Original Article

Effect of Phycoerythrin on Antimicrobial Activity and Shelf-life Extension of the Nile Tilapia (Oreochromis niloticus) at Refrigerator Temperature

Nowruzi, B[1*], Jafari Porzani, S[1], Ali Anvar, A.A[2]

1. Department of Biotechnology, Faculty of Converging Sciences and Technologies, Islamic Azad University, Science and Research Branch, Tehran, Iran
2. Department of food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran

How to cite this article: Nowruzi B, Jafari Porzani S, Anvar AA. Effect of Phycoerythrin on Antimicrobial Activity and Shelf-life Extension of the Nile Tilapia (Oreochromis niloticus) at Refrigerator Temperature. Archives of Razi Institute. 2023;78(6):1811-21.

DOI: 10.32592/ARI.2023.78.6.1811

Article Info:
Received: 20 June 2023
Accepted: 09 July 2023
Published:30 December 2023

Corresponding Author's E-Mail:
bahareh.nowruzi@srbiau.ac.ir

ABSTRACT

The present study was performed to evaluate the effect of phycoerythrin (PE) treatment extracted from *Nostoc* sp. on the shelf-life extension of the Nile Tilapia (*Oreochromis niloticus*) fillet at 4°C and 8°C. After extraction and purification of pigment in BG-110 medium, the pigment PE was extracted and purified with 56% ammonium sulfate followed by dialysis. After that, the effect of pigment on *Escherichia coli* and *Staphylococcus aureus* were investigated. The fillet samples were immersed in pigment solution, and their physicochemical, microbiological and sensory properties were examined. The results showed that the concentration and purity of the pigments increased after the dialysis. The results from performed chemical tests and total number of living mesophilic bacteria, psychrotrophic bacteria, *Staphylococcus aureus* coagulase positive, and coliform bacteria of the samples compared to the blank sample showed that sample treated with algae extracts were able to control the increase in these parameters. In these tests, the highest levels belonged to Nile Tilapia fillet sample Nile Tilapia fillet coated with PE solution at a temperature 8°C and the lowest amount was observed with fillet coated with PE solution at a temperature of 4˚C ($P \leq 0.05$). The results of sensory evaluation showed that the highest score of taste, texture, color, and total acceptance were observed for Nile Tilapia fillet coated with PE solution at temperature 8°C. In conclusion, the extract pigments from *Nostoc* sp. has strong antimicrobial activity and can maintain the quality parameters for controlling of spoilage bacteria and extend the shelf-life of *Oreochromis niloticus*.

Keywords: Nile tilapia, Nostoc sp, Phycoerythrin, Refrigerator temperature, Shelf-life

1. Introduction

The growing demand for high-quality ready-to-cook fish products with a long shelf-life has prompted the development of numerous novel approaches to preserve the quality and produce secure products. Among the many ways, utilizing microalgae extracts as natural preservatives appears to be a viable option. Because of their well-balanced chemical components, microalgae have great potential as a biological resource for developing novel goods and uses, including the enhancement of the nutritional content of food and animal feed. Unsaturated fatty acids, pigments, antioxidants, pharmaceutical compounds, and other biologically active chemicals are among the many important substances found in them. The pigments found in microalgae are becoming increasingly valuable to businesses due to their versatility and ease of extraction. It has been demonstrated that incorporating microalgae extracts into seafood goods can be an efficient method of minimizing microbial growth, boosting oxidative stability, and protecting sensory characteristics, consequently extending shelf-life (1-4).

The principal cause of deterioration in many fishing products is microorganisms. Seafoods have more free amino acids, more water, and a higher pH postmortem than most land animal products, making them sensitive to microbes such as gram-positive and gram-negative bacteria, yeasts, and molds that cause seafood spoiling. Other mechanisms for food spoilage are caused by changes via biological reactions, such as oxidation of unsaturated fat, hydrolysis of fats, as well as spoilage caused by protein compounds and enzymes (5, 6). It seems that the use of natural pigments from cyanobacteria is a suitable way to control spoilage bacteria and extend the shelf-life of the processed food. Therefore, due to the importance of natural pigments such as phycoerythrin (PE), the impact of PE to increase the shelf-life of Nile Tilapia (*Oreochromis niloticus*) was evaluated.

2. Materials and Methods

2.1. Chemicals

All chemicals and protein molecular weight markers utilized in this investigation were of analytical grade and were obtained from Hi-Media, Merck, and Sigma. All buffers and reagents were made with double-distilled water.

2.2. Cyanobacterial isolation and growth conditions

Isolates of *Nostoc* sp. (FSN) were found in rice paddies in the Iranian region of Golestan (36° 54′ 41″ N, 54° 47′ 25″ W). Soil samples were inoculated in sterile Petri dishes with liquid BG-110 (two 10-day-old cyanobacterial strains) medium (Allen 1968), without a nitrogen source, pH 7.1, and incubated in a growth chamber (Merck, Germany) for two weeks at 28 ± 1 ºC under constant cool white fluorescent light (100-150 E/m2 s) to obtain a cyanobacterial monoculture. After 14 days, the strongest colonies were picked and placed onto new solid BG-110 media. Rajabpour et al. (2019) reported that dextrose-peptone broth and caseinate-glucose agar media were used to examine colonies for bacterial contamination in bacteria-free cultures (7). The bacteria-free colony was kept alive on several agar slants medium. The isolate was rinsed with sterile deionized water after 20 days and transferred to 1L of freshly made liquid BG-110 media.

