Original Article

Production of Cheese and Ice Cream Enriched With Biomass and Supernatant of *Spirulina platensis* With Emphasis on Organoleptic and Nutritional Properties

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ABSTRACT

Background: *Spirulina platensis* contains natural pigments along with phenolic and flavonoid compounds that may be excreted to the supernatant in addition to biomass during culture.

Objectives: We prepared enriched cheese and ice cream using biomass and supernatant of *S. platensis* microalgae extract. We aimed to evaluate these algae's organoleptic and nutritional properties.

Methods: After preparing a 30-day culture of Spirulina, different concentrations of its biomass and supernatant extract were prepared to enrich cheese and ice cream. In the next step, the physicochemical and antioxidant properties of the products were measured by FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenyl 1-picryl hydroxyl) methods. Expert evaluators performed the sensory analysis according to the Iranian national standard method in terms of color, odor, taste, texture, and general acceptance on a scale of 0 to 5. Finally, statistical analysis of the data obtained from each experiment was performed with a one-way analysis of variance three times using SPSS software, version 24.

Results: The results showed that the content of protein, fat, ash, and biomass was higher in enriched foods. However, in enriched ice creams, the content of protein, fat, sugar, and aeration rate increased significantly compared to the supernatant. In addition, cheese and ice cream fortified with supernatant had significantly greater antioxidant activity than biomass. The results of the hedonic test showed that with increasing concentrations of these agents in fortified cheeses and ice creams, the level of satisfaction in odor, taste, color, texture, and general acceptance factors was increased.

Conclusion: The study results showed that adding biomass and supernatant of *S. platensis* to ice cream and cheese increased the nutritional value and improved the taste of these foods. Because these substances play an essential role in children's diet, this microorganism can be used to combat malnutrition and a diet without micronutrients.

Keywords: Cheese, Ice cream, Microalgae

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Introduction

Arthrospira platensis (brand name Spirulina) is a phototrophic and filamentous cyanobacterium, used as a potential food additive due to its high protein (65%-70% dry matter), vitamins, and minerals among microalgae (Nowruzi et al., 2022; Shamloo et al., 2019). Besides high protein levels, Spirulina cells also contain significant amounts of phycocyanin, an antioxidant used in various products developed in the cosmetics and pharmaceutical industries (Szmejda et al., 2018). Spirulina is a low-cost dietary supplement with no side effects. The effects of its various substances on weight loss and fat have been evaluated. The nutritional benefits of Spirulina go beyond the above benefits and include anti-viral, anti-cancer, anti-oxidant, anti-diabetic, anti-inflammatory, liver-strengthening, heart-protecting, and strengthening properties (Rajasekar et al., 2019). Most studied bioactive compounds in Spirulina include essential amino acids, vitamins (especially B12), mineral salts, pigments (carotenoids, phycocyanins, and chlorophylls), unsaturated fatty acids, including omega-3 fatty acids, and other active omega-3 fatty acids. In addition, its nutrients include iron, manganese, zinc, and copper (Nowruzi et al., 2021).

Phycocyanin-c is one of the most important components of Spirulina (Kumar et al., 2014). *S. platensis*, as an excellent source of phycobiliproteins, has been commercialized in the food and nutrient industries, such as the use of phycocyanin as a colorant in ice cream, soft drinks and soda (Chew et al., 2019; Chew et al., 2017; Hu, 2019). So far, *S. platensis* biomass powder has been used to produce various food products such as soups, sauces, snacks, beverages, chocolate, candies, biscuits, bread, cakes, and fortified flour (Anvar et al., 2022). In addition, many bioactive compounds in cyanobacteria are released into the culture medium as exopolysaccharides. They are available as supernatants in the culture medium. In recent years, the trend of using natural polymers has boosted research into the production of exopolysaccharides (EPSs) from bacteria. Special attention has been paid to the unique structural properties of bacterial EPS in chemistry, medicine, and the food industry (Nguyen et al., 2020). EPSs can increase water retention, so they are widely used in the food industry as adhesives, stabilizers, and emulsifiers to improve the rheological properties, texture, and sensitivity of bread and fermented milk products such as yogurt and cheese (Garai-Ibabe et al., 2010; Singh & Saini, 2017). In addition to these properties, EPSs have shown potential health benefits, such as anti-oxidant, anti-cancer, anti-viral, anti-inflammatory, and cholesterol-lowering effects (Noori et al., 2013). So far, the biological activities of EPSs, such as prebiotics, antioxidants, and anti-inflammatory, are related. These compounds exhibit different therapeutic effects of monosaccharides (Wang et al., 2017; Alishahi et al., 2018).

Using dietary supplements derived from natural sources seems to be an excellent solution to meet the nutritional needs of different segments of society. In this regard, the exploitation of microalgae resources is of particular importance. Compared to other natural resources of bioactive compounds, microalgae have a greater advantage due to their incredible biodiversity (Mohammadi et al., 2012; Misaghi., 2017).

The nutritional properties of Spirulina make it suitable for a variety of uses, but in many cases, it tastes unpleasant. However, its combination with other foods makes healthy, nutritious, and economical products available to individuals. In this regard, due to the bioactive compounds of Spirulina that can improve the value of food, its extract can be used to enrich heat-sensitive foods such as cheese and ice cream (Hoseini et al., 2013; Abdulrahman et al., 2018).

Milk and dairy products play an essential role in the human diet due to the nutritional benefits of proteins, minerals, and vitamins. Recently, food scientists have tested the enrichment of dairy products using natural products to improve overall food intake with minimal effects (Terpou et al., 2019).

Cheese is a processed milk product made using whole or part of the milk protein, skim milk, buttermilk, and curd cream using rennet or other coagulants. Cheese is one of the foods high in protein and essential amino acids. Compared to other fermented dairy products, such as yogurt and milk, cheese maintains its biological activity in all stages of machine digestion due to some features such as high fat, with its dense and cohesive tissue as food-carrying probiotics. Adding microalgae such as Spirulina to cheese doubles its nutritional value (Hoseini et al., 2013; Anvar et al., 2022).

Ice cream is also semi-solid, made by freezing milk, fat, and sugar mixture with or without food additives. Commercial ice creams are deficient in protein, have antioxidant properties, are easy to melt, and are high in fat. Therefore, it is necessary to improve the quality of ice cream. Adding nutrients with high nutritional value, such as Spirulina, can enrich the nutrient values of ice cream and cream cheese. Since cheese and ice cream may be a significant part of the diet, especially for children, the use of eatable algae paves the way to produce food rich in essential nutrients and improve their quality (Anvar et al., 2022; Pourbaba et al., 2022).

