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Industrial application of Natural Phycocyanin Edible Pigment isolated from *Spirulina platensis* in Preparation of fortified ice cream with emphasize on microbial and antioxident properties

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ABSTRACT

Due to its unique role , phycocyanin pigment extracted from the cycanobacterium *Spirulina platensis* can play an important role in enriching traditional cheese. In this study , the amount of protein , fat , total sugar, aeration, melting speed of fortified ice cream with different concentrations of phycocyanin pigment were determined. In addition , the counting of bacteria was done along with the assessment of antioxidant properties. Also , GC/MS test was performed to identify volatile compounds . The results of tests showed that the amount of fat , total sugar , melting speed , DPPH of fortified ice cream has decreased significantly compared to the control . Also , the amount of protein , aeration , FRAP and ABTS significantly compared to the control . sensory evaluation, the acceptability of the smell decreased with increasing concentration, but there was no significant difference in other parameters compared to the control . In addition no sighns of *Escherichia coli*, *Staphylococcus aureus* and *salmonella* were found during different days . However , the presence of *coliform bacteria* 42 to 56 days in enriched and control ice cream . The presence of mold and yeast on days 28 to 56 was evident only in the control sample . The presence of *phychrophilic bacteria* on days 14 to 56 of fortified ice cream has decreased significantly compared to the control. Also, the results of the vaolatile compounds obtained from GC/MS test in control ice cream and enriched ice cream with 2% phycocyanin pigment show the presence of antioxidant and antimicrobial properties that played an important role in the shelf life and quality of ice cream. It is hoped that the results of this study will be the basis for empowering the food industry in using of pigments obtained from cyanobacteria

1. Introduction

In recent years, communicable poisonings and infections transmitted through food, have become the most important research topic for researchers and scientists. With the increasing attention given to these diseases, people have been asking for sufficient and new information regarding the methods of overcoming these diseases. Dairy-based products hold significant importance in maintaining daily dietary requirements as they serve as primary sources of calcium, vitamin D, phosphorus, potassium, manganese, riboflavin, and niacin [1].

Ice cream is a dairy product, and according to the Nielsen definition, it is a frozen dairy product characterized by a complex matrix where small air bubbles are dispersed within a continuous phase that is partially frozen. In this phase, emulsified fats and non-fat solids act as texturizers, while sugars and salts form a true solution. Ice cream enjoys immense popularity among various age groups, especially children [2].

This frozen favorite has numerous variations worldwide, differing in additives and manufacturing processes [3].

Several bacteria have been known as pathogens responsible for diarrhea, vomiting, infection, and food poisoning [4]. Ice cream, being a dairy-based product, provides an ideal medium for microbial growth due to its nutrient content, desirable pH, and prolonged shelf life even in frozen state. The quality of ice cream is influenced by various factors including production methods, stages, and ingredients. Primary sources of microbial contamination in ice cream include raw milk and water, while secondary sources encompass flavoring agents, containers, and transportation [5]. Pathogenic bacteria during ice cream production can lead to contamination. Pathogenic bacteria that potentially contaminate dairy products include Staphylococcus aureus, coliforms, Escherichia coli, Salmonella, psychrophilic bacteria, etc. [6]. Cyanobacteria, also known as blue-green algae, belong to a group of microorganisms closely related to bacteria but capable of photosynthesis. Therefore, cyanobacteria are generally classified as microalgae, although they are prokaryotes, and the term algae should be restricted to eukaryotes [7].

Currently, cyanobacteria are cultivated on a large scale, indicating their high economic value as a protein source capable of meeting human dietary needs and obtaining other consumer products. Industrial and commercial use of microalgae as nutritional supplements, antioxidants, anti-inflammatory drugs, antibiotics, and various toxins is rapidly expanding [8].

Spirulina, a type of blue-green algae, is a branch of cyanobacteria renowned worldwide as a dietary supplement due to its richness in protein (50-60%), antioxidants, essential fatty acids, etc. [9]. The potential application of phycocyanin, generally recognized as safe (GRAS), has attracted attention not only in cosmetics and personal care formulations, but also in food products [10]. Phycocyanins, predominant pigments in Spirulina platensis, possess inhibitory properties against various harmful free radicals such as alkoxyl, hydroxyl, and peroxide, albeit diminishing over time [11]. With their coloring, antioxidant, and antimicrobial properties,

phycocyanins find utility in various food formulations such as yogurt, cheese, ice cream, etc., with their efficacy well-established in numerous studies [12]. The potential applications of Spirulina platensis as a nutritional component to enhance the health properties of products like dietary supplements, beverages, fermented sweets, grains, bakery products, desserts, cakes, confectionery products, biscuits, snacks, soups, salad dressings, and dairy products like ice cream, yogurt, and milk-based beverages have been explored [13]. Despite their inherent instability, derived colorants from Spirulina offer potential health benefits during consumption [14] functioning as antioxidants and anti-cancer agents. Furthermore, rich in minerals and vitamins, notably potassium, calcium, magnesium, selenium, iron, zinc, and B-group vitamins, Spirulina presents valuable nutritive properties. The water-soluble B-group vitamins play pivotal roles in DNA repair, electron transfer, fatty acid synthesis, and one-carbon metabolism.

The antioxidant and anti-inflammatory activities of microalgae can significantly contribute to human health. They can activate cellular antioxidant enzymes, inhibit lipid peroxidation and DNA damage, scavenge free radicals, and enhance the activity of superoxide dismutase and catalase [16]. In alignment with the trend towards increased dietary intake and healthy lifestyles, consumers are shifting preferences from artificial additives to natural substances. Natural colors derived from plants, fruits, or animals are extracted and purified, showcasing beneficial biological activities as antioxidants and anticancer agents [14].

According to researchers, ice cream, while being a high-calorie and nutritious product, often lacks sufficient amounts of antioxidants or dietary fibers. This deficiency can be compensated by incorporating plants or vegetables [17]. Spirulina's value lies in its easily digestible nature due to the absence of cellulose in its cell wall. Its high nutrient absorption capability, especially for minerals, recommends its use in the diets of pregnant women and malnourished individuals. The World Health Organization has hailed Spirulina as the most nutritious food on Earth, and NASA utilizes it as a compact food source for space missions [18]. The production of nutrient-rich products can pave the way for the practical application of food coloring phycocyanin to enhance the quality of food items, not only innovatively but also economically. Since there has been no research conducted in Iran regarding the extraction, isolation, purification, antioxidant activity evaluation, and antimicrobial properties of phycocyanin colorants and their impact on ice cream, this study aims to use natural phycocyanin colorants to enrich ice cream and increase its nutritional factors, comparing the enriched ice cream's nutritional factors with control ice cream. The results of this research can facilitate the introduction of natural cyanobacteria-derived food colorants for use in the food industry and prolonging the shelf life of dairy products in Iran.

2-Materials and Methods

2.1. Materials

The materials utilized in this study include liquid Zarrouk culture medium, phosphate buffer (pH 7.2), sodium phosphate buffer, acetic acid, rent tablets, TPTZ reagent

(2,4,6-Tripyridyl-s-triazine), Griess reagents 1 and 2, DPPH reagent (1,1-diphenyl-2-picrylhydrazyl), catalysts (copper sulfate, sodium sulfate), sulfuric acid, 85% acetone, methanol, hydrochloric acid, monovanadate reagent, sodium periodate reagent, hydrochloric acid amine reagent, phenanthroline reagent, manganese sulfate standard, ammonium iron sulfate standard, potassium chloride, and 10 millimolar nitroprusside solution, etc.

2.2. Cultivation of Spirulina Cyanobacteria and Extraction of Phycocyanin Pigment

Spirulina cyanobacteria were cultured in liquid Zarrouk medium in a growth chamber at a temperature of 28 ± 2°C and continuous fluorescent lighting with an intensity of 300 micro Einstein per square meter per second for thirty days [19]. For the extraction process, 500 milliliters of the fourteen-day-old culture medium were centrifuged at 4000 rpm and the resulting sediment was washed with phosphate buffer (pH 7.2) and lyophilized. Two grams of freeze-dried biomass were suspended in 500 milliliters of sodium phosphate buffer (pH 7.2, 1.0 M). Phycocyanin pigment was extracted by repeating the freeze-thaw method at minus twenty degrees Celsius followed by thawing at room temperature and darkness. The resulting mixture was centrifuged at 10,000 rpm for thirty minutes at 5 ± 0°C, and the phycocyanin content was collected and freeze-dried [20].

2.3. Ice Cream Preparation

To prepare the ice cream, egg yolks (3%) and granulated sugar (16%) were thoroughly mixed until a paste was formed. Milk, comprising 45.5%, 44.9%, and 44.3% whole milk, skim milk, and 0.5% gelatin, respectively, was heated to 60 degrees Celsius. The milk mixture was then poured into the egg yolk and sugar mixture, and essence (10%) was added. The mixture was pasteurized at 85 degrees Celsius for 10 minutes. While the resulting creamy mixture was still hot, it was homogenized for 10 minutes at 1500 rpm. Subsequently, pigment powder at various concentrations (0.5%, 1%, and 2%) was added. The final ice cream mixture was then placed in the refrigerator, allowing the milk fat to crystallize to a certain extent and giving time for the proteins to hydrate. Only 15 minutes are required for the production of ice cream. Ice cream packaging was immediately done after removal from the refrigerator/freezer. The product was stored in the freezer at a temperature of minus eighteen degrees Celsius for 24 hours.