2.3. Extraction and purification of analytical grade of phycoerythrin (PE)

The extraction of pigment was carried out from a 500 mL homogenized culture in its log phase, which was 14 days old. The obtained pellet was achieved through centrifugation at 4,000 rpm. The sediment was resuspended in a solution of 20 millimolar acetate buffer (pH 5.1) with a volume of 100 milliliters. The method employed for extraction involved a repeated cycle of freezing at a temperature of -200°C and thawing at room temperature over a period of four days until the cell biomass attained a dark purple hue, as reported by Afreen and Fatma (2018) (8). The process of centrifugation at 5,000 rpm for 10 min was

employed to eliminate cellular debris, resulting in the acquisition of a raw extract. The purification process was conducted following the methodology outlined by Afreen and Fatma (2018) (8). The crude extract was subjected to the gradual addition of solid ammonium sulphate while being continuously stirred until a saturation level of 65% was attained. The resultant solution was permitted to remain at room temperature for a duration of 12 h, followed by centrifugation at a rate of 4,500 rpm for 10 min. The pellets were re-suspended in a limited quantity of 50 mM acetic acid-sodium acetate buffer (pH 7.1) and subjected to overnight dialysis. The sample was retrieved from the dialysis membrane and subsequently passed through a 0.45 μm filter, as documented in references (9, 10). The absorption spectrum was ascertained through the utilization of a Specord 200 spectrophotometer (Analytik Jena, Germany) by scanning the sample within the wavelength range of 300-750 nm (11, 12). The quantification of PE was performed by means of absorbance measurements at 565 nm, utilizing equations (1) (13). The purity of PE was determined at every stage using a purity ratio (A555/A280).

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

2.4. Preparation and treatment of Fish Fillets

Fresh Forty specimens of Nile Tilapia (*Oreochromis niloticus*; average weight: 600±650 g) were taken from the Caspian Sea and were kept in ice throughout the transportation to the laboratory (14). A Total of 60 fillets were used for the study. After washing the fillets in tap water, they were divided into two lots. The first lot contained 40 fillets each, with the size of 5 cm × 5 cm × 1 cm (length × width × thickness) and weighing 10 g were used for microbiological analysis (20 fillets), control and sensory evaluation (20 fillets). The second one contained 20 fillets each, with the size of 8 cm × 8 cm × 1 cm (length × width × thickness) and weighing 25 g were used for the detection of *Salmonella* sp. One separate fillet of fish was used for each day of the experiment (0, 3, 7, 14, and 21 days) to evaluate the shelf-life at different temperatures of storage (4 and 8°C). The study carried out a MIC test on 30 fillets subjected to marination using purified PE of analytical grade. The PE was prepared by incorporating preservatives such as citric acid, sucrose, sodium chloride, and calcium chloride at a concentration of 0.193 mg/mL. The marination was carried out at varying temperatures of 4°C and 8°C, and the absorption spectrum of the fillets was recorded for a period of 30 days. A separate set of fillets was used as a control without PE addition. The fillets that underwent treatment were subsequently segregated and enclosed in polyethylene zipper bags. These bags were then placed in a refrigerator at two different temperatures, namely 4°C and 8°C, for varying durations of time (0, 3, 7, 14, and 21 days). Extraction of each fish fillet was performed separately on the day of the experiment expect for *salmonella* sp. where the fillets were used directly for testing. For extraction, sterile physiological serum (90 ml) was added to each fillet and homogenized in a Waring blender (Waring Products, Torrington, Conn., USA) for 1 min.

2.5. Estimation of the minimum inhibitory concentration (MIC) of the purified Phycoerythrin (PE)

The bacterial strains and culture conditions utilized for lyophilized bacterial pathogens sourced from food items encompass *Escherichia coli* and *Staphylococcus aureus*. These strains were procured from the Persian type culture collection of Iran Science and Technology Research Organization. The antibacterial evaluation was conducted in accordance with the methodology outlined by Sarada et al. (2011), albeit with certain adjustments. The bacterial strains were introduced into Brain Heat Infusion (BHI) broth, sourced from Merck in Germany, and subjected to incubation at 35°C for E. coli and S. aureus, and at 30°C for a duration of 18 h in a shaking incubator operating at 150 rpm. The bacterial cell suspension was standardized to a turbidity of 0.5 McFarland at a wavelength of 580nm, indicating a concentration of

1.5 x 108 colony-forming units per milliliter (1.5 x 108 CFU ml-1). The determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was conducted through the utilization of the serial dilution method. The PE was subjected to serial dilutions and subsequently adjusted to attain final concentrations of 50, 100, 200, 400, and 500 µg/ml BHI. Following this, 1 ml of each dilution was transferred into a 5 ml test tube. Inoculation of 100 µL of 24-h culture bacteria was carried out in each tube, followed by incubation for 24 h at 37°C and 30°C. The measurement of turbidity was conducted at a wavelength of 600 nm utilizing a UV/VIS spectrophotometer subsequent to a 24-h duration. Upon completion of the incubation period, the MIC was determined as the concentration of PE that exhibited the lowest level of visible growth inhibition. The MBC was determined as the concentration of PE at which all the bacteria inoculated were completely eradicated. The experimental procedures were conducted in triplicate. Negative and positive controls were employed in the study, with sterile distilled water serving as the former and doxycycline as the latter (15).

2.6. Microbiological analysis

2.6.1. Total viable microorganisms count

The quantification of viable psychrophilic bacteria was conducted following the methodology outlined by Mari and Antonini (16). The microbiological loads were quantified by determining the number of colony-forming units (cfu) per gram over 14 days.

2.6.2. Total psychrophilic bacteria count

The quantification of psychrophilic bacteria was conducted over a period of 14 days, following the methodology outlined by Raeisi et al. (17).