Many studies have been conducted on using Spirulina extract to enrich cheese and ice cream with nutrient factors (Saranraj & Sivasakthi, 2014). This study tried to evaluate the effect of biomass and supernatant of Spirulina extract in different concentrations separately. We assessed the protein, fat, water, ash, and β-carotene content of the enriched cheese, as well as fat, protein, total sugar, aeration rate, and the melting point of the enriched ice cream along with sensory evaluation. Finally, the results of this research were compared with commercial cheese and ice cream available in the market.

Materials and Methods

In this study, Spirulina powder was prepared by the manufacturer of organic algae located in Rasht City, Iran. Culture medium (Zarrouk, USA), phosphate buffers, sodium phosphate, acetic acid, lubricating reagent, gelatin, and 2, 2-diphenyl 1-picryl hydroxyl (DPPH and Rent tablet) were all purchased from Pars Co. (Iran).

Culture and extraction of cyanobacterium spirulina

The culture was performed in a 10-L Zarrouk culture medium and inoculated with 800 mL of stock Spirulina for two months. Aeration was performed daily by an air pump in a culture chamber under fluorescent light for 16 hours alternating with 8 hours in darkness.

Extraction and preparation of different concentrations of biomass and Spirulina supernatant

The collected biomass from the *S. platensis* strain was sonicated for 10 minutes and then centrifuged at 10000 rpm for 10 minutes. After centrifugation, biomass and supernatant were collected. Then different biomass and Spirulina supernatant concentrations were prepared to produce fortified cheese (0.5%, 1%, and 1.5%) and fortified ice cream (at concentrations of 0.6% and 1.2%).

Cheese preparation

To prepare the cheese, fresh milk (1 L) was heated at 73-75°C for 15 minutes (pasteurization) and then cooled to 40°C. After adding the starter, Rennet tablets were added to the desired mixture for milk clotting and cheese production (Figure 1A). In the next step, fresh cow's milk was fermented, and hot water was drained to accelerate whey production. Afterward, different concentrations of biomass and Spirulina supernatant (0.5%, 1%, and 1.5%) were added to the cheese. Treatments were prepared in 3 replications. Finally, the resulting cheese was stored at refrigerator temperature for further analysis (Stafilov et al., 2020).

Determination of the protein and fat content of cheese

Kjeldahl's method determined the amount of crude protein after acidic digestion. The fat content of the samples was determined using the Soxhlet method. Fat cells were extracted with diethyl ether by extraction unit (Buchi, Switzerland) (Stafilov et al., 2020).

Determination of moisture and water content in cheese

The water content of the samples was measured using the standard methods (Chen et al., 2020).

Determination of cheese ash

The amount of cheese ash was obtained the standard methods (Chen et al., 2020).

Determination of β-carotene and elements concentration in cheese

β-carotene concentrations were measured by spectrophotometric analysis using dye adsorption levels. The extraction was performed using the Association of Official Agricultural Chemists (AOAC) method (Grootaert et al., 2021). The optical absorption of the supernatant of the solution obtained from β-carotene extraction was read using a UV/VIS spectrophotometer at 436 nm. The concentration of β-carotene in the samples was obtained using the Equation 1, and the values were calculated in μg/g.

1. $C(\mu g/L)=A/EL$

Elemental analysis was carried out using the Agilent 720 spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) through axial plasma configuration (Agilent Technologies, Switzerland) as previously described by Šnirc et al. (2020). The detection limits of the monitored elements were as follows (mg/kg): Cr, 0.0033, Fe, 0.0087; Ca, 0.036; Cu, 0.0027; K, 0.31; Mg, 0.0078; Mn, 0.0023; Mo, 0.041; Na, 0.28; Se, 0.013; and Zn, 0.0028. Its recovery values (considering the water

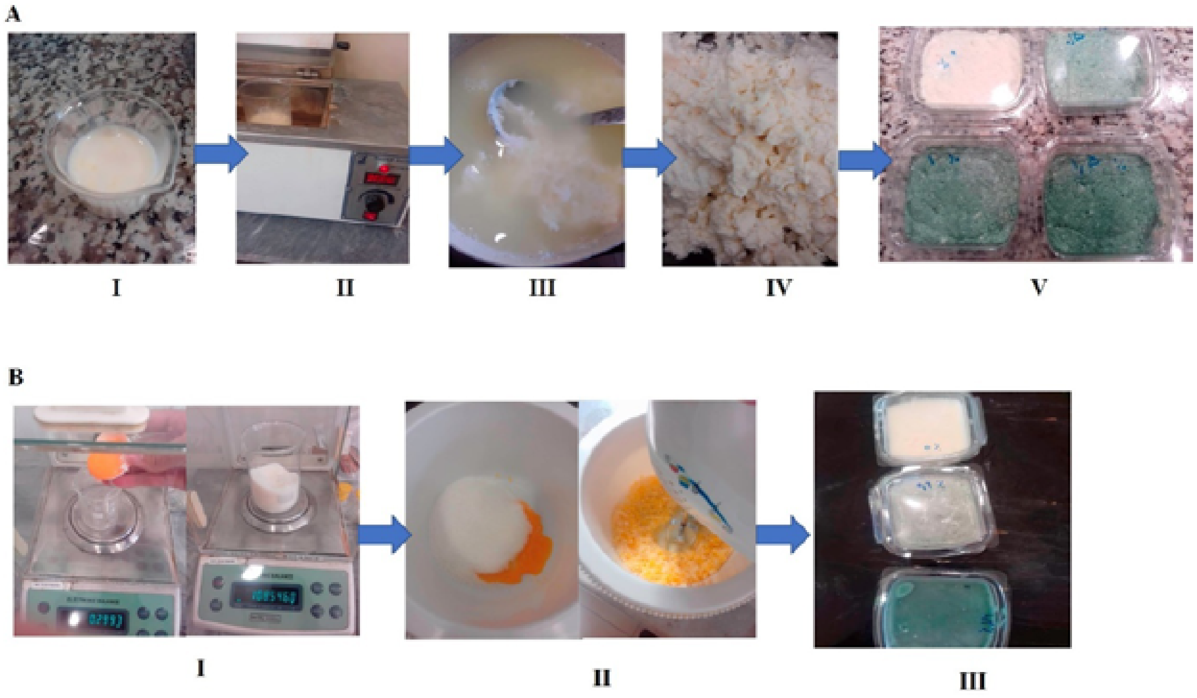

Figure 1. A) The steps of preparing cheese, B) The steps of preparing ice cream

content) ranged from 85% to 123% for most of the determined elements.

