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Investigation of The effect of Phycocyanin Extracted From *Spirulinaplatensis* and Persimmon Powder on Physicochemical and Sensory Characteristics of Yogurt

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YOGURT is one of the most widely consumed fermented products obtained from milk that has a positive effect on health due to its high nutritional value. Yogurt enrichment can play a major role in the developmentof consumers' health level. *Spirulina platensis*is a blue-green microalga and is rich in protein, essential fatty acids, vitamins, minerals and antioxidant compounds, such as phycocyanin. The aim of the present study was to investigate the effect of phycocyanin extracted from *S. platensis* and persimmon powder on physicochemical and sensory characteristics of yogurt. For this purpose, the yoghurt samples were analyzed for their physicochemical characteristics. Samples without persimmon powder and containing higher amounts of phycocyanin (1% and 1.5%) had the highest acidity. The acidity values of all samples increased over time. The highest pH belonged to the control sample and the addition of different amounts of phycocyanin and persimmon powder caused a significant reduction in pH.In all time intervals, with the increase of persimmon powder and phycocyanin, the syneresis rate of samplesincreased though it was not significant compared to the control sample. Samples containing higher amounts of persimmon powder (codes 6 and 7) had higher dry matter, protein, and water holding capacity (WHC).On both studied days, the highest and the lowest viscosity belonged to treatment code 7 andcode 2, respectively ($p \leq 0.05$). Additionally, the viscosity of all treatments increased significantly from the first day to the fifteenth day over time ($p \leq 0.05$). The control sample had the highest lightness (L*) and the treatment code 8 had the highest redness (a *). In all time intervals, the highest hardness and springiness belonged to the control sample ($p \leq 0.05$). The results of sensory evaluation of the samples showed that on the first day, the color score of the control sample and treatment codes 5, 6 and 7 was significantly higher than other treatments ($p \leq 0.05$). On all days studied, the taste scores of the control sample and treatment codes 5 and 6 and the texture score of the control sample and treatment codes 6, 7, and 8 were significantly higher than other treatments ($p \leq 0.05$).Considering the sensory evaluation and protein content of the yogurt samples, treatment code 6 was selected as the best treatment. The results showed that phycocyanin extracted from *S. platensis* and persimmon powder is effective on the physicochemical and sensory properties of yogurt and can be used as an effective additive.

Keywords: *Spirulina platensis*, Phycocyanin, Yogurt.

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Introduction

Yogurt is one of the most popular dairy products, which is widely consumed worldwide. Milk and its products consumption is growing rapidly across the world. Yogurt is one of the widely consumed fermented milk products, which has a positive effect on health due to its high nutritional value[1]. Yogurt's high reputation and consumption is due to its nutritional value and beneficial effects of starter bacteria [2]. This product is more nutritious than milk owing to its higher concentration, better fat digestibility and absorption, lactose, protein and minerals, and is among sources rich in calcium, phosphorus, riboflavin, vitamin B12, nicotinic acid (niacin), vitamin B5, zinc and magnesium[3]. Yogurt is also a more easily digestible product than milk and is capable of preserving the gastrointestinal microbial flora [4].

Microalgae are important biological resources for production and application of new products due to the equilibrium of chemical compounds. They contain highly valuable substances, such as unsaturated fatty acids, pigments, antioxidants, pharmaceutical compounds and other biologically-active compounds [5].

Spirulina platensis, a blue-green microalga, with sever alnutritional and therapeutic effects has been usedin the enrichment of various food products[5]. Spirulina is one of the richest additives in terms of protein, essential fatty acids, such as gamma-linolenic acid (GLA), vitamins, especially vitamin B12 and vitamin A precursor, minerals, especially iron and calcium and antioxidant compounds such as phycocyanin.*S. platensis* is a potential drug agent to treatoxidative stress-induced diseases due to having antioxidant components and compounds, such as phycocyanin, selenium and carotenoids [5].

Phycocyanin is one of the main constituents of spirulina microalgae, which is used as a dietary supplement. Research studies show that phycocyanin extracted from *S. platensis* is used as a natural pigment in various foods, such as dairy products, jellies, gum and pastilles. Despite its low resistance to heat and light, this pigment is more versatile to gardenia and indigo and gives a bright color to pastilles and coated candies [6].

Phycocyanin has antioxidant and anti-inflammatory properties. It is used in the treatment of Parkinson'sdisease, Alzheimer'sdisease, cancer, renal diseases, hypertension, central nervous system disorders and skin-care compounds and its therapeutic effects have been well proven [6].

Numerous research studies have been conducted to improve the physicochemical and rheological properties of yogurts e.g.,the addition of compounds such as inulin, whey protein, gum tragacanth, basil gum, etc., for better texture and consistency of yogurt [7].