2.6.3. Staphylococcal coagulase-positive bacteria count

Staphylococcal coagulase-positive bacteria count were carried out according to Junior et al. during 14 days (18).

2.6.4. Enumeration of Total Coliforms

The Most Probable Number technique was utilized to quantitatively assess the total coliforms over a period of 14 days (19, 20)

2.6.5. Total fecal count (E. coli) Test

About one loopful from each gas positive LTB was inoculated into a test tube of sterilized Brilliant Green Bile Broth (BGLBB) and L-EMB agar plate and a test tube of sterilized 10 mL (20).

2.6.6. Detection of Salmonella sp.

Salmonella spp. was detected following the procedure described by Sanjee and Karim (20). The identified *Salmonella* spp. was serogrouped using a BD Difco™ Salmonella O Antisera in slide agglutination tests during 14 days (20).

2.7. Sensory evaluation

The quality assessment of fish fillets was conducted by ten panelists based on the results of the microbial count. The fillets were marinated in an analytical solution of PE, while control fillets were not treated with PE. The evaluation was carried out on four different days, namely the 0th, 3rd, 7th, and 14th days. The sensory attributes, namely color, odor, appearance, flavor, texture, and overall acceptability, were evaluated utilizing a 5-point hedonic scale as per the methodology proposed by Altug and Elmacı (21). The specimens were subjected to a thermal treatment in an oven using an oven bag at a temperature of 180°C for 10 min directly before their presentation for consumption.

2.8. Statistical analysis

The present study employed SPSS (version 24) to investigate the impact of the categorical variables "additive," "temperature," and "storage time," along with their respective interactions, on the numeric parameters under examination. A statistical significance threshold of 95% was utilized to determine the presence of significant differences. The Duncan test was performed to assess the statistical significance of differences in mean values subsequent to the detection of a significant variation ($P<0.05$) by means of the ANOVA test. In addition, Levene's tests and t-tests were used at a significance level of 0.05 to assess the equivalence of means between the

independent variables, namely the Control and Treated fillets, with the addition of Citric acid and PE. Three replicated measurements were conducted for each treatment, from which the mean values ± standard error of mean were derived.

3. Results

3.1. Extraction, purification, and characterization of Phycoerythrin (PE)

Table 1 was consulted to verify the concentration and purity of PE at each stage of purification. The purity absorption rate of PE increased from 0.797 nm to 3.200 nm during consecutive purification steps. The purity ratio was observed to increase after each purification procedure. The present study demonstrates the effectiveness of a purification method involving sequential steps of solid ammonium sulphate precipitation and dialysis in achieving a nearly four-fold increase in the purity of PE, from crude extract to purified form.

3.2. Evaluation of the minimal inhibitory concentration (MIC) of the purified Phycoerythrin (PE)

The comparison results of the average minimum inhibitory concentration, minimum lethal concentration, and the diameter of the inhibition zone of phycocyanin are evaluated and presented in Table 2. In addition, the results showed that the minimum inhibitory concentration and minimum lethal concentration of phycocyanin against *Escherichia coli* were significantly higher, with values of 2.6 and 3.9 mg of extract per milliliter, respectively ($P \leq 0.05$). Furthermore, the results showed that the maximum diameter of the inhibition zone of PE was significantly higher than that of *Staphylococcus aureus* (10.66 cm) ($P \leq 0.05$).

3.3. Microbiological analysis

3.3.1. Total viable microorganisms and mesophilic bacteria

The results of the comparison of the average number of live mesophyll bacteria of the samples in the time intervals of 0, 3, 7, 14, and 21 days are displayed in Figure 1. According to the results of the mean comparison, on day zero, no significant statistical difference was observed in the total number of living mesophilic bacteria in the samples with and without PE at 4°C and 8°C, While over time, the total number of living mesophilic bacteria in the treated samples with PE increased significantly ($P \leq 0.05$). The highest total number of live mesophilic bacteria belonged to sample Nile Tilapia fillet coated with PE solution at a temperature of 8°C and the lowest amount of total number of live mesophilic bacteria (trout fillet) coated with PE solution at a temperature of 4˚C was observed ($P \leq 0.05$).

3.3.2. Total number of psychrotrophic bacteria

The results of the comparison of the average number of total psychrotrophic bacteria of the samples in the time intervals of 0, 3, 7, 14, and 21 days are presented in Figure 2. According to the results of the mean

Table 1. Stepwise purification of PE.

Nostoc sp. strain	Step	Peak	PE (µg/mL)	Purity of PE (OD555/OD280)
FSN	Crude extract	566.2 - 616.9	0.108	0.797
	Ammonium sulphate precipitation	565.5 - 617.4	0.152	1.559
	Dialysis	567.6 - 617.7	0.193	3.20

Table 2. Comparison of the minimum inhibitory concentration, the minimum lethal concentration and the diameter of the inhibition zone of PE against *Escherichia coli* and *Staphylococcus aureus*. Results are Means ± SE.