Sensory evaluation of cheese

The sensory analysis was performed according to the Iranian national standard method number 4938 (sensory test method) based on previous studies. All 15 evaluators were food industry experts and well-acquainted with the characteristics of cheese. The samples were taken out of the cold storage sometime before the test and were given to the judges after reaching the ambient temperature. The evaluators evaluated the samples of cheeses prepared with biomass and supernatant in terms of color, odor, taste, texture, and general acceptance on a scale of 0 to 5 (0: Very bad, 2: A little bad, 3: Neither good nor bad 4: A little good, and 5: Very good).

Ice cream production

To make ice cream, egg yolk (3%), milk (3% fat), and sugar cane (16%) were thoroughly mixed to form a foam. The mixture was pasteurized at 84°C for 10 minutes and homogenized at 1500 rpm while the creamy dough was still hot. Biomass and Spirulina supernatant were added to the ice cream in 0.5% and 2% concentrations, respectively. The final ice cream mixture was then refrigerated, allowing the milk fat to crystallize somewhat and the proteins to hydrate. It took only 15 minutes to make the ice cream to get the final product. The product was stored in the freezer at -18°C for 24 hours (Figure 1B).

To prepare the cheese, milk was placed in a saucepan (A-I), and for clotting and cheese production, acetic acid and rent were added (A-III). Finally, different concentrations of Spirulina powder were added to the cheese produced (A-V). In the preparation of ice cream (B-I), ice cream was produced by adding egg yolk to sugar cane, milk, and gelatin, and (B-III) different concentrations of Spirulina powder were added.

Measurement of ice cream protein content

The protein content of ice cream was measured according to the Yan et al by the Kjeldahl method (Yan et al., 2022).

Measurement of ice cream fat content

The fat content of ice cream was measured according to Iranian standard 2450 by the Gerber method (ISIRI, 2009).

Measurement of ice cream sugar content

Total sugar was measured by the Fehling method according to Iranian standard, No. 2450 (ISIRI, 2009).

Measurement of the amount of aeration or increase in the volume of ice cream

The amount of aeration or increase in the volume of ice cream was measured according to the 2450 standard (ISIRI, 2009).

Measurement of ice cream melting point

The melting strength of ice cream samples was determined based on previous studies. Ice cream at 18˚C to 30˚C was placed on the Buchner funnel at ambient temperature (25˚C), and the weight of the melted substance was recorded after 15 minutes and expressed as a percentage of melted weight. This test was performed by considering two replications.

Sensory evaluation of ice cream

The sensory analysis was performed according to the ISIRI method number 4938 (sensory test method) (ISIRI, 1999). The three evaluators were food industry experts who were well-acquainted with the characteristics of cheese. The samples were taken out of the cold storage sometime before the test and were given to the judges after reaching the ambient temperature. The evaluators tasted the ice cream samples prepared with biomass and supernatant in terms of color, odor, taste, texture, and general acceptance on a scale of 0 to 5 (0: Very bad, 2: A little bad, 3: neither good nor bad 4: A little good, and 5: Very good).

Measurement of antioxidant activity by DPPH method in cheese and ice cream

One milliliter of stock DPPH is poured into the coat, and a spectrophotometer reads the absorbance at 515 nm. Then 0.1 mL of the sample extract was added, and the resulting mixture was shaken well and incubated for 15 minutes in a dark room. The adsorption of the mixture was read by a spectrophotometer at 515 nm versus the control (Devi et al., 2009).

Evaluation of nitric oxide trapping in cheese and ice cream

Nitric oxide radical scavenging activity was measured using a grease reagent. Sodium nitroprusside (10 mM) was mixed in the phosphate buffer with the extract of the samples dissolved separately in water and then incubated for 150 minutes at room temperature. After incubation, 0.5 mL of lubricating reagent (1% sulfanyl hope and 0.1% naphthyl ethylenediamine dihydrochloride in 2% phosphoric acid) was added to the samples. Mixed adsorption (OD) was then obtained at 545 nm (Green et al., 1982; Kalim et al., 2010). Potassium nitrate was used as a positive control, and the decrease in uptake indicated more inhibitory activity. The experiments were performed in three replications, and the percentage of inhibitory activity was calculated as follows.

Evaluation of antioxidant potential by ferric reducing antioxidant power (FRAP) method in cheese and ice cream

The antioxidant capacity was estimated by the iron reduction method (FRAP), which is based on the ability of an antioxidant to reduce Fe^{+3} to Fe^{+2}, performed according to the method described by Benzie and Strain (1996). After preparation of the reagent, ice cream was enriched with biomass and supernatant of Spirulina at concentrations of 0.6% and 1.2%, respectively. In addition, cheese was enriched with biomass and supernatant prepared with Spirulina at concentrations of 0.5%, 1%, and 1.5%. Then, the absorption changes were measured at 593 nm and 37˚C for 4 minutes. After blank absorption, the amount of FRAP obtained using the Trolox standard was converted to μmol Trolox equivalent/L.

Methods and tools of data analysis

Statistical analysis of the data obtained from each experiment was performed with SPSS software, version 24 and Excel software, version . All measurements were repeated three times. Significant differences between the measured factors were performed by one-way analysis of variance with a 95% confidence interval. The means were compared with the Tukey test, and the comparisons' results were shown graphically with Excel.

Results

Results of physicochemical analysis of inoculated cheeses

Analysis of data on protein and fat content of inoculated cheeses

The results of measuring the protein and fat content of inoculated cheeses were consistent with each other, so the protein and fat content of cheeses enriched with Spirulina supernatant increased with the concentration of Spirulina, with a significant ($P<0.05$) difference compared to the control of 1.91 and 1.5, respectively, increasing 1 fold (Figures 2A and 2B). In general, using Spirulina supernatant increases cheese's protein and fat content by 1.22 and 1.12 times more than biomass.