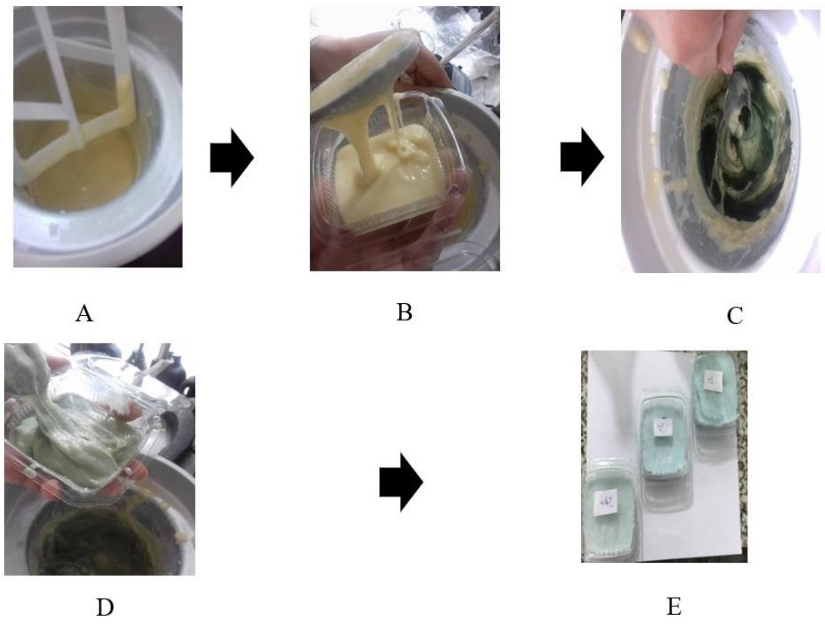


Figure 1. Putting ice cream ingredients in the ice cream maker. (A), Shahid ice cream preparation (B), add concentrations of 0/5 % ,1% and 2% phycocyanin pigment to ice cream (C), Preparing ice creams and placing them in containers (D, E)

1.4. Chemical Properties of the Produced Ice Cream Sample

1.4.1. Determination of Protein Content

The protein content was determined according to the Iranian Standard 13483 using the Kjeldahl method in three stages: digestion, distillation, and titration. The percentage was measured using the following formula [21]:

(Formula 1)

Protein Percentage = 6.25 × Nitrogen Percentage

Fat Content

The fat content of the ice cream was determined according to the Iranian Standard 2450 using the Gerber method. It was performed using a Gerber butyrometer or fatometer, and the percentage was measured using the following formula [22]:

(Formula 2)

$$\text{Fat Percentage} = \frac{\text{Fat with balloon weight} - \text{Empty balloon weight}}{\text{Dry sample weight}} \times 100$$

Total Sugar Content

The measurement of total sugar content was carried out by the Fehling method based on the Iranian Standard 2685. For this purpose, a solution from the previous stages, hydrochloric acid solution, concentrated sodium hydroxide solution, and Fehling solutions were utilized, and the percentage was measured using the following formula [23]:

(Formula 3)

$$\text{Total Sugar} = \frac{\text{Fehling Factor} \times 100 \times 100 \times 100}{\text{Volume of Consumed Solution} \times 25 \times 25}$$

Overrun

The measurement of overrun or volume increase of ice cream was conducted

according to Standard 2450. The percentage of ice cream volume increase was determined by two methods, volumetric and weight, using the following formulas [22]:

(Formula 4)

$$\text{Overrun Percentage (per Weight)} = \frac{Ice\text{-}Cream\ Mixture\ Weight - Prepared\ Frozen\ Ice\text{-}Cream\ Weight}{Prepared\ Frozen\ Ice\text{-}Cream\ Weight} \times 100$$

(Formula 5)

$$\text{Overrun Percentage (per Volume)} = \frac{Ice\text{-}Cream\ Mixture\ Volume - Prepared\ Frozen\ Ice\text{-}Cream\ Volume}{Prepared\ Frozen\ Ice\text{-}Cream\ Volume} \times 100$$

Melting Point Measurement

For the measurement of the melting point by the micro method, a capillary tube and a Thiele tube were used. The percentage was measured using the following formula:

(Formula 6)

$$\text{Melting Point Resistance Percentage} = \frac{30 - (\text{Ice Cream Weight after Melting} - \text{Empty Erlan Weight}}{30} \times 100$$

1.5. GC/MS Technique and Its Application in Identifying Ice Cream Volatile Compounds

This experiment was conducted using the Purge _Trap method on the (GC/MS) device. Considering that ice cream samples have a dense texture and cannot be directly injected into the GC device, the samples were first homogenized in a mortar with distilled water and solvent (3-methyl heptanone) to obtain a homogeneous solution. After centrifugation, the supernatant was taken, resulting in a clear extract, which was diluted 20 times and introduced into the concentrator section. This section is connected to a gas chromatography (GC) and mass spectrometry (MS) system. Separation of sample volatile compounds was carried out using an HP INNOWax column with a polyethylene glycol coating (0.25mm × 60m). Then, helium carrier gas was injected at a constant rate of one milliliter per minute and a ratio of 30:1 at 200°C. The temperature program started with a 7-minute hold at 32°C, followed by an increase from 6 to 220°C at a rate of degrees per minute, and finally held for 5 minutes at 220°C. The transfer path (from gas chromatography to mass spectrometry) was conducted at 220°C. Detection operations in scan mode (3 scan/s) were performed from 19 to 250 atomic mass units (amu), and ionization was carried out by electron ionization at 70 eV. The data were then registered and analyzed by the device on a Vectra XM 5.166PC computer [24].

2-6- Microbiological Analyses

Total Bacteria Count:

To identify the total bacterial count according to Iranian Standard 5272-1, the pour plate method was used in count agar culture medium and incubated for 72 hours at 30°C. The microbial load was expressed as colony-forming units (CFU) per gram.

Identification of Escherichia coli Bacteria:

For the identification of Escherichia coli, the MPN method was employed on EC Broth culture medium. A specific amount of the initial suspension was inoculated into the culture medium and incubated at 33°C for 41 hours. Gas production was examined after 94 and 41 hours. If turbidity or gas was observed in the tube containing EC Broth, it was inoculated. Then, the indole production test was performed [25].

Identification of Coliform Bacteria:

Identification of coliform bacteria was conducted using Violet Red Bile Agar (VRBA) culture medium for 24 hours at 37°C. The microbial load was expressed as MPN colony-forming units per gram [26].

Identification of Salmonella Bacteria:

Salmonella identification was carried out using Salmonella Shigella Agar, Brilliant Agar, and Bismuth Sulfite Agar culture media [27].

Identification of Staphylococcus aureus Bacteria:

Isolation and counting of Staphylococcus aureus bacteria were performed using surface culture and Parker agar culture medium. The coagulase test was conducted to confirm the Staphylococcus aureus colonies that had grown on Parker agar culture medium [28].

Identification of Pseudomonas aeruginosa Bacteria:

Psychrophili Total Count (PTC), expressed in CFU units, and its measurement method were in accordance with Standard Method 2629, using the surface culture technique [29].

Identification of Yeasts and Molds:

The contamination level of samples with molds and yeasts was determined using the mixed culture method and Yeast Extract Chloramphenicol Dextrose Agar culture medium, incubated at 25°C for 5 days [30].

2-7- Assessment of Antioxidant Activity

Evaluation of Antioxidant Potential Using the Ferric Reducing Antioxidant Power (FRAP) Method:

The assessment of antioxidant potential via the FRAP method utilized the Benzie and Strain method. In this method, the reduction of ferric (Fe^{3+}) to ferrous (Fe2+) ions by antioxidants present in the samples was carried out. Different concentrations of standard iron ion solution (Fe2+) were prepared using a 1000 micromolar iron stock solution. Subsequently, 1/5 milliliters of the FRAP solution (containing TPTZ and FeCl3) were added to a test tube along with 50 microliters of each concentration. After incubation for 10 minutes at 37°C, the color intensity was measured at 593 nanometers against a blank (50 microliters of solvent extract (water or methanol) + 1/5 milliliters of FRAP), and the FRAP level in unknown samples was calculated based on the standard curve [31].

Assessment of Antioxidant Activity Using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Method:

This method relies on the scavenging of free radicals by compounds, employing 1,1-diphenyl-2-picrylhydrazyl (DPPH). The change in the solution color from purple to colorless, and hence its absorption change was measured in the spectrophotometer at 520 nanometers. Initially, a mixture of 1 milliliter of phycocyanin pigment with 1 milliliter of 0.002% DPPH in methanol was prepared. After incubation for thirty minutes in darkness at room temperature, the absorption at 517 nanometers was measured. Ascorbic acid was used as a positive control, and the antioxidant activity level was evaluated using the formula provided [32].

