Ca}$ | $0.00 \pm 0.00^{Ca}$ | $1.67 \pm 0.19^{Ba}$ | $3.11 \pm 0.01^{Aa}$ |
| T1 | $0.00 \pm 0.00^{Ca}$ | $0.00 \pm 0.00^{Ca}$ | $0.00 \pm 0.00^{Ca}$ | $1.69 \pm 0.09^{Ba}$ | $3.08 \pm 0.01^{Aa}$ |
| T2 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Bb}$ | $2.90 \pm 0.04^{Ab}$ |
| T3 | $0.00 \pm 0.00^{ABa}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Bb}$ | $0.00 \pm 0.00^{Bb}$ | $2.76 \pm 0.02^{Acd}$ |
| T4 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Bb}$ | $2.50 \pm 0.06^{Ae}$ |
| T5 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Bb}$ | $3.06 \pm 0.02^{Aa}$ |
| T6 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Bb}$ | $0.00 \pm 0.00^{Bb}$ | $2.84 \pm 0.06^{Abc}$ |
| T7 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Bb}$ | $2.69 \pm 0.01^{Ad}$ |
| T8 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Bb}$ | $2.45 \pm 0.12^{Ae}$ |
| | **Mold and Yeast Count (Log CFU.ml)** | | | | |
| Treatments | 0 Day time | 4 Day | 7 Day | 14 Day | 28Day |
| Co | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $2.98 \pm 0.01^{Aa}$ |
| T1 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $2.85 \pm 0.02^{Ab}$ |
| T2 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $2.63 \pm 0.02^{Ac}$ |
| T3 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $2.40 \pm 0.10^{Ad}$ |
| T4 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $2.09 \pm 0.05^{Ae}$ |
| T5 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $2.83 \pm 0.01^{Ab}$ |
| T6 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $2.53 \pm 0.04^{Ac}$ |
| T7 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $2.20 \pm 0.14^{Ae}$ |
| T8 | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $0.00 \pm 0.00^{Ba}$ | $1.88 \pm 0.09^{Af}$ |

Different lowercase and uppercase letters indicate statistically significant differences in each row and column ($p<0.05$).
*Co: Control sample (low-fat milk). T1: Control + 0.1% PE. T2: Control + 0.5% PE. T3: Control + 1% PE. T4: Control + 2% PE. T5: Control + 0.1% PC. T6: Control + 0.5% PC. T7: Control + 1% PC. T8: Control + 2% PC.

**Results of Sensory Evaluation**

According to the results, different treatments and storage duration had a significant effect on the sensory evaluation of taste and flavor in milk samples containing PC and PE ($p<0.05$). The highest sensory evaluation score for taste and flavor was observed in the control sample ($p<0.05$). The addition of PC and PE significantly reduced the sensory evaluation of taste and flavor in the milk samples ($p<0.05$). This significant reduction in sensory evaluation of taste and flavor was dependent on the percentage of PC and PE ($p<0.05$). No significant difference was found in the sensory evaluation of taste and flavor between milk samples containing PE and PC (Table 7).
According to the results, different treatments and storage duration had a significant effect on the sensory evaluation of aroma and odor in milk samples containing PC and PE ($p<0.05$). The highest sensory evaluation score for aroma and odor was observed in the control sample ($p<0.05$). The addition of PC and PE significantly reduced the sensory evaluation of aroma and odor in the milk samples ($p<0.05$). This reduction was significantly greater in milk samples containing PC, and the significant reduction in sensory evaluation of aroma and odor was dependent on the percentage of PC and PE ($p<0.05$) (Table 7).
According to the results, different treatments and storage duration had a significant effect on the sensory evaluation of color in milk samples containing PC and PE ($p<0.05$). The lowest sensory evaluation score for color was observed in the control sample ($p<0.05$). The addition of PC and PE significantly increased the sensory evaluation of color in the milk samples ($p<0.05$). This increase was significantly greater in samples containing PE compared to PC. The significant increase in sensory evaluation of color was dependent on the percentage of PC and PE ($p<0.05$). However, a significant reduction in sensory evaluation of color was observed in all tested groups during 28 days of storage ($p<0.05$) (Table 7).
According to the results, different treatments and storage duration had a significant effect on the sensory evaluation of mouthfeel in milk samples containing PC and PE ($p<0.05$). The lowest sensory evaluation score for mouthfeel was observed in the control sample ($p<0.05$). The addition of PC and PE significantly increased the sensory evaluation of mouthfeel in the milk samples ($p<0.05$), and no significant difference was found in the sensory evaluation of mouthfeel between samples containing PC and PE. This significant increase in sensory evaluation of mouthfeel was dependent on the percentage of PC and PE ($p<0.05$). However, a significant reduction in sensory evaluation of mouthfeel was observed in all tested groups during 28 days of storage ($p<0.05$) (Table 7).
According to the results, different treatments and storage duration had a significant effect on the sensory evaluation of overall acceptance in milk samples containing PC and PE ($p<0.05$). On the first and fourth days, no statistically significant difference was observed in the overall acceptance of the samples, and all samples had the highest sensory evaluation scores for overall acceptance ($p>0.05$). According to the results, on the seventh day, the lowest sensory evaluation score for overall acceptance was observed in the control sample ($p<0.05$). The addition of PC and PE significantly increased the sensory evaluation of overall acceptance in the milk samples ($p<0.05$), and no significant difference was found in the sensory evaluation of overall acceptance between samples containing PC and PE. This significant increase in sensory evaluation of overall acceptance was dependent on the percentage of PC and PE ($p<0.05$). However, a significant reduction in sensory evaluation of overall acceptance was observed in all tested groups during 28 days of storage ($p<0.05$) (Table 7).