	Minimum inhibitory concentration (mg/ml)	Minimum lethal concentration (mg/ml)	The diameter of inhibition zone (mm)
E. coli	2.6 ±0.05	3.9 ±0.04	7.66 ±0.01
Staphylococcus aureus	1.29 ±0.05	2.6 ±0.03	10.66 ±0.02

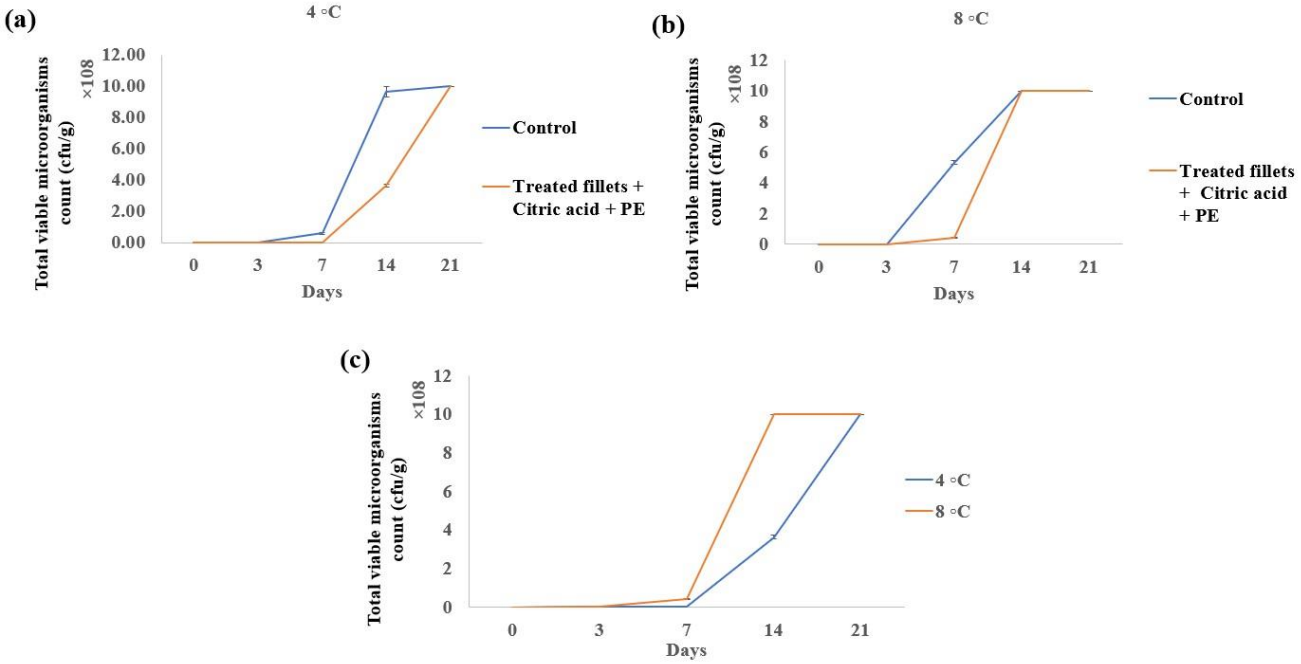

Figure 1. Total viable microorganisms and mesophilic bacteria at (a) 4˚C; (b) 8˚C; (c) comparison of two temperature

comparison, on day zero, no significant statistical difference was observed in all psychrotrophic bacteria in the samples with and without PE at 4°C and 8°C, While with the passage of time, the total number of living psychrotrophic bacteria in the treated samples with PE increased significantly (P≤0.05). In total, the differences between sample Nile Tilapia fillet coated with PE solution at the temperature of 8°C and 4˚C

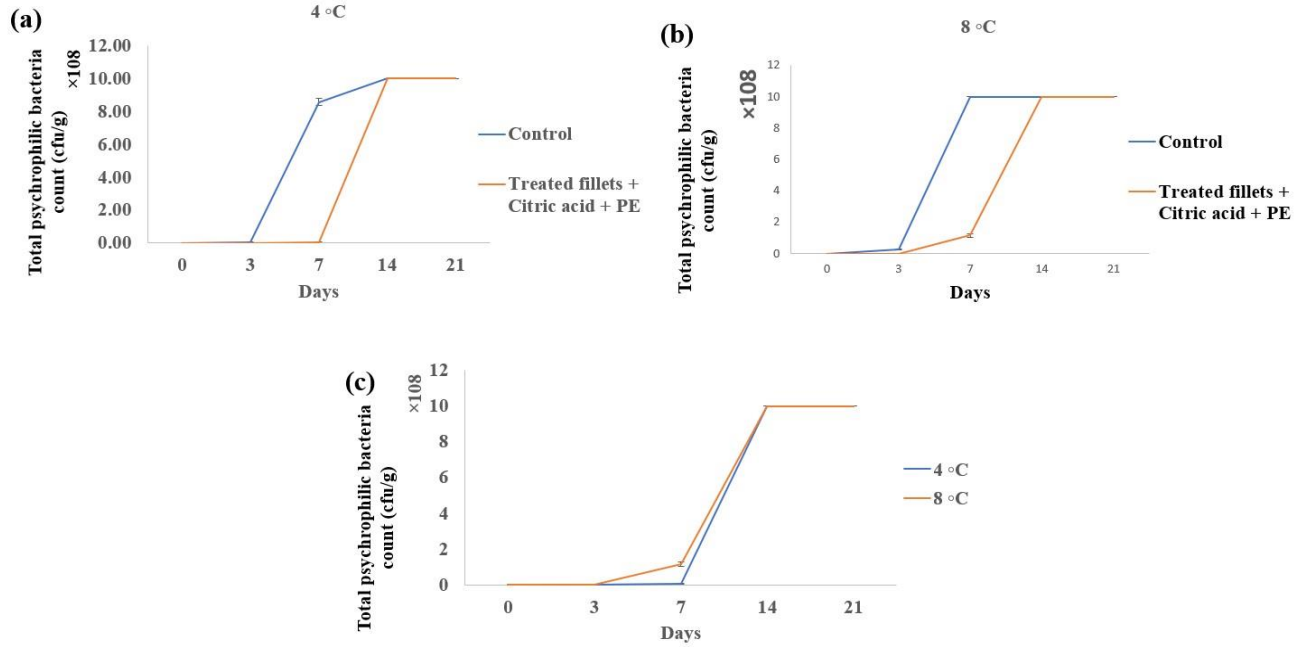

Figure 2. The total number of psychrotrophic bacteria at (a) 4˚C; (b) 8˚C; (c) comparison of two temperature