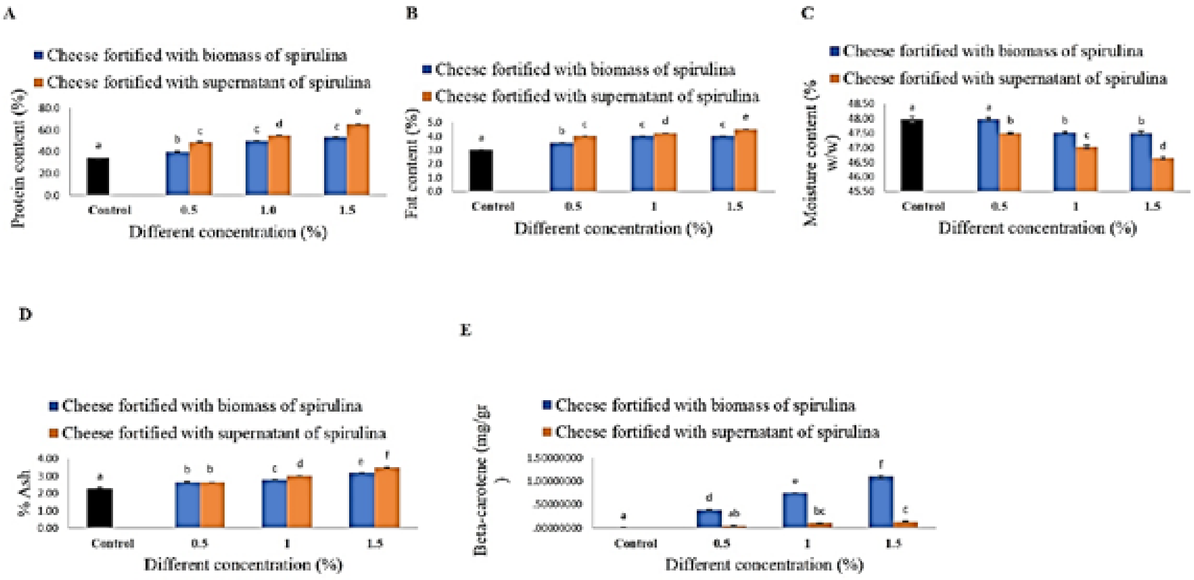

Figure 2. Comparing protein (a), fat (b), humidity (c), ash (D), and β-carotene (e) concentrations of cheese fortified with different concentrations of biomass and Spirulina supernatant

The moisture content of cheese

The results of moisture measurement showed that the moisture content in cheeses enriched with Spirulina supernatant decreased significantly by increasing the concentration of Spirulina compared to the control by 0.98 times ($P<0.05$) (Figure 2C). In general, using Spirulina supernatant reduces cheese's moisture content by 0.989 times more than biomass.

Ash content of cheese

The results of measuring the amount of ash showed that in cheeses enriched with Spirulina supernatant, the ash content increased significantly ($P<0.05$) by 1.52 times.

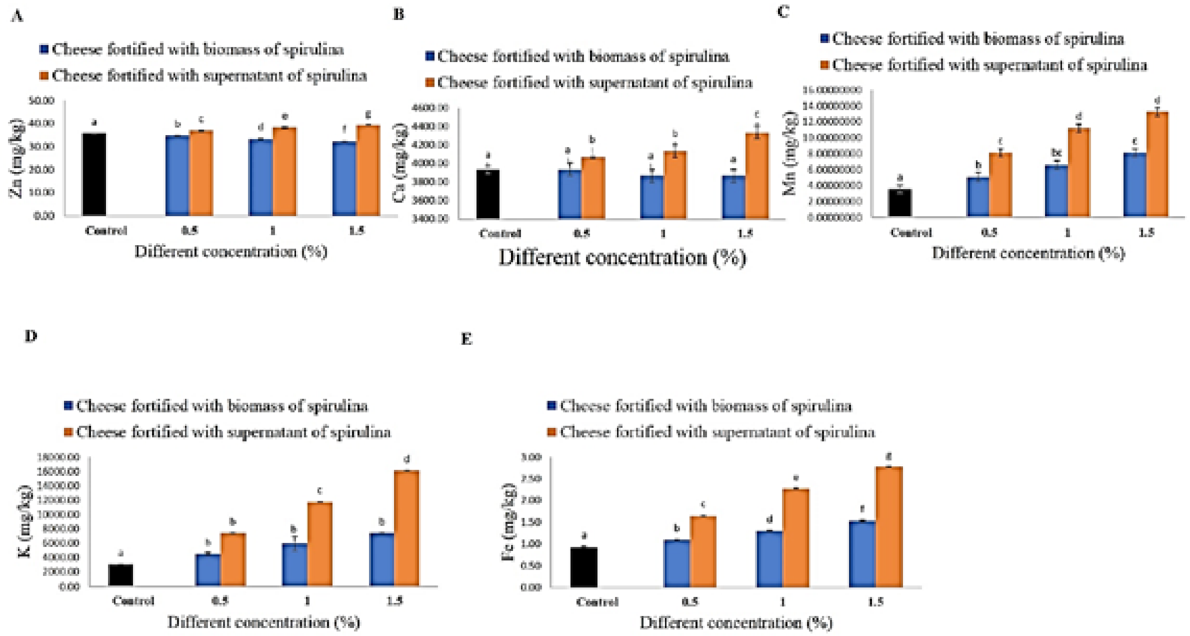

Figure 3. Examining the content of zinc (a), calcium (b), magnesium (c), potassium (d), and iron (e) in cheese enriched with biomass and Spirulina supernatant