**Table 7** Mean results of sensory evaluation of samples containing PC and PE during 28 days of storage

| **Treatments** | **0 Day** | **4 Day** | **7 Day** | **14 Day** | **28Day** |
|---|---|---|---|---|---|
| | **Sensory Evaluation of Taste and Flavor** | | | | |
| Co | $5.00 \pm 0.00^{Aa}$ | $4.00 \pm 0.00^{Ba}$ | $2.67 \pm 0.58^{Ca}$ | $2.00 \pm 0.00^{Da}$ | $1.00 \pm 0.00^{Ea}$ |
| T1 | $4.67 \pm 0.58^{Aab}$ | $3.67 \pm 0.58^{Bab}$ | $3.00 \pm 0.00^{Ca}$ | $2.00 \pm 0.00^{Da}$ | $1.00 \pm 0.00^{Ea}$ |
| T2 | $4.67 \pm 0.58^{Aab}$ | $3.67 \pm 0.58^{Bab}$ | $3.33 \pm 0.58^{Ba}$ | $2.00 \pm 0.00^{Ca}$ | $1.00 \pm 0.00^{Da}$ |
| T3 | $4.33 \pm 0.58^{Aab}$ | $3.67 \pm 0.58^{Bab}$ | $3.00 \pm 0.00^{Ca}$ | $2.00 \pm 0.00^{Da}$ | $1.00 \pm 0.00^{Ea}$ |
| T4 | $3.00 \pm 0.00^{Ac}$ | $2.67 \pm 0.58^{Ac}$ | $3.00 \pm 0.00^{Aa}$ | $2.33 \pm 0.58^{Aa}$ | $1.33 \pm 0.58^{Ba}$ |
| T5 | $4.33 \pm 0.58^{Aab}$ | $3.67 \pm 0.58^{Aab}$ | $2.67 \pm 0.58^{Ba}$ | $2.00 \pm 0.00^{Ba}$ | $1.00 \pm 0.00^{Ca}$ |
| T6 | $4.00 \pm 0.00^{Ab}$ | $3.00 \pm 0.00^{Bbc}$ | $3.00 \pm 0.00^{Ba}$ | $2.00 \pm 0.00^{Ca}$ | $1.00 \pm 0.00^{Da}$ |
| T7 | $4.00 \pm 0.00^{Ab}$ | $3.33 \pm 0.58^{Babc}$ | $3.00 \pm 0.00^{Ba}$ | $2.00 \pm 0.00^{Ca}$ | $1.00 \pm 0.00^{Da}$ |
| T8 | $3.00 \pm 0.00^{Ac}$ | $3.00 \pm 0.00^{Abc}$ | $3.00 \pm 0.00^{Aa}$ | $2.00 \pm 0.00^{Ba}$ | $1.00 \pm 0.00^{Ca}$ |
| | **Sensory Evaluation of Aroma and Odor** | | | | |
| **Treatments** | **0 Day** | **4 Day** | **7 Day** | **14 Day** | **28Day** |
| Co | $5.00 \pm 0.00^{Aa}$ | $3.67 \pm 0.58^{Ba}$ | $2.67 \pm 0.58^{Ca}$ | $2.00 \pm 0.00^{Db}$ | $1.00 \pm 0.00^{Eb}$ |