Many plant-based yogurts have been prepared to improve the texture and physicochemical properties of yogurt, such as soy yogurt, peanut butter yogurt, and hawk tea yogurt [8]. Persimmon (Diospyros Thunb kaki called Persimmon in English) belongs to the genus Ebenaceae, and is a subtropical fruit. Persimmon fruit contains significant amounts of vitamins B1, B2, B3 and C and beta-carotene and lycopene pigments and is able to contribute to making the human body immune against cardiovascular diseases and some cancers [9].

In this research, different ratios of phycocyan in extracted from *S. platensis* and persimmon powder were used as the thickening agent and masking unfavorable taste of algae in yogurt production and the effect of these compounds on the physicochemical and sensory properties of yogurt was evaluated.

Materials and Methods

Preparation of algae

*S. platensis*was identified and isolated in the Caspian Sea (northern Iran). Phycocyanin, which is a natural pigment with protein and antioxidant properties, was extracted from *S. platensis* by the knowledge-based Zhen Fanavar Kio Co. Phycocyanin confirmation by HPLC analysis and standard were purchased from Sigma brand by the above mentioned company and approved.

Yogurt preparation method

First, the whole cow's raw milk (containing 3% fat) was concentrated to increase the dry matter percentage of yogurt with the addition of skim milk powder (2% w/w). The milk was then heated at 90 °C for 5 minutes and after cooling to 43 ±1 °C was divided into seven equal parts. Then phycocyanin extracted from *S. platensis* and persimmon powder was added to it.

TABLE 1. Characteristics of the tested treatments.

Code	Phycocyanin (%)	Persimmon powder (%)
CY	0	0
PY1	0.5	0
PY2	1.0	0
PY3	1.5	0
PPY1	0.5	2
PPY1	1.0	2
PPY3	1.5	2

Yogurt starter (3%) was added to all of the above mentioned parts and poured into 200 ml plastic glasses. In the next step, all samples were kept at 42±1°C until the pH of the samples reached 4.7. They were then transferred to the refrigerator at a temperature of 5±1°C and stored at this temperature for 21 days. All experiments were done on days 1, 8, and 5 of the storage period. The production steps and necessary experiments were repeated three times.

Physicochemical tests

Titratable acidity

The titratable acidity was analyzed according to the national standard of Iran No. 2852.Acidity was calculated according to the percentage of lactic acid using the following formula and reported based on the Dornick degree [10].

Percentage of acidity=a×N×F×Meq LA×100

a=amount of 0.1 N NaOH in ml

N=Normality of consumed NaOH

F= Factor of consumed NaOH

M= Yogurt sample weight

Meq LA= Milli equivalent (mEq) gram of lactic acid = 0.09

pH measurement

pH was measured based on Iran's national standard No. 2852. The pH meter's electrode was calibrated before the test using standard pH buffers of pH = 7 and pH = 4. Then the pH meter temperature was set based on the sample temperature and was in contact with the product for about 45 s (the readings become steady). The sample pH was then read [11].

Calculating dry matter

Dry matter and fat-free dry matter were obtained according to standards 2852, 5222 and 695 [12].Dry matter percentage was calculated from oven drying to constant weight. Dry matter percentage was calculated using the following formula:

Dry matter percentage

$$= \frac{100\times\text{container weight}-\text{final weight (weight of the sample and container after drying)}}{\text{wet sample weight}}$$

Fat determination

Fat percentage was determined using the Gerber method based on standards 2852, 5222 and 695 [12].

Measuring protein

The amount of protein in the milk used and the yogurts produced were calculated using the micro-Kjeldahl method.

Measuring the syneresis rate of samples

The syneresis rates of samples were determined using the following relation. For this purpose, 25 grams of yogurt was poured into Erlenmeyer using Whatman No.43 filter paper and funnel and kept in the refrigerator at 4 ° C for 120 minutes. The extracted liquid was then weighed [13].

Water holding capacity (WHC)

The yogurt samples WHC was determined by centrifugation. For this purpose, 5 grams of sample was placed in acentrifuge (4000 rpm) for 30 minutes at 10 ºC. After centrifugation, the resulting liquid was removed and the remaining solids were weighed at the end of the centrifuge tube and expressed as a percentage of yogurt weight.

Viscosity percentage measurement

The viscosity of yogurt samples was determined using Brookfield viscometer and IV Spindle for rotation speed of 100 rpm at 4°C. The viscosity was recorded 30 seconds after the spindle rotation.The analyses were conducted three times for each sample of yogurt and the results were expressed in cp (centipoise).

