JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

STUDYING THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF PHYCOERYTHRIN TO INCREASE THE SHELF LIFE OF HAMBURGERS AT REFRIGERATOR TEMPERATURE

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https://doi.org/10.55251/jmbfs.11625

ARTICLE INFO

Received 31. 7. 2024
Revised 3. 2. 2025
Accepted 11. 2. 2025
Published xx.xx.201x

Regular article

OPEN ACCESS

ABSTRACT

Cyanobacteria represent promising sources of active biochemical compounds and natural products with antioxidant and antimicrobial properties. The present study investigated the antimicrobial and antioxidant capabilities of phycoerythrin (PE) from *Nostoc* sp., used it to coat hamburger samples with 40%, 75%, and 95% meat, and examined the physicochemical and sensory characteristics of the hamburger samples over 21 days. The results showed that the antioxidant activity of phycoerythrin was 2.71±0.03 mg/mL using the DPPH method. The minimum inhibitory concentration (MIC) of PE against *Pseudomonas aeruginosa* was significantly higher, and in *Bacillus subtilis* and then *Bacillus cereus*, it was noticeably lower than the other samples ($p \leq 0.05$). Also, PE did not show a lethal effect against any bacteria ($p \leq 0.05$). Findings indicated that no significant variations were seen in the inhibition zone diameter of PE against the examined bacteria ($p < 0.05$). In both the investigated temperatures and in all the time intervals, the maximum and minimum of the thiobarbituric acid and peroxide values, total bacterial counts for *mesophilic*, *psychrophilic*, E. *coli*, *Staphylococcus aureus*, and total *mold* and *yeast* observed in codes 5 and 2, respectively. In hamburger samples containing identical meat percentages, the incorporation of PE resulted in a significant enhancement of antioxidant activity ($p \leq 0.05$).

Keywords: Phycoerythrin, Hamburger, Coating, Shelf life

INTRODUCTION

Perishable food products' shelf life is crucial due to potential issues like microbial growth, protein denaturation, and lipid oxidation, which can alter the product's appearance, taste, texture, and color during storage **(Benjakul *et al.*, 2005)**. Synthetic preservatives are significantly restricted for consumers due to associated risks, including carcinogenicity; thus, an appropriate substitute should be selected. Conversely, hamburgers with elevated nutrient levels and delicate packaging create optimal conditions for microbial proliferation **(Duan *et al.*, 2010)**. Hamburger is a perishable product owing to its elevated nutritional composition and inadequate packaging, making it susceptible to spoilage at low temperatures. Refrigerator temperatures limit microbial growth, but this alone is insufficient. Low temperatures, antimicrobial compounds, and modified atmosphere packaging are recommended for preservation.

Microalgae, including pigments, polysaccharides, and phenolic compounds, have biologically active compounds suitable for food formulations. Phycoerythrin (PE), a natural antioxidant, has anti-inflammatory and antioxidant properties, protecting against physiological changes caused by oxidative stress. Synthetic antioxidants like butylhydroxytoluene and butylhydroxynisol pose potential risks due to their toxicity, so safer natural antioxidants like phycoerythrin are essential **(Torrieri *et al.*, 2006; Vazquez and Sanchez, 2022; Kumar *et al.*, 2015).**

Three variants of phycoerythrin, namely B-PE, C-PE, and R-PE, have been classified according to their fluorescence spectra and absorption properties. Many studies have shown the role of cyanobacterial extracts in the enhancement of the longevity of food products **(Kumar *et al.*, 2015; Malairaj *et al.*, 2016)**. **Haghdoost *et al.*** (2022) examined the effect of algal phycobiliprotein and nanoliposomes on prolonging the lifespan of carp fish burgers under refrigeration. The findings showed that both free and nano-encapsulated phycobiliproteins effectively delayed spoilage by decreasing levels of PV, TBA, TVB-N, TVC, and PTC.

Agregán Pérez *et al.* (2019) studied the antioxidant properties of marine macroalgae (*Fucus vesiculosus*, *Bifurcaria bifurcata*, and *Ascophyllum nodosum*) and microalgae (*Spirulina platensis* and *Chlorella vulgaris*) in meat products. They found that macroalgae have a rich nutritional profile with high levels of polyunsaturated fatty acids and valuable proteins. *Fucus vesiculosus* extract showed the highest antioxidant capacity, providing protection against oxidation in hamburger samples. **Hentati et al. (2019)** found that fish burgers with 1% algae, such as *Jania adhaerens* and *Cystoseira compressa*, had improved sensory attributes as well as enhanced antioxidant activity, suggesting algae could be a nutritious supplement for fish-based products **(Hentati, *et al.*, 2019).** Hence, this research utilized PE pigment, the predominant pigment of *Nostoc* cyanobacteria, to enhance the preservation time of hamburgers.