| | | | | | |
|---|---|---|---|---|---|
| T1 | 5.00 ± 0.00 $^{Aa}$ | 4.00 ± 0.00 $^{Ba}$ | 3.00 ± 0.00 $^{Ca}$ | 1.00 ± 0.00 $^{Eb}$ | 1.00 ± 0.00 $^{Eb}$ |
| T2 | 5.00 ± 0.00 $^{Aa}$ | 4.00 ± 0.00 $^{Aba}$ | 3.33 ± 0.58 $^{Ca}$ | 2.00 ± 0.00 $^{Db}$ | 1.00 ± 0.00 $^{Eb}$ |
| T3 | 3.00 ± 0.00 $^{Ab}$ | 3.33 ± 0.58 $^{Aa}$ | 2.33 ± 0.58 $^{Aab}$ | 2.33 ± 0.58 $^{Aab}$ | 1.33 ± 0.58 $^{Bab}$ |
| T4 | 3.67 ± 0.58 $^{Ab}$ | 3.00 ± 0.00 $^{Abb}$ | 3.00 ± 0.00 $^{Aba}$ | 2.67 ± 0.58 $^{Ba}$ | 1.67 ± 0.58 $^{Ca}$ |
| T5 | 3.67 ± 0.58 $^{Ab}$ | 3.33 ± 0.58 $^{Abab}$ | 2.67 ± 0.58 $^{Bca}$ | 2.00 ± 0.00 $^{Cb}$ | 1.00 ± 0.00 $^{Db}$ |
| T6 | 3.67 ± 0.58 $^{Ab}$ | 3.33 ± 0.58 $^{Aab}$ | 3.00 ± 0.00 $^{Aa}$ | 2.00 ± 0.00 $^{Bb}$ | 1.00 ± 0.00 $^{Cb}$ |
| T7 | 3.67 ± 0.58 $^{Ab}$ | 3.33 ± 0.58 $^{Aab}$ | 3.00 ± 0.00 $^{Aa}$ | 2.00 ± 0.00 $^{Bb}$ | 1.00 ± 0.00 $^{Cb}$ |
| T8 | 3.33 ± 0.58 $^{Ab}$ | 3.33 ± 0.58 $^{Aab}$ | 2.67 ± 0.58 $^{Aba}$ | 2.00 ± 0.00 $^{Bb}$ | 1.00 ± 0.00 $^{Cb}$ |
| | **Sensory Evaluation of Color** | | | | |
| **Treatments** | **0 Day** | **4 Day** | **7 Day** | **14 Day** | **28Day** |
| Co | 4.00 ± 0.00 $^{Ab}$ | 3.00 ± 0.00 $^{Bb}$ | 2.00 ± 0.00 $^{Cd}$ | 1.67 ± 0.58 $^{Cd}$ | 1.00 ± 0.00 $^{Db}$ |
| T1 | 4.33 ± 0.58 $^{Aab}$ | 3.67 ± 0.58 $^{ABab}$ | 2.67 ± 0.58 $^{BCbcd}$ | 2.33 ± 0.58 $^{Cbcd}$ | 1.67 ± 0.58$^{Cab}$ |
| T2 | 4.67 ± 0.58 $^{Aab}$ | 4.00 ± 0.00 $^{Ba}$ | 3.00 ± 0.00 $^{Cabc}$ | 2.67 ± 0.58 $^{Cabc}$ | 2.00 ± 0.00 $^{Da}$ |
| T3 | 5.00 ± 0.00 $^{Aa}$ | 4.00 ± 0.00 $^{Ba}$ | 3.33 ± 0.58 $^{Cab}$ | 3.00 ± 0.00 $^{Cab}$ | 1.33 ± 0.58 $^{Bab}$ |
| T4 | 4.67 ± 0.58 $^{Aab}$ | 3.67 ± 0.58 $^{ABab}$ | 3.33 ± 0.58 $^{BCab}$ | 2.67 ± 0.58 $^{BCabc}$ | 2.33 ± 0.58 $^{Ca}$ |
| T5 | 4.33 ± 0.58 $^{Aab}$ | 3.33 ± 0.58 $^{Bab}$ | 2.33 ± 0.58 $^{Ccd}$ | 2.00 ± 0.00 $^{Ccd}$ | 1.00 ± 0.00 $^{Db}$ |
| T6 | 4.67 ± 0.58 $^{Aab}$ | 4.00 ± 0.00 $^{ABa}$ | 3.33 ± 0.58 $^{Bab}$ | 3.33 ± 0.58 $^{Ba}$ | 2.33 ± 0.58 $^{Ca}$ |
| T7 | 4.67 ± 0.58 $^{Aab}$ | 3.67 ± 0.58 $^{Bab}$ | 3.33 ± 0.58 $^{Bab}$ | 3.00 ± 0.00 $^{BCab}$ | 2.33 ± 0.58 $^{Ca}$ |
| T8 | 5.00 ± 0.00 $^{Aa}$ | 4.00 ± 0.00 $^{Ba}$ | 3.67 ± 0.58 $^{Ba}$ | 3.00 ± 0.00 $^{Cab}$ | 2.00 ± 0.00 $^{Da}$ |
| | **Sensory Evaluation of Mouthfeel** | | | | |
| **Treatments** | **0 Day** | **4 Day** | **7 Day** | **14 Day** | **28Day** |