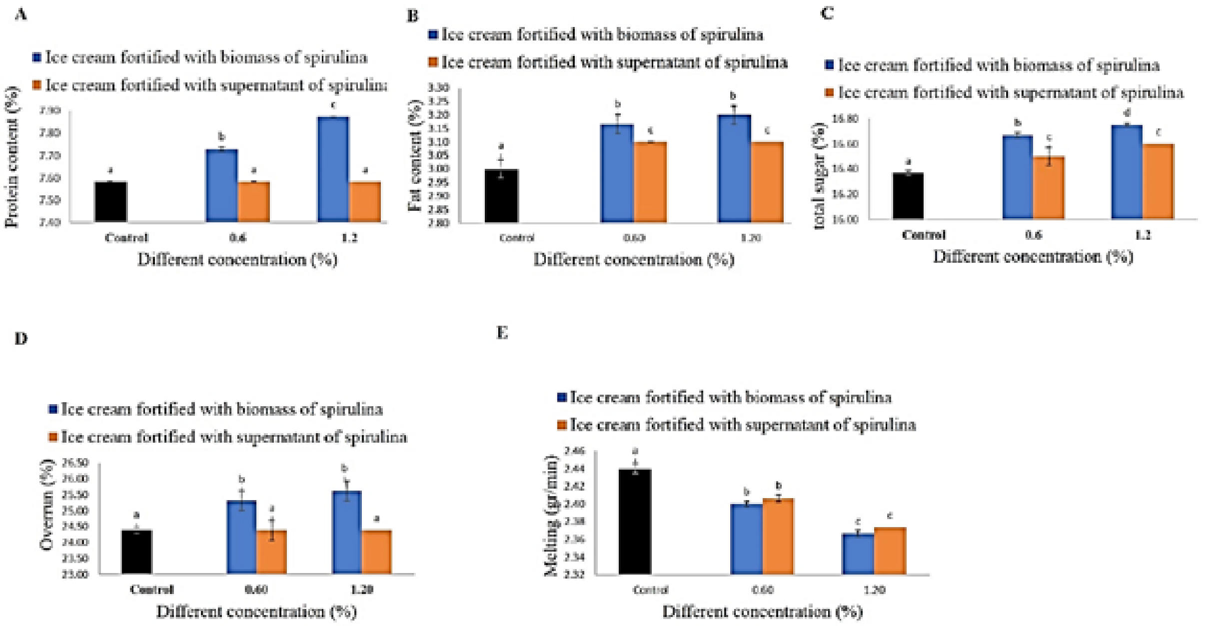

Figure 4. Comparing the content of protein (a), fat (b), sugar (c), aeration (d), and melting point (e) of ice cream enriched with different concentrations of biomass and Spirulina supernatant

Using Spirulina supernatant reduced the cheese ash content by 1.09 times more than biomass (Figure 2D).

The concentration of β-carotene in cheese

The results of measuring the β-carotene content showed that, unlike other factors, fortified cheese with Spirulina biomass increased significantly ($P<0.05$) by raising the concentration compared to the control by 68.16 times. In general, using Spirulina biomass increases the β-carotene content of cheese by 7.86 times more than that of supernatant (Figure 2E).

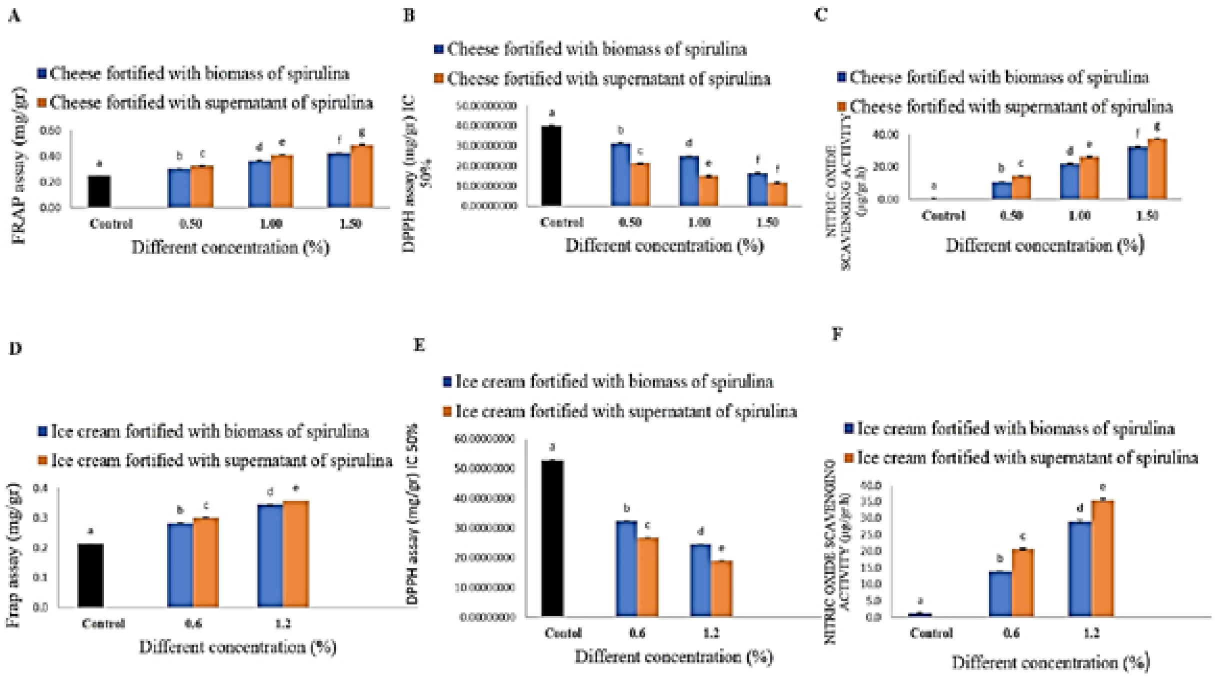

Figure 5. Measurement and comparison of FRAP (a & d), DPPH (b & e) and nitric oxide cleaning activity (c & f) in cheese and ice cream enriched with different concentrations of biomass and Spirulina supernatant

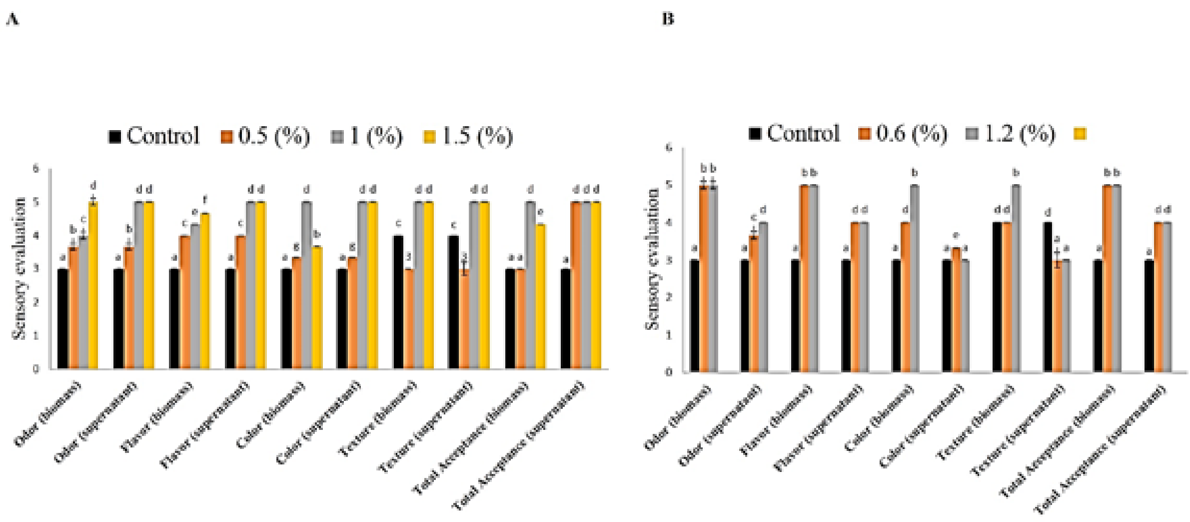

Figure 6. Evaluation of hedonic test (A) in cheese and (B) fortified ice cream enriched with different concentrations of biomass and Spirulina supernatant

Results of zinc, calcium, magnesium, potassium, and iron content in cheese samples

The results of zinc, calcium, magnesium, potassium, and iron content in cheeses fortified with Spirulina supernatant showed that with increasing the concentration of Spirulina, compared to the control, their contents were increased by 1.34, 1.1, 1.62, 1.58 and 1.81 times, respectively. However, the content of these elements in biomass-enriched cheeses with increasing concentration, in many cases, do not differ significantly (Figure 3).

