Turkish Journal of Agriculture - Food Science and Technology, 14(2): 514-518, 2026
DOI: https://doi.org/10.24925/turjaf.v14i2.514-518.8608

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Investigation of Quality Criteria and Antioxidant Capacity of Ice Cream Enriched with *Chlorella vulgaris*

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ARTICLE INFO

Research Article

Received : 10.12.2025
Accepted : 27.01.2026

Keywords:
Goat milk
Chlorella vulgaris
Diabetic ice cream
Nutritional profile
Antioxidant capacity

ABSTRACT

Microalgae stand out due to their essential compound content and rich nutritional profile, as well as their ease of production and environmentally friendly nature. *C. vulgaris*, one of the popular microalgae, offers various important health benefits, including antioxidant, antitumor, antihypertensive, and antidiabetic properties, thanks to its protein content and bioactive compounds. In this context, the effect of using *C. vulgaris* in different proportions in ice cream formulation on physicochemical properties and antioxidant capacity was investigated in the current study. In this context, the current study investigated the effects of *C. vulgaris* added at different concentrations to an ice cream formulation containing 1.5% stevia instead of sugar, on the quality criteria and functional properties of the ice cream. Adding 2.5%, 5%, and 7.5% *C. vulgaris* to ice cream samples was found to cause very significant differences in fat, protein, pH, first dripping time, complete melting time, overrun, DPPH free radical scavenging activity IC50 values, ABTS radical scavenging activity IC50 values, and TPC values. The inclusion of *C. vulgaris* in diabetic ice cream production has been found to have positive effects on quality criteria and antioxidant capacity. Consequently, it is believed that further detailed studies will provide a new perspective to the dairy industry in order to offer a healthier and more functional product.

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Introduction

The world's population, which reached 8 billion in 2022, is projected to reach approximately 9.7 billion by 2050. Despite the rapid growth of the world's population, reaching the limits of food production capacity to meet population needs while simultaneously managing food security is putting pressure on global food systems. Furthermore, the global population's protein consumption in 2017 is known to be 202 million tons, and it is projected that the population in 2050 will require approximately 250-361 million tons of protein. Therefore, to support the population while simultaneously implementing the United Nations Sustainable Development Goals, a need has arisen for alternative protein sources that can replace animal-based foods in daily diets. In this context, it is reported that the inclusion of microalgae in the daily diet as a protein source will make a significant contribution to many Sustainable Development Goals, including Zero Hunger (2), Good Health and Well-being (3), Clean Water and Sanitation (6), Responsible Consumption and Production (12), Climate Action (13), Life Below Water (14), Life on Land (15) and Partnerships for the Goals (17) (Severo et al., 2024; Williamson et al., 2024). In recent years, it has been reported that the microalgae industry has a large market share with its nutraceutical properties and has exhibited rapid development (Batista et al., 2017). Microalgae, which date back 3.4 billion years and are known to be fossilized in Western Australian rocks, are members of the cyanobacteria group (Safi et al., 2014). *Chlorella vulgaris*, which belongs to the green microalgae family, are unicellular microscopic eukaryotic organisms that thrive under both heterotrophic, autotrophic and mixotrophic growth conditions. While *C. vulgaris* attracts attention with its protein content (61.6%), it also offers unique nutritional elements to consumers in terms of potassium, iron, calcium, magnesium and phosphorus minerals; inositol, biotin, thiamine, folic acid, provitamin-A, C, D, E and K vitamins and α-tocopherol, lycopene, α-carotene, β-carotene, chlorophyll and essential fatty acids content (Blas-Valdivia et al., 2011; Graça et al., 2018; Guo et al., 2024). While studies on ice cream production using various microalgae sources are almost non-existent in the literature (Durmaz et al., 2020; Rodrigues et al., 2020), this study aims to investigate the potential of using *C. vulgaris*, which has high biological value, in ice cream production. In this context, this study aims to investigate the effect of *C. vulgaris* addition on the physicochemical and antioxidant capacity properties of ice cream and to design the ice cream formulation accordingly. In this context, the

current study modified the standard ice cream formulation by using stevia instead of sugar. *C. vulgaris* was added at 2.5%, 5%, and 7.5% levels to improve nutritional value and functional properties. In addition to physicochemical analyses, DPPH free radical scavenging activity IC50 value, ABTS radical scavenging activity IC50 value, and TPC analyses, which represent antioxidant activity, were performed on the produced ice cream samples.