| Co | 4.33 ± 0.58 $^{Aa}$ | 3.33 ± 0.58 $^{Ba}$ | 2.33 ± 0.58 $^{Cb}$ | 1. 67 ± 0.58 $^{CDb}$ | 1.00 ± 0.00 $^{Db}$ |
| T1 | 4.33 ± 0.58 $^{Aa}$ | 3. 67± 0.58 $^{Aba}$ | 3.00 ± 0.00 $^{Bcab}$ | 2.33 ± 0.58 $^{Cab}$ | 1.33 ± 0.58 $^{Db}$ |
| T2 | 4.67 ± 0.58 $^{Aa}$ | 4.00 ± 0.00 $^{Ba}$ | 3.00 ± 0.00 $^{Cab}$ | 2.67 ± 0.58 $^{Cab}$ | 2.00 ± 0.00 $^{Da}$ |
| T3 | 5.00 ± 0.00 $^{Aa}$ | 4.00 ± 0.00 $^{Ba}$ | 4.00 ± 0.00 $^{Ba}$ | 3.00 ± 0.00 $^{Ca}$ | 2.00 ± 0.00 $^{Da}$ |
| T4 | 5.00 ± 0.00 $^{Aa}$ | 4·00 ± 0.00 $^{Ba}$ | 3.67 ± 0.58 $^{Ba}$ | 3.00 ± 0.00 $^{Ca}$ | 2.00 ± 0.00 $^{Da}$ |
| T5 | 4.33 ± 0.58 $^{Aa}$ | 3.33 ± 0.58 $^{Ba}$ | 3.00 ± 0.00 $^{Bab}$ | 3.00 ± 0.00 $^{Ba}$ | 2.00 ± 0.00 $^{Ca}$ |
| T6 | 4.67 ± 0.58 $^{Aa}$ | 3.67 ± 0.58 $^{Ba}$ | 3.33 ± 0.58 $^{Bab}$ | 3.00 ± 0.00 $^{Ba}$ | 2.00 ± 0.00 $^{Ca}$ |
| T7 | 5.00 ± 0.00 $^{Aa}$ | 4.00 ± 0.00$^{ABa}$ | 3.67 ± 1.15 $^{ABCa}$ | 2.67 ± 0.58 $^{BCab}$ | 2.33 ± 0.58 $^{Ca}$ |
| T8 | 5.00 ± 0.00 $^{Aa}$ | 4.00 ± 0.00 $^{Ba}$ | 3.67 ± 0.58 $^{Ba}$ | 2.67 ± 0.58 $^{Cab}$ | 2.00 ± 0.00 $^{Da}$ |
| | **Evaluation of Overall Acceptance** | | | | |
| **Treatments** | **0 Day** | **4 Day** | **7 Day** | **14 Day** | **28Day** |
| Co | 4.33 ± 0.58 $^{Aa}$ | 3.33 ± 0.58 $^{Ba}$ | 2.33 ± 0.58 $^{Cb}$ | 1. 67 ± 0.58 $^{CDb}$ | 1.00 ± 0.00 $^{Db}$ |
| T1 | 4.67 ± 0.58 $^{Aa}$ | 3.67± 0.58 $^{Ba}$ | 3.00 ± 0.00 $^{Bcab}$ | 2.33 ± 0.58 $^{Cab}$ | 1.33 ± 0.58$^{Dcb}$ |
| T2 | 4.67 ± 0.58 $^{Aa}$ | 4.00 ± 0.00 $^{Aba}$ | 3.33 ± 0.58 $^{Bcab}$ | 2.67 ± 0.58 $^{CDa}$ | 2.00 ± 0.00 $^{Ca}$ |
| T3 | 4.67 ± 0.58 $^{Aa}$ | 4.00 ± 0.00 $^{Aba}$ | 3.67 ± 0.58 $^{Ba}$ | 2.67 ± 0.58 $^{Ca}$ | 2.00 ± 0.00 $^{Da}$ |
| T4 | 4.33 ± 0.58 $^{Aa}$ | 4·00 ± 0.00 $^{Aba}$ | 3.33 ± 0.58 $^{Bcab}$ | 2.67 ± 0.58 $^{CDa}$ | 2.00 ± 0.00$^{ADa}$ |
| T5 | 4.33 ± 0.58 $^{Aa}$ | 3.67 ± 0.58 $^{Ba}$ | 3.00 ± 0.00 $^{Bcab}$ | 2.67 ± 0.58 $^{Ca}$ | 1. 67 ± 0.58$^{Dbc}$ |
| T6 | 4.33 ± 0.58 $^{Aa}$ | 3.67 ± 0.58 $^{Aba}$ | 3.33 ± 0.58 $^{Bab}$ | 3.00 ± 0.00 $^{Ba}$ | 2.00 ± 0.00 $^{Ca}$ |
| T7 | 4.67 ± 0.58 $^{Aa}$ | 3.67 ± 0.58 $^{Ba}$ | 3.33 ± 0. 58 $^{Bab}$ | 3.00 ± 0.00 $^{BCb}$ | 2.33 ± 0.58 $^{Ca}$ |
| T8 | 4.33 ± 0.58 $^{Aa}$ | 4.00 ± 0.00 $^{Ba}$ | 3.67 ± 0.58 $^{Aa}$ | 3.00 ± 0.00 $^{Ba}$ | 2.00 ± 0.00 $^{Ca}$ |