Results of physicochemical analysis of inoculated ice cream

Ice cream protein content

The results of measuring the protein content in ice cream showed that the protein content of ice creams enriched with Spirulina biomass increased significantly (P<0.05) by raising the concentration of Spirulina compared to the control by 1.03 fold. However, in enriched ice creams with different concentrations of supernatant compared to the control, no significant (P<0.05) difference was observed (Figure 4A).

The fat content of ice cream

The results showed that the fat content of ice creams fortified with Spirulina biomass increased significantly (P<0.05) by increasing the concentration of Spirulina compared to the control by 1.06 times. However, in enriched ice creams with different concentrations of supernatant compared to the control, no significant difference (P<0.05) was observed (Figure 4B).

Evaluation of the total sugar of ice cream

The results showed that the sugar contents of ice creams fortified with Spirulina biomass increased significantly (P<0.05) by increasing the concentration of Spirulina by 1.2 times compared to the control (Figure 4C).

Ice cream aeration

The results showed that aeration in ice creams fortified with Spirulina biomass increased significantly (P<0.05) by increasing the concentration of Spirulina by 1.5 times compared to the control. However, in enriched ice creams with different concentrations of supernatant compared to the control, no significant (P<0.05) difference was observed (Figure 4D).

Ice cream melting point

The results showed that the melting point of ice creams fortified with biomass and Spirulina supernatant decreased significantly (P<0.05) by increasing the concentration of Spirulina compared to the control by 0.98 times. However, the significant differences in concentrations of biomass did not reduce the melting point (Figure 4E).

Results from the antioxidant activity of cheese and ice cream

The results of measurement of antioxidant potential by FRAP, DPPH, and nitric oxide trapping in cheeses and ice creams enriched with biomass and Spirulina supernatant were consistent with each other and significant (P<0.05) antioxidant potential with increasing concentration of Spirulina supernatant, compared to the control increased by 1.96, 0.52 and 2.59 times in cheese and by 2, 0.5 and 29.75 times in ice cream. In addition, the level of antioxidant activity in supernatant increased by 1.16, 1.47, and 1.16, and in ice cream by 1.33, 1.2, and 1.23 times compared to biomass (Figure 5).

Results of hedonic test of inoculated cheese and ice cream

The results of the hedonic test in cheeses enriched with biomass and Spirulina supernatant showed a direct relationship between satisfaction and sensory evaluations with increasing concentrations of biomass and supernatant ($P<0.05$). However, increasing the concentration of Spirulina biomass led to decreased color satisfaction and overall acceptance of ice cream and cheese (Figure 6A). The results of the hedonic test in ice cream inoculated with different concentrations of biomass and supernatant showed that the satisfaction level with increasing the biomass concentration was significantly higher than that of supernatant. Most sensory analyses showed a significant difference between the different concentrations (Figure 6B).

Discussion

A dairy enrichment-based approach can be one of the most important, appropriate, and practical methods to prevent malnutrition. The presence of unique bioactive compounds has made microalgae one of the most functional and useful foods. Compared to other natural resources with bioactive compounds, microalgae have a greater advantage due to their vast biodiversity. The present study investigated the production of cheese and ice cream fortified with biomass and cyanobacteria *S. platensis* supernatant with emphasis on organoleptic and nutritional properties.

The high protein content of microalgae is one of the main reasons for considering them as the main source of protein. Spirulina contains high amounts of protein (60%-70% of its dry weight). The protein in this microalga includes all the essential amino acids, such as valine, leucine, and isoleucine, and since this microalga has no cell wall, its protein is highly digestible. According to the statistical results (Figure 2), after adding different concentrations of biomass and Spirulina supernatant to cheese and ice cream samples, fortified cheeses with Spirulina supernatant had higher protein, fat, and ash content than biomass. However, the amount of protein and fat was higher for ice cream samples enriched with Spirulina biomass than for the samples enriched with supernatant. However, in the Varga et al. (2002) study, the benefits of Spirulina biomass-enriched milk were reported. The results showed that adding Spirulina biomass increased the content of amino acids, vitamins, and fatty acids. This result may be because the presence and abundance of bioactive substances in the biomass of Spirulina algae are of great nutritional importance for producing functional dairy foods (Varga et al., 2002).

Akalin et al. (2009), in the study of the effect of Spirulina biomass on the microbiological activity of traditional yogurts and probiotics during refrigerated storage, showed that the protein content of algae-containing samples increased significantly compared to the control sample. The results of this study are consistent with the present research and led to a significant increase in protein content in traditional cheeses by 73%, 86%, and 93% compared to the control group. Similar results were obtained in the study of Bosnea et al. (2021) on the effect of concentrations of 0.25, 0.5, and 1 g/kg of *S. platensis* powder on traditional Greek cheese (Bosnea et al., 2021).

Microalgae contain significant amounts of fat with a composition like vegetable oil. Under certain conditions, microalgae have up to 85% fat; however, fat is usually between 20%-40% in its dry weight. Microalgae fat is usually the glycerol ester and 14-22 carbon fatty acids. Spirulina contains 5%-7% of lipids, mainly composed of essential fatty acids such as linoleic acid and γ-linolenic acid (Ötleş & Pire, 2001). Spirulina is cholesterol-free and rich in unsaturated fatty acids, which makes it suitable for treating and preventing atherosclerosis, obesity, and high blood pressure. Due to the direct effects of γ-linolenic acid on the immune system and the treatment of many diseases, there has always been great interest in producing high concentrations of γ-linolenic acid (Sajilata et al., 2008). In the study of Bosnea et al. (2021) on the effect of concentrations of 0.25, 0.5, and 1 g/kg *S. platensis* powder on traditional Greek cheese, it was shown that by increasing the concentration of Spirulina, the amount of fat increases but the moisture decreases (Bosnea et al., 2021). However, the Augustini results in 2016 showed that the application of *S. platensis* powder in concentrations of 0%, 1%, and 1.5% on ice cream and soft cheese did not change the fat content but reduced the moisture content (Agustini et al., 2016). The increased fat content of Spirulina-enriched cheese was observed in studies by Beheshtipour et al. (2013). Also, the amount of cheese ash in their study similar to the present study, increased between 10% and 11%. This increase in cheese ash is due to the addition of powder of this microalgae due to the high percentage of Spirulina ash content.