Materials and Methods

Material

Goat milk (14.1% dry matter, 4.8% fat, 3.4% protein, 0.8% ash, and 6.7 pH) used in ice cream production was supplied by local farms in Erzurum (Türkiye), while cream, stevia (Egepak Gıda ve Ambalaj San. A.Ş., İzmir, Türkiye), emulsifier (mono and diglyceride), stabilizer (salep), and milk powder were purchased from local companies in Erzurum (Türkiye). *C. vulgaris* powder (1.5% fat, 66% protein, 0.16% ash) was purchased from Naturiga Natural Foods (Istanbul, Türkiye).

Method

Ice Cream Production

In ice cream production, cream (38% fat) was added to adjust the fat content of goat milk to an average of 6%. The ice cream mix was homogenized by adding 4% milk powder, 0.90% stabilizer, 0.25% emulsifier, and 1.5% stevia. It was then heat-treated at 90ºC for 10 minutes. Similarly, following heat-treatment, *C. vulgaris* was added to the prepared ice cream mixes at different rates (2.5%, 5%, and 7.5%). All prepared ice cream mixes were kept at 4ºC for 24 hours, then mixed in an ice cream machine for 45 minutes to produce ice cream. Ice cream samples were placed in storage containers and stored at -18ºC. Images of the ice cream samples are presented in Figure 1.

Physicochemical Analyses

In the physicochemical analysis of ice cream samples, first dripping time, complete melting time and overrun (Çakmakçı et al., 2016) analyses were performed in addition to dry matter, fat, protein, ash and pH values (Sayar et al., 2022).

Antioxidant Capacity Analyses

In order to analyze the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity IC50 values and ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity IC50 values of ice cream samples, firstly, extracts were prepared. For this purpose, the volume of 3 mg of ice cream sample was made up to 30 mL with methanol, then kept for 30 min, centrifuged at 6000 rpm for 15 min, and filtered (Whatman No. 2) (Ciniviz & Yildiz, 2020). In the DPPH free radical scavenging activity IC50 value, the volume of the solutions prepared at different concentrations (25-75 µg/µL) was made up to 2 mL with methanol and homogenized by adding DPPH solution (0.5 mL). Then, absorbance measurement was carried out at 537 nm on a spectrophotometer (Optizen, Optizen POP, South Korea). For ABTS radical scavenging activity IC50 value, ABTS solution (7 mM) was prepared with $K_2S_2O_8$ (2.45 nM) and diluted with 0.1 M buffer solution, pH 7.4, and then the absorbance at 734 nm was adjusted to 0.700.

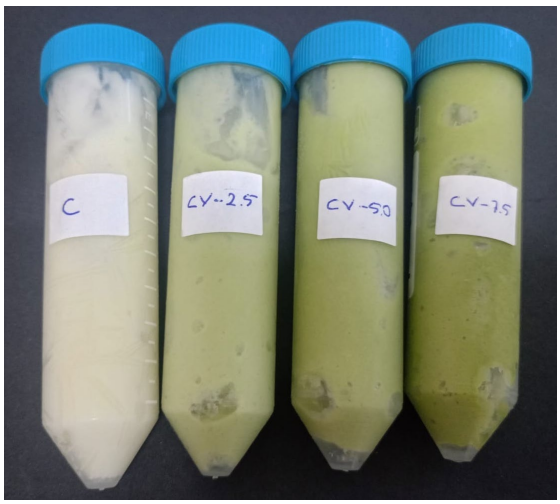

Figure 1. Images of ice cream samples

Different concentrations (10-30 µg/mL) were obtained from extracts prepared from ice cream samples, and their volumes were made up to 2 mL with 0.5 mL of ABTS solution. Absorbance measurements were then performed on a spectrophotometer (Zor et al., 2022).