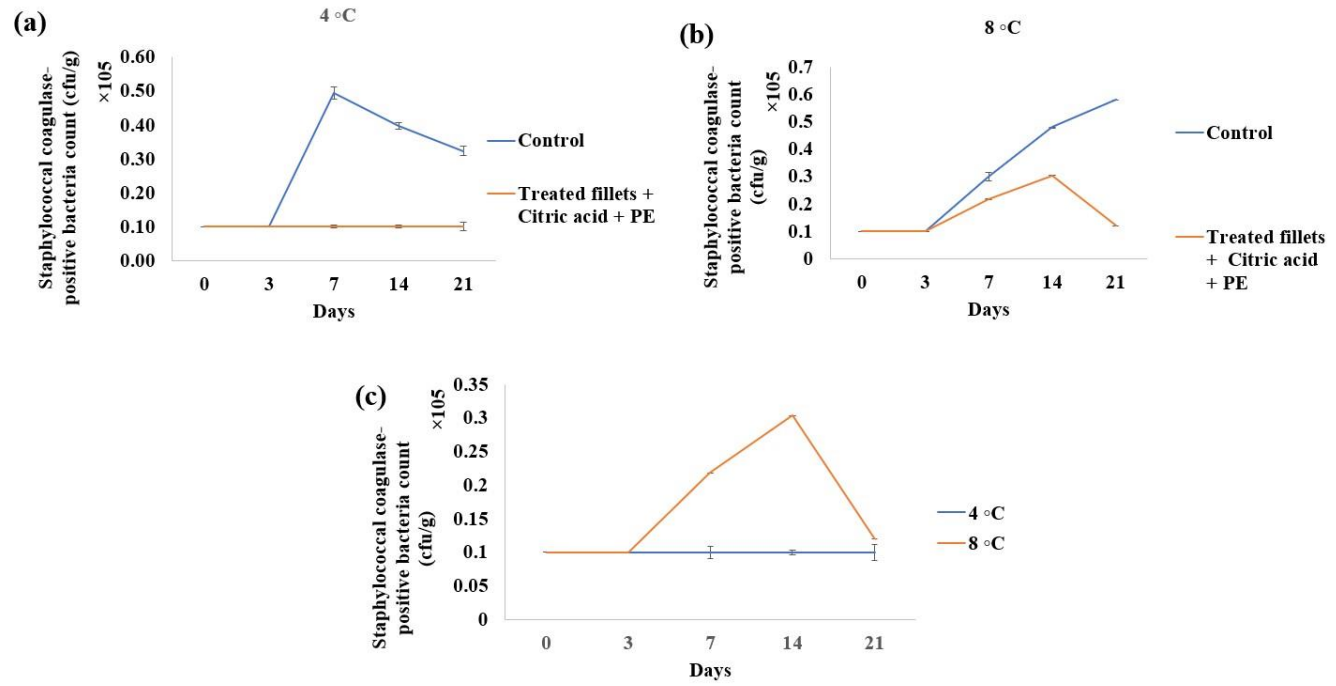

Figure 3. The number of *Staphylococcus aureus* coagulase positive bacteria at (a) 4˚C; (b) 8˚C; (c) comparison of two temperature

followed the same path with slightly higher for Nile Tilapia fillet coated with PE solution at the temperature of 8°C (P≤0.05).

3.3.3. Number of Staphylococcus aureus coagulase positive bacteria

The results of the comparison of the average number of *Staphylococcus aureus* bacteria in the samples in the time intervals of 0, 3, 7, 14, and 21 days are presented in Figure 3. According to the results of the mean comparison, on day zero, there was no significant statistical difference in the number of *Staphylococcus aureus* bacteria in the samples with and without PE at 4°C and 8°C (P>0.05). The highest total number of *Staphylococcus aureus* bacteria belonged to sample Nile Tilapia fillet coated with PE solution at a temperature of 8°C and the lowest amount of total number of *Staphylococcus aureus* bacteria (trout fillet) coated with PE solution at a temperature of 4˚C was observed (P≤0.05).

3.3.4. Number of coliform bacteria

The results of the comparison of the average number of coliform bacteria of the samples in the time intervals of 0, 3, 7, 14, and 21 days are presented in Figure 4. According to the results of the mean comparison, on day zero, no significant statistical difference was observed in the number of coliform bacteria in the samples with and without PE at 4°C and 8°C (P>0.05). The highest total number of coliform bacteria belonged to sample Nile Tilapia fillet coated with PE solution at a temperature of 8°C and the lowest amount of total number of coliform bacteria (Nile Tilapia fillet) coated with PE solution at a temperature of 4˚C was observed (P≤0.05).