Different lowercase and uppercase letters indicate statistically significant differences in each row and column ($p<0.05$).
*Co: Control sample (low-fat milk). T1: Control + 0.1% PE. T2: Control + 0.5% PE. T3: Control + 1% PE. T4: Control + 2% PE. T5: Control + 0.1% PC. T6: Control + 0.5% PC. T7: Control + 1% PC. T8: Control + 2% PC.

## DISCUSSION

Food products are susceptible to spoilage through microbiological, enzymatic, physical, or chemical processes, which can reduce their quality, nutritional value, and safety, and lead to undesirable changes in their physicochemical and sensory properties. Due to the potential health risks associated with synthetic preservatives, consumers and companies are increasingly seeking to replace them with natural preservatives derived from sources such as plants, bacteria, fungi, animals, and algae, which are considered safer for human health and the environment.

Phycobiliproteins (PBPs), are pigment-protein complexes with intense colors. PBPs, due to their coloring properties and physiological activities, can be used as natural food colorants, fluorescent agents, and bioactive compounds in food applications. In this regard, this study aimed to investigate the industrial application of phycobiliproteins extracted from the cyanobacteria *Desmonostoc ablorizicum* and *Neowestiellopsis persica A1387* as edible colorants in low-fat milk.

The results of this study showed that PE extracted from *Desmonostoc ablorizicum* exhibited maximum absorption at a wavelength of 567 nm, while PC extracted from *Neowestiellopsis persica* showed maximum absorption at 620 nm. In this context, **Carrigee *et al*. (2022)** demonstrated that PEs, due to their high quantum efficiency of bands emission in the visible spectrum, are recognized as fluorescent proteins with multiple applications, with maximum absorption at 565, 539, and 498 nm **(Carrigee *et al*., 2022)**. The results of this study are consistent with reports by **Rahman *et al*. (2017)** and **Yu *et al*. (1999)** regarding the absorption and emission characteristics of PC. Rahman *et al*. (2017), during the extraction of PC from the microalga *Cyanidioschyzon merolae*, showed an *in vitro* absorption spectrum (300 to 800 nm) with three main absorption maxima at 430, 620, and 680 nm. The maxima at 430 and 680 nm are typical for chlorophyll, while the 620 nm maximum is characteristic of PC. The amount of PC per cell appeared stable, with approximately 17.5 to 18 mg of PC per unit absorbance at 800 nm across all time points **(Rahman *et al*., 2017)**. **Yu *et al*. (1999)** determined the maximum absorption peak and fluorescence emission peak at 625 and 648 nm, respectively **(Yu *et al*., 1999)**.