MATERIAL AND METHODS

Chemicals

Nostoc sp. cyanobacteria were purified from the Azad University Cyanobacteria Culture Collection (CCC) Herbarium Alborz. Antimicrobial tests were conducted on Gram-positive bacteria *Staphylococcus aureus* (PTCC 1112), *Bacillus cereus* (PTCC 1015), and *Bacillus subtilis* (PTCC 1023), as well as three Gram-negative bacteria *Pseudomonas aeruginosa* (PTCC 1310), *Escherichia coli* (PTCC 1047), and *Salmonella typhi* (PTCC 1609). Additionally, a pathogenic fungus *Aspergillus niger* (ATCC 16404) and a yeast *Candida albicans* (ATCC 10231) were included. All bacterial strains were sourced from Iran's Scientific and Industrial Research Organization, and all chemicals utilized were obtained from Merck, Germany.

Cultivation and Selection of Cyanobacterial Samples

The cyanobacterial strain *Nostoc* sp., obtained from the Cyanobacteria culture collection (CCC) of the ALBORZ herbarium at the Science and Research Branch of Islamic Azad University in Tehran, was cultivated in BG_{110} media and exposed to illumination (300 m-2 s-1) and maintained at 28±2°C for a duration of 30 days **(Kawachi, 2005)**.

Extraction and purification of analytical grade of phycoerythrin

The extraction and purification of PE were conducted according to the methodology described by **Nowruzi *et al.* (2020)**. The pigments were isolated from a 14-day-old log-phase culture and subsequently subjected to centrifugation at 4,000 rpm to get a pellet. The pellet was subsequently suspended in a potassium phosphate buffer at pH 7.1. The cell biomass was subsequently frozen and thawed over a period of four days until it exhibited a dark purple coloration. The crude extract was acquired using centrifugation at 5,000 rpm for 10 min. Purification was

conducted with solid ammonium sulfate to get 65% saturation (**Afreen and Fatma, 2018)**. The solution was subsequently centrifuged at 4,500 × g for 10 minutes. The pellets were resuspended in a 50 mM acetic acid-sodium acetate solution and underwent overnight dialysis. The extracts were filtered, and the absorption spectrum was acquired using a Specord 200 spectrophotometer. The amounts of PE were ascertained using absorbance measurements at 565 nm utilizing the purity ratio A555/A280 **(Mishra and Mishra, 2014).**

$$PC\ (\mu g\ mL^{-1}) = \frac{(OD\ 620nm - 0.7OD\ 650nm)}{7.38} \quad (1)$$

$$APC\ (\mu g\ mL^{-1}) = \frac{(OD\ 650nm - 0.19OD\ 620nm)}{5.65} \quad (2)$$

$$PE\ (\mu g\ mL^{-1}) = \frac{(OD\ 565nm - 2.8[PC] - 1.34[APC])}{12.7} \quad (3)$$

Antimicrobial activity of PE

Disc diffusion method

The study used Mueller-Hinton agar as a culture medium for gram-negative and gram-positive bacteria. Petri dishes were infected with a bacterial suspension at 37°C for 24 hours (0.5 McFarland standard) **(Østensvik *et al*., 1998)**. Discs coated with different amounts of PE were placed on the plates. After 24 h, the growth of the target bacteria was evaluated **(Rodriguez-Tudela *et al*., 2008)**. Results were quantified in micrograms per milliliter (µg/mL). Sabouraud dextrose agar medium was used to examine antifungal efficacy. A fungal suspension was created using a 30 g/l sabouraud dextrose broth liquid culture medium **(Mishra *et al.*, 2008)**. The antibacterial efficacy of the tested sample was evaluated with the following standard (antimicrobial index).
Antimicrobial index= (sample inhibition zone ×100)/ (standard inhibition zone). (4)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) analysis

The study used a broth microdilution method to evaluate the Mueller Hinton Broth cell suspension. The extracts were dissolved in DMSO, and 100 µl of each dilution and bacterial suspension were added to microwells, and the mixture was incubated at 37°C for 24 hours. The MIC represents the minimum concentration needed to suppress microorganism proliferation, while the MBC is the antimicrobial agent that decreases colony count by 99.9% **(El Hamdaoui *et al*., 2018; Fernández-López *et al*., 2006; Re *et al*., 1999)**.

Hamburger Preparation and Treatments

Following the determination of the suitable concentration of PE pigment, a total of 80 ml of pigment solution was prepared for treatment applications. Fresh hamburgers were utilized, comprising 5 pieces of 10 g and 5 pieces of 25 g of meat, which were immersed in 80 ml of PE solution for 20 min and stored at 8 °C and 4 °C. Microbial and chemical properties were assessed at intervals of 0, 3, 7, 14, and 21-days post-storage. The compliance of these properties with established standards facilitated the determination of the acceptable preservation duration. The optimal concentration and type of extract were identified to maximize preservation duration. Furthermore, each test was conducted on two separate occasions.

Hamburger Tests

pH

The pH of the samples was measured using a digital pH meter, which was cleaned and calibrated before testing **(Alizadeh Khaledabad *et al.*, 2020)**. The temperature was adjusted to match the sample's temperature, and the electrode was submerged in the sample for 45 seconds until a stable pH measurement was obtained **(Ky-Dembele *et al*., 2010; Mishra and Tripathi, 2008)**.