3.4. Results of Sensory evaluation

The results of the comparison of the average sensory evaluation, including flavor, texture, color, and Total acceptance score of the samples in the time intervals of 0, 3, 7, 14, and 21 days are presented in Figure 5. According to the results of the mean comparison, on day zero, no significant statistical difference was observed in the taste score in the samples with and without PE at 4°C and 8°C (P>0.05). Totally, for all of these sensory analyses, the highest score belonged

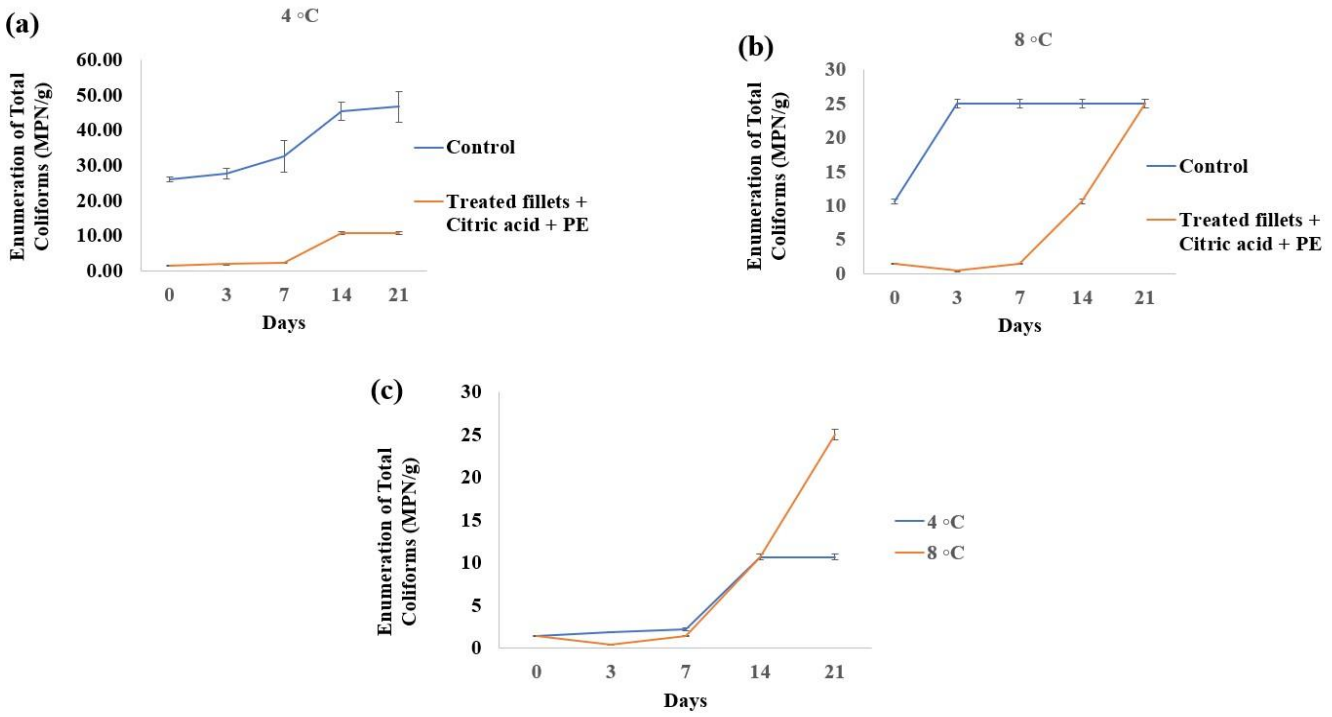

Figure 4. The number of coliform bacteria at (a) 4˚C; (b) 8˚C; (c) comparison of two temperature

to sample Nile Tilapia fillet coated with PE solution at a temperature of 8°C and the lowest score for Nile Tilapia fillet coated with PE solution at a temperature of 4˚C was observed (P≤0.05).

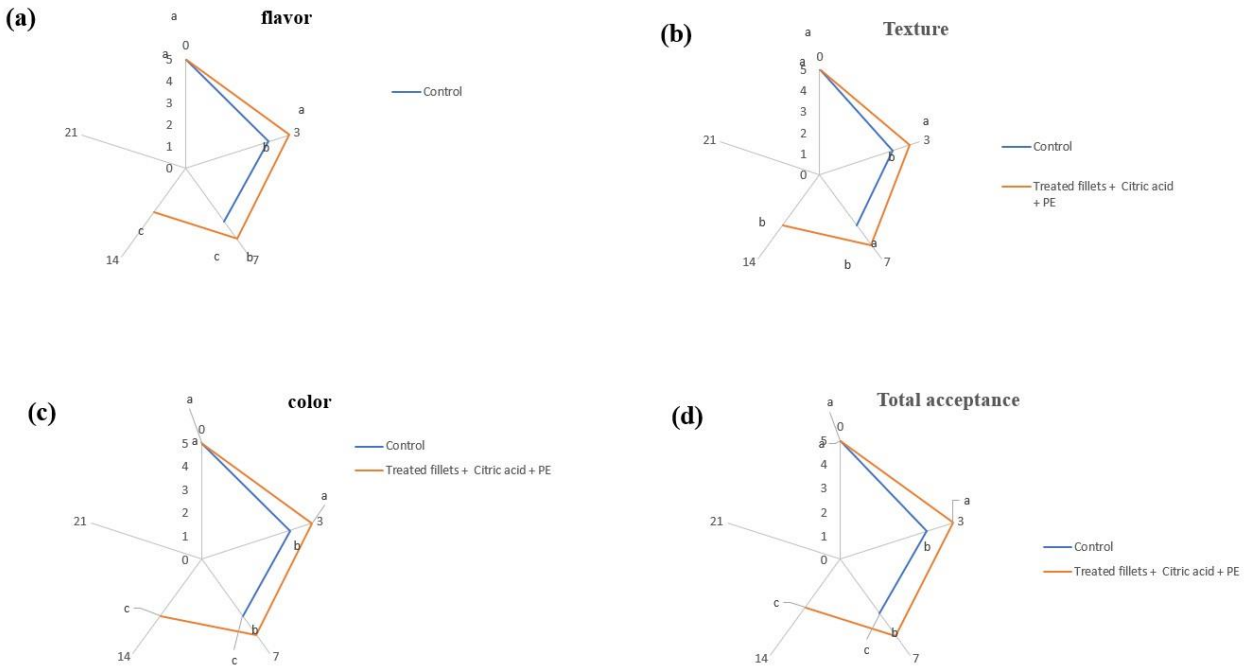

Figure 5. The sensory evaluation of (a) Flavor; (b) Texture; (c) Color and (d) Total acceptance score

