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Development of microalgae-enriched ice milk: Nutritional and sensory evaluation

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ABSTRACT

Microalgae are increasingly recognized as sustainable food ingredients due to their richness in proteins, vitamins, minerals, flavonoid, phenolic compounds, and antioxidants. This study investigated the incorporation of *Chlorella vulgaris* and *Spirulina platensis* powders at 0.5%, 1.0%, and 1.5% into ice milk formulations to develop functional dairy products. The fortified samples were evaluated for physicochemical, phytochemical, antioxidant, microbiological, and sensory characteristics. Results showed that the addition of microalgae did not significantly affect proximate composition, including total solids, fat, protein, carbohydrate, ash, acidity, or pH ($p \leq 0.05$). However, significant improvements were observed in functional bioactive properties, including antioxidant activity, total phenolic content, and flavonoid content levels, alongside improvements in density, weight per gallon, and overrun, indicating enhanced product structure and functional quality. Sensory evaluation confirmed strong sensory acceptability by trained panelists, with favorable ratings for appearance, color, flavor, texture, and overall quality. These findings highlight *Chlorella vulgaris* and *Spirulina platensis* as valuable natural sources of bioactive compounds and colorants, supporting the development of functional ice milk with enhanced antioxidant potential rather than changes in basic macronutrient composition, aligning with current trends in sustainable and health-oriented dairy innovation.

1. Introduction

Diet is an important determinant of health, physical status, and overall well-being; consequently, increasing attention has been paid to the development of functional foods in recent years. Among these, milk and dairy products enriched with probiotic cultures or functional ingredients have gained considerable interest due to their ability to enhance the nutritional and health-promoting properties of the final products [1–3].

Although millions of microalgal species are estimated to exist in nature, biotechnological research has focused mainly on a limited number of genera, including *Arthrospira* (Spirulina), *Chlorella, Porphyridium, Nannochloropsis, Haematococcus*, and *Dunaliella* [4,5]. In recent years, the incorporation of microalgae into food products has gained significant attention due to their rich composition of macronutrients and micronutrients. In particular, *Spirulina platensis* is known for its high nutritional value, containing approximately 60% protein on a dry weight basis [6].

Moreover, *Spirulina platensis* is a rich source of bioactive compounds with potential health benefits, including essential vitamins, minerals, polyunsaturated fatty acids (PUFAs), and antioxidant pigments such as phycocyanin. These compounds exhibit nutraceutical properties and may contribute to disease prevention and overall health improvement [7,8]. Similarly, *Chlorella vulgaris* and *Spirulina platensis* have been widely investigated as functional food ingredients capable of enhancing the nutritional and antioxidant properties of various food and dairy

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products [9,10].

Ice cream is one of the most popular dairy products worldwide, valued for its sensory appeal, nutritional content, and refreshing properties, particularly during warm seasons. It is typically formulated using milk, sweeteners, stabilizers, emulsifiers, flavorings, and colorants [11]. Therefore, fortification of ice cream with functional ingredients may improve its nutritional profile and contribute to meeting dietary requirements for proteins, vitamins, and minerals. In recent years, increasing concern regarding the potential health risks associated with synthetic food colorants has driven demand for natural alternatives. In this context, microalgae represent a promising source of natural pigments and bioactive compounds that can enhance both the functional and visual properties of ice cream. Previous studies have demonstrated the successful application of microalgal biomass (e.g., spray-dried *Nannochloropsis oculata, Porphyridium cruentum*, and *Diacronema vlkianum*) in ice cream formulations due to their pigment content and functional properties [11].

However, despite these advances, limited research has compared the combined use of *Chlorella vulgaris* and *Spirulina platensis* across different incorporation levels in ice milk formulations with a comprehensive evaluation of physicochemical, phytochemical, rheological, microbiological, and sensory properties.

Therefore, the present study aims to develop a novel functional ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis*. This work investigates the effect of different concentrations of these microalgae on the nutritional, functional, and sensory characteristics of the final product, with the goal of identifying an optimal formulation that balances enhanced bioactivity with consumer acceptability.

2. Materials and methods

2.1. Materials and reagents

Fresh buffalo's milk (6% fat) was obtained from the milk production unit of Al-Azhar University (Assiut Branch, Egypt). Skim milk powder (AGRI, BEST; Holland) was purchased from the local market in Cairo, Egypt. Carboxymethyl cellulose (CMC) of Indian origin (high viscosity, minimum assay 95.5%) was supplied by El Nasr Pharmaceutical Chemicals Co., Egypt. Vanillin was obtained from Jia Xing Zhonghua Chemical Co., Ltd., China, and sugar was purchased from the local market in Assiut, Egypt. Freeze-dried microalgae powders (*Chlorella vulgaris* and *Spirulina platensis*) were provided by the Algal Biotechnology Unit, National Research Centre, Dokki, Giza, Egypt. All chemicals used in this study were of analytical grade and supplied by international chemical companies, including VWR Chemicals BDH (Wharf Road, London, UK), Sigma (ON L6H 6J8, Canada), and Difco (La Pointe de Claix, France). Distilled water and Pyrex glassware were used throughout all experimental procedures.