Determination of peroxide value (PV)

The 50 g samples were dissolved in a chloroform and methanol solution. Then, the mixture filtered and added to a potassium chloride solution at 35°C. The oil was used for peroxide number testing, calculated using the formula V = mq of H_2O_2 per kilogram of fat. The normality solution was N, and the fish oil's weight was W **(Soltanizadeh and Ghiasi-Esfahani, 2015).**

$$PV = \frac{(V \times N \times 1000)}{W} \quad (5)$$

Determination of thiobarbituric acid reactive substance (TBARS)

This experiment quantified TBAR using a colorimetric technique. 20 mg of minced fish meat was placed in a flask filled with 1-butanol and then transferred to a test tube with TBA reagent. The tubes were placed in an oven at 95°C for 2 h, and the absorbance was measured at 530 nm using a spectrophotometer. The quantity of TBA (mg of malondialdehyde per kg of hamburger) was determined using the equation (6), where As and Ac represent sample and blank absorption, respectively **(Soltanizadeh and Ghiasi-Esfahani, 2015).**

$$TBA = \frac{(VAs - Ac) \times 50)}{200} \quad (6)$$

Measurement of antioxidant activity of hamburgers coated with phycoerythrin extract

DPPH assay

The study used the DPPH method at 0 and 72-hour intervals, using 96-well microtiter plates. The reaction mixtures were kept in a dark atmosphere at 30°C for 30 min. Absorbance measurements were taken at a wavelength of 517 nm using a UV-Vis spectrophotometric plate reader. The experiment was conducted according to **Afreen *et al.* (2018)**.

ABTS

The study assessed the antioxidant activity of ABTS radical scavenging using a Re et al. (1999) method, measuring absorbance at 734 nm and calculating Trolox equivalent antioxidant capacity **(Re *et al*., 1999)**:

100 x (absorption control/absorption control-absorption sample) = (%) ABTS radical removal activity

Microbiological analysis of formulated hamburgers

The microbial analysis such as total viable psychrophilic bacteria counts **(Raeisi *et al.*, 2016)**, total mesophilic bacteria count **(Lalitha and Surendran, 2006)**, enumeration of total *E. coli* **(Rahman *et al.*, 2019; Sanjee and Karim, 2016)**, staphylococcal coagulase-positive bacteria count **(Junior *et al.*, 2014)** and total mold and yeast count were performed over 14 days at 37 °C **(Junior *et al.*, 2014)**

Sensorial evaluation

Thirty trained panelists evaluated a product's sensory qualities using a 5-point hedonic scale. They assessed odor, texture, color, and acceptability. The IRB approved the study, and no compensation was provided. Tests were conducted at 8-10 ºC **(Golmakani e*t al*., 2019)**.

Statistical analysis

The experiment's outcomes were assessed using ANOVA with SPSS version 24, with a 95% significance level. The Tukey test assessed changes in mean values after a significant variation. Three duplicate measurements were performed for each treatment **(Nowruzi *et al*., 2013)**.

RESULTS

Antioxidant activity of extracted PE

The extracted PE had an antioxidant activity of 2.71±0.03 mg/ml.

Antimicrobial tests

The results of the MIC of PE was significantly elevated in *Pseudomonas aeruginosa*, while it was significantly reduced in *Bacillus subtilis* and *Bacillus cereus* in comparison to other samples ($p \leq 0.05$) (Table 1). The results indicated that PE did not exhibit a lethal effect on any of the bacteria ($p \leq 0.05$). No significant statistical difference was observed in the inhibition diameter of PE in relation to the studied microorganisms ($p < 0.05$). The lowest levels of inhibition were recorded against *Pseudomonas aeruginosa*, while the highest inhibition was noted against *Bacillus subtilis* ($p \leq 0.05$) (Table 2).

Table 1 Comparison of MIC i.e., the minimum inhibitory concentration of phycoerythrin against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*.

Bacteria	MIC	MBC	Inhibition Zone (mm)	growth inhibition (%)
Escherichia coli	33.3±1.44 ^{bc}	negative	11.66±0.57 ^{ab}	38.59±3.03
Salmonella typhi	4.16±1.44 ^{b}	negative	10.66±0.57 ^{ab}	40.35±3.03
Pseudomonas aeruginosa	8.33±2.88 ^{a}	negative	11±0.00 ^{a}	29.82±3.03 ^{c}
Staphylococcus aureus	3.33±1.44 ^{bc}	negative	11.66±0.57 ^{a}	40.74±3.20 ^{b}
Bacillus cereus	2.50±0.00 ^{bc}	negative	10.66±0.57 ^{ab}	43.33±2.88
Bacillus subtilis	0.83±0.36 ^{c}	negative	11.33±0.57^{a}	48.33±2.88 ^{a}

Different small letters show an important difference in the column ($p \geq 0.05$).

Table 2 Comparing the inhibition diameter of PE against *Candida albicans* and *Aspergillus niger*

Species	Inhibition zone diameter
Candia albicans	7.66±0.57 ^{a}
Aspergillus niger	6.33±0.57 ^{b}

Various small letters show an important difference in the column ($p>0.05$).