4. Discussion

Preservatives have been employed in the seafood industry to prolong the shelf-life of commercial products. Microalgae are a valuable source of extracts and bio-preservative compounds that may not be available in other raw materials. Microalgae are a rich source of major bioactive compounds such as proteins, fatty acids (primarily omega-3), pigments, carotenoids, polysaccharides, and vitamins. These compounds have significant value in seafood production, as they can improve the quality and extend the shelf-life of the product. According to a study (22), extracts of microalgae possess bioactive compounds that have the potential to compromise the structural integrity of bacterial cell membranes. The active compounds have the potential to enhance the permeability of cell membranes, leading to the significant loss of essential ions, including potassium and other cytoplasmic components. This phenomenon has the potential to ultimately result in cellular demise.

Phycobiliproteins are hydrophilic protein-pigment complexes that exhibit hydrophilic properties. They are found in various cyanobacterial species, including *Phorphyridium*, *Spirulina*, and *Oscillatoria*. The mentioned pigments, which are primarily composed of proteins, have been categorized into three distinct groups: phycocyanins (blue pigments), allophycocyanins (pale-blue pigments), and phycoerythrins (red pigments). The utilization of these pigments as natural colorants and bioactive agents in seafood processing has been documented (23, 24).

The utilization of microalgae biomass in seafood has been observed to improve quality and prolong shelf-life by mitigating microbial growth and chemical reactions. In a recent investigation conducted by Ben Atitallah et al. (2019) (25), it was demonstrated that the inclusion of *Chlorella minutissima*, *Isochrysis galbana*, and *Picochlorum* sp. powder in canned fish burgers made from common barbel (Barbus barbus) resulted in a significant enhancement of the overall sensory acceptability, texture analysis parameters (namely, hardness, chewiness, gumminess, and cohesiveness), nutritional value, and functional characteristics (specifically, water and oil holding capacities) in comparison to the control group. The results of the microbial contamination analysis indicate the absence of foodborne pathogens, mold, or yeast growth over two months at a temperature of 4˚C.

Compared to our study, total viable microorganisms such as mesophilic, psychrotrophic, and *Staphylococcus aureus* coagulase positive, coliform, and salmonella bacteria were analyzed. In all of these performed tests, the highest total number of bacteria belonged to sample Nile Tilapia fillet coated with PE solution at a temperature of 8°C and the lowest amount of bacteria (Nile Tilapia fillet) coated with PE solution at a temperature of 4˚C was observed ($P \leq 0.05$).

In addition to the chemical and microbial attributes, the sensory characteristics of seafood play a significant role in shaping consumers' preferences. The sensory quality of seafood is significantly influenced by the appropriate color, which is considered an integral attribute. The perception of a pale coloration is frequently indicative of inferior quality, whereas the presence of natural and vibrant hues is subconsciously linked to premium seafood (23). To attain the desired aesthetic qualities and coloration preferred by consumers, it is imperative to optimize the dietary levels of pigments for aquaculture species (23).

Moreover, sensory evaluations, including flavor, texture, color, and total acceptance, were performed. According to the results of the mean comparison, on day zero, no significant statistical difference was observed in the taste score in the samples with and without PE at 4°C and 8°C ($P>0.05$). Totally, for all of these sensory analyses, the highest score belonged to sample Nile Tilapia fillet coated with PE solution at a temperature of 8°C and the lowest score for Nile

Tilapia fillet coated with PE solution at a temperature of 4˚C was observed (P≤0.05).

The interest of seafood manufacturers and consumers has been increasingly drawn toward microalgae biomass and its derivatives, including extracts and bioactive compounds. The aforementioned products exhibit diverse functionalities, including but not limited to natural pigments, potential antimicrobial agents, antioxidants, health-promoting nutritional compounds, and ingredients with enhanced technological attributes that can be incorporated into seafood. Notwithstanding, numerous obstacles exist in the manufacture and application of microalgae biomass or its byproducts in the food sector. The challenges primarily pertain to the cost of production, evaluation of safety, and determination of optimal concentrations for application. Therefore, we evaluated the effect of natural pigments PE on antimicrobial activity, as well as the shelf-life extension of the Nile Tilapia (*Oreochromis niloticus*) at the two refrigerator temperatures of 4°C and 8°C. Overall, the results have shown that the highest total number of bacteria and sensory evaluation belonged to sample Nile Tilapia fillet coated with PE solution at the temperature of 8°C and the lowest score was observed for Nile Tilapia fillet coated with PE solution at the temperature of 4˚C (P≤0.05). In conclusion, microalgae-based products can be a potential option for application in food safety.

Acknowledgment

Not applicable**.**

Authors' Contribution

BN: wrote the first draft, supervised, editted; SJP: co-wrote and editted; AAA: editted

Ethics

Not applicable.

Conflict of Interest

Authors have no conflict of interest on this work.

Funding

No funding was received for this work.