(Formula 7)

$$\text{Optical Absorption} = \frac{Sample\ Absorption - Control\ Sample\ Absorption}{Control\ Sample\ Absorption} \times 100$$

Evaluation of Antioxidant Properties Using the ABTS Method:

ABTS radical is a stable synthetic radical with high sensitivity used for evaluating the antioxidant activity of various compounds. This method is based on the scavenging of ABTS radicals, which exhibit high absorption at 734 nanometers. It involves the production of the chromophore ABTS in the presence of an oxidant (usually potassium persulfate). Initially, a cation radical was produced by mixing a 7 mM ABTS stock solution with a 2.45 mM potassium persulfate solution, followed by incubation for 4 to 16 hours until complete reaction and stable absorption. The ABTS solution was diluted with ethanol, and absorption at 734 nanometers was measured. The photometric test was performed with 0.9 milliliters of ABTS solution and 0.1 milliliters of test samples, mixed for 45 seconds, and read at 734 nanometers after one minute. Antioxidant activity was determined based on the reduction in absorption and the respective equations [33].

(Formula 8)

$$\text{ABTS Radical Scavenging Activity} = \frac{Control\ Sample\ Absorbtion - Sample\ Absorbtion}{Control\ Sample\ Absorbtion} \times 100$$

2-8- Sensory Evaluation

Sensory evaluation of ice cream was conducted according to Iranian Standard 4937. The evaluation was performed by a trained semi-skilled group of five evaluators. Factors assessed in this evaluation included appearance, texture and shape, aroma and flavor, color and appearance, texture, and overall acceptance of ice cream mixtures, rated on a 5-point sensory evaluation scale [34].

2-9- Data Analysis Methods and Tools

Statistical analysis of the data from each experiment was performed using SPSS software (version 16) and Excel. All data were obtained from three repetitions. Significance difference between measured

factors was determined using one-way analysis of variance with 95% confidence intervals, and mean comparisons were conducted using the Tukey test. The results of comparisons were presented graphically using Excel.

3-Results and Discussion

3.1. Results of Physicochemical Analysis of Enriched Ice Cream

3.1.1. Fat Content of Enriched Ice Cream

The results obtained from the assessment of fat content showed a significant decrease in fat levels at concentrations of 0.5%, 1%, and 2% of phycocyanin pigment compared to the control, with a confidence level of $P < 0.05$, such that the fat content at a concentration of 2% phycocyanin pigment decreased by 1.13 times compared to the control. (Figure 2-A)

3.1.2. Protein Content of Enriched Ice Cream

The results of the protein content evaluation demonstrated a significant increase in protein levels at concentrations of 0.5%, 1%, and 2% of phycocyanin pigment compared to the control, with a confidence level of $P < 0.05$, such that the protein content at a concentration of 2% phycocyanin pigment increased by 0.85 times compared to the control. (Figure 2-B)

3.1.3. Total Sugar Content of Enriched Ice Cream

The results of the evaluation of total sugar content showed a significant decrease in total sugar levels at concentrations of 0.5%, 1%, and 2% of phycocyanin pigment compared to the control, with a confidence level of $P < 0.05$, such that the total sugar content at a concentration of 2% phycocyanin pigment decreased by 1.05 times compared to the control. (Figure 2-C)

3.1.4. Overrun of Enriched Ice Cream

The results obtained from the assessment of overrun indicated a significant increase in overrun levels at concentrations of 0.5%, 1%, and 2% of phycocyanin pigment compared to the control, with a confidence level of $P < 0.05$, such that the overrun at a concentration of 2% phycocyanin pigment increased by 0.86 times compared to the control. (Figure 2-D)

3.1.5. Melting Rate of Enriched Ice Cream

The results of the evaluation of melting rate showed a significant decrease in melting rate at concentrations of 0.5%, 1%, and 2% of phycocyanin pigment compared to the control, with a confidence level of $P < 0.05$, such that the melting rate at a concentration of 2% phycocyanin pigment decreased by 1.02 times compared to the control. (Figure 2-E)

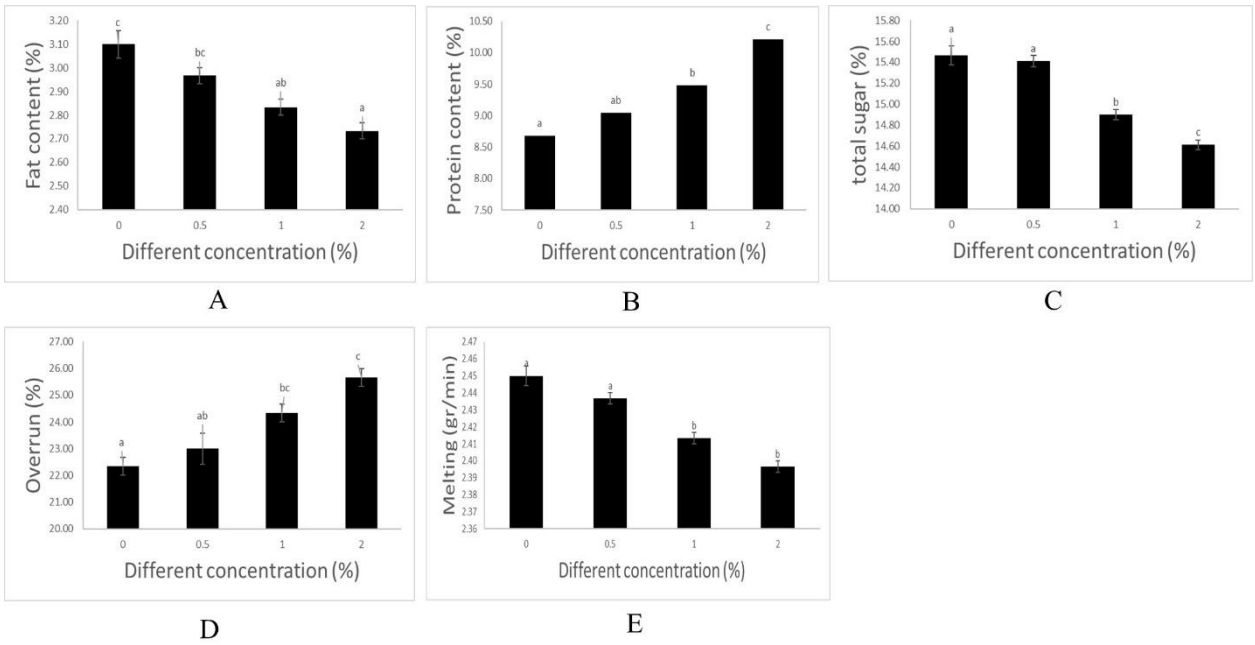


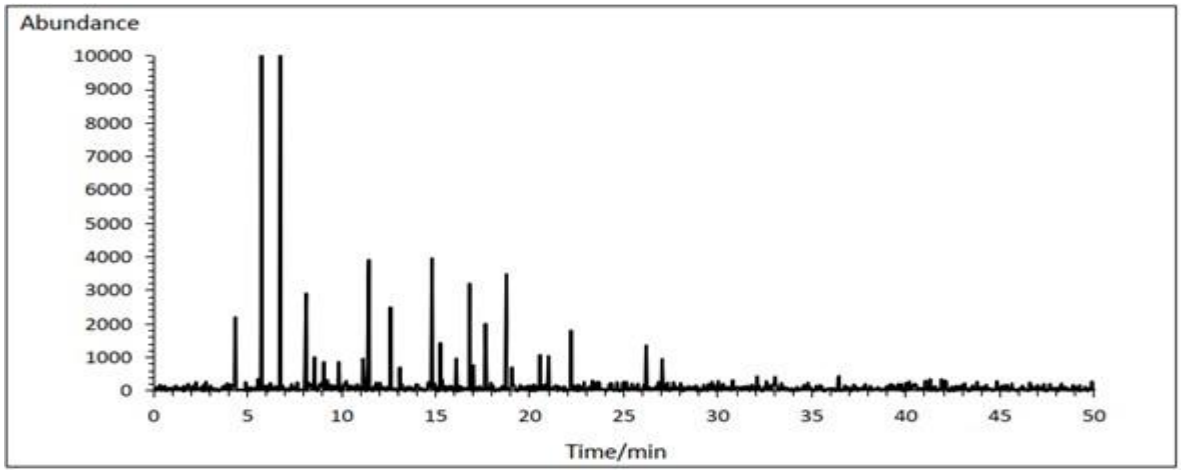
Figure 2. Investigation of fat content (A), protein (B), total sugar (C), overrun (D) and melting (E) fortified ice cream with phycocyanin pigment

3.2. Results of Analysis of Ice Cream Volatile Compounds by GC/MS Technique

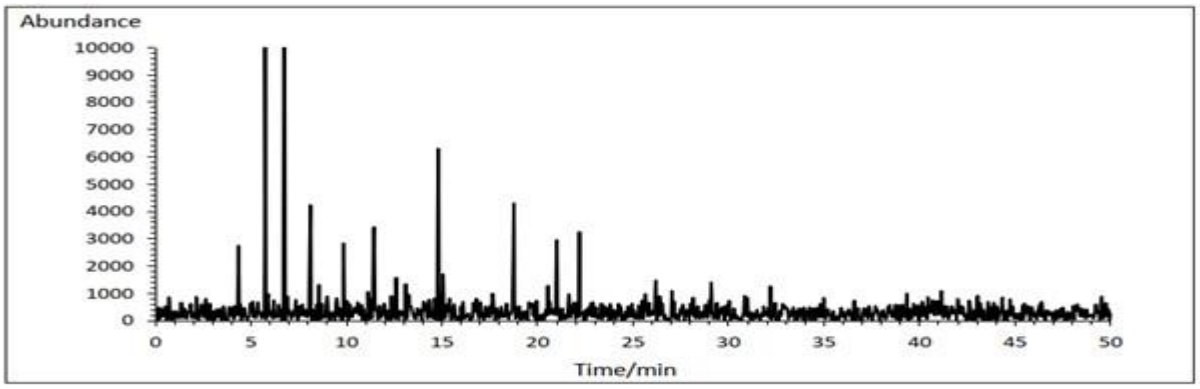
In this study, classification of common volatile compounds in control ice cream and ice cream enriched with 2% concentration of phycocyanin pigment revealed the presence of 7 aldehydes, 4 alcohols, 6 ketones, 3 esters, 2 alkanes, 1 benzene, 1 hexane, and 1 acid. Aldehydes such as acetaldehyde, hexanal, 3-methylbutanal, and 2-methylbutanal, alcohols like ethanol, 3-methylbutanol, and 1-pentanol, esters such as ethyl acetate and ethyl propionate, benzene derivative toluene, acid derivative dimethyl sulfone, alkanes like hexane, acetone and 2-butanone, were found to be more abundant in the control ice cream.