According to the results, the highest purity index (0.173) and concentration (100%) for PC and the highest purity index (1.75) and concentration (100%) for PE were observed at zero hours. A significant reduction in the purity index and concentration of PE and PC was observed over 72 hours of storage. The results showed that 99% of PE and 93% of PC retained their concentration after 72 hours. This stability indicates that the pigments' properties are preserved, making them suitable for use in the food and cosmetic industries. **Kovaleski *et al*. (2022)** demonstrated that the purity index, expressed as the A565.A280 nm ratio for PE and A615.A280 nm for PC, indicates suitability for various applications, with a value of 0.7 indicating food-grade purity, 3.9 indicating reactive grade, and above 4 indicating analytical grade **(Kovaleski *et al*., 2022)**. **Munier *et al*. (2014)** stated that the concentration of PE from *Grateloupia turuturu* remains stable up to 40°C, suggesting its potential use as a natural colorant in food and cosmetic industries **(Munier *et al*., 2014)**. Additionally, **Galland-Irmouli *et al*. (2000)** showed that the optimal concentration of PE from *Palmaria palmata* is stable up to 60°C **(Galland-Irmouli *et al*., 2000)**.

The results of this study indicated that the addition of PC and PE significantly reduced the pH (and significantly increased acidity) in milk samples compared to the control. These changes were more pronounced with increasing concentrations of PC and PE from 0.1% to 2%. This result was attributed to the high protein content of phycobiliproteins, which promoted the growth of lactic acid bacteria in milk. Comparison between treatments containing PC and PE showed that samples with PC had higher acidity. Consistent with these findings, **Pan-utai and Iamtham (2019)** demonstrated that proteins in pigments promote the growth of lactic acid bacteria **(Pan-utai & Iamtham, 2019)**. **Debbabi *et al*. (2017)** and **Pan-utai *et al*. (2019)** showed that yogurt fortified with *Spirulina* significantly reduced pH and increased acidity, with these changes dependent on the concentration of *Spirulina* extract used **(Debbabi *et al*., 2019; Pan-utai & Iamtham, 2019)**. Similarly, **Shaban *et al*. (2021)** reported that adding *Porphyridium* extract to raw milk significantly reduced pH and increased acidity during storage.

The results showed that the addition of PC and PE had no significant effect on the Brix level of milk samples, with all samples exhibiting the same Brix level and no statistically significant differences. In this regard, **Ramos *et al*. (2017)** showed that

adding extract of sweet potato pulp significantly increased the Brix level of fermented milk samples **(Ramos *et al*., 2017)**. In comparison, PC and PE lack such an effect.

The fat content results of milk samples containing PC and PE indicated that different treatments had no significant effect on Brix levels, with all samples showing the same Brix level and no statistically significant differences. **Nefasa *et al*. (2022)** reported that adding up to 5% PC had no significant effect on the fat content of pasteurized milk samples **(Nefasa *et al*., 2022)**. **Arslan and Aksay (2022)** showed that adding pigment extracted from *Spirulina* had no effect on the fat content of yogurt samples **(Arslan & Aksay, 2022)**. **Sangian *et al*. (2022)** also confirmed that adding PC had no effect on the fat content of yogurt samples **(Sangian *et al*., 2022)**. Additionally, **Panutai *et al*. (2019)** stated that adding fresh *Spirulina* extract to yogurt had no statistically significant effect on the fat content of yogurt samples **(Panutai & Iamtham, 2019)**.

The protein content results of milk samples containing PC and PE in this study showed that adding these pigments significantly increased the protein content of milk samples. This increase in protein content was dependent on the percentage of PC and PE added. No statistically significant difference in protein content was observed between samples with the same percentages of PC and PE. Consistent with our results, **Ganesan and Shanmugam (2020)** showed that adding PE increased the protein content in ice cream **(Ganesan & Shanmugam, 2020)**. **Arslan *et al*. (2021)** also reported that adding *Spirulina* to yogurt increased the protein content, with the increase dependent on the percentage of *Spirulina* used **(Arslan *et al*., 2021)**. Similarly, **Sangian *et al*. (2022)** showed that adding PC significantly increased the protein content in yogurt samples **(Sangian *et al*., 2022)**. **Nefasa *et al*. (2022)** confirmed that adding PC significantly increased the protein content of pasteurized milk **(Nefasa *et al*., 2022)**.