Colorimetric test

Colorimetry is one of the important indicators in food quality. With the progress of the process and over storage time, it is essential to control color quickly. In this study, the color indices L*, a*, and b were assessed using the HunterLab device [14]. First, it was standardized using a white and black calibration plate. The samples were then inserted into the device one by one and assessed. The test was conducted for each product with 3 replications.

Texture analysis

Yogurt samples texture was analyzed with Brookfield CT3 Texture Analyzerusing a bar probe with a thickness of 13 mm (Analyzer Brennan and Tuderica Model)based on the test conducted by Brennan using Texture Analyzer [15]. To determine the texture properties, samples were put underthe aluminum probe of the device with a circular cross section of 70 mmin diameter and the two-phase compression test was conducted with a pretest probe speed of 5 mm/s at time intervals of 10 sand the yogurt texture related factors (hardness, springiness, cohesiveness, adhesivness) were investigated.

Lactic acid bacteria count test

MRS Agar culture medium and the method proposed by Hekmat et al. were used to count lactic acid bacteria [16]. For analysis, 1 ml of the sample was added to 0.1% sterile peptone water and diluted up to 6, 7, and 8 dilutions and 1 ml of each of these dilutions was transferred to the plates and cultured using the purplate method. MRS Agar and 0.1% sterile Peptone water medium were prepared based on the method specified by the manufacturer on the can containing these materials. Plates with 20-200 colonies were counted

Analysis of sensory properties

For sensory analysis of yogurt samples, a seven-member panel of the students and professors of the Food Industry Department of Islamic Azad University of Medical Sciences of Tehran, who used yogurt in their diet, was formed. The results of the sensory properties analysis of the produced yogurt samples are presented in Tables 2-4, and sensory analysis was conducted based on appearance and color (5 points), texture and consistency (5 points), flavor (10 points) and overall score (20 points). The final indicator of the analysis was the overall analysis.

Statistical analysis method

The results of the experiments carried out on the samples from three production times were evaluated using SPSS software (SPSS package program, version 16.0, SPSS Inc., USA). Duncan's mean comparison test was used to compare the means.The significance of differences between means was investigated at the levels of $\alpha = 0.05$ or $\alpha = 0.01$.

Results

Based on the obtained results, all the measured factors of milk consumption were at the level of the national standard of Iran with standard No. 103. Table 2 shows the results from comparing the mean pH of samples. As shown in table 2, with the addition of persimmon powder and an increase in the amount of phycocyanin, the pH value of samples significantly reduced ($p \leq 0.05$). The lowest pH on day 1 belonged to the treatment code 4, on day 8 belonged to the treatment code 3 and code 7, and on day 15 belonged to the treatment code 4 ($p \leq 0.05$). Moreover, the pH of samples significantly reduced over time ($p \leq 0.05$).

Values are reported based on the mean ± standard deviation. Uppercase and lowercase letters indicate a significant difference between columns and rows at the 5% probability level, respectively.

Samples: Code 1: control sample (without persimmon powder and phycocyanin), Code 2: Sample containing 0% persimmon powder and 0.5% phycocyanin, Code 3. Sample containing 0% persimmon powder and 1% phycocyanin, Code 4. Sample containing 0% persimmon powder and 1.5% phycocyanin, Code 5. Sample containing 2% persimmon powder and 0.5% phycocyanin, Code 6: Sample containing 2% persimmon powder and 1% phycocyanin, code 7. Sample containing 2% persimmon powder and 1.5% phycocyanin (items of code 1 to code 7 are common in Tables (1-17)

TABLE 2. Titratable acidity, pH, physical and textural properties of persimmon and phycocyanin-supplemented yogurts during storage period (Mean ± SD)