3-methylbutanol, hexanal from the aldehyde group, acetone, and 2-butanone from the ketone group, α-pinene from the terpene group, ethanol, and 3-methyl-1-butanol from the alcohol group, bis (methylthio) methane from the hexanal group, ethyl acetate from the ester group, 1-hexane from the alkane group, and toluene from the benzene group were found to be more abundant in ice cream enriched with 2% concentration of phycocyanin pigment.

Nonanal, 6-methyl-5-hepten-2-one, α-pinene, and n-butanol are volatile compounds found only in ice cream enriched with 2% concentration of phycocyanin pigment and are not present in the control ice cream. Additionally, n-butanol from the alcohol group and α-pinene from the terpene group exhibit a higher abundance in this enriched ice cream. (Table 3)



Graph 1. Frequency of volatile compounds in control ice cream in time (min)



Graph 2. Frequency of volatile compounds of ice cream enriched with 2% phycocyanin pigment in time (min)

Tabel 1. Volatile compounds of control ice cream extracted from gas chromatography mass spectrometry.

Name of Compounds	Molecular Formula	Molecular Weight	RT (min)	Nature of Compounds	Abundance (%)
Acetaldehyde	CH_3CHO	44.05	4.35	Aldehyde	3.315076
Hexanal	$C_6H_{12}O$	100.15888	22.2	Aldehyde	2.714409
3_Methylbutanal	$C_5H_{10}O$	86.13	14.81	Aldehyde	5.955264

3_methyl_1_butanole	$C_5H_{12}O$	88.148	18.77	Alcohol	5.247783
2_Butanone	C_4H_8O	72.11	11.45	Ketone	5.891043
2_Pentanone	$C_5H_{10}O$	86.13	16.1	Ketone	1.454777
Ethanol	C_2H_5OH	46.07	5.74	Alcohol	15.47327
Acetone	C3H6O	58.08	6.74	Ketone	23.55803
Bis(methylthio)methane	C3H8S2	108.23	8.12	Hexanal	4.38388
Methyl acetate	C3H6O2	74,08	8.56	Ester	1.523138
dimethyl sulfone	C2H6O2S	94.14	9.06	Acid	1.30112
Isobutyraldehyde	C4H8O	11.72	9.85	Aldehyde	1.3067492
2 3-butanedione	C4H6O2	86.09	11,13	Ketone	1,448706
1-hexene	C6H12	84,16	11,4	Alkan	2,726569
Ethyl acetate	C4H8O2	88,11	12,6	Ester	3,760377
Isobutanol	C4H10O	74,12	13,1	Alcohol	1,059414
2-methylbutanal	C5H10O	86,13	15,25	Aldehyde	2,1599
1-hexene	C6H12	84,16	16,81	Alkan	4,825948
Pentanal	C5H10O	86,13	17,02	Aldehyde	1,15374
Ethyl propionate	C6H12	84,16	17,65	Ester	3,021329
2-hexanone	C6H12O	100,16	19,05	Ketone	1,618284
1-pentanol	C5H12O	88,15	20,55	Alcohol	1,618284

Toluene	C7H8	92,14	21,01	Benzene	1,564111
2-heptanone	C7H14O	114,19	26,2	Ketone	2,043201
Heptanal	C7H14O	114,19	27,05	Aldehyde	1,431417

Table 2. Volatile compounds of ice cream enriched with 2% concentration of phycocyanin pigment extracted from gas chromatography mass spectrometry.

Name of Compounds	Molecular Formula	Molecular Weight	RT (min)	Nature of Compounds	Abundance (%)
Acetaldehyde	CH_3CHO	44.05	4,35	Aldehyde	3,669985
Hexanal	$C_6H_{12}O$	100.15888	22,2	Aldehyde	4,340712
2_Butanone	C_4H_8O	72.11	11,45	Ketone	4,57819
alpha-Pinene	C10H16	136,23	29,1	Terpene	1,872935
Ethanol	C_2H_5OH	46.07	5,74	Alcohol	14,05928
3_Methylbutanal	C5H10O	86,13	14,81	Aldehyde	8,417297
3_methyl_1_butanole	$C_5H_{12}O$	88.148	18,77	Alcohol	5,732798
n-butanol	C4H10O	74,12	15,05	Alcohol	2,277339
Acetone	C3H6O	58.08	6,74	Ketone	18,83208
Bis(methylthio)methane	C3H8S2	108.23	8,12	Hexanal	5,652246
Methyl acetate	C3H6O2	74,08	8,56	Ester	1,750601
dimethyl sulfone	C2H6O2S	94.14	9,06	Acid	0,424906

Isobutyraldehyde	C4H8O	72.11	9,85	Aldehyde	3,78157
2 3-butanedione	C4H6O2	86.09	11,13	Ketone	1,408533
1-hexene	C6H12	84,16	16,81	Alkan	1,073748
Ethyl propionate	C5H10O	102,13	17,65	Ester	1,322523
1-hexene	C6H12	84,16	11,4	Alkan	3,338512
2-pentanone	C5H10O	86,13	16,1	Ketone	0,918915
Ethyl acetate	C4H8O2	88,11	12,6	Ester	2,101259
Isobutanol	C4H10O	74,12	13,1	Alcohol	1,789126
2-methylbutanal	C5H10O	86,13	15,25	Aldehyde	0,800746
1-pentanol	C5H12O	88,15	20,55	Alcohol	1,705805
Pentanal	C5H10O	86,13	17,02	Aldehyde	0,902051
2-hexanone	C6H12O	100,16	19,05	Ketone	0,702598
Toluene	C7H8	92,14	21,01	Benzene	3,946342
2-heptanone	C7H14O	114,19	26,2	Ketone	1,962821
Heptanal	C7H14O	114,19	27,05	Aldehyde	1,457009
6-methyl-5-hepten-2-one	C8H14O	126,20	30,04	Keton	0,97138
Nonanal	C9H18O	142,24	35,35	Aldehyde	0,208693

Table 3. Volatile compounds enriched only in ice cream with 2% concentration of phycocyanin pigment extracted gas chromatography mass spectrometry.

Name of Compounds	Molecular Formula	Molecular Weight	RT (min)	Nature of Compounds	Abundance (%)
Nonanal	C9H18O	142,24	35,35	Aldehyde	0,208693
6-methyl-5-hepten-2-one	C8H14O	126,20	30,04	Keton	0,97138
alpha-Pinene	C10H16	136,23	29,1	Terpene	1,872935
n-butanol	C4H10O	74,12	15,05	Alcohol	2,277339

1.6. Results of Microbiological Analysis of Enriched Ice Cream

1.6.1. Results of Total Bacterial Count in Enriched Ice Cream

The results of total bacterial count showed that there was no significant difference in the total bacterial count on the first day between the 2% phycocyanin pigment concentration and the control. However, significantly lower levels of total bacteria were observed on the fourteenth, twenty-eighth, forty-second, and fifty-sixth days for the 2% phycocyanin pigment concentration compared to the control, with reductions of 1.04, 0.13, 1.17, and 1.17 times, respectively. (Figure 4-A)

Results of Coliform Bacteria Count in Enriched Ice Cream

No evidence of coliform bacteria was observed on the first, fourteenth, and twenty-eighth days. The level of coliform bacteria on the forty-second day decreased by 3.6 times compared to the control in the 0.5% concentration. The level of coliform bacteria on the fifty-sixth day decreased by 5.13 times compared to the control in the 1% concentration. (Figure 4-B)