The DPPH and FRAP antioxidant activity results of milk samples containing PC and PE showed that the lowest antioxidant activity was observed in the control sample. Adding PC and PE significantly increased the antioxidant activity of milk samples, with this increase dependent on the percentage of PC and PE used. In this regard, **García *et al*. (2021)** demonstrated increased antioxidant activity in milk-based products containing phycobiliproteins **(García *et al*., 2021)**. **Silva *et al*. (2019)** showed that adding encapsulated PC extracted from *Spirulina* to yogurt increased its antioxidant activity, attributing this to the presence of α-tocopherol in the pigment **(da Silva *et al*., 2019)**. Studies by **Tan *et al*. (2022)** indicated that antioxidant activity depends on the amount and type of PE obtained from marine microalgae **(Tan *et al*., 2022).** de **Amarante *et al*. (2020)** also demonstrated increased antioxidant activity in ice cream samples containing PC **(de Amarante *et al*., 2020)**. **Davoodi *et al*. (2023)** showed increased antioxidant activity in Iranian cheese samples containing PC **(Davoodi *et al*., 2023)**.

The results of this study showed that adding PC and PE significantly affected the color indices of milk samples. The brightness index L* was highest in the control samples, while adding PC and PE significantly reduced the brightness index. This reduction was dependent on the percentage of PC and PE used. This was entirely expected due to the blue color of PC and the red color of PE. **Arslan *et al*. (2021)** showed that adding PC extracted from *Spirulina* significantly reduced the brightness index of yogurt samples **(Arslan *et al*., 2021). Prasantha and Wimalasiri (2019)** indicated that changes in the L* component of pasteurized milk during storage may be due to Maillard browning reactions, the formation of various organic compounds during thermal degradation from pasteurization, and increased activity of mesophilic bacteria **(Prasantha & Wimalasiri, 2019)**. Examination of the redness index a* showed that the lowest redness index was observed in the control sample, and adding PC and PE significantly increased the redness of milk samples. This increase in the redness index was dependent on the percentage of PC and PE used. Comparison between the two pigments showed that treatments containing PE produced higher redness. The redness of the samples was directly influenced by PE extracted from *Desmonostoc ablorizicum*. **Moreira *et al*. (2012)**, during a study on the use of *Nostoc* cyanobacterial pigments in yogurt, reported similar results, attributing the redness of yogurt samples to the PE in the extracted pigment **(Moreira *et al*., 2012)**. The yellowness index b* results showed that the lowest yellowness was observed in the control sample, and adding PC and PE significantly increased yellowness. This increase in the yellowness index was dependent on the percentage of PC and PE used. Comparison between the two pigments showed that treatments containing PE increased the yellowness of milk samples. The b* index indicates the blueness of the extract and is influenced by PC, the blue protein of the *Neowestiellopsis persica* extract. **Moreira *et al*. (2012)** reported similar results during a study on the use of *Nostoc* cyanobacterial pigments in yogurt, identifying PC as the factor responsible for the yellowness of the samples **(Moreira *et al*., 2012)**.

The overall color change results showed that adding PC and PE significantly increased the overall color change index of milk samples. This increase was dependent on the percentage of PC and PE used. The results also showed that greater color changes were observed over time. However, a significant increase in the yellowness index of milk samples was observed in all tested groups over time. Nevertheless, a reduction in the brightness of yogurt samples was observed during 28 days of storage, which may be attributed to changes in sample acidity.

**García *et al*. (2021)** showed that phycobiliproteins are unstable at acidic pH (below 3.8) and alkaline pH (above 11), becoming completely disrupted. They also stated that when the coloring extract is mixed with phosphate buffer at pH values below 3, proteins immediately precipitate **(García *et al*., 2021)**.

The results of this study showed that adding PC and PE had a significant effect on inhibiting microbial growth in milk samples. According to the results, no bacterial growth was observed in any of the treatments during seven days of storage. However, on the 14th day, bacterial growth was observed only in the control sample and T1, while other treatments showed no bacterial growth. This trend continued until the 28th day, with the highest growth recorded in the control and T1 samples. Regarding the growth of Enterobacteriaceae bacteria, no bacterial growth was observed in any of the tested groups during 28 days of storage. Additionally, the results showed that no bacterial growth was observed in any of the treatments during 14 days of storage. On the 28th day, mold and yeast growth was observed in all tested groups, with the highest mold growth in the control treatment and the lowest in the T8 treatment. **Mohammadi-Gouraji *et al*. (2019)** showed that adding 8% PC to yogurt samples completely prevented the growth of *Escherichia coli*, *Staphylococcus aureus*, and *coliforms*, and significantly reduced the growth of molds and yeasts **(Mohammadi-Gouraji *et al*., 2019)**. **Safari *et al*. (2020)** demonstrated that increasing PC concentration significantly reduced pathogenic bacteria in food **(Safari *et al*., 2020)**. Additionally, **Karuppannan *et al*. (2024)** showed the antibacterial effect of PE extracted from the red alga *P. hornemannii* against pathogens such as *Bacillus subtilis*, *Bacillus cereus*, *Shigella*, and *E. coli* **(Karuppannan *et al*., 2024)**.