Similar results to the present study were found in Agustini et al. (2016) study on the application of *S. platensis* powder on ice cream and soft cheese. The Tańska et al. (2017) study on the sensory, physicochemical properties and water absorption of extruded corn enriched with Spirulina powder and also the Arslan and Aksay (2021) study on the sensory and physicochemical properties of yogurt stained with phycocyanin obtained from *S. platensis* revealed an increase the amount of ash.

Fadaei et al. (2015) also investigated the effect of different concentrations of microalgae *S. platensis* (0%, 0.3%, 0.5%, 0.8%) for some physicochemical properties (pH and acidity, protein and texture) and sensory properties of cheese containing 0.5 and 1 Oregano and found a significant increase in protein content, acidity, texture hardness, and acceptance and sensory characteristics compared to control. In a similar study, Golmakani et al. (2019) investigated the effects of Spirulina (*Arthrospira platensis*) on the growth of the probiotic *Lactobacillus casei* in bacterial fatty acid cheese. The results of the chemical, textural, and sensory properties of cheese showed a statistically significant difference in the protein content of samples containing algae compared to the control sample (Golmakani et al., 2019).

Cheese texture is one of its basic quality characteristics that play an essential role in the desirability of cheese. The structure of cheese consists of a protein network that includes the fat and soluble phases. In the present study, the moisture content of supernatant-enriched cheeses was reduced. In the studies of Fadaei et al. (2015) and the studies of Jeon (2006), the use of algae-fortified cheeses increases the hardness of the tissue. It is possible that as the moisture content decreases, the hardness of the algae-enriched specimens increases (Jeon, 2006). Şahin et al. (2020) showed that the moisture content of these Spirulina-enriched cookies decreased, and their protein content and sensory acceptance increased (Şahin, 2020).

Bensehaila et al. (2015) reported that Spirulina contains all the essential minerals for the body, such as calcium, iron, magnesium, manganese, phosphorus, potassium, selenium, and zinc. Based on the results of ion content, with increasing the concentration of Spirulina in cheeses enriched with supernatant, the amount of these ions also increased in the samples (Figure 3). *S. platensis* is one of the richest ion sources. For example, this algae has twice as much iron as plants and most meats, so it prevents anemia better than these plant and animal sources (Nakano et al., 2005).

Spirulina can also be used to improve ice cream's nutritional quality and functional properties. Due to the rich chemical composition with beneficial nutritional characteristics for health (Qiang et al., 1996) and the functional properties of Spirulina, in a study conducted by Malik et al. (2013), for partial or complete stabilizer replacement in ice cream is made with natural resources. Due to the rich nutritional properties of Spirulina, the replacement of stabilizers with microalgae in ice cream resulted in increased protein, minerals, essential fatty acids, and trace elements of natural origin (Malik et al., 2013). Their results are consistent with our research that in ice cream samples enriched with Spirulina biomass, protein content, fat, sugar, and aeration rate of ice cream are significantly increased by 1.039, 1.03, 1.009, and 1.05, respectively. As mentioned, the aeration rate of ice cream fortified with Spirulina has increased. This condition can be attributed to the surface-active properties of the protein and the fat content of Spirulina, which has an emulsifying capacity of 1.13 mL fat/g protein, a foam capacity of 207%, and a foam stability of 27% (Malik et al., 2013).

The melting point property is attributed to water holding capacity, which refers to the interaction between protein, product, and water, resulting in some water remaining with the product. In a study by Malik et al. (2013), adding Spirulina to ice cream increased the melting resistance, which was consistent with the results of our research. This outcome may be due to the protein and fat in ice cream fortified with Spirulina biomass (Sofjan & Hartel, 2004). In another study, Rasouli et al. (2017) optimized the formulation of traditional Iranian ice cream containing Spirulina microalgae. Their study showed that enriching ice cream with Spirulina increases viscosity and melting resistance and reduces ice cream aeration. Based on the sensory evaluation results, with increasing the amount of Spirulina in the ice cream formulation, consumers' acceptance of the taste, color, texture, and overall acceptance of the product decreased. Finally, their conclusion showed that enriching ice cream with Spirulina microalgae can achieve a product with desirable physical and organoleptic properties (Rasouli et al., 2017).

Agustini et al. (2016) enriched the cheese with Spirulina to investigate the micronutrient effect of this microalgae. The results showed that enrichment with Spirulina increased protein content, water, fat, β-carotene, and cheese texture. Increased β-carotene was one of the most important properties of adding Spirulina to cheese, consistent with our study. Spirulina strain contains large amounts (27%) of β-carotene, amounting to 700 to 1700 mg/kg of dry biomass of this cyanobacterium.

Compounds containing antioxidant properties may not respond equally to different sources of free radicals. Therefore, using multiple assays to measure antioxidant capacity is necessary for appropriate assessments (Alizadeh Khaledabad et al., 2020). Thus, the present study studied antioxidant properties in three different ways. Another study evaluated the antioxidant properties of phycobiliproteins and phenolic compounds extracted from Bangia atropurpurea. It was shown that the reducing power of the FRAP method is dose-dependent, and with increasing the concentration of phycocyanin, the antioxidant activity increases (Punampalam et al., 2018). Similar results were observed in the study of the antioxidant and nutritional potential of cookies enriched with biomass *S. platensis* at concentrations of 2% and 5% by the FRAP method by Egea et al. (2014). Gabr et al. (2020) studied the antioxidant activity of phycocyanin extracted from *S. platensis* at concentrations of 5, 10, 15, 20, 25, and 30 µg/mL by the DPPH method. They showed that as the concentration of phycocyanin increases, its antioxidant activity increases. Shalaby et al. (2013) attributed the antioxidant activity of Spirulina extracts by DPPH to the presence of phenols with high levels of biologically active phytochemicals (sterols, flavonoids, reducing sugars, tannins, and anthraquinones). In addition, in a study on the evaluation of antioxidant activity by nitric oxide trapping method of *S. platensis* ethanolic extract in vitro, it was shown that its antioxidant activity is dose-dependent, and with increasing the concentration of the extract, the antioxidant activity increases (Anbarasan et al., 2011). Similar results were obtained in the study of extraction and purification of phycocyanin from the dry powder of *S. platensis* and evaluation of its antioxidant, anticoagulant activity, and prevention of DNA damage (Kamble et al., 2013).