References

1. Nowruzi B, Porzani SJ. Toxic compounds produced by cyanobacteria belonging to several species of the order Nostocales: A review. J Appl Toxicol. 2021;41(4):510-48.
2. Rahmati M, Morovvati H, Abdi R. Histomorphometric Analysis of Skin and Stress Indices of Nile Tilapia (Oreochromis niloticus) Exposed to Different Concentrations of Ammonia. Iran J Vet Med. 2022;16(3).
3. Frazaneh M, Fadaei V, Gandomi Nasrabadi H. Study of the effect of aqueous extract of Agave tequilana on antioxidant, syneresis and sensorial properties of probiotic yogurt. Iran J Vet Med. 2022.
4. Baghkheirati AA, Javan AJ, Naeimi S, Ghazvinian K. Usability Evaluation of Camel Thorn (Alhagi maurorum) in Broiler Diet and Its Effects on Lipid and Protein Oxidation of Broiler Breast Fillets During Frozen Storage. Iran J Vet Med. 2021;15(3).
5. Alighazi N, Noori N, Gandomi H, Basti AA. Effect of Ziziphora clinopodioides Essential Oil Stress on Viability of Lactobacillus acidophilus and Bifidobacterium bifidum Microencapsulated with Alginate-Chitosan and Physicochemical and Sensory Properties of Probiotic Yoghurt. Iran J Vet Med. 2021;15(2).
6. Peidaei F, Ahari H, Anvar A, Ataei M. Nanotechnology in Food Packaging and Storage: A Review. Iranian Journal of Veterinary Medicine. 2021;15(2).
7. Rajabpour N, Nowruzi B, Ghobeh M. Investigation of the toxicity, antioxidant and antimicrobial activities of some cyanobacterial strains isolated from different habitats. Acta Biologica Slovenica. 2019;62(2):3-14.
8. Afreen S, Fatma T. Extraction, purification and characterization of phycoerythrin from Michrochaete and its biological activities. Biocatalysis and agricultural biotechnology. 2018;13:84-9.
9. Chakdar H, Pabbi S. Extraction and purification of phycoerythrin from Anabaena variabilis (CCC421). Phykos. 2012;42(1):25-31.
10. Tiwari ON, Devi WI, Silvia C, Devi AT, Oinam G, Singh OA, et al. Modulation of phycobiliprotein production in Nostoc muscorum through culture manipulation. J Appl. Biol. 2015;3(4):011-6.

11. Mishra SK, Shrivastav A, Mishra S. Effect of preservatives for food grade C-PC from Spirulina platensis. P Process Biochem. 2008;43(4):339-45.
12. Mishra SK, Shrivastav A, Pancha I, Jain D, Mishra S. Effect of preservatives for food grade C-Phycoerythrin, isolated from marine cyanobacteria Pseudanabaena sp. Int. J. Biol. Macromol. 2010;47(5):597-602.
13. Nowruzi B, Fahimi H, Lorenzi AS, editors. Recovery of pure C-phycoerythrin from a limestone drought tolerant cyanobacterium Nostoc sp. and evaluation of its biological activity. Anales de Biología; 2020: Servicio de Publicaciones de la Universidad de Murcia.
14. Sáez M, Suárez M, Martínez T. Effects of alginate coating enriched with tannins on shelf life of cultured rainbow trout (Oncorhynchus mykiss) fillets. LWT. 2020;118:108767.
15. AbdulGhani AA. Antibacterial phycocyanin from Anabaena oryzae SOS13. Int. J. Appl. Res. Nat. Prod. 2015.
16. Mari A, Antonini G. Validation of the micro biological survey method for total viable count and E. coli in food samples. J. Food Technol. 2011;6(11):951-62.
17. Raeisi S, Sharifi-Rad M, Quek SY, Shabanpour B, Sharifi-Rad J. Evaluation of antioxidant and antimicrobial effects of shallot (Allium ascalonicum L.) fruit and ajwain (Trachyspermum ammi (L.) Sprague) seed extracts in semi-fried coated rainbow trout (Oncorhynchus mykiss) fillets for shelf-life extension. LWT-Food Sci. Technol. 2016;65:112-21.
18. Junior PG, Assunção AW, Baldin JC, Amaral LA. Microbiological quality of whole and filleted shelf-tilapia. Aquac. 2014;433:196-200.
19. Rahman MA, Ahmad T, Mahmud S, Barman N, Haque M, Uddin M, et al. Isolation, identification and antibiotic sensitivity pattern of Salmonella spp. from locally isolated egg samples. Am J Pure Appl Sci. 2019;1(1):1-11.
20. Sanjee SA, Karim M. Microbiological quality assessment of frozen fish and fish processing materials from Bangladesh. Int. J. Food Sci. 2016;2016.
21. Altuğ Onoğur T, Elmacı Y. Sensory evaluation in foods. İzmir, Turkey: Sidaş AOAC(1990) Official methods of analysis (15th ed) Association of Official Analytical Chemists, Washington DC, USA. 2011.
22. Schuelter AR, Kroumov AD, Hinterholz CL, Fiorini A, Trigueros DEG, Vendruscolo EG, et al. Isolation and identification of new microalgae strains with antibacterial activity on food-borne pathogens. Engineering approach to optimize synthesis of desired metabolites. Biochem. Eng. J. 2019;144:28-39.
23. Guedes AC, Sousa-Pinto I, Malcata FX. Application of microalgae protein to aquafeed. Handbook of marine microalgae: Elsevier; 2015. p. 93-125.
24. Levasseur W, Perré P, Pozzobon V. A review of high value-added molecules production by microalgae in light of the classification. Biotechnol. Adv. 2020;41:107545.
25. Atitallah AB, Barkallah M, Hentati F, Dammak M, Hlima HB, Fendri I, et al. Physicochemical, textural, antioxidant and sensory characteristics of microalgae-fortified canned fish burgers prepared from minced flesh of common barbel (Barbus barbus). Food Biosci. 2019;30:100417.