2.2. Ice milk mix preparation

The ice milk mixes were formulated to contain 4% fat, 12% milk solids-not-fat (MSNF), 15% sugar, and 0.3% carboxymethyl cellulose (CMC) (Table 1). Liquid ingredients were first combined in a mixing vessel and heated to 40 °C using a water bath. Dry ingredients, except sugar and stabilizer, were gradually incorporated into the heated mixture under gentle stirring. Subsequently, sugar and stabilizer were added, and the mixture was homogenized using a Moulinex® mixer (France) at 55 °C for 2 min.

Six experimental ice milk formulations were prepared in addition to the control (without algae). These formulations were obtained by partially replacing skim milk powder with microalgae powder at different levels as follows: T1 (0.5% *Chlorella vulgaris*), T2 (1.0% *Chlorella vulgaris*), T3 (1.5% *Chlorella vulgaris*), T4 (0.5% *Spirulina platensis*), T5 (1.0% *Spirulina platensis*), and T6 (1.5% *Spirulina platensis*), compared with the control (0% algae).

All mixes were pasteurized at 85 °C for 5 min, rapidly cooled to 10 °C, and aged at 4 °C for 18 h. After aging, preliminary physical evaluation of the mixes was conducted to assess initial quality. The mixes were then flavored with 0.01% vanillin, whipped, and frozen using a batch ice cream maker (STARMATIC V 500, Italy) at low speed for 12 min, allowing controlled air incorporation and development of the desired texture and overrun.

The resulting ice milk was packaged into 120 mL high-density polyethylene cups and hardened at −26 °C for 24 h to stabilize structure and minimize ice crystal growth. Finally, the hardened samples were subjected to comprehensive physicochemical, microbiological, rheological, and sensory analyses to evaluate product quality, texture, flavor, and overall acceptability [12].

2.3. Physicochemical characterization

2.3.1. Proximate analysis

The total solids (TSs) by oven drying method, total protein (TP) by Kjeldahl method, ash contents using a muffle furnace, fat by Gerber method, and titratable acidity (TA) by titration of ice milk samples were determined according to the AOAC [13]. Total carbohydrates were determined by the difference method [100 - (proteins + fats + moisture + ash in percentage)] [14]. The pH values of all treatments were measured electronically using a glass electrode connected to a pH meter (Hanna model 8417 digital pH meter, USA).

2.3.2. Color properties

The color parameters of the ice cream samples were measured using a colorimeter. (Hanter lab colour Flex). Color measurements of the ice cream treatments were obtained using a Hunter Lab Color Flex instrument. The L*, a*, and b* values were recorded, representing lightness, redness/greenness, and yellowness/blueness, respectively, as described by Francis [15].

2.3.3. Physical properties

Density and weight per gallon of the samples were determined according to the procedures outlined by Arbuckle [16]. Moreover, the overrun was measured according to Goff [17].

Table 1
Ingredients used in preparing functional ice milk fortified with different types and ratios of microalgae powder (*Chlorella vulgaris* and *Spirulina platensis*).

Ingredients %	Control	Ice milk treatments					
		T1	T2	T3	T4	T5	T6
Whole buffalo milk	75.99	75.99	75.99	75.99	75.99	75.99	75.99
Skim milk powder	8.7	8.2	7.7	7.2	8.2	7.7	7.2
Microalgae powder	-	0.5	1	1.5	0.5	1	1.5
Sugar	15	15	15	15	15	15	15
Stabilizer (CMC)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vanillin	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100	100	100

CMC: carboxymethyl cellulose.

The melting properties of the resultant ice cream were determined according to the method described by Goff [17]. A 70 g ice cream sample was carefully placed on a wire mesh (1.5 mm × 1.5 mm) positioned within a glass funnel mounted on a conical flask. The melting ice cream was collected in the conical flask at room temperature (25 ± 2 °C). The weight of the melted ice cream was recorded at 10–50 min intervals and expressed as a percentage of the initial ice cream block weight.

2.4. Antioxidant activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, according to the method described by Salman et al. [18]. The total phenol content of the treatments was determined using the Folin–Ciocalteu method, as described by Abd El-Aziz et al. [19].