Results of the tests of hamburger samples covered with phycoerythrin and stored at 4°C and 8°C

PH

The findings indicated that the greatest and lowest pH values were associated with codes 1 and 6 at both examined temperatures, respectively. The results indicated that as the quantity of meat in hamburgers increased, the pH dramatically decreased ($p \leq 0.05$) (Table 3).

Table 3 pH changes of hamburger samples coated with phycoerythrin solution at 4°C

Test Sample	Storage time (day)				
	Day 1	Day 3	Day 7	Day 14	Day 21
Code (1)	00/0±12/6 ^{aE}	01/0±18/6 ^{aD}	00/0±24/6 ^{aC}	00/0±28/6 ^{aB}	01/0±37/6 ^{aA}
Code (2)	01/0±12/6 ^{aE}	00/0±15/6 ^{bD}	01/0±19/6 ^{bC}	01/0±24/6 ^{bB}	01/0±31/6 ^{bA}
Code (3)	00/0±98/5 ^{bE}	00/0±05/6 ^{cD}	00/0±14/6 ^{cC}	01/0±23/6 ^{bB}	01/0±30/6 ^{bA}
Code (4)	00/0±98/5 ^{bE}	00/0±00/6 ^{dD}	01/0±07/6 ^{dC}	00/0±12/6 ^{cB}	01/0±18/6 ^{cA}
Code (5)	00/0±83/5 ^{cE}	01/0±90/5 ^{eD}	01/0±99/5 ^{eC}	01/0±08/6 ^{dB}	01/0±18/6 ^{cA}
Code (6)	01/0±83/5 ^{cE}	00/0±86/5 ^{fD}	01/0±94/5 ^{fC}	01/0±01/6 ^{eB}	00/0±06/6 ^{dA}

Various small letters show an important difference in the column and various small letters show a remarkable difference in the row ($p<0.05$).
Code (1): Hamburger 40% meat without coating, Code (2): Hamburger (40% meat) coated with phycoerythrin solution, Code (3): Hamburger 75% meat without coating, Code (4): Hamburger 75% meat coated with phycoerythrin solution, code (5): hamburger 95% meat without coating, code (6): hamburger 95% meat coated with phycoerythrin solution.

TBARS and PV

The findings showed that in both of the studied temperatures, the highest and lowest amounts of TBARS and PV belonged to codes 5 and 2, respectively ($p \leq 0.05$) (Table 4).

Table 4 Thiobarbituric Acid (TBARS) and Peroxide value (PV) changes of hamburger samples coated with phycoerythrin solution at 4°C.

Test Sample	Thiobarbituric Acid (TBARS)				
	Day 1	Day 3	Day 7	Day 14	Day 21
Code (1)	01/0±27/0 ^{cA}	00/0±33/0 ^{eA}	01/0±74/0 ^{eA}	01/0±92/0 ^{eB}	02/0±08/1 ^{eA}
Code (2)	00/0±27/0 ^{cA}	00/0±30/0 ^{fA}	00/0±66/0 ^{fA}	01/0±82/0 ^{fB}	01/0±99/0 ^{fA}
Code (3)	00/0±36/0 ^{bA}	00/0±43/0 ^{cA}	01/0±99/0 ^{cA}	00/0±18/1 ^{cB}	00/0±41/1 ^{cA}
Code (4)	00/0±36/0 ^{bA}	00/0±40/0 ^{dA}	00/0±90/0 ^{dA}	01/0±07/1 ^{dB}	01/0±26/1 ^{dA}
Code (5)	00/0±44/0 ^{aA}	00/0±51/0 ^{aA}	01/0±16/1 ^{aA}	01/0±44/1 ^{aB}	01/0±69/1 ^{aA}
Code (6)	00/0±43/0 ^{aA}	00/0±48/0 ^{bA}	01/0±06/1 ^{bA}	01/0±27/1 ^{bB}	01/0±47/1 ^{bA}
	Peroxide value (PV)				
	Day 1	Day 3	Day 7	Day 14	Day 21
Code (1)	02/0±14/0 ^{cA}	01/0±30/0 ^{dA}	01/0±46/0 ^{eA}	01/0±59/0 ^{eA}	02/0±74/0 ^{dA}
Code (2)	01/0±14/0 ^{bcA}	01/0±24/0 ^{eA}	02/0±40/0 ^{fA}	00/0±48/0 ^{fA}	02/0±62/0 ^{eA}
Code (3)	01/0±17/0 ^{bA}	02/0±50/0 ^{bA}	02/0±60/0 ^{cA}	02/0±77/0 ^{cA}	02/0±02/1 ^{bA}
Code (4)	01/0±16/0 ^{bcA}	01/0±41/0 ^{cA}	01/0±53/0 ^{dA}	01/0±64/0 ^{dA}	02/0±79/0 ^{cA}
Code (5)	02/0±28/0 ^{aA}	01/0±60/0 ^{aA}	01/0±79/0 ^{aA}	02/0±98/0 ^{aA}	02/0±28/1 ^{aA}
Code (6)	01/0±27/0 ^{aA}	02/0±48/0 ^{bA}	01/0±68/0 ^{bA}	01/0±82/0 ^{bA}	02/0±03/1 ^{bA}