Total Phenolic Content (TPC)

TPC analysis of ice cream samples were carried out based on the Folin-Ciocalteu method. 0.1 g of ice cream sample was diluted to 50 mL with pure water and filtered through Whatman No. 1. 0.5 mL of the filtrate, 2.5 mL of 2 N Folin-Ciocalteu reagent, and 2 mL of 75 g/L Na_2CO_3 were homogenized. After the homogenates were left at room temperature for 2 hours, their absorbance at 760 nm was measured in a spectrophotometer, and gallic acid was used in drawing the calibration curve (Meda et al., 2005).

Statistical Analysis

The current study was conducted according to a completely randomized trial design. The data obtained were subjected to analysis of variance using the SPSS 25 package program, and differences between samples were determined using the Duncan multiple comparison test.

Results And Discussion

Physicochemical Analysis Results

The physicochemical analysis results of the control group and *C. vulgaris* added ice cream samples are given in Table 1. It was observed that the addition of *C. vulgaris* to the ice cream samples did not have a statistically significant effect on the total dry matter and ash contents ($p>0.05$). However, it was found that the addition of *C. vulgaris* to the ice cream samples caused statistically very significant ($p<0.01$) differences in fat, protein, pH, first dripping time, complete melting time and overrun values. It was found that dry matter, protein, first dripping time, complete melting time and overrun values increased while fat content and pH values decreased in ice cream samples added to *C. vulgaris* compared to the control group.

In the current study, it was determined that the addition of *C. vulgaris* provided a very significant increase in the protein content of the ice cream samples, and in ice cream samples with the highest protein content of 7.5% *C. vulgaris* added, the protein content increased by approximately 30% compared to the control group.

Table 1. Physicochemical analysis of ice cream samples.

Samples		Total Solids (%)	Fat (%)	Protein (%)	Ash (%)
Plain Ice cream		25.20±1.07^{a}	6.03±0.13^{a}	3.47±0.06^{c}	0.81±0.03^{a}
C. vulgaris	CV-2.5	25.67±0.97^{a}	5.80±0.10^{a}	3.98±0.21^{b}	0.82±0.01^{a}
	CV-5.0	26.26±1.04^{a}	5.55±0.06^{b}	4.32±0.06^{a}	0.80±0.06^{a}
	CV-7.5	26.69±1.20^{a}	5.35±0.04^{b}	4.49±0.08^{a}	0.79±0.02^{a}
Sig.		ns	**	**	ns
Samples		pH	First Dripping Time (s)	Complete Melting Time (s)	Overrun (%)
Plain Ice cream		6.34±0.05^{a}	2078.00±185.26^{c}	7752.00±237.59^{c}	31.41±2.49^{c}
C. vulgaris	CV-2.5	6.20±0.08^{ab}	2534.00±168.29^{bc}	8595.50±194.45^{b}	36.26±1.65^{bc}
	CV-5.0	5.99±0.16^{b}	2917.50±65.76^{ab}	9174.00±96.17^{ab}	42.11±3.85^{ab}
	CV-7.5	5.69±0.11^{c}	3190.00±237.59^{a}	9527.50±296.28^{a}	47.65±1.51^{a}
Sig.		**	**	**	**

Sign.: Significant; ns: not significant, p>0.05; **: p<0.01; P: Plain ice cream; CV-2.5: Ice cream with 2.5% *C. vulgaris*; CV-5.0: Ice cream with 5% *C. vulgaris*; CV-7.5: Ice cream with 7.5% *C. vulgaris*

Table 2. DPPH IC50, ABTS IC50 and TPC analyses results of ice cream samples.