2.5. Total flavonoid content

Total flavonoid content was determined using the aluminum chloride method, as described by El Sohaimy et al. [20].

2.6. Microbiological analysis

For the total bacterial count, standard plate count agar was utilized [21]. Coliform bacterial counts are carried out according to the method of ISO 4832 [22] using MacConkey agar media and incubated at 37 °C/24 h. The counts of yeast and molds were assessed according to the method described in ISO 21,527–2 [23] using plate count agar media and incubated at 25 °C/120 h. Psychrotroph counts were measured using the Standard Plate Count (SPC) method. Diluted milk samples were plated and incubated at 7 °C for 10 days [24].

2.7. Sensory evaluation

Sensory evaluation of ice milk treatments was conducted following the hardening process by 12 panelists of experienced staff members of the Dairy Dept., Fac., of Agric., Al-Azhar University (Assiut Branch, Egypt). Before sensory evaluation, the ice cream was tempered at a temperature ranging from −15 °C to −12 °C. Sensory evaluation was according to the scheme of Khalil and Blassy [25], which considered flavor (30 points), body and texture (30 points), melting rate (20 points), and appearance (20 points).

2.8. Statistical analysis

All measurements were performed in triplicate, and data are expressed as mean ± standard error (SE). Prior to analysis, data were checked for normality and homogeneity of variance. Statistical analysis was conducted using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS). One-way analysis of variance (ANOVA) was used to evaluate differences among treatments, and when significant differences were detected, means were separated using Duncan's multiple range test. Differences among means were considered statistically significant at $P \leq 0.05$.

3. Results and discussion

3.1. Chemical composition

The properties of the ice cream fortified with various algae powder concentrations are illustrated in Table 2. The data showed that the fortification of algae powder exhibited very slight differences in the chemical composition of ice milk, but the variation did not statistically differ ($p > 0.05$). Therefore, the findings exhibited non-significant differences of functional ice milk fortified with algae powder in total solids content (%), ash (%), fat (%), protein (%), carbohydrate (%), and % SNF compared with the control sample. This may be due to the substitution between the components of the mixture of ice milk fortified by algae. The higher the percentage of algae added, the lower the percentage of skim milk powder added. Furthermore, the limited impact observed may result from the relatively low proportion of algae powder added to the ice milk formulation. These results agree with those of Jadhav and Chawla [26], who reported that adding algae to ice milk decreased total solid content in all treatments compared to the control.

Moreover, data presented in Table 2 revealed an increase in acidity, expressed as % lactic acid, in ice milk samples containing *Chlorella vulgaris and Spirulina platensis* compared to the control. However, this increase was not statistically significant ($p > 0.05$). Titratable acidity increased slightly with the addition of microalgae, ranging from 0.16 ± 0.02 in the control sample to 0.18% in the T3 sample (highest algae concentration). This indicates a positive trend between increasing algae concentration and titratable acidity. These findings align with those reported by Malik et al. [27]. No significant differences in pH were observed among the measured samples ($p > 0.05$).

3.2. Antioxidant activity (DPPH)

Antioxidant activity describes a compound or mixture's ability to inhibit or neutralize reactive oxygen species (ROSs) [28]. Several in vitro assays evaluate antioxidant capacity, including DPPH, ABTS, FRAP, CUPRAC, and ORAC. These methods differ in their underlying mechanisms, providing insights into various aspects of antioxidant function.

Table 3 shows the DPPH radical scavenging activity (%) of ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis*. The results indicate significant differences ($p \leq 0.05$) between all fortified treatments and the control, confirming that microalgae addition enhances antioxidant activity. In general, both algae improved free radical scavenging capacity compared with the control, which can be attributed to their naturally high content of bioactive compounds such as phenolics,

Table 2
Chemical composition of functional ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis*.