Parameters	Storage period (days)	Yogurts						
		CY	PY1	PY2	PY3	PPY1	PPY2	PPY3
Titratable acidity (lactic acid %)	1	1.18±0.02^{cB}	1.31±0.02^{abB}	1.33±0.01^{aC}	1.37±0.02^{aC}	1.24±0.01^{bcC}	1.30±0.01^{abC}	1.33±0.02^{aC}
	8	1.30±0.07^{deA}	1.34±0.01^{cdB}	1.43±0.00^{abB}	1.47±0.01^{aB}	1.37±0.02^{cB}	1.39±0.01^{bcB}	1.43±0.02^{abB}
	15	1.39±0.02^{dA}	1.50±0.01^{cA}	1.59±0.02^{aA}	1.61±0.01^{aA}	1.49±0.01^{cA}	1.53±0.01^{bA}	1.55±0.02^{bA}
pH	1	4.53±0.02^{aA}	4.41±0.02^{cA}	4.19±0.01^{eA}	4.01±0.01^{gA}	4.45±0.01^{bA}	4.28±0.01^{dA}	4.15±0.02^{fA}
	8	4.37±0.01^{aB}	4.28±0.01^{cB}	4.03±0.03^{eB}	3.88±0.02^{fB}	4.33±0.02^{bB}	4.10±0.01^{dB}	4.04±0.03^{eB}
	15	4.40±0.11^{aC}	4.20±0.01^{bC}	4.13±0.00^{dC}	3.90±0.10^{fC}	3.75±0.03^{aC}	4.18±0.00^{dC}	3.92±0.15^{eC}
Syneresis (%)	1	6.20±0.01^{aA}	7.77±0.13^{aA}	7.59±0.06^{aA}	7.35±0.02^{aA}	6.50±0.01^{aA}	6.38±0.05^{aA}	6.37±0.06^{aA}
	8	6.18±0.04^{aB}	7.60±0.05^{aB}	7.42±0.07^{aB}	7.19±0.05^{aB}	6.26±0.05^{aB}	6.26±0.06^{aB}	6.16±0.06^{aB}
	15	6.13±0.11^{aC}	7.45±0.02^{aB}	7.23±0.06^{aC}	7.04±0.03^{aC}	6.21±0.05^{aB}	6.13±0.07^{aC}	5.92±0.07^{aC}
Water-holding capacity (%)	1	35.57±0.12^{abC}	31.23±0.13^{bA}	31.23±0.06^{bA}	32.93±0.02^{bB}	34.25±0.01^{bC}	37.98±0.05^{abC}	39.63±0.06^{aC}
	8	36.45±0.27^{bB}	32.44±1.63^{bA}	32.44±1.63^{bA}	34.32±0.64^{bAB}	35.70±0.41^{bB}	37.98±0.28^{abB}	38.30±0.57^{aB}
	15	37.60±0.20^{bA}	33.63±1.34^{bA}	33.63±1.34^{bA}	35.65±0.84^{bA}	37.08±0.58^{bA}	39.17±0.29^{abA}	39.63±0.61^{aA}
Viscosity (cP)	1	9070±30^{dB}	8990±50^{fB}	9870±30^{bB}	91100±50^{dB}	8980±60^{eB}	8980±70^{eB}	1039±70^{aB}
	15	14280±30^{dA}	12930±80^{fA}	1531±25^{bA}	14523±120^{cA}	1454±120^{fA}	1294±100^{fA}	11631±0.00^{aA}
Hardness (g)	1	0.31±0.00^{aA}	0.27±0.07^{bA}	0.23±0.01^{cB}	0.20±0.00^{dA}	0.18±0.01^{eA}	0.16±0.01^{fB}	0.15±0.00^{fB}
	15	0.32±0.01^{aA}	0.28±0.00^{bA}	0.25±0.00^{cA}	0.21±0.01^{dA}	0.19±0.01^{eA}	0.18±0.01^{efA}	0.17±0.00^{fA}
Springiness (%)	1	6.81±0.51^{aA}	6.05±0.29^{bA}	4.90±0.01^{cdA}	5.56±0.18^{bcA}	6.02±0.51^{bA}	4.31±0.38^{dA}	4.29±0.42^{dA}

$^{a–g}$: Means in the same row with different letters were significantly different at $p < 0.05$.

$^{A-D}$: Means in the same column with different letters were significantly different at $p < 0.05$.

The results obtained from the comparison of the mean acidity of samples are presented in table 2. As shown in table 2, the acidity rates ofsamples increased significantly (p≤0.05) with the addition of persimmon powder and increasing the amount of phycocyanin.

On day 1, the acidity rate of treatment codes 3, 4, 6, and 7 was significantly higher than others (p≤0.05). On day 8, the highest acidity rate in treatments code 3, code 4, and code 7 was significantly higherand the acidity rate of the control sample was significantly lower than others (p≤0.05). On day 14, the highest acidity rates in treatments code 3, and code 4 were significantly higher and the acidity rate of the control sample was significantly lower (p≤0.05). Moreover, the samples› acidity rates increased significantly over time (p≤0.05).

Results obtained from the comparison of the mean dry matter of samples are shown in table 2, indicating that the samples' dry matter increased significantly ((p≤0.05) with the addition of persimmon powder and increasing the amount of phycocyanin. The dry matter amount in treatments code 5 and code 6 was significantly greater and the dry matter amount in treatments code 3 and code 4 was significantly lower than other treatments (p≤0.05). Table 5 shows the results from the comparison of the mean fat of samples. As shown in this table, the dry matter of samples increased significantly (p≤0.05) with the addition of persimmon powder and increasing the amount of phycocyanin. The fat amount of treatments code 6 and code 7 was significantly higher than other treatments (p≤0.05).