The antioxidant properties of yogurt and cheese enriched with Spirulina were investigated by Barkallah et al. (2017). In their study, after adding Spirulina to yogurt in different concentrations, they studied its effect on the fermentation process, texture, and nutritional and sensory properties of yogurt. The data showed that adding Spirulina biomass accelerates fermentation and preserves our tissue properties and sensory acceptance. These data align with the increase in acceptability and the increase in antioxidant activity in line with the findings of our study. Our study also showed that the results increased the antioxidant potential of cheese and ice cream fortified with supernatant more than biomass.

Sensory characteristics play an important role in consumers' overall acceptance of food products. Actually, consumers avoid functional foods if the combined ingredients produce unpleasant tastes.

A decrease in color satisfaction and overall acceptance was observed in the hedonic test analyses of cheeses fortified with Spirulina biomass. The decreased sensory score may be due to the probiotic metabolic by-products (Alizadeh Khaledabad et al., 2020). A study by Bosnea et al. (2021) showed that with increasing Spirulina concentration, the olfactory satisfaction of traditional Greek cheese decreased significantly, which was in line with the present study evaluating the smell of traditional cheeses. Adding *S. platensis* creates an unpleasant odor similar to fish due to its mineral content (Agustini et al., 2016). Researchers have studied methods to reduce the odor associated with Spirulina algae using activated charcoal adsorption, heating, enzymatic hydrolysis of lysozyme, the inclusion of β-cyclodextrin, fermentation and solvent extraction, and fermentation and extraction of ethanol as the most effective method to reduce the odor associated with Spirulina has also been proved (Bao et al., 2018).

Microalgae biomass is considered a sustainable source of protein that can meet the growing global demand for these biomolecules. Spirulina is one of the most nutritious microalgae and can provide up to 70% of dry biomass to protein. Spirulina has great potential for use as a functional enhancer in dairy products. Similarly, in the present study, protein enrichment, microbiological stability, and consumer acceptance in producing Spirulina-enriched cheese and ice cream could be promising in terms of nutritional value and considered an alternative to enrichment.

Conclusion

The study revealed that the amount of protein, fat, and ash in cheeses enriched with Spirulina supernatant increased significantly by 1.22, 1.12, and 1.09, respectively, more than biomass, also, the moisture content of cheese with a significant difference decreased by about 0.989 times more than biomass. Conversely, in ice cream samples enriched with Spirulina biomass, the protein content, fat, sugar, and aeration rate of ice cream increased significantly by 1.039, 1.03, 1.009, and 1.05 times, respectively. In addition, a decrease in point melting was observed in ice cream enriched with biomass and Spirulina supernatant significantly compared to controls. The results of antioxidant potential in cheese and ice cream enriched with Spirulina supernatant showed that the antioxidant activity towards biomass in FRAP methods and nitric oxide trapping compared to control increased significantly. The results of the hedonic test in cheeses enriched with Spirulina biomass showed that at a concentration of 1.5% compared to the control, the satisfaction of odor and taste factors increased significantly.

In contrast, for ice cream samples, color and texture increased. In addition, ice cream samples enriched with supernatant at a concentration of 1.5% compared to the control, the odor factor was observed to be the highest satisfaction. The tissue factor in supernatant-enriched ice cream also decreased significantly compared to the control.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization, methodology and supervision: Seyed Amir Ali Anvar; Data collection: Bahareh Nowruzi and Leila Golestan; Investigation, data analysis, funding acquisition and writing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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چکیده

زمینه مطالعه: اسپیرولیناپلاتنسیس جلبکی حاوی رنگدانه‌های طبیعی و همچنین ترکیبات فنلی و فلاونوئیدی است که ممکن است علاوه‌بر زیست توده در طی کشت به مایع رویی نیز منتقل شود.

هدف: در این تحقیق برای اولین بار پنیر و بستنی غنی‌شده با استفاده از زیست توده و مایع رویی عصاره ریز جلبک اسپیرولینا پلاتنسیس با تأکید بر خواص ارگانولپتیک و تغذیه تولید شد.

روش کار: پس از کشت ۳۰ روزه اسپیرولینا، غلظت‌های مختلف زیست توده و مایع رویی عصاره اسپیرولینا تهیه و برای پنیر و بستنی استفاده شد. سپس ویژگی‌های فیزیکوشیمیایی و پتانسیل آنتی‌اکسیدانی با روش‌های Frap و DPPH اندازه‌گیری شد. درنهایت، تجزیه‌وتحلیل آماری داده‌های به‌دست‌آمده از هر آزمایش با استفاده از نرم‌افزار SPSS نسخه ۲۴ با تحلیل واریانس یک‌طرفه و در سه تکرار انجام شد.

نتایج: نتایج نشان داد محتوای پروتئین، چربی، خاکستر و همچنین زیست توده در غذاهای غنی‌شده بیشتر بود. بااین‌حال، در بستنی‌های غنی‌شده، محتوای پروتئین، چربی، قند و سرعت هوادهی به‌طور قابل‌توجهی در مقایسه با مایع رویی افزایش یافت. بااین‌حال، پنیر و بستنی غنی‌شده با مایع رویی، فعالیت آنتی‌اکسیدانی بیشتری نسبت به زیست توده داشتند. نتایج آزمایش لذت در پنیرهای غنی‌شده با زیست توده و مایع رویی اسپیرولینا نشان داد که با افزایش غلظت این عوامل در پنیرها و بستنی‌های غنی‌شده، سطح رضایت ازنظر بو، طعم، رنگ، بافت و عوامل پذیرش عمومی افزایش یافت.

نتیجه‌گیری نهایی: داده‌های به‌دست‌آمده از این مطالعه نشان داد که افزودن زیست توده و مایع رویی اسپیرولینا پلاتنسیس به بستنی و پنیر باعث افزایش محتوا و بهبود خواص ارگانولپتیک و حسی این غذاها شد. به نظر می‌رسد چون این مواد نقش مهمی در رژیم غذایی کودکان دارند، می‌توان از این میکروارگانیسم برای مبارزه با سوءتغذیه و رژیم غذایی بدون ریزمغذی‌ها به نحو مطلوب استفاده کرد.

کلیدواژه‌ها: Spirulina platensis، پنیر، بستنی، ریز جلبک

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