Properties	Control	Treatments					
		T1	T2	T3	T4	T5	T6
TS (%)	35.00±0.12^{A}	35.00±0.04^{A}	34.99±0.15^{A}	34.98±0.02^{A}	35.00±0.42 ^{A}	34.99±0.18^{A}	34.98±0.09^{A}
Ash (%)	1.17±0.01^{A}	1.11±0.06^{A}	1.12±0.04^{A}	1.12±0.01^{A}	1.10±0.04^{A}	1.10±0.03^{A}	1.11±0.05^{A}
Fat (%)	4.53±0.04^{A}	4.55±0.01 ^{A}	4.56±0.02^{A}	4.57±0.03^{A}	4.54±0.03^{A}	4.55±0.04^{A}	4.56±0.06^{A}
Protein (%)	5.50±0.11^{A}	5.55±0.04^{A}	5.62±0.03^{A}	5.69±0.07^{A}	5.59±0.04^{A}	5.70±0.03^{A}	5.83±0.02^{A}
Acidity (%)	0.16 ± 0.02^{A}	0.16±0.01^{A}	0.17±0.02^{A}	0.18±0.05^{A}	0.16±0.03^{A}	0.17±0.01^{A}	0.18±0.04^{A}
pH values	6.77 ± 0.03^{A}	6.76±0.02^{A}	6.72±0.11^{A}	6.68±0.04^{A}	6.76±0.03^{A}	6.72±0.04^{A}	6.68±0.08^{A}
Carbohydrate (%)	23.82±0.17^{A}	23.79±0.42^{A}	23.70±0.15^{A}	23.64±0.02^{A}	23.79±0.00 ^{A}	23.70±0.33^{A}	23.64±0.12^{A}
S.N.F (%)	30.47±0.52^{A}	30.45±0.61^{A}	30.43±0.26^{A}	30.41±0.45 ^{A}	30.46±0.18 ^{A}	30.44±0.74^{A}	30.42±0.35^{A}

S.N.F: solid not fat. ^{A} Means in the same row with same capital litters are non-significantly different ($p > 0.05$).
Where, C: ice milk without any microalgae powder (Control treatment). T1, T2, T3: ice milk fortified with 0.5%, 1.0%, and 1.5% *Chlorella vulgaris* respectively. T4, T5, T6: ice milk fortified with 0.5%, 1.0%, and 1.5% *Spirulina platensis* respectively.

Table 3
Antioxidant activity, total phenolic, and total flavonoid content of functional ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis.*

Treatments	Mean (%) ± SE		
	DPPH* inhibition (%)	TPC (mg GAE/g)	TFC (mg QE /g)
C	19.46±0.04 ^{F}	0.126±0.01 ^{E}	0.030±0.03 ^{F}
T1	35.19±0.07 ^{E}	0.159±0.06 ^{D}	0.042±0.05 ^{E}
T2	51.65±0.01 ^{D}	0.193±0.04 ^{C}	0.054±0.01 ^{D}
T3	67.88±0.03 ^{C}	0.227±0.11 ^{AB}	0.066±0.00 ^{C}
T4	52.83±0.09 ^{D}	0.168±0.09 ^{D}	0.064±0.07 ^{C}
T5	84.16±0.05 ^{B}	0.210±0.02 ^{B}	0.098±0.04 ^{B}
T6	97.68±0.01 ^{A}	0.252±0.08 ^{A}	0.132±0.09 ^{A}

TPC: Total phenolic content, TFC: Total flavonoid content. $^{A, B, C, D, E, F}$: Means with the different capital letters in the same column are significantly different between the treatments ($p < 0.05$).
See Table 2 caption for the abbreviations meaning of the treatments.

pigments, and other antioxidant molecules.

When comparing the two algal types, *Spirulina platensis* consistently showed higher antioxidant activity than *Chlorella vulgaris* at the same or comparable incorporation levels, with the highest DPPH scavenging observed in the 1.5% *Spirulina* treatment. This difference may be explained by the higher concentration of phycocyanin and other antioxidant pigments in *Spirulina platensis*, which are known to contribute strongly to radical scavenging activity.

These findings agree with Boyanova et al. [29], who reported a significant increase in antioxidant capacity ($p \leq 0.05$) in ice cream samples fortified with spirulina powder. Overall, the results suggest that while both microalgae improve antioxidant properties, *Spirulina platensis* exhibits a stronger effect under the studied conditions.

3.3. Total phenolic compounds

Total phenol contents of functional ice milk fortified with microalgae powder *Chlorella vulgaris* and *Spirulina platensis* are presented in Table 3. The data showed that the addition of *Chlorella vulgaris and Spirulina platensis* to functional ice milk resulted in higher total phenol content compared to the control (without algae powder). On the other hand, the total phenol content was increased in functional ice milk with increasing algae content, and this resulted from the high total phenol content in algae, which was added to functional ice milk. Where the functional ice milk fortified with 1.5% *Spirulina platensis* showed higher total phenols content compared to all treatments, these results agree with Boyanova et al. [29], who also reported a significant increase in total phenolic content in ice cream fortified with Spirulina platensis. However, in our study, the increase at 1.5% Spirulina platensis was higher compared to their findings, which may be due to differences in the ice milk matrix, the concentration of algae used, or the extraction solvent employed for phenolic compounds."