Results obtained from the comparison of the mean protein content of samples shows that the protein content in treatments code 5, code 6, and code 7 was significantly higher than other treatments (p≤0.05).

Results obtained from the comparison of the mean syneresis rate of samples showed that there has been no significant difference in syneresis rates of treatments in all time intervals, and the syneresis rates of samples decreased significantly over time. Results obtained from the comparison of the mean WHO of samples are shown in table 7, indicating that on day 1, the syneresis rates of control sample and code 7, and on days 8 and 15, the syneresis rates of treatments code 6 and code 7 were significantly greater than other treatments (p≤0.05).

Results from the comparison of viscosity of samples on days 1 and 15 are shown in table 2. According to the results, on both days studied, the highest viscosity belonged to the treatment code 7 and the lowest viscosity belonged to the treatment code 2 (p≤0.05). Moreover, from the first day to 15th day, the viscosity of the samples increased significantly (p≤0.05).

Table 2 shows the results obtained from the comparison of mean springiness of samples. As shown in table 2, the springiness of the control sample was significantly higher than other treatments in all time intervals (p≤0.05). Moreover, the lowest amount of springiness on day 1 belonged to treatments code 6 and code 7, and on day 8 to treatment code 4 (p≤0.05).

Results obtained from the comparison of the meanadhesiveness of samples are presented in table 2. According to table 2, on day 1, the adhesiveness rate of samples code 5 and code 8 was significantly higher than other treatments (p≤0.05). On day 8, the highest springinessrate was observed in treatment code 7 (p≤0.05).

Results obtained from the comparison of the mean cohesiveness of samples show that on day 1, the cohesivenessrate of sample code 8 was significantly higher than other treatments (p≤0.05). On day 8, the highest springiness rate was observed in treatment code 7 (p≤0.05).

Results obtained from the comparison of the mean color component showed that the color component value in treatment code 1 and treatment code 8 was significantly higher and lower than other treatments, respectively (p≤0.05).

The results from the comparison of the mean color component (b*) of samples are shown in Table 3, indicating that the color component b* in treatment code 2 was significantly higher and in treatment code 8 was significantly lower than other treatments (p≤0.05), respectively.

Results obtained from the texture analysis of samples showed that on all days studied, the lowest microbial population was found in treatmentscode 1 and code 5 (p≤0.05).

TABLE 3. Color analysis of persimmon and phycocyanin-supplemented yogurts (Mean (± SD)).

Parameter	Value	Yogurts						
		CY	PY1	PY2	PY3	PPY1	PPY2	PPY3
Color	L	95.09±0.27^{a}	93.69±0.49^{b}	93.48±0.10^{bc}	92.66±0.12^{d}	93.05±0.20^{cd}	91.90±0.40^{e}	90.90±0.27^{f}
	a	-2.24±0.11^{a}	-3.65±0.03^{a}	-3.85±0.01^{a}	-4.09±0.28^{a}	-2.67±0.53^{a}	-2.49±0.00^{a}	-3.40±0.01^{a}
	b	-0.04±0.30^{a}	-1.05±0.01^{c}	-1.15±0.00^{c}	-1.43±0.04^{d}	-0.56±0.00^{b}	-0.65±0.00^{b}	-1.53±0.00^{d}

$^{a–f}$: Means in the same row with different letters were significantly different at $p < 0.05$.

Table 5 shows the results obtained from the comparison of the mean color score of samples, showing that on day 1, the color scores of the control sample and treatments code 5, code 6, and code 7 were significantly higher and the color scores of treatments code 2 and code 3 were significantly lower than other treatments (p≤0.05).

On day 8, the color score of the control sample was significantly higher than other treatments (p≤0.05). On day 14, the color scores of samples code 2 and code 3 and control sample were significantly higher than other treatments (p≤0.05). No statistical significant differences were observed in the color scores of samples over time, and only in treatment code 1 and code 2 the color scores of samples reduced significantly from day 1 to day 8 (p≤0.05).

Results obtained from the comparison of the mean scores of the taste of samples showed that on all days studied, the taste scores of the control sample and treatments code 5 and code 6 were statistically higher, and the taste scores of treatment codes 4 and 8 were significantly lower than other treatments (p≤0.05).

The results obtained from the comparison of the mean texture score of samples show that on all days studied, the taste scores of the control group and treatments code 6, 7, and 8 were significantly higher than other treatments (p≤0.05).