Samples		DPPH IC_{50} (µg/mg)	ABTS IC_{50} (µg/mg)	TPC (mg GA/kg)
Plain Ice cream		61690.42±1382.78^{a}	43152.46±3780.39^{a}	45.17±13.87^{d}
C. vulgaris	CV-2.5	47887.14±2915.21^{b}	35258.16±3014.13^{ab}	476.10±8.95^{c}
	CV-5.0	40251.62±1897.54^{c}	27590.40±4661.62^{bc}	541.11±19.05^{b}
	CV-7.5	32850.76±3685.88^{d}	18758.05±1716.64^{c}	684.92±13.87^{a}
Sig.		**	**	**

Sign.: Significant; **: p<0.01; P: Plain ice cream; CV-2.5: Ice cream with 2.5% *C. vulgaris*; CV-5.0: Ice cream with 5% *C. vulgaris*; CV-7.5: Ice cream with 7.5% *C. vulgaris*

Similarly, Faresin et al. (2022) reported that the protein content of ice cream samples produced by adding *Spirulina platensis* increased compared to the control group. Atallah et al. (2022) reported that the total dry matter content of ice cream samples with stevia addition was 28.01%, protein content was 3.54%, ash content was 0.86%, and overrun value was 57.99%, and that the use of sweetener instead of sucrose altered the physicochemical analyses. Srinu et al. (2022) reported that the pH value of ice creams produced by adding different amounts of spices decreased compared to the control group, varying between 6.58 and 6.68. Shelke et al. (2020) reported that the fat content of ice creams produced by adding jamun pulp decreased depending on the pulp ratio compared to the control group (11.15%), varying between 9.40 and 10.72%. They also reported that fat content in ice cream is important in terms of texture, stability, and consistency. One of the primary quality criteria of ice cream is melting speed, which can vary particularly with the number and size of fat globules, dry matter content, and ice crystal size. In this case, the initial ice cream mix is closely related to melting (Góral et al., 2018). Indeed, the current study observed that the dry matter content of ice cream samples paralleled the first dripping and complete melting times. Overrun, on the other hand, characterizes the amount of air in ice cream mixes stabilized by surfactants and foaming. It is another important quality criterion affecting production efficiency, product texture, structure, and flavor (Atallah et al., 2022). Similarly, Malik et al. (2013) and Winarni Agustini et al. (2016) also reported that the use of *Spirulina* in ice cream production positively contributes to melting time and overrun values. Additionally, Malik et al. (2013) reported that the effect on volume increase is related to the protein content of *Spirulina* and consequently to the surface-active properties of the proteins. It is reported that the dry matter of *C. vulgaris* consists of an average of 51-58% protein, 14-22% fat, and 12-17% carbohydrates (Koyande et al., 2019). Generally, the increase in dry matter, protein, first dripping time, complete melting time, and overrun values of ice creams supplemented with *C. vulgaris* can be attributed to the unique composition of *C. vulgaris* (Hernández et al., 2022).

Antioxidant Capacity Characteristics

Reactive oxygen species (ROS) are associated with cancer, neurological disorders, cardiovascular disease, and diabetes. Therefore, the use of antioxidants has become a necessity to prevent these diseases, neutralize ROS, and maintain homeostasis. However, despite the potential toxic effects of synthetic antioxidants, the use of natural antioxidants, such as polyphenols, carotenoids, flavonoids, isoflavones, and vitamins, is recommended, as they are considered safe, effective, and harmless (Khan et al., 2022). Antioxidant activity can be defined as the ability of a product or compound to neutralize or capture ROS (Boyanova et al., 2022). Because the antioxidant activity values of foods are affected by many parameters, using methods with different reaction mechanisms, i.e., different mechanisms of action against radicals, is crucial for providing accurate results (de Amarante et al., 2020). In this context, the current study utilized both DPPH free radical scavenging activity IC50 value and ABTS radical scavenging activity IC50 value analyses to more clearly observe the contribution of *C. vulgaris* to the antioxidant activity of ice cream samples, given the mixed ice cream matrix. The results for DPPH free radical scavenging activity IC50 value, ABTS radical scavenging activity IC50 value, and total phenolic compound content (TPC) of the control group and *C. vulgaris*-added ice cream samples are presented in Table 2.