Various small letters show a meaningful discrepancy in the column and various small letters show a meaningful discrepancy in the row ($p<0.05$).
Code (1): hamburger 40% meat without coating, code (2): hamburger 40% meat coated with phycoerythrin solution, code (3): hamburger 75% meat without coating, code (4): hamburger 75% Meat coated with phycoerythrin solution, code (5): 95% hamburger meat without coating, code (6): 95% hamburger meat coated with phycoerythrin solution

Antioxidant Activity

The study found that adding PE to hamburger samples with matching meat percentages significantly enhanced their antioxidant activity ($p \leq 0.05$). However, the antioxidant activity of these samples significantly diminished over time ($p \leq 0.05$), as indicated by the highest and lowest antioxidant activity levels measured by DPPH and ABTS corresponding to codes 6 and 1, respectively ($p>0.05$) (Table 5).

Table 5 Changes in antioxidant activity (DPPH and ABTS) of hamburger samples coated with phycoerythrin solution at 4°C

Test Sample	DPPH				
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Code (1)	36/0±22/32 ^{aA}	65/0±18/35 ^{aA}	23/0±23/39 ^{aA}	15/0±64/44 ^{aA}	36/0±11/49 ^{aA}
Code (2)	22/0±85/28 ^{bA}	55/0±42/30 ^{cA}	00/0±27/31 ^{cA}	00/0±82/33 ^{cA}	09/0±74/35 ^{cA}
Code (3)	14/0±78/28 ^{bA}	30/0±57/31 ^{bA}	08/0±56/33 ^{bA}	29/0±33/36 ^{bA}	26/0±23/42 ^{bA}
Code (4)	44/0±09/25 ^{cA}	05/0±22/27 ^{dA}	12/0±45/28 ^{dA}	13/0±73/29 ^{dA}	07/0±64/31 ^{eA}
Code (5)	34/0±88/20 ^{dA}	15/0±02/23 ^{eA}	14/0±87/24 ^{eA}	12/0±95/38 ^{eA}	39/0±42/32 ^{dA}
Code (6)	37/0±78/15 ^{eA}	32/0±41/16 ^{fA}	11/0±51/17 ^{fA}	24/0±81/18 ^{fA}	03/0±43/20 ^{fA}
	ABTS				
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Code (1)	47/0±35/25 ^{aA}	30/0±75/25 ^{aA}	48/0±03/28 ^{aA}	30/0±14/30 ^{aA}	15/0±49/32 ^{aA}
Code (2)	43/0±48/22 ^{bA}	14/0±63/22 ^{bA}	09/0±26/23 ^{bA}	33/0±31/24 ^{cA}	36/0±22/25 ^{cA}
Code (3)	49/0±42/22 ^{bA}	24/0±01/23 ^{bA}	20/0±37/23 ^{bA}	08/0±91/26 ^{bA}	22/0±89/29 ^{bA}
Code (4)	09/0±33/19 ^{cA}	34/0±49/20 ^{cA}	26/0±60/21 ^{cA}	04/0±11/23 ^{dA}	27/0±83/23 ^{dA}
Code (5)	03/0±90/15 ^{dA}	17/0±25/16 ^{dA}	43/0±37/18 ^{dA}	00/0±08/19 ^{eA}	08/0±09/21 ^{eA}
Code (6)	12/0±84/11 ^{eA}	16/0±15/12 ^{eA}	36/0±90/12 ^{eA}	34/0±75/13 ^{fA}	18/0±60/14 ^{fA}

Various small letters show an important difference in the column and various small letters show a meaningful difference in the row ($p<0.05$).
Code (1): hamburger 40% meat without coating, code (2): hamburger 40% meat coated with phycoerythrin solution, code (3): hamburger 75% meat without coating, code (4): hamburger 75% Meat coated with phycoerythrin solution, code (5): hamburger 95% meat without coating, code (6): hamburger 95% meat coated with phycoerythrin solution

Microbial Analyses

The findings revealed that codes 5 and 2 exhibited the highest and lowest total bacterial counts for mesophilic, psychrophilic, E. coli, Staphylococcus aureus, and total mold and yeast at both examined temperatures. The results indicated that an increase in the percentage of hamburger meat correlated with a significant rise in the total bacterial count of *mesophilic*, *psychrophilic*, E. *coli*, *Staphylococcus aureus*, and total *mold* and *yeast* ($p\leq0.05$). The use of PE in hamburger samples led to a significant decrease in microbial populations, while over time, the total bacterial counts of *mesophilic*, *psychrophilic*, E. *coli*, *Staphylococcus aureus*, mold, and yeast increased significantly ($p\leq0.05$) (Table 6).