According to Iran National Standard No. 695, the measurable acidity of yogurt should not be less than 0.7% (in terms of weight percentage - lactic acid weight). The results of comparing the mean acidity rates of the samples shown in Table 4 indicate that on day 1, the acidity rates of treatments code 3, code 4, code 6 and code 7 were significantly higher and the acidity rate of treatment code 8 was significantly lower than other treatments (p≤0.05).On day 8, the highest acidity rates in treatments code 3, code 4, and code 7 were significantly higher and the acidity rates of the control sample and treatment code 8 were significantly lower (p≤0.05). On day 14, the highest acidity rates in treatment codes 3 and 4 were significantly higher and the acidity rates of control sample and treatment code 8 were significantly lower (p≤0.05).

Moreover, the sensory analysis results of persimmon and phycocyanin-supplemented yogurts during storage period are shown in Table 5.

Generally, it can be stated that the yogurt samples without persimmon powder and containing higher amounts of phycocyanin (1 and 1.5%) had higher acidity rates. Researchers have attributed the increase in acidity rate of yogurt samples over time to the fermentation of lactose by bacteria and the production of lactic acid. The results of the present study were consistent with those obtained from the observations of Agustini et al. (2017) in the study of physicochemical, microbiological and sensory properties of *S. platensis*-supplemented yogurts, which showed that by increasing the amount of spirulina, the acidity ratesof the samples increased significantly in terms of lactic acid[17]. Kavimandan (2015) also investigated the effect of *S. platensis* on fermented dairy products and its effect on their physicochemical, microbiological, and sensory properties, and found thatthe yogurt durability, probiotic properties and acidity rate increase with the addition of *S. platensis*[18].

TABLE 4. Counts of lactic acid bacteria of persimmon and phycocyanin-supplemented yogurts during storage period (Mean (± SD)).

Parameter	Storage period (days)	Yogurts						
		CY	PY1	PY2	PY3	PPY1	PPY2	PPY3
Lactic acid bacteria	1	$1.19\times 10^7 \pm 0.11^{cA}$	$6.30\times 10^7 \pm 0.22^{aA}$	$6.03\times 10^7 \pm 1.72^{aA}$	$6.00\times 10^7 \pm 3.13^{aA}$	$2.13\times 10^7 \pm 0.09^{bcA}$	$5.60\times 10^7 \pm 2.43^{abA}$	$3.36\times 10^7 \pm 2.48^{abcA}$
	8	$5.20\times 10^7 \pm 0.43^{aA}$	$4.00\times 10^7 \pm 0.37^{aA}$	$4.50\times 10^7 \pm 0.36^{aA}$	$4.32\times 10^7 \pm 0.13^{aA}$	$2.71\times 10^7 \pm 0.46^{aA}$	$6.08\times 10^7 \pm 0.78^{aA}$	$5.05\times 10^7 \pm 0.18^{aA}$
	15	$4.05\times 10^7 \pm 2.77^{aB}$	$6.09\times 10^7 \pm 0.37^{aA}$	$6.05\times 10^7 \pm 0.38^{aA}$	$6.26\times 10^7 \pm 0.56^{aA}$	$3.33\times 10^7 \pm 2.31^{aB}$	$4.43\times 10^7 \pm 0.78^{aA}$	$6.58\times 10^7 \pm 0.36^{aB}$

$^{a–c}$: Means in the same row with different letters were significantly different at $p < 0.05$.

$^{A-B}$: Means in the same column with different letters were significantly different at $p < 0.05$.

TABLE 5. Sensory analysis results of persimmon and phycocyanin-supplemented yogurts during the storage period (Mean (± SD)).