It was observed that the addition of *C. vulgaris* to the ice cream samples at different rates caused very significant ($p<0.01$) differences in the results of DPPH free radical scavenging activity IC50 value, ABTS radical scavenging activity IC50 value and TPC values. As can be seen in Table 2, it was determined that the use of *C. vulgaris* in ice cream production contributed positively to DPPH free radical scavenging activity IC50 values, ABTS radical scavenging activity IC50 values and TPC value compared to the control group. Indeed, it was determined that the best DPPH free radical scavenging activity IC50 value (32850.76 µg/mg), ABTS radical scavenging activity IC50 value (18758.05 µg/mg) and TPC value (684.92 mg GA/kg) were in the samples with 7.5% *C. vulgaris* added. In the present study, it was observed that the addition of *C. vulgaris* at 2.5-7.5% levels increased the TPC value approximately 10-15 times compared to the control group. Pradhan et al. (2021) reported that *C. vulgaris* is a valuable source of bioactive antioxidant agents through its high content of phenolics, flavonoids and other active compounds. In addition, researchers reported that *C. vulgaris* has a superior ability in reducing or neutralizing ROS as a hydrogen atom donor in scavenging free radicals due to its rich phenolic compound profile. Abdel-Karim et al. (2019) reported that the dominant phytochemical groups of *C. vulgaris* are phenols, alkaloids and flavonoids and that *C. vulgaris* also has glycoside, saponin and steroid content. The researchers reported a significant correlation between the antioxidant activity analyses (DPPH, total antioxidant capacity and reducing power) of *C. vulgaris* and the phenolic compound content. In the literature, it has been recorded by other researchers that the use of *Spirulina platensis* (Boyanova et al., 2022); camu-camu pulp (Curti et al., 2021); C-phycocyanin (de Amarante et al., 2020); pomegranate, grape and sesame seed powder and oils (Akca & Akpinar, 2021) in ice cream production exhibited positive results in terms of antioxidant activity and phenolic compound content. Szmejda et al. (2018) determined DPPH inhibition in mint ice cream enriched with *Spirulina* extract to be 39.7%, while Hacıbektaşoğlu and Gündoğdu (2024) found DPPH inhibition to be 27.98% in ice creams with 10% sucrose and 15% beetroot added. In conclusion, it was found that the use of *C. vulgaris* in ice cream production is a good strategy for improving antioxidant activity.

Conclusion

Goat milk, with its composition, textural properties, and functional compounds, holds a significant place in the diet, meeting a high proportion of essential nutrients needed by humans and even being used in the treatment of certain diseases. Among dairy products, ice cream is considered the most suitable for formulation enrichment, and in recent years, the production of functional ice cream by adding various additives has become increasingly common. The use of stevia instead of sugar in ice cream production is of great importance for the production of diabetic products. Among the additives added to enhance the functionality and nutritional value of dairy products, microalgae have recently attracted attention. In addition to their rich nutritional profile, microalgae impart significant properties to the product to which they are added, thanks to their unique compounds. In this context, the current study found that *C. vulgaris*, added at different concentrations to the formulation of diabetic ice cream samples produced from goat milk using stevia, improved various quality criteria expected for ice cream. Furthermore, the use of *C. vulgaris*, as a rich protein source and antioxidant agent in diabetic ice cream production, has been shown to increase antioxidant capacity and functionality.

Declarations

Acknowledgements

We would like to thank Atatürk University Scientific Research Projects Coordination Unit (Project No: FBA-2025-15220) for their support in conducting this study.

Declaration of interests

The author states no conflict of interest.

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