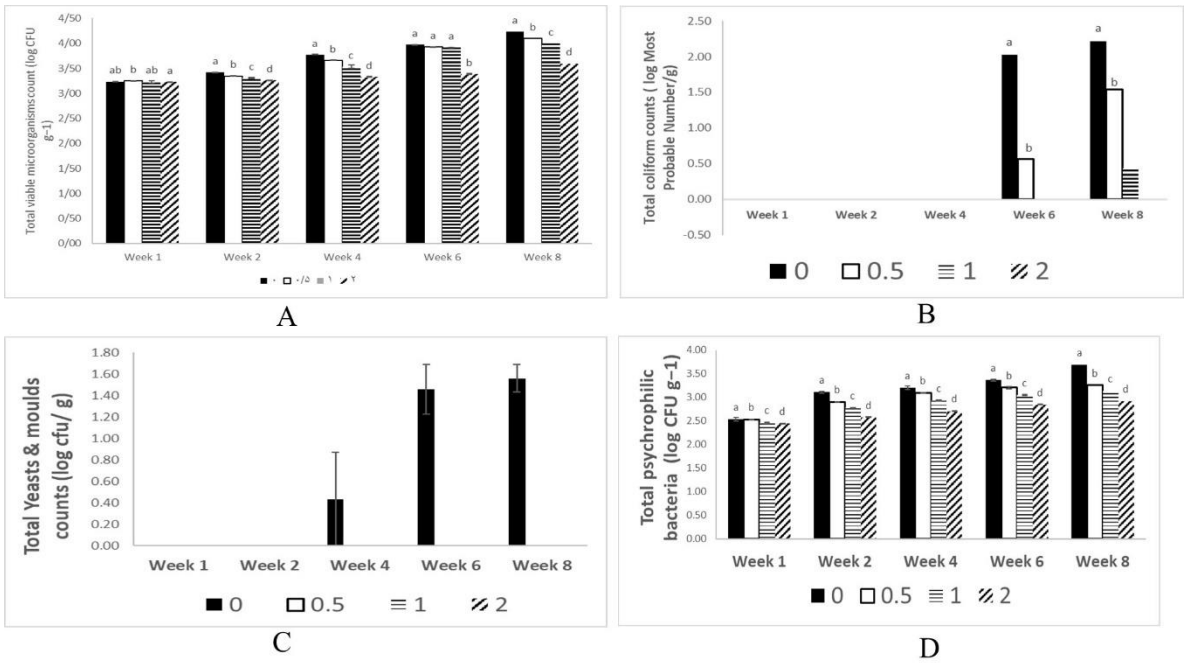


Figure 4. The effect of different concentrations of the phycocyanin pigment reducing the total number of bacteria (A), coliforms (B), yeasts & molds (C) and psychrophilic (D) in ice cream on the 1,3,7,14,21 days

Results of Counting Escherichia coli, Staphylococcus aureus, and Salmonella in Enriched Ice Cream

No evidence of total coliforms, Staphylococcus aureus, and Salmonella bacteria was found on the first, fourteenth, twenty-eighth, forty-second, and fifty-sixth days in the 5.0%, 1%, and 2% concentrations of phycocyanin pigment.

Results of Counting Psychrophilic Bacteria

The results of counting psychrophilic bacteria showed that the level of psychrophilic bacteria on the first day did not have a significant difference. The level of psychrophilic bacteria significantly decreased on the fourteenth, twenty-eighth, forty-second, and fifty-sixth days compared to the control by 1.21, 1.18, 1.18, and 1.26 times, respectively. (Figure 4-D).

Results of Counting Yeast and Mold

No evidence of yeast and mold was found on the first and fourteenth days. The level of yeast and mold on the forty-second and fifty-sixth days in the control was 0.43 and 1.56, respectively. (Figure 4-C).

1.7. Results of Antioxidant Analysis of Enriched Ice Cream

1.7.1. Results of Measurement of Antioxidant Potential by FRAP Method

The results of evaluating the antioxidant potential by the FRAP method showed that on the first, fourteenth, twenty-eighth, forty-second, and fifty-sixth days, the antioxidant potential in the 0.5%, 1%, and

2% concentrations of phycocyanin pigment compared to the control significantly increased at P<0.05 confidence level. The antioxidant potential in the 2% concentration on the first, fourteenth, twenty-eighth, forty-second, and fifty-sixth days increased by 0.71, 0.75, 0.66, 0.63, and 0.54 times, respectively, compared to the control. (Figure 5-A)

Results of Assessment of Antioxidant Activity by DPPH Method

The results of evaluating antioxidant activity by the DPPH method showed a significant decrease in antioxidant activity on the first, fourteenth, twenty-eighth, forty-second, and fifty-sixth days in the 0.5%, 1%, and 2% concentrations of phycocyanin pigment compared to the control, at P<0.05 confidence level. The antioxidant activity on the first, fourteenth, twenty-eighth, forty-second, and fifty-sixth days in the 2% concentration decreased by 1.43, 1.52, 1.52, 1.7, and 2.07 times, respectively, compared to the control. (Figure 5-B)

Results of Assessment of Antioxidant Activity by ABTS Method

The results of evaluating antioxidant activity by the ABTS method showed a significant decrease in antioxidant activity on the first, fourteenth, twenty-eighth, forty-second, and fifty-sixth days in the 0.5%, 1%, and 2% concentrations of phycocyanin pigment compared to the control, at P<0.05 confidence level. The antioxidant activity on the first, fourteenth, twenty-eighth, forty-second, and fifty-sixth days in the 2% concentration decreased by 1.31, 1.48, 1.67, 1.84, and 2 times, respectively, compared to the control. (Figure 5-C)

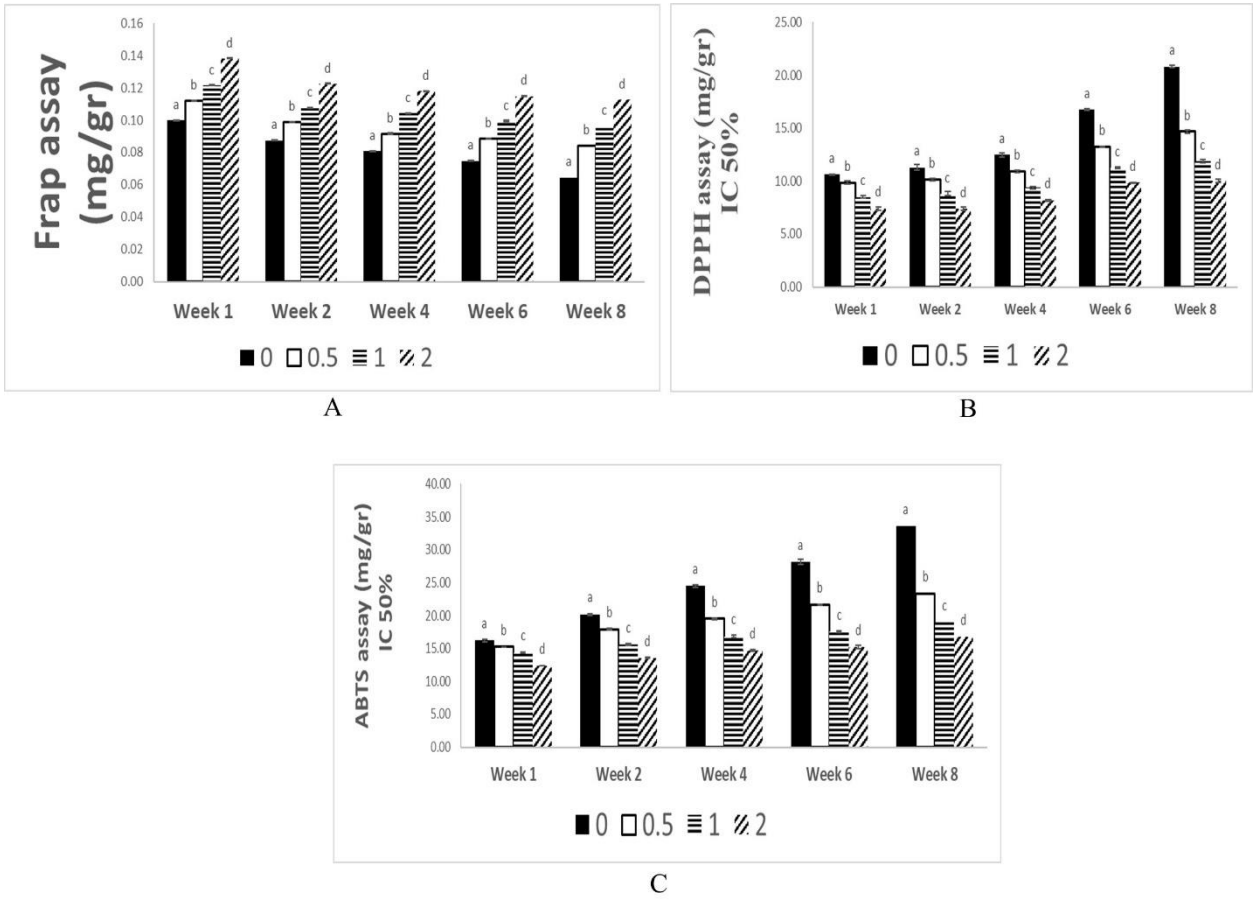


Figure 5. Assessment of FRAP(A), DPPH(B) and ABTS (C) in fortified ice cream with phycocyanin pigment.

1.8. Results of Sensory Evaluation of Enriched Ice Cream

The sensory evaluation results of ice cream enriched with phycocyanin pigment showed no significant difference in taste, color, smell, texture, and consistency of ice cream in the 0.5%, 1%, and 2% concentrations of phycocyanin pigment compared to the control at confidence level P<0.05. However, there was a significant difference in aroma evaluation at the 2% concentration compared to the 0.5% concentration and the control, at confidence level P<0.05. There was no significant difference in overall acceptance between the 0.5%, 1%, and 2% concentrations of phycocyanin pigment compared to the control at confidence level P<0.05. In fact, the satisfaction level decreased with the increase in phycocyanin concentration, leading to reduced satisfaction with aroma and overall acceptance. (Figure 6).