3.4. Total flavonoid content

The effect of algae powder addition on the total flavonoid content (mg QE/g) of ice milk fortified with microalgae powder *Chlorella vulgaris and Spirulina platensis* is shown in Table 3. Data indicated that supplementation with microalgae *Chlorella vulgaris and Spirulina platensis* resulted in a significant increase ($p \leq 0.05$) in the total flavonoid content of functional snow milk compared to the control (without algae powder). The functional ice milk fortified with 1.5% *Spirulina platensis* showed higher total flavonoid content than all treatments. The total flavonoid content was increased in functional ice milk with increasing algae content; this could be due to the high total flavonoid content in *C. vulgaris* and *S. platensis,* which were added to functional ice milk.

Similar findings were reported by Darwish [30], who observed a significant increase in total flavonoid content in Karish cheese fortified with Spirulina platensis. The consistency between our results and those of Darwish [30] suggests that the dairy matrix does not interfere with the release or detection of flavonoids from microalgae. However, the magnitude of increase in our study differed, likely due to the different dairy products (ice milk vs. cheese) and the higher moisture content of ice milk, which may enhance flavonoid extractability.

3.5. Physical properties

Table 4 presents the physical properties of ice milk produced using various types and ratios of *Chlorella vulgaris and Spirulina platensis* microalgae powders. The results showed that both density and weight per gallon of the functional ice milk decreased significantly ($p \leq 0.05$) with increasing levels of algae powder. Density values decreased from 0.770 to 0.628 g/cm^3 when using *Chlorella vulgaris* and from 0.770 to 0.632 g/cm^3 when using *Spirulina platensis*. Similarly, weight per gallon decreased from 6.42 to 5.24 with *Chlorella vulgaris* and from 6.42 to 5.27 with *Spirulina platensis*. These findings demonstrate that the addition of algae powder to ice milk significantly affects both density and weight per gallon ($p < 0.05$) as the additive ratio of microalgae powder increases.

Overrun is defined as the percentage increase in volume of the ice cream mix due to the incorporation of air during freezing. The rate of air incorporation is directly influenced by the total solids content of the mix, including the presence and concentration of additives, stabilizers, and emulsifiers [31].

From the same Table, it was noted that the overrun of the resultant ice milk increased significantly ($p \leq 0.05$) due to the addition of *C. vulgaris* and *S. platensis.* This increase in overrun may be attributed to the presence of microalgae powder, which can enhance the air retention capacity of the ice milk mixture [32]. These results agreed with the findings observed by Malik et al. [27].. These findings corroborate the work of Akesowan [33], who observed an increase in foam volume during homogenization when *S. platensis* powder was added compared to a control without the *S. platensis*. Incorporating protein-rich substances into ice cream can enhance foam volume by facilitating air entrapment within the ice cream mixture, resulting in a higher overall volume.

3.5.1. Melting rate

The melting rate in ice milk is influenced by various factors, including its thermal diffusivity, which represents the ability of heat to penetrate the product. A direct relationship exists between thermal diffusivity and melting rate. Milk fat acts as a thermal insulator, hindering heat transfer within the ice milk. Consequently, as the fat content of the ice milk increases, the melting rate decreases.

The impact of different microalgae powders on ice milk varied depending on the addition percentage and specific properties of the algae used. Incorporating microalgae powder showed the highest resistance to melting. Incorporating microalgae powder can increase the

Table 4
The density, weight per gallon, and overrun of functional ice milk are fortified with *Chlorella vulgaris* and *Spirulina platensis.*

Treatments	Properties		
	Density (g/cm^3)	Weight per gallon (lb)	Overrun
C	0.770±0.00 ^{A}	6.42±0.02 ^{A}	42.69±0.04 ^{C}
T1	0.723±0.08 ^{A}	6.03±0.09 ^{AB}	53.55±0.01 ^{B}
T2	0.681±0.12 ^{A}	5.68±0.01 ^{B}	55.74±0.03 ^{AB}
T3	0.628±0.03 ^{B}	5.24±0.05 ^{C}	57.31±0.16 ^{A}
T4	0.726±0.16 ^{A}	6.05±0.08 ^{AB}	53.10±0.09 ^{B}
T5	0.687±0.04 ^{A}	5.73±0.01 ^{B}	55.35±0.03 ^{AB}
T6	0.632±0.08 ^{B}	5.27±0.01 ^{C}	57.13±0.00 ^{A}

$^{A, B, C}$: Means with the different letters in the same column are significantly different between the treatments ($p < 0.05$).
See Table 2 caption for the abbreviations meaning of the treatments.

viscosity of the ice milk mix by forming hydrogen bonds with water molecules. This increased viscosity enhances the ice milk's resistance to melting [34,35]. Compared to the control, the higher melting rate observed in ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis* can likely be attributed to the increased microalgae powder content. Microalgae powders exhibited a significant increase in ice milk melting resistance compared to the control, but with different rates.