Parameters	Storage period (days)	Yogurts						
		CY	PY1	PY2	PY3	PPY1	PPY2	PPY3
Appearance	1	4.74 ± 0.17^{aA}	3.14 ± 0.14^{cA}	3.35 ± 0.21^{cA}	4.35 ± 0.21^{bA}	4.35 ± 0.21^{bA}	4.40 ± 0.14^{abA}	4.50 ± 0.22^{abA}
	8	4.39 ± 0.17^{bB}	2.57 ± 0.43^{cB}	3.35 ± 0.21^{bA}	4.28 ± 0.14^{bA}	4.28 ± 0.14^{bA}	4.42 ± 0.14^{bA}	4.58 ± 0.03^{bA}
	15	4.42 ± 0.20^{aAB}	2.99 ± 1.34^{bAB}	3.21 ± 0.21^{bA}	4.21 ± 0.07^{aA}	4.21 ± 0.00^{aA}	4.28 ± 0.00^{aA}	4.42 ± 0.14^{aA}
Flavour	1	9.85 ± 0.14^{aA}	9.27 ± 0.13^{bA}	9.23 ± 0.08^{bA}	9.00 ± 0.14^{cA}	9.71 ± 0.14^{aA}	9.64 ± 0.07^{aA}	9.35 ± 0.07^{bA}
	8	9.71 ± 0.14^{aA}	9.21 ± 0.21^{bcA}	9.18 ± 0.21^{bcA}	9.18 ± 0.21^{dA}	8.90 ± 0.08^{aB}	9.64 ± 0.07^{aA}	9.28 ± 0.14^{bA}
	15	9.56 ± 0.20^{aA}	8.93 ± 0.07^{cA}	9.95 ± 0.21^{cA}	8.86 ± 0.07^{cB}	9.49 ± 0.07^{aA}	9.49 ± 0.07^{aA}	9.11 ± 0.11^{bA}
Texture	1	4.35 ± 0.07^{aA}	3.64 ± 0.07^{cA}	3.85 ± 0.14^{bA}	3.92 ± 0.07^{bC}	4.21 ± 0.07^{bA}	4.21 ± 0.07^{aA}	4.28 ± 0.00^{aA}
	8	4.57 ± 0.15^{aA}	3.64 ± 0.07^{eA}	3.85 ± 0.14^{dA}	4.00 ± 0.00^{cdB}	4.35 ± 0.07^{bA}	4.42 ± 0.14^{abA}	4.49 ± 0.07^{abA}
	15	4.65 ± 0.06^{aA}	3.78 ± 0.07^{dA}	3.99 ± 0.14^{cA}	4.07 ± 0.07^{cA}	4.42 ± 0.14^{bA}	4.57 ± 0.00^{abA}	4.57 ± 0.00^{abA}

$^{a–d}$: Means in the same row with different letters were significantly different at $p < 0.05$.

$^{A-C}$: Means in the same column with different letters were significantly different at $p < 0.05$.

The pH scale is shown as the negative logarithm of the hydrogen-ion molarconcentration. The higher the strength of the acid or hydrogen ion, the lower the pH scale, and it thus tends toward zero. The pH determines the density of H ions or ionized hydrogen in the sample. According to the national standard of Iran No. 695 [19], the pH of yogurt should not be more than 4.6. The samples produced on day zero had such a feature. The results from comparing the mean pH of the samples show that in all time intervals the pH of treatment scode 1 was significantly higher than other treatments ($p\leq0.05$). The lowest pH on day 1 belonged to treatment code 4, on day 8 to treatments code 3 and code 7, and on day 15 belonged to treatment code 4 ($p\leq0.05$). Generally, it can be said that the highest pH belonged to the control group and the addition of different values of phycocyanin and persimmon powderled to a significant reduction in pH. In the present research, the addition of the extractedphycocyanin led to a reduction in pH, but in the mentioned articles, *S. platensis* powder has been added to samples. Therefore, the difference in the obtained results can be attributed to the difference in the compounds used.

Based on the national standard of Iran No. 695 [19], dry matter of yogurt should not be less than 9.5% (in terms of the weightpercentage-lactic acid weight). In the present study it was in the standard range in all samples and on all days studied. The results from the comparisonof the mean dry matter of the samples showed that the amount of dry matter in treatments code 5 and code 6 was significantly higher and the amount of dry matter in treatments code 5 and code 6 was significantly lower than other treatments ($p\leq0.05$). In other words, samples containing higher amounts of persimmon powder (codes 6 and 7) had higher dry matter. According to Iranian National Standard No. 695 [19], the fat content of low-fat yogurt should be between 0.5-1.5%. In the present study, yogurt fat content was within the standard range in all samples and on all days studied. The results from comparing the mean fat of the samples showed that the fat content of treatments code 6, code 7 and code 8 was significantly higher than other treatments ($p\leq0.05$).

Results from the comparison of the mean protein content of samples showed that the protein content of treatments code 5, code 6, code 7, and code 8 was significantly higher than other treatments ($p\leq0.05$). In other words, samples containing higher amounts of persimmon powder (codes 6, and 7) had higher amount of protein. Agustini et al (2017) investigated the physicochemical, microbiological, and sensory properties of yogurt samples enriched with *S. platensis*. They found that the protein content of samples increased significantly by increasing the amount of spirulina [20]. In the study on doughnuts containing Spirulina microalgae, Rabelo et al. (2013) stated that the protein content of samples has increased with the addition of microalgae powder [21]. Moreover, Salas-Mellado (2014) reported that the protein content of bread samples from rice flour with Spirulina has increased.

A decline in pH at the end of the storage period causes a change in the natural form of the protein and the water attached to it is released and thesyneresis rate increases due to the denaturation of the protein [3]. Other researchers have attributed the increased syneresis rate over time to the increased rate of acidity, as well as severe contraction of the gel network due to cooling[3]. Results from the comparison of the mean syneresis rates of samples show that there has been no statistically significant difference in the syneresis ratesof treatments in all time intervals.