The sensory evaluation results for taste and odor of milk samples containing PC and PE showed that the control sample had the highest sensory scores across all parameters. However, adding PC and PE significantly reduced the sensory evaluation of taste and odor in milk samples. This significant reduction in sensory evaluation of taste and odor was dependent on the percentage of PC and PE used. In contrast, the results for mouthfeel and color evaluation showed that the lowest sensory scores for mouthfeel and color were observed in the control sample, and adding PC and PE significantly increased the sensory evaluation of mouthfeel and color in milk samples. This significant increase in sensory evaluation of mouthfeel and color was dependent on the percentage of pigments used. The significant increase in sensory evaluation of overall acceptance was dependent on the percentage of PC and PE. Up to 14 days, higher percentages of samples containing PC and PE had the highest overall acceptance scores. **Arslan and Aksay (2022)**, during the fortification of yogurt with *Spirulina* extract, showed that the color of yogurt changed from white to blue due to increased *Spirulina* content. When yogurts were compared for taste, the taste score decreased with increasing *Spirulina*, resulting in an unpleasant intensification of flavor. They recommended using 0.5% *Spirulina* extract for producing functional yogurt without affecting sensory parameters **(Arslan & Aksay, 2022)**. **Pehlivan *et al*. (2023)**, during the fortification of ayran (a salted, frothy yogurt-based beverage in Turkey) with the microalgae *Chondrus crispus* and *Chlorella vulgaris*, showed that all sensory scores of ayran samples decreased with the addition of algae.

However, among algae- containing samples, those with both C. *vulgaris* and C. *crispus* had slightly higher overall acceptance scores **(Pehlivan *et al*., 2023)**. Additionally, Mohammadi-Gouraji *et al*. (2019) showed that adding 2 to 4% PC to yogurt resulted in higher sensory evaluation by the sensory panel **(Mohammadi-Gouraji *et al*., 2019)**.

## CONCLUSION

This study was conducted to investigate the industrial application of phycobiliproteins extracted from the cyanobacteria *Desmonostoc ablorizicum* and *Neowestiellopsis persica A1387* as edible colorants in low-fat milk. After extracting PE from *Desmonostoc ablorizicum* and PC from *Neowestiellopsis persica A1387*, these pigments were evaluated for spectroscopy, purity index, and concentration over 72 hours of storage. Spectroscopic results showed that PE and PC had maximum absorption at wavelengths of 567 nm and 620 nm, with absorbance values of 1.83 and 0.653, respectively. The purity index evaluation of PE and PC over 72 hours of storage revealed that the highest purity indices were observed at zero hours, with values of 0.173 and 1.75, respectively, confirming the suitability of the extracted pigments for food applications. However, a significant reduction in the purity index was observed for both pigments over 72 hours. Concentration results for PE and PC showed that both pigments exhibited the highest stability (100%) at zero hours. After 72 hours, the concentration of PE remained at 93%, and PC at 98.26%.

Physicochemical analysis of milk samples showed that the addition of PC and PE significantly reduced the pH (or significantly increased acidity) compared to the control sample. Comparison between treatments containing PC and PE indicated that samples with PC had lower pH, possibly due to the inherent pH (6.55) of PC. The addition of PC and PE had no significant effect on the Brix and fat content of the produced milk samples; however, a significant increase in protein content was reported in samples containing PC and PE. Antioxidant activity evaluation of milk samples using DPPH and FRAP methods showed that the addition of PC and PE significantly increased antioxidant activity compared to the control sample. Comparison between treatments containing PC and PE revealed that treatments with PE exhibited higher antioxidant properties. Color index analysis showed that the addition of PC and PE significantly reduced the brightness index (L*) of milk samples, while the redness and yellowness indices significantly increased. Additionally, increasing the percentage of PC and PE led to greater overall color

changes in the milk samples. The results indicated that color changes were more pronounced in samples containing PE compared to PC. Microbial analysis of the produced milk samples showed that the addition of PC and PE inhibited the total microbial count as well as mold and yeast growth. No Enterobacteriaceae growth was observed in any of the tested groups during 28 days of storage. Sensory evaluation results indicated that the addition of PE and PC reduced the sensory acceptability of taste and odor parameters but increased the sensory evaluation of mouthfeel and color.

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