Table 5 describes the functional ice milk fortified with different types and ratios of microalgae powder, *Chlorella vulgaris,* and *Spirulina platensis* rate of melting resistance. The results showed that the highest values of melting resistance after 10 and 50 min were found in T6 and T3 samples, which contained 1.5% algae powder, followed by treatment T5 and T2, which contained 1.0% algae powder, followed by treatment T4 and T1, which contained 0.5% algae powder. These findings concur with those reported by Ertugay et al. [36] & Xavier and Ramana [37].

Using algae powder in ice milk formulations increased the melting point. The melting point, a crucial determinant of ice cream quality, represents the temperature at which the ice cream completely melts. Melting rate is influenced by numerous factors, including total solids content, the concentration of stabilizers, emulsifiers, salts, and other components, as well as specific processing and storage conditions [24].

Adding Chlorella vulgaris and Spirulina platensis powders resulted in greater melting resistance compared with the control without algae. Moreover, increasing the concentration of microalgae powders generally enhanced the melting resistance of the functional ice milk, with the highest melting resistance observed in the treatments containing the higher algae concentrations. These results are consistent with the findings of Agustini et al. [38].

3.5.2. Color parameters

The findings in Table 6 present the color parameters of the ice cream samples. The L* coordinate represents luminosity, measuring the amount of light reflected by a color. It ranges from 0 (black) to 100 (white). Treatments of ice creams containing *Chlorella vulgaris and Spirulina platensis* lost their luminosity due to the darker color of the microalgae. Significant color differences were observed between all samples compared to the control (without algae), negative * denotes a shift towards green, whereas positive* values indicate a shift towards red.

Parameter a* indicated a tendency towards green in the treatments containing *Chlorella vulgaris and Spirulina platensis*. T1 and T4 exhibited lower a* values, as they contained lower ratios of algae and consequently displayed the least intense green color. Fortification with algae powder resulted in a less intense green color.

Table 5
Melting resistance of functional ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis.*

Treatments	Melting down (%) after various times				
	10 min	20 min	30 min	40 min	50 min
C	8.90 ±0.02 ^{A}	13.95 ±0.04 ^{A}	36.00 ±0.05 ^{A}	58.40 ±0.04 ^{A}	80.10 ±0.07 ^{A}
T1	8.45 ±0.01 ^{A}	13.50 ±0.05 ^{A}	35.49 ±0.08 ^{A}	52.96 ±0.01 ^{AB}	78.72 ±0.05 ^{A}
T2	8.10 ±0.05 ^{AB}	12.15 ±0.01 ^{B}	31.76 ±0.03 ^{AB}	49.81 ±0.05 ^{B}	76.81 ±0.01 ^{A}
T3	7.70 ±0.01 ^{B}	11.10 ±0.05 ^{C}	26.79 ±0.02 ^{C}	44.64 ±0.07 ^{C}	73.24 ±0.03 ^{BCE}
T4	8.40 ±0.03 ^{A}	13.45 ±0.02 ^{A}	34.30 ±0.01 ^{A}	51.45 ±0.01 ^{AB}	77.75 ±0.07 ^{AB}
T5	8.00 ±0.06 ^{AB}	12.00 ±0.06 ^{B}	30.60 ±0.07 ^{AB}	48.70 ±0.04 ^{B}	74.50 ±0.05 ^{B}
T6	7.50 ±0.04 ^{BCE}	10.60 ±0.01 ^{C}	25.35 ±0.05 ^{C}	43.90 ±0.03 ^{C}	72.10 ±0.08 ^{C}

$^{A, B, C}$: Means with the different letters in the same column are significantly different between the treatments ($p < 0.05$).
See Table 2 caption for the abbreviations meaning of the treatments.

Negative b* values indicate while, blue Positive b* values indicate yellow. All samples showed positive b* values, indicating a yellow color tendency.

The results indicated a significantly higher difference in color scores between all samples and the control. All samples containing algae powder were significantly darker. As anticipated, ice cream with 1.5% algae showed the lowest L* value. All samples exhibited slightly increased green and yellow hues compared to the control; these results align with the findings reported by Tiepo et al. [39].

3.6. Microbiological analyses

A result in Table 7 illustrates the microbiological evaluation of functional ice milk fortified with different types of microalgae powder (total viable counts, yeast and mould counts, psychrotrophic bacteria counts, and coliform bacteria counts). The data showed that the percentages of algae powder in the functional ice milk impacted the total bacterial counts.