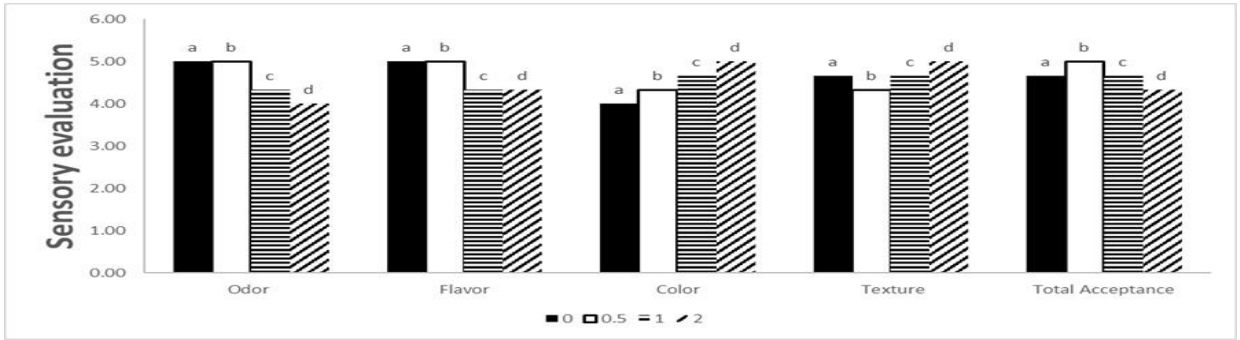


Figure 6. Sensory evaluation of phycocyanin pigment enriched ice cream

Ice cream is one of the most popular frozen dairy products worldwide, consisting of a complex food emulsion with ice crystals, dispersed fat, air cells, protein-hydrocolloid structures, and an unfrozen aqueous phase. There are various types of ice cream, differing in additives and manufacturing methods [35]. Dairy products, such as ice cream, can act as carriers for pathogenic bacteria [36]. Pathogenic bacteria that can potentially contaminate food products such as milk include Staphylococcus aureus, Coliform, Escherichia coli, Salmonella, psychrophilic bacteria, etc., causing many foodborne illnesses and human diseases [37].

Contamination of ice cream with Staphylococcus aureus occurs through raw milk contamination or improper storage. This bacterium is capable of producing enterotoxins, leading to food poisoning [38]. The distribution and prevalence of Staphylococcus aureus in various food items pose a significant threat to food safety [39].

Studies conducted on 144 samples of traditional ice cream in the Urmia County revealed a very high level of contamination, with 78% of traditional ice cream production centers in Urmia exceeding the permissible bacterial limit of 4.2×10^7 CFU/g [40]. Additionally, 37.08% of the ice cream samples tested in the surrounding areas of Shiraz were reported to have exceeded the national standard limit for total bacterial count in Iran [41]. Staphylococcus aureus is considered one of the most common etiological factors in bacterial diseases. Food poisoning caused by this bacterium has been cited as the third leading cause of food poisoning worldwide [42]. Staphylococcus aureus is also one of the most common causes of foodborne illnesses in the United States [43]. In Libya, 19% of the ice creams examined were found to be contaminated with Staphylococcus aureus, with no significant difference observed between unpasteurized and packaged ice creams [44].

In some studies, higher percentages of contamination with this bacterium have been reported, namely 57% (2) and 36.6% [45]. Similar investigations have been conducted in other countries as well. For instance, in Turkey, 55% of ice cream samples were found to be contaminated with Staphylococcus aureus [46], and in a study conducted in Senegal, 45% of ice cream samples were contaminated with Staphylococcus aureus [47].

Under certain conditions, milk and its derivatives can promote the growth of pathogenic agents, including Escherichia coli, which is the most significant food contaminant [48]. According to research, infections related to Escherichia coli represent the most common type of foodborne infections. Escherichia coli producing Shiga toxin (STEC) as a pathogen can be transmitted through food, causing symptoms such as diarrhea or bloody diarrhea [49]. Based on reports by Salehian et al. in the city of Sari, the contamination rate of traditional ice cream with this bacterium was reported at 52% [50]. This study indicates that the prevalence of bacterial species isolated from ice creams related to Escherichia coli is 50%, indicating poor hygiene practices during production and post-production processes.

Salmonella is one of the major causes of food poisoning. Salmonella infection can lead to complications and fatalities worldwide. The bacteria causing this disease can actively reside in the bodies of animals and humans [51]. Furthermore, in an examination of traditional ice creams in Urmia, it was found that 40% of the samples tested were contaminated with Salmonella [52]. The growth of mold in dairy products causes economic damage by weakening texture and altering color, aroma, and taste. More seriously, some molds are capable of producing mycotoxins such as Aflatoxin, Patulin, and Citrinin, some of which are known carcinogens [53]. In a study conducted by Behbahani et al. on the microbial contamination of ice creams in Tehran, mold contamination was reported in 73% of traditional ice creams [54]. Investigations on ice creams in Baqubah, Iraq, showed that these ice creams were free of mold and yeast [55]. A report by Barman et al. on ice creams in Kolkata revealed a significant number of fungi and yeast, ranging from 1.75×102 to 1.95×104 cfu/g [56]. A study conducted in Nigeria observed contamination with various types of molds in the ice cream samples examined [57].

Psychrophilic bacteria play a role in the spoilage of food products such as meat, poultry, fish, and dairy products, and they can proliferate and grow even at temperatures close to zero [58]. In the research by Fallah Rad et al., it was determined that the contaminating bacteria in raw milk in Mashhad were mostly psychrophilic bacteria [59]. A study conducted on ice cream in Nigeria showed that the examined ice creams were contaminated with psychrophilic bacteria of the Pseudomonas genus [60]. A study in Sweden aimed to identify the factors contributing to the spoilage of raw and pasteurized milk, revealing that the main bacteria causing spoilage in raw milk are psychrophilic bacteria [61]. If the pasteurization conditions and personal hygiene are not fully observed in the production and distribution of these products, the ice cream produced will be contaminated by various bacteria, leading to numerous economic and health problems at the community level [62]. In recent years, there has been an unprecedented increase in demand for fermented dairy products due to medical recommendations and lifestyle changes [63]. With its coloring, antioxidant, and antimicrobial properties, Phycocyanin has the potential for use in various food formulations such as yogurt, cheese, ice cream, etc., and its use and effects have been proven in various studies [12].

In the current study, by adding concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment to the ice cream, the levels of Staphylococcus aureus, Escherichia coli, Salmonella, mold, and yeast were not observed. The total counts of coliforms, psychrophilic bacteria, and total bacterial counts decreased compared to the control. A study in 2021 reported a high antibacterial property for spirulina phycocyanin pigment against Staphylococcus aureus [64].

In this study, on the first day (Day 1), the total bacterial counts in the control sample and concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment showed no significant difference. On the fourteenth

day (Day 14), the total bacterial counts in the control sample were 3.42, and with the addition of spirulina phycocyanin pigment, the total bacterial counts decreased. The total bacterial counts in concentrations of 0.5%, 1%, and 2% were 3.35, 3.31, and 3.26, respectively. On the twenty-eighth day (Day 28), the total bacterial counts in the control sample were 3.77, and with the addition of spirulina phycocyanin pigment, the total bacterial counts decreased. The total bacterial counts in concentrations of 0.5%, 1%, and 2% were 3.66, 3.53, and 3.33, respectively. On the forty-second day (Day 42), the total bacterial counts in the control sample were 3.97, and the total bacterial counts decreased with the addition of spirulina phycocyanin pigment. The total bacterial counts in a concentration of 2% were 3.39. On the fifty-sixth day (Day 56), the total bacterial counts in the control sample were 4.23, and the total bacterial counts decreased with the addition of spirulina phycocyanin pigment. Specifically, the total bacterial counts in concentrations of 0.5%, 1%, and 2% were 4.10, 3.99, and 3.59, respectively. Similar to the studies by Takyar et al. [65], adding spirulina phycocyanin pigment had an inhibitory effect on the total microbial count.

In this study, on days one (Day 1), fourteen (Day 14), and twenty-eight (Day 28), the total coliform counts in the control sample and concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment were negative and not observed. On the forty-second day (Day 42), the total coliform counts in the control sample were 2.2, and with the addition of spirulina phycocyanin pigment, the total coliform counts decreased. The total coliform counts were negative and not observed in samples with concentrations of 1% and 2%, and only in the 0.5% concentration sample, it was 0.56. On the fifty-sixth day (Day 56), the total coliform counts in the control sample were 2.21, which decreased with the addition of spirulina phycocyanin pigment. Specifically, the total coliform counts in concentrations of 0.5% and 1% were 1.53 and 0.43, respectively, and were negative and not observed in the 2% concentration sample. Similar to the findings of Kheradmand and Khandagh (2021), it had an inhibitory effect on total coliform counts [66].