Table 6 Changes in the total bacterial counts for *mesophilic*, *psychrophilic*, E. *coli*, *Staphylococcus aureus*, and total *mold* and *yeast* in hamburger samples coated with phycoerythrin solution at 4°C

Test sample	**Total *mesophilic* count**				
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Code (1)	00/0±19/4 ^{aE}	01/0±19/5 ^{bD}	02/0±45/5 ^{cC}	04/0±21/8 ^{aB}	04/0±82/10 ^{cA}
Code (2)	00/0±17/4 ^{aE}	01/0±96/4 ^{cD}	01/0±27/5 ^{eC}	06/0±62/6 ^{eB}	01/0±34/9 ^{fA}
Code (3)	00/0±08/4 ^{cE}	02/0±30/5 ^{aD}	03/0±96/5 ^{bC}	04/0±13/8 ^{bB}	01/0±10/11 ^{bA}
Code (4)	00/0±07/4 ^{cE}	04/0±82/4 ^{dD}	02/0±29/5 ^{eC}	02/0±85/6 ^{dB}	07/0±57/9 ^{eA}
Code (5)	02/0±08/4 ^{cE}	01/0±22/5 ^{bD}	02/0±34/6 ^{aC}	02/0±05/8 ^{cB}	01/0±33/11 ^{aA}
Code (6)	04/0±12/4 ^{bE}	06/0±59/4 ^{eD}	01/0±40/5 ^{dC}	07/0±83/6 ^{dB}	04/0±90/9 ^{dA}
	Total *psychrophilic* count				
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Code (1)	00/0±19/4 ^{aE}	04/0±19/5 ^{bD}	05/0±45/5 ^{cC}	04/0±21/8 ^{aB}	03/0±82/10 ^{cA}
Code (2)	00/0±17/4 ^{aE}	02/0±96/4 ^{cD}	02/0±27/5 ^{eC}	04/0±62/6 ^{eB}	04/0±34/9 ^{eA}
Code (3)	00/0±08/4 ^{cE}	02/0±30/5 ^{aD}	03/0±96/5 ^{bC}	04/0±13/8 ^{bB}	01/0±10/11 ^{bA}
Code (4)	00/0±07/4 ^{cE}	00/0±82/4 ^{dD}	02/0±29/5 ^{eC}	02/0±85/6 ^{dB}	00/0±57/9 ^{eA}
Code (5)	00/0±08/4 ^{cE}	01/0±22/5 ^{bD}	02/0±34/6 ^{aC}	02/0±05/8 ^{cB}	01/0±33/11 ^{aA}
Code (6)	02/0±12/4 ^{bE}	00/0±59/4 ^{eD}	01/0±40/5 ^{eC}	01/0±83/6 ^{dB}	00/0±90/9 ^{dA}
	Total E. *coli* count				
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Code (1)	00/0±00/1 ^{aE}	02/0±51/3 ^{bD}	02/0±60/4 ^{cC}	03/0±14/6 ^{bB}	03/0±81/7 ^{cA}
Code (2)	00/0±00/1 ^{aE}	00/0±08/3 ^{dD}	01/0±96/3 ^{fC}	06/0±67/4 ^{eB}	04/0±12/7 ^{dA}
Code (3)	00/0±00/1 ^{aE}	00/0±72/3 ^{aD}	05/0±73/4 ^{bC}	00/0±91/5 ^{cB}	01/0±14/8 ^{bA}
Code (4)	00/0±00/1 ^{aE}	02/0±32/3 ^{cD}	01/0±26/4 ^{eC}	01/0±39/5 ^{dB}	00/0±05/7 ^{eA}
Code (5)	00/0±00/1 ^{aE}	03/0±81/3 ^{aD}	00/0±04/5 ^{aC}	00/0±28/6 ^{aB}	01/0±38/8 ^{aA}
Code (6)	00/0±00/1 ^{aE}	02/0±39/3 ^{cD}	00/0±37/4 ^{dC}	00/0±38/5 ^{dB}	00/0±08/7 ^{eA}
	Total *Staphylococcus aureus* count				
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Code (1)	00/0±00/1 ^{aE}	03/0±08/3 ^{aD}	02/0±08/3 ^{aC}	03/0±22/5 ^{aB}	02/0±33/7 ^{aA}
Code (2)	00/0±00/1 ^{aE}	00/0±79/2 ^{cD}	00/0±79/2 ^{cC}	03/0±93/4 ^{dB}	03/0±36/6 ^{dA}
Code (3)	00/0±00/1 ^{aE}	01/0±98/2 ^{bD}	02/0±98/2 ^{bC}	03/0±08/5 ^{bB}	00/0±25/7 ^{bA}
Code (4)	00/0±00/1 ^{aE}	00/0±60/2 ^{dD}	00/0±60/2 ^{dC}	02/0±43/4 ^{eB}	03/0±21/6 ^{eA}
Code (5)	00/0±00/1 ^{aE}	02/0±91/2 ^{bD}	01/0±91/2 ^{eC}	00/0±33/4 ^{cB}	01/0±63/6 ^{cA}
Code (6)	00/0±00/1 ^{aE}	00/0±58/2 ^{dD}	02/0±58/2 ^{fC}	00/0±88/3 ^{fB}	01/0±78/5 ^{fA}
	total *mold* and *yeast* count				
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Code (1)	00/0±00/1 ^{aE}	00/0±95/2 ^{aD}	01/0±34/4 ^{aC}	02/0±17/5 ^{aB}	01/0±76/6 ^{aA}
Code (2)	00/0±00/1 ^{aE}	00/0±83/2 ^{bD}	01/0±98/3 ^{dC}	03/0±34/4 ^{dB}	01/0±23/6 ^{cA}
Code (3)	00/0±00/1 ^{aE}	01/0±79/2 ^{abD}	01/0±27/4 ^{bC}	02/0±86/4 ^{bB}	02/0±45/6 ^{bA}
Code (4)	00/0±00/1 ^{aE}	01/0±72/2 ^{cD}	01/0±76/3 ^{dC}	05/0±34/4 ^{dB}	03/0±20/6 ^{cA}
Code (5)	00/0±00/1 ^{aE}	02/0±46/2 ^{dD}	03/0±54/3 ^{cC}	00/0±48/4 ^{cB}	03/0±74/5 ^{dA}
Code (6)	00/0±00/1 ^{aE}	02/0±24/2 ^{eD}	01/0±11/3 ^{eC}	03/0±10/4 ^{eB}	03/0±15/5 ^{eA}