Results from the comparison of mean viscosity of samples on days 1 and 15 showed that the highest and the lowest amount of viscosity on both days studied belonged to treatment code 7 and treatment code 2, respectively ($p\leq0.05$). Moreover, the viscosity of all treatments increased significantly over time from the first day to the15th day ($p\leq0.05$). The color parameter L*represents lightnessranging from zero (black) to 100 (100%reflectance). The results related to the lightness (L* component) of samples showed that the highest and the lowest rate of lightness belonged to the treatment code 1 and treatment code 8, respectively. Evaluating the effect of adding spirulina microalgae on kiwi pastille properties, Khazaiypool et al. (2015) stated that by increasing the percentage of spirulina in formulation, parameter L^* of samples decreased significantly [22]. The results from the comparison of b^* (blue to yellow) of samples showed that the color component b^* is significantly higher and significantly lower than other treatments in treatment code 2 and treatment code 8, respectively ($p\leq0.05$). The results from the comparison of the mean adhesiveness of samples showed that on day 1, the adhesiveness rate of samples code 5 and code 8 was significantly higher than other treatments ($p\leq0.05$). On day 8, the highest amount of springiness was observed in treatment code 7 ($p\leq0.05$). Hardness index is related to the sample'ssoftness or stiffness. It is the maximum height of the force curve at the first compression and shows the maximal force applied during the act of biting. In sensorially terms, hardness is the maximum force required to compress food between molar teeth until a certain deformation is attained. Springiness is related to the plastic properties and springiness of the body. Springiness (return after being compressed) represents the duration of the pressure cycle during the second bite. In other words, it is the amount of height that is retrieved at the time between the end of the first bite and the second bite. In sensorially terms, the return amount of deformed material to its original state (without deformation) once the chewing force was removed is called springiness. Results from texture analysis of samples indicate that on all studied days, the lowest microbial population was observed in treatments code 1 and code 5 (($p\leq0.05$). In the study of physicochemical,

microbiological and sensory properties of *S. platensis*-enriched yogurts,Agustini et al. (2017) found that by increasing the amount of spirulina, the population of lactic bacteria has increased significantly [20]. The release of flavor and taste-producing compounds from foods is a determining and complex factor for understanding flavor and taste. High fat and oil content reduces the volatility of hydrophobic aromatic substances, including long-chain aldehydes. Polysaccharides can be bonded with volatile compounds in various ways. For example, some carbohydrates can bond volatile compounds through hydrogen bonding between appropriate functional groups. Other components, such as starch, which are composed of three-dimensional structures with hydrophobic regions, are capable of forming complexes with various volatile compounds. Volatile flavor compounds react extremely with milk fat [23,24]. According to Iran National Standard No. 695, yogurt should not have bad taste, acidification, undesirable acidification and over-acidification in taste or smell, and should be free of any unnatural taste, including bitter, metallic, tasteless / bland, musty, crud-like, rancid, cooked, burnt, and alcoholic taste.

In a study on using spirulina in formulation of kiwi puree-based fruit pastille, Khazaiypool et al. (2015) stated that increasing the concentration of spirulina in the studied range (0.25-1%) had no significant undesirable effect on taste as expected. In interpreting this finding, the researchers stated thatperhaps kivi has been well capable of masking the taste effect of spirulina[22]. Medicinal plants and natural substances and active herbal substances can have nutritional, health and medicinal applications [25, 26].

Conclusion

The physicochemical analyses of yogurt samples showed that samples without persimmon powder and containing higher amounts of phycocyanin (1 and 1.5%) had acidity and all samples acidity increased over time. The highest pH belonged to the control sample and the addition of different amounts of phycocyanin and persimmon powder led to a significant reduction in pH. In all time intervals, syneresis rate of samples increased by increasing phycocyanin and persimmon powder though it was insignificant compared to the control. Samples containing higher amounts of persimmon powder (code 6 and code 7) had higher amounts of dry matter, protein, and WHC. The control sample had the highest lightness (L*) and the treatment code 8 had the highest redness (a*). In all time intervals, the highest rate of hardness and springiness belonged to the control group. The results from sensory analysis of samples showed that on day 1, the color scores of the control group and treatment codes 5, 6, and 7 were significantly higher and the color scores of treatments code 2 and code 3 were significantly lower than other treatments ($p \leq 0.05$). On all studied days, the taste scores of the control group and treatments code 5 and code 6 and the texture scores of the control group and treatment codes 6, 7, and 8 were significantly higher than other treatments ($p \leq 0.05$).

Conflict of Interest
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