In the present study, the counts of Escherichia coli at all concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment were negative and not observed. Zanganeh et al. (2022) reported the inhibitory effect of different concentrations of spirulina on the growth of Escherichia coli [67]. Similarly, in this study, the counts of Salmonella at all concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment were negative and not observed. Consistent with the findings of Rasouli et al. (2017), it showed an inhibitory effect on Salmonella [68]. In this study, on the first day (Day 1), the count of psychrophilic bacteria in the control sample and the sample with a concentration of 0.5% was 2.53, indicating a decrease with the addition of spirulina phycocyanin pigment. The counts of psychrophilic bacteria in concentrations of 1% and 2% were 2.46 and 2.44, respectively. On the fourteenth day (Day 14), the count of psychrophilic bacteria in the control sample was 3.11, decreasing with the addition of spirulina phycocyanin pigment. The counts of psychrophilic bacteria in concentrations of 0.5%, 1%, and 2% were 2.90, 2.78, and 2.57, respectively. On the twenty-eighth

day (Day 28), the count of psychrophilic bacteria in the control sample was 3.20, decreasing with the addition of spirulina phycocyanin pigment. The counts of psychrophilic bacteria in concentrations of 0.5%, 1%, and 2% were 3.09, 2.93, and 2.84, respectively. On the forty-second day (Day 42), the count of psychrophilic bacteria in the control sample was 3.36, decreasing with the addition of spirulina phycocyanin pigment. The counts of psychrophilic bacteria in concentrations of 0.5%, 1%, and 2% were 3.20, 3.05, and 2.84, respectively. On the fifty-sixth day (Day 56), the count of psychrophilic bacteria in the control sample was 3.68, decreasing with the addition of spirulina phycocyanin pigment. The counts of psychrophilic bacteria in concentrations of 0.5%, 1%, and 2% were 3.25, 3.13, and 2.91, respectively. Similar to the results of Elschon Jaber et al., it demonstrated an inhibitory effect on psychrophilic bacteria [69].

In the present study, the counts of mold and yeast on the first day (Day 1) and the fourteenth day (Day 14) at all concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment were negative and not observed. However, on the twenty-eighth day (Day 28), forty-second day (Day 42), and fifty-sixth day (Day 56), mold and yeast were only observed in the control sample, with counts of 0.43, 1.45, and 1.56, respectively. Similar to the findings of Souza et al. (2011), the addition of spirulina phycocyanin pigment exhibited an inhibitory effect on mold and yeast [70].

Spirulina is rich in protein and can be utilized as a complete and beneficial food source [71]. Tahami et al. (2019) investigated the effect of spirulina nutrition on the characteristics of processed cheese, showing that an increase of up to 6% spirulina leads to enhancements such as increased protein content [72]. In the present study, the protein content in the control ice cream was 8.86%, while the addition of 0.5%, 1%, and 2% concentrations of spirulina phycocyanin pigment increased the protein content to 9.04%, 9.48%, and 10.21%, respectively. Therefore, it can be used to enhance the protein content of food products with low protein content. Di Marco et al. (2014) used Spirulina platensis algae in pasta formulation, resulting in a significant increase in protein content in enriched samples [73]. Spirulina contains essential fatty acids such as linoleic acid (LA), gamma-linolenic acid (GLA), and palmitic acid [74]. GLA plays a crucial role in improving human body performance, and spirulina is one of the richest sources of GLA [75]. In the present study, the fat content in the control ice cream was 3.10%, while the addition of 0.5%, 1%, and 2% concentrations of spirulina phycocyanin pigment reduced the fat content to 2.97%, 2.83%, and 2.73%, respectively.

Faresin et al. reported a reduction in fat content in ice cream by up to 50% with the addition of inulin (2%) and spirulina (1%) [76].

Air is an essential component of ice cream. The air present in ice cream creates a light texture and affects its physical properties, melting, and hardness [77]. There are numerous factors that influence the growth of air cells in ice cream [78]. Axon (2009) acknowledged that adding spirulina microalgae to ice cream leads to an increase in the overrun volume of the ice cream mixture during homogenization [79]. In the

present study, the overrun in the control ice cream was 22.33%, while adding concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment increased the overrun to 23%, 24.33%, and 25.67%, respectively. Physical analysis results, such as overrun in ice cream, which leads to an increase in the volume of ice cream due to trapped air during mixing and freezing inside the ice cream maker, showed that the overrun in ice cream with spirulina powder is higher compared to overrun without adding powder [80]. According to the evaluation results, the addition of phycocyanin caused a significant reduction ($p>0.05$) in the melting rate at concentrations of 1.5% and 0.2% phycocyanin, compared to the control ice cream [81]. In this regard, Malick et al. (2013) reported that substituting stabilizers with spirulina in ice cream improves its resistance to melting, increasing the melting resistance from 13 minutes in the control sample to 17.8 minutes. This is due to the high protein content of spirulina, which helps stabilize air molecules, thereby increasing the resistance to melting of ice cream [82]. In the present study, the melting rate in the control ice cream was 2.45%, while adding concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment reduced the melting rate to 2.44%, 2.41%, and 2.40%, respectively. Safari et al. reported a significant decrease in the melting percentage of ice cream by adding phycocyanin and maltodextrin and sodium caseinates coatings [12].

Agostini et al. reported that the minimum sugar content (sucrose) in ice cream should be 8%. Before adding Spirulina platensis, this amount was completely exceeded, and after adding Spirulina platensis to the ice cream, there was a significant difference in reducing the total sugar content. The results showed that adding 1% and 1.2% Spirulina platensis had a significant effect on reducing the total sugar content [83]. In the present study, the total sugar in the control ice cream was 15.47%, while adding concentrations of 0.5%, 1%, and 2% of spirulina phycocyanin pigment reduced the total sugar to 15.41%, 14.90%, and 14.61%, respectively.

Due to the undesirable effects such as mutagenicity and carcinogenicity associated with synthetic antioxidants, some of them have gradually been removed from the list of consumable antioxidants. Therefore, the preparation and production of natural antioxidants as suitable substitutes are necessary, which also increases their nutritional value [84]. Nowadays, the use of a wide range of medicinal plants and their aromatic compounds as natural sources with antioxidant properties has attracted the attention of researchers [85]. Therefore, research on plant essences as a safe alternative is progressing. These substances are used in Iran and worldwide to improve the performance and stability of various products such as ice cream.

Li et al. (2022) investigated the composition and antibacterial activity of Amomum tsao-ko essence based on different regions using GC-MS and GC-IMS. The volatile compounds mainly included terpenes and aldehydes, with 1-8-cineole, (E)-dec-2-enal, citral, α-pinene, and α-terpineol being the main components. Amomum tsao-ko essence exhibited strong antimicrobial properties. Moreover, it showed activity against Staphylococcus aureus (S. aureus) and demonstrated minimum inhibitory

concentration and minimum bactericidal concentration. In the present study, α-pinene was found in ice cream enriched with 2% phycocyanin pigment [86].

Qadiry et al. (2014) conducted research on the GC-MS analysis and antibacterial, antioxidant, and anticancer activities of Pinus roxburghii oil essence from Kashmir, India. α-pinene and β-pinene were the major compounds present in this oil. This essence showed significant antibacterial and anticancer activities, with negligible antioxidant activity. In the present study, α-pinene was found in ice cream enriched with 2% phycocyanin pigment [87].

Ferdosi et al. (2021) conducted an analysis of n-butanol from the extract of CASSIA FISTULA flowers through GC-MS and identified antimicrobial compounds. The volatile compounds included cyclohexene, 1-butyl-9-heptadecanol, behenic alcohol, decane, 3-methyl, 1-methyl-3(1-methylethenyl), cis-, 3-hexanol, 5-methyl, acytaldehyde isopentyl propyl acetal, undecane, 1,3-dioxane, 2-ethyl-5-methyl, acetaldehyde butyl pentyl acetal, acetaldehyde dipentyl acetal, cycloheptasiloxane, tetradecamethyl cyclooctassiloxane, hexadecamethyl cyclononasiloxane, octadecamethyl, n-hexadecanoic acid tetracosamethyl-cyclododecasiloxane. Investigations showed that cyclononasiloxane, hexadecamethyl cyclononasiloxane, octadecamethyl, behenic alcohol, n-butanol, and butyl-cyclooctasiloxane have antibacterial, antifungal, and antiviral properties. In the present study, n-butanol was found in ice cream enriched with 2% phycocyanin pigment [88].

Qureshi et al. (2019) investigated the total polyphenolic compounds, total flavonoids, GC-MS analysis of volatile compounds, antioxidant activity, and antimicrobial activity of Prunus dulcis seeds. Volatile compounds in the hexane and chloroform ethanol extracts of almond skin included 1,1,3,3-tetramethyl cyclopentane and 6-octadecenoic acid, respectively. The results showed that only n-butanol extract exhibited slight activity against the gram-negative bacterium E. coli, and all extracts showed very low antioxidant activity [89]. In the present study, n-butanol was found in ice cream enriched with 2% phycocyanin pigment.

Fausto et al. (2020) researched the phytochemical composition, antioxidant, cytotoxic, and antimicrobial activities of ethanol extract of Brown Propolis from Mexico. The main volatile compounds included nonanal, α-pinene, and neryl alcohol. The study demonstrated antioxidant and antimicrobial activities in Brown Propolis [90]. In the present study, nonanal was found in ice cream enriched with 2% phycocyanin pigment.

Esmaeili et al. (2018) studied the essential oil composition, total phenolic content, flavonoid content, and antioxidant activity of Oliveria decumbens Vent. at different phenological stages. The main volatile compounds during the vegetative stage were γ-terpinene, thymol, carvacrol, and n-nonanal. The results indicated that the essential oil possessed antioxidant and antimicrobial capacities [91]. In the present study, nonanal was found in ice cream enriched with 2% phycocyanin pigment.