Various small letters show an important difference in the column and various small letters show a meaningful difference in the row ($p<0.05$).
Code (1): Hamburger 40% meat without coating, Code (2): Hamburger (40% meat) coated with phycoerythrin solution, Code (3): Hamburger 75% meat without coating, Code (4): Hamburger 75% meat coated with phycoerythrin solution, code (5): hamburger 95% meat without coating, code (6): hamburger 95% meat coated with phycoerythrin solution

Sensorial evaluation

The study found no significant difference in sensory scores like odor, texture, color, and overall acceptance at different temperatures. However, an increase in the meat percentage of hamburgers significantly enhanced these factors. The addition of PE to identical meat samples resulted in a significant increase in these factors. Over time, these factors decreased within the samples. The maximum and minimum values were associated with codes 6 and 1 ($p \leq 0.05$) (Figure 1).

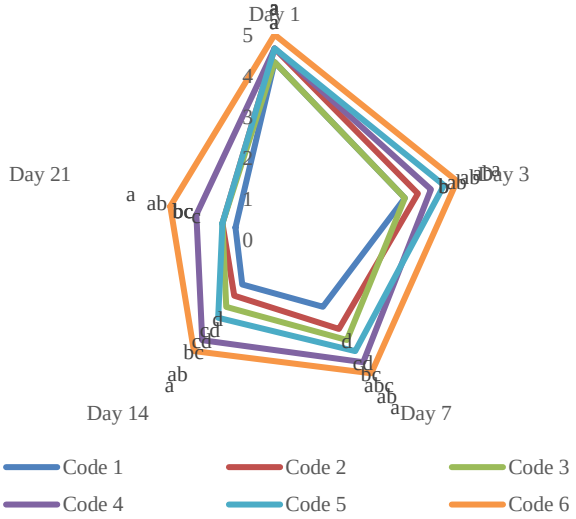

Figure 1 Sensorial evaluation of hamburger samples coated with phycoerythrin solution at 4°C

DISCUSSION

The extracted PE had an antioxidant activity of 2.71±0.03 mg/ml. Phycoerythrin exhibits potential antioxidant activity **(Basheva *et al.*, 2018)**. Antioxidants inhibit the detrimental chain reactions initiated by free radicals by neutralizing the effects of oxidation. Their interaction occurs safely, effectively neutralizing free radicals prior to inflicting further cellular damage. Cyanobacteria pigments have antioxidant activity equal to or greater than other non-enzymatic antioxidants, such as ascorbic acid, ferrous sulfate, uric acid, alpha-tocopherol, and phycocyanin **(Ren *et al.*, 2015)**. Nowruzi et al. (2020) demonstrated that PE from *Nostoc* sp. FA1 had potent free radical scavenging properties, considerably enhanced with higher pigment concentrations **(Nowruzi, 2020)**.

It is shown that *Pseudomonas aeruginosa* had a higher minimum inhibitory concentration than *Bacillus subtilis* and *Bacillus cereus*, and phycoerythrin did not have a lethal effect on any of the bacteria studied. There was no significant difference in the inhibition zone diameter of phycoerythrin against the microorganisms studied, with the lowest levels of inhibition observed against *Pseudomonas aeruginosa* and the highest inhibition against *Bacillus subtilis*. Several studies demonstrated antimicrobial activity comparable to the findings of the current research. **(Arun *et al.*, 2012)**. For example, Afreen and Fatma (2018) demonstrated that *Candida albicans* exhibited more excellent resistance to *Aspergillus niger* at 0.2 mg/ml of phycoerythrin **(Afreen *et al.*, 2018)**.