According to the findings shown in the same table, the total bacterial counts of the functional ice milk samples increased from 4.16 log cfu/g in control samples to 4.48 log cfu/g in T6 samples. Incorporating algae Powder in functional ice milk leads to a significant increase ($p \leq 0.05$) in total bacterial counts with increased algae Powder concentrations. These findings agree with Kashi et al. [40].

No yeast, mold, psychrotrophic, or coliform bacteria were detected in any functional ice milk samples. Furthermore, *Spirulina platensis* enhanced the final products' nutritional and sensory properties and demonstrated antifungal activity against spoilage yeasts and molds [41].

3.7. Sensory evaluation

Experienced panelists used the scoring sheet to score samples of functional ice milk fortified with algae powder. Recent research has demonstrated that panel acceptance of healthy food products is strongly influenced by their sensory characteristics [42].

Fig. 1 shows the sensory evaluation of functional ice milk fortified with different types and ratios of microalgae powder *Chlorella vulgaris* and *Spirulina platensis*. The data obtained indicated that functional ice milk samples containing algae powder had lower flavor scores compared with control samples (without algae). Likewise, flavor scores decreased gradually with increased algae powder ratios in the functional ice milk blends. The same trend has been reported by Agustini et al. [38] and Rushikesh and Varsha [26].

Trained panelists preferred the control ice milk (without adding *S. platensis*). Adding *S. platensis* may introduce an off flavor, often described as fishy or unpleasant. This fishy odor in *S. platensis* is attributed to its mineral content [43].

The results also indicated a difference in body and texture property score between all samples. The addition of algae powder in functional ice milk caused a higher body and texture score, improving the final product's body and texture properties. This was due to the chemical composition of algae. Functional ice milk fortified with algae powders, i.e., T3, T5, and T6, received the highest score for body and texture, followed by T1, T2, and T4, while the lowest score was perceived with the control sample. Likewise, functional ice milk fortified with 1.5% algae powder *Chlorella vulgaris* (T3) and *Spirulina platensis* (T6) clustered around a higher score of body and texture as illustrated in Fig. 2. The same trend has been reported by Agustini et al. [38] and Rushikesh & Varsha [26].

The data in Fig. 1 demonstrated that functional ice milk fortified with algae powder had superior melting properties to the control. When algae powder was added, the melting quality of the ice milk improved. This suggests that adding algae powder makes frozen milk softer. Functional ice milk fortified with 1 and 1.5% algae powder *Spirulina platensis* (T5 and T6) received the highest score for melting quality, while the lowest score was perceived by the control sample.

Table 6
Color parameters of functional ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis*.

Treatments	Color parameters					
	L	a	b	I	Chroma	Hue
C	82.06±1.15^{A}	−14.38±0.54^{C}	6.08±0.72^{D}	83.12±2.12^{A}	15.73±0.02^{C}	157.04±0.74^{A}
T1	65.76±0.48^{B}	−22.39±0.57^{AB}	13.94±0.14^{C}	72.51±1.5^{B}	26.38±0.02^{B}	144.08±2.52^{B}
T2	59.20±0.82^{C}	−23.45±0.42^{A}	15.92±0.06^{B}	65.65±0.02^{C}	28.37±0.02^{AB}	145.86±0.69^{B}
T3	54.94±1.9^{C}	−25.00±0.35^{A}	16.85±0.45^{B}	62.67±2.32^{C}	30.15±0.02^{A}	146.01±1.32^{B}
T4	56.22±1.12^{C}	−20.50±0.18^{B}	18.88±0.47^{A}	62.76±1.17^{C}	27.89±0.02^{AB}	137.30±0.61^{C}
T5	47.85±0.87^{D}	−21.63±0.47^{B}	19.22±0.54^{A}	55.92±0.92^{D}	28.94±0.02^{AB}	138.36±0.48^{C}
T6	42.62±1.85^{DE}	−24.31±0.33^{A}	19.83±0.73^{A}	53.07±1.81^{D}	31.41±0.02^{A}	140.32±0.39^{BC}

$^{A, B, C}$: Means with the different letters in the same column are significantly different between the treatments ($p < 0.05$).
See Table 2 caption for the abbreviations meaning of the treatments.

Table 7
Microbiological counts (log CFU/mL) of functional ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis*.