Ould Bellahcen et al. (2019) investigated the chemical composition and antibacterial

activity of Spirulina platensis essence from Morocco. The volatile compounds included heptadecane, tetradecane, ethyl benzene, 6-methyl-5-hepten-2-one, and geosmin. The S. platensis essence demonstrated effective antimicrobial properties, exhibiting complete inhibition against Staphylococcus aureus [92]. In the present study, 6-methyl-5-hepten-2-one was found in ice cream enriched with 2% phycocyanin pigment.

Alavi et al. (2011) studied the effect of thermal processing on the chemical composition and antioxidant properties of Lippia citriodora essence. The volatile compounds included R-curcumene, caryophyllene oxide, 6-methyl-5-hepten-2-one, and spathulenol. The essence exhibited significant antioxidant properties [93]. In the present study, 6-methyl-5-hepten-2-one was found in ice cream enriched with 2% phycocyanin pigment.

According to recent studies and research, Spirulina cells in combination with other products can even act as effective antioxidants [16]. Spirulina extract exhibits significant and potent performance in scavenging hydroxyl radicals, which are among the strongest oxygen radicals [94]. Positive effects of antioxidant activity of the compounds present in Spirulina, including phycocyanins, selenium, and carotenoids, have been reported in a study using the FRAP (Ferric Reducing Antioxidant Power) method. These compounds have shown notable radical scavenging ability. Systematic investigations have demonstrated that this alga has been able to improve various symptoms and may even possess anticancer, antiviral, and antiallergic effects, playing a significant role in the treatment of allergy-related diseases, inflammations, oxidative stress, and viruses [96]. The results obtained from evaluating the antioxidant potential using the FRAP method have shown a significant increase in antioxidant potential at concentrations of 0.5%, 1%, and 2% phycocyanin pigment compared to the control, at confidence level $P<0.05$. Specifically, the antioxidant potential at a concentration of 2% has increased significantly on days one, fourteen, twenty-eight, forty-two, and fifty-six, with values of 0.71, 0.75, 0.66, 63.0, and 0.54, respectively, compared to the control.

Spirulina activates cellular antioxidant enzymes, inhibits lipid peroxidation and DNA damage, scavenges free radicals, and increases the activity of superoxide dismutase and catalase [16]. In a study conducted in 2017, researchers reported the high antioxidant potential of ice cream by adding Spirulina, attributing this antioxidant activity to the presence of phycocyanins, the predominant pigment in Spirulina. According to the results of a study on the effect of Spirulina on the quality parameters of ice cream using the DPPH method, it was shown that the antioxidant power decreases over time [98].

The results obtained from evaluating the antioxidant activity using the DPPH method showed a significant decrease in antioxidant activity on days one, fourteen, twenty-eight, forty-two, and fifty-six at concentrations of 0.5%, 1%, and 2% phycocyanin pigment compared to the control, at confidence level $P<0.05$. Specifically, the antioxidant activity on days one, fourteen, twenty-eight, forty-two, and fifty-six at a concentration of 2% decreased by 1.43, 1.52, 1.52, 1.7, and 2.07 times, respectively, compared to the

control. Takyar et al. (2019) reported a statistically significant increase in antioxidant activity of Spirulina in higher concentrations in the DPPH assay [65].

The ABTS radical is a stable artificial radical used with high sensitivity to evaluate the antioxidant activity of various compounds. This method is based on the reduction of the ABTS radical, which has high absorbance at 734 nanometers. This method requires the production of the ABTS chromophore in the presence of an oxidizing agent (usually potassium persulfate). In a study in 2015, the antioxidant and antimicrobial activity of butanolic extract of Spirulina platensis was reported using the ABTS method [97]. The results obtained from evaluating the antioxidant activity using the ABTS method showed a significant decrease in antioxidant activity on days one, fourteen, twenty-eight, forty-two, and fifty-six at concentrations of 0.5%, 1%, and 2% phycocyanin pigment compared to the control, at confidence level $P<0.05$. Specifically, the antioxidant activity on days one, fourteen, twenty-eight, forty-two, and fifty-six at a concentration of 2% decreased by 1.31, 1.48, 1.67, 1.84, and 2 times, respectively, compared to the control. Shalaby and Shanab also found in their study on the antioxidant activity of aqueous and methanolic extracts of Spirulina platensis using the ABTS method that the methanolic extract of this alga exhibits about 50% higher antioxidant activity compared to its aqueous extracts [99].

Sensory characteristics play a crucial role in the acceptance of food products. Kheradmand and Khandagh (2021) investigated the effect of Spirulina algae on the sensory properties of dairy desserts. In this study, Spirulina algae were used at concentrations of 0.5%, 1%, and 1.5%. Sensory analysis results showed that the highest scores were allocated to control samples and desserts with lower amounts of Spirulina [66]. Statistical analyses using one-sided variance and Tukey's post hoc test showed no significant difference in the evaluation of taste, aroma, color, texture, and consistency of ice cream at concentrations of 0.5%, 1%, and 2% phycocyanin pigment compared to the control, at confidence level $P<0.05$. However, there was a significant decrease in aroma acceptance at a concentration of 2% compared to 0.5% concentration and the control, at confidence level $P<0.05$. There was no significant difference in overall acceptance among concentrations of 0.5%, 1%, and 2% phycocyanin pigment compared to the control, at confidence level $P<0.05$.

3-Conclusion:

The results of the study demonstrated that adding concentrations of 0.5%, 1%, and 2% of phycocyanin pigment to ice cream over days 1, 14, 28, 42, and 56, and with longer duration, resulted in a reduction in contamination of ice cream by bacteria such as Escherichia coli, Staphylococcus aureus, Salmonella, and Coliform. Moreover, the levels of mold and yeast were only found in control samples on days 28, 42, and 56, and it reduced the total bacterial count. The increase in antioxidant activity, protein content, and aeration, as well as the reduction in fat, melting rate, and total sugar, were observed outcomes of this study. Furthermore, the results of volatile compounds obtained from GC-MS analysis

in the control sample and ice cream enriched with 2% phycocyanin pigment included 7 aldehydes, 4 alcohols, 6 ketones, 3 esters, 2 alkanes, 1 benzene, 1 hexane, and 1 acid, some of which, like nonanal, alpha-pinene, n-butanol, and 6-methyl-5-hepten-2-one, have antimicrobial properties. Based on the results obtained in the aroma evaluation, increasing the phycocyanin pigment reduced its acceptability, but the acceptability of other parameters may meet the demand level of the community for enriched products. Therefore, it calls for optimal performance, realization, and development in the use of phycocyanin pigment in the food industry of Iran.

5-Acknowledgments:

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کاربرد صنعتی رنگدانه طبیعی خوراکی فیکوسیانین مستخرج از سیانوباکتری *Spirulina platensis* در تهیه بستنی با تاکید بر ویژگی های میکروبی و آنتی اکسیدانی

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چکیده

رنگدانه فیکوسیانین مستخرج از سیانوباکتری *اسپیرولینا پلاتنسیس* به دلیل خواص منحصر به فرد، می تواند نقش مهمی در غنی سازی بستنی داشته باشد. در این مطالعه میزان پروتئین، چربی، قند کل، هوادهی، نقطه ذوب بستنی غنی شده با غلظت های متفاوت رنگدانه فیکوسیانین تعیین شد. علاوه برآن شمارش باکتری ها به همراه ارزیابی خاصیت آنتی اکسیدانی انجام گردید. همچنین آزمایش GC/MS جهت شناسایی ترکیبات فرار انجام گردید. نتایج حاصل از آزمایشات نشان داد که میزان چربی و قند کل ، نقطه ذوب ، ABTS و DPPH بستنی های غنی شده کاهش معناداری نسبت به کنترل داشته است. همچنین میزان پروتئین، هوادهی، FRAP و به افزایش معناداری نسبت به کنترل داشته است. ارزیابی حسی نیز تفاوت معناداری در طعم ، مزه ، رنگ و قوام بستنی نسبت به کنترل نداشته است جز حس بویایی که نسبت به کنترل کاهش داشته است. علاوه بر آن، هیچ نشانی از حضور *اشرشیا کلی* ،استافیلوکوکوس *ارئوس و سالمونلا* در طی روزهای مختلف یافت نگردید. با این حال، حضور باکتری *کلی فرم* از روزهای ۴۲ تا ۵۶ در بستنی های غنی شده و کنترل مشهود بود. همچنین حضور باکترهای *کپک و مخمردر* طی روز های ۲۸ تا ۵۶ فقط در بستنی کنترل مشاهده گردید. میزان باکتری های *سرمادوست* از هفته ی ۲ تا هفته ۸ کاهش پیدا کرد . نتایج ترکیبات فرار حاصل از آزمایش GC/MS در بستنی شاهد و بستنی غنی شده با ۲٪ رنگدانه فیکوسیانین نشان از حضور ترکیباتی با خاصیت ضد میکروبی و آنتی اکسیدانی دارد که نقش مهمی در ماندگاری و کیفیت بستنی دارد. امید است نتایج حاصل از این مطالعه، زمینه ساز توانمندسازی صنایع غذایی در استفاده از رنگدانه های حاصل از سیانوباکتری ها باشد.