Our research found that hamburger samples' highest and lowest pH values were in codes 1 and 6, respectively. The pH of the meat affects meat color, taste, aroma, crispness, and overall edible quality, as well as spoilage caused by microbial enzymes and microorganism proliferation **(Kinsella and Melachouris, 1976)**. The addition of phycoerythrin to hamburger samples decreased pH, indicating that phycoerythrin extract effectively inhibits nitrogenous and amine compounds that degrade hamburger samples **(Soltanizadeh and Ghiasi-Esfahani, 2015)**. Hentati et al. (2019) investigated the impact of incorporating *Jania adhaerens* and *Cystoseira compressa* into fish burgers. They showed that 1% C. *compressa* can potently decrease the pH of the samples **(Hentati, Barkallah, *et al.*, 2019).**

Thiobarbituric acid (TBA) is a key indicator of lipid oxidation levels in food products, indicating the secondary phase of oxidation where peroxides are converted into ketones, alcohols, and aldehydes **(Hentati, Barkallah, *et al.*, 2019; Sheard *et al.*, 2000)**. The TBA index increases over storage due to meat accumulation of other peroxides and free iron. Aldehydes are formed as secondary oxidation products, and the rising prevalence of hydroperoxides may contribute to this issue **(Ozyurt and Bayari, 2008; Shahidi and Zhong, 2005)**. The outcome of the current research showed that in both of the studied temperatures, the highest and lowest amounts of TBARS and PV belonged to codes 5 and 2, respectively. The results indicated that as the percentage of meat in hamburgers increased, there was a significant rise in the levels of TBARS and PV ($p \leq 0.05$). The study found that adding phycoerythrin to hamburger samples reduced TBARS and PV levels, attributed to the antioxidant properties of phycoerythrin. However, over time, the levels increased. This finding aligns with Haghdoost et al. (2022), who found that adding phycobiliprotein in carp fish burgers increased TBA and PV factors. The presence of fat in meat may also contribute to this effect **(Haghdoost *et al.*, 2022)**.

The study found that codes 6 and 1 were associated with the highest and lowest antioxidant activity at different temperatures. Phycoerythrin incorporation increased antioxidant activity in hamburger samples. Compared to our results, Takyar *et al*. (2019) also showed that lipid oxidation and extended rainbow trout fillet shelf life decreased **(Takyar *et al.*, 2019)**.

The study analyzed hamburger samples' total bacterial counts for mesophilic, psychrophilic, E. coli, Staphylococcus aureus, and mold and yeast. Codes 5 and 2 showed the highest and lowest measurements. The addition of PE significantly decreased these factors in hamburger samples. Similar results were observed by Haghdoost et al. (2022), Sudhakar et al. (2024), Afreen et al. (2018), and Nowruzi et al. (2023).

Sudhakar et al. (2024) found that PE exhibited significant growth inhibition against *Candida albicans*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Shigella sonae*. **Nowruzi et al. (2023)** found that aggregate counts of *psychrotrophic*, *mesophilic*, coagulase-positive *Staphylococcus aureus*, and *coliform* bacteria were lower in samples with PE coating compared to the control condition. The pigments derived from *Nostoc* sp. exhibited significant antimicrobial activity, maintaining quality parameters in fish samples **(Nowruzi and SA, 2023)**.

The outcomes of the sensory evaluation indicated that the greatest and lowest values were associated with codes 6 and 1, respectively ($p < 0.05$). The findings of Nowruzi et al. (2023) aligned with our study. Haghdoost *et al*. (2022) also found a significant decline in sensory evaluation scores and an increase in chemical spoilage. The acceptance scores for hamburgers with 5% encapsulated phycobiliprotein were superior. Encapsulated and non-encapsulated phycobiliproteins can maintain the quality of treated hamburgers without affecting aroma, color, or texture. Nanoliposome-treated samples showed superior sensory characteristics, aligning with algal phycobiliproteins' antibacterial and antioxidant properties **(Haghdoost *et al.*, 2022)**.

CONCLUSION

The primary aim of the present investigation was to assess the antimicrobial and antioxidant properties of PE extracted from the cyanobacterium *Nostoc* sp., apply it as a coating on hamburger samples containing 40%, 75%, and 95% meat, and analyze the physicochemical and sensory attributes of the hamburger samples over durations of 1, 3, 7, 14, and 21 days. The antioxidant activity of pigment showed a value of 2.71±0.03 mg/ml. The investigation revealed that PE exhibited a greater MIC against *Pseudomonas aeruginosa*, lower doses against *Bacillus subtilis* and *Bacillus cereus*, and no fatal impact on any bacterium, with no significant difference in inhibitory zone width ($p \leq 0.05$). The pH outcome indicated that the most outstanding value was associated with code 1, while the lowest value corresponded to code 6. The TBARS and amicrobial analysis findings indicated that codes 5 and 2 exhibited the maximum values, respectively ($p \leq 0.05$). The antioxidant activity and sensory assessment of formulated hamburgers with PE indicated that the greatest and lowest values were recorded in codes 6 and 1, respectively ($p \leq 0.05$). In summary, incorporating PE can improve prepared hamburgers' antioxidant, microbiological, and organoleptic qualities.

Declarations

Ethics approval and consent to participate: The study followed rules and was authorized by the Tehran Medical Sciences, Islamic Azad University ethics committee. No human experiments or tissue samples were used. Fahimeh Nemati and Sarvenaz Falsafi were the ethical committees that authorized the study."

Consent for publication: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article

Competing interests: The authors declare no competing financial or non-financial conflicts of interest.

Funding: Not applicable

Acknowledgments: Not applicable

Authors' contributions: MB: writing original draft and investigation; **MA:** methodology, software; **BN:** supervision, formal analysis, review, and editing; **SAAA:** visualization, editing; **HA:** resources, software

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