Treatments	Microbial type			
	Total bacterial count	Psychrotrophic	Coliform group	Yeast and moulds
C	4.16±0.09^{A}	ND	ND	ND
T1	4.21±0.04^{B}	ND	ND	ND
T2	4.33±0.02^{C}	ND	ND	ND
T3	4.45±0.07^{D}	ND	ND	ND
T4	4.23±0.0^{B}	ND	ND	ND
T5	4.36±0.10^{C}	ND	ND	ND
T6	4.48±0.03^{D}	ND	ND	ND

ND: Not detected. $^{A, B, C}$: Means with the different letters in the same column are significantly different between the treatments ($p < 0.05$).
See Table 2 caption for the abbreviations meaning of the treatments.

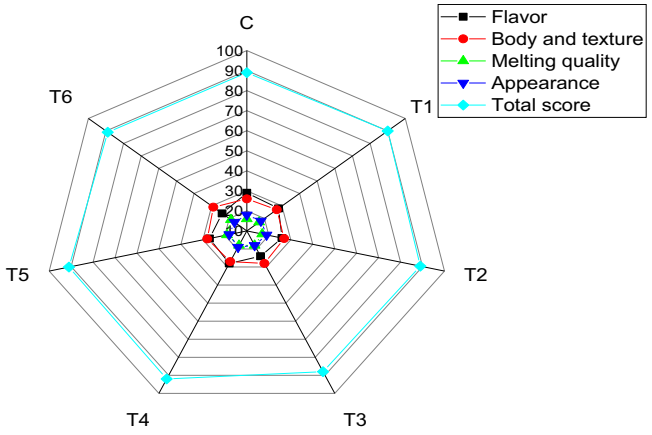


Fig. 1. Mean sensory evaluation (%) of functional ice milk fortified with *Chlorella vulgaris* and *Spirulina platensis* C: ice milk without any microalgae powder (Control treatment).T1, T2, T3, ice milk fortified with 0.5%, 1.0%, 1.5% *Chlorella vulgaris,* respectively. T4, T5, T6, ice milk fortified with 0.5%, 1.0%, 1.5% *Spirulina platensis* respectively.

In addition, appearance and color are considered the dominant factors for food quality. In terms of appearance and color, the data show that adding algae powder to ice milk changes the color to light, medium, or dark green depending on the percentage of algae addition, which caused the trained panelists' opinions to differ according to the data in Fig. 1. Results indicated that, while Spirulina addition enhanced the ice cream mixture body and texture properties, trained panelists preferred the control sample in terms of color [29].

In relation to the overall score as displayed by principal component analysis (PCA) results in Fig. 2, PC1 and PC2 showed 59.67% and 27.48%, respectively, and both components represented 87.15% of the data variability. Moreover, the T4 sample of functional ice milk fortified

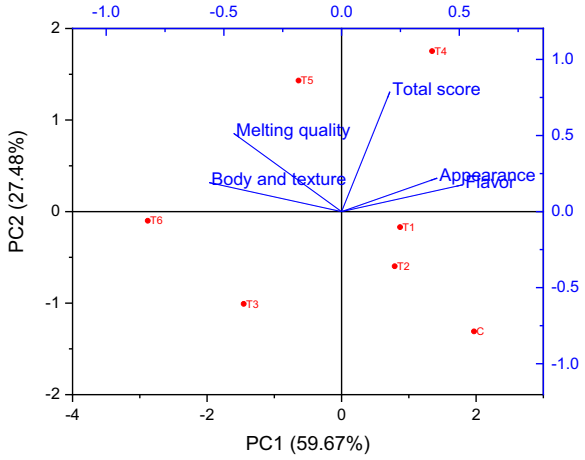


Fig. 2. Principal component analyses (PCA) representation biplot (scores and loading) of sensorial properties of developed functional ice milk fortified with *C. vulgaris* and *S. platensis*. C: ice milk without any microalgae powder (Control treatment). T1, T2, and T3, ice milk fortified with 0.5%, 1.0%, and 1.5% *Chlorella vulgaris* respectively. T4, T5, and T6, ice milk fortified with 0.5%, 1.0%, and 1.5% *Spirulina platensis* respectively.

with algae powder treatment containing 0.5% *Spirulina platensis* had higher scores (92%) as well as clustered around the higher overall acceptability (Fig. 2), followed by functional ice milk fortified with 1.0% *Spirulina platensis* (T5, with a value of 91%).

In general, the enrichment of ice milk by *Chlorella* and *Spirulina* improved the functional and nutritional quality as well as the health benefits. According to the total sensorial score findings, the data show significant differences ($p \leq 0.05$) between all treatments. These results agree with those obtained by Agustini et al. [38] and Lucas et al. [44], who observed significant color differences in snacks supplemented with *Spirulina*, as expected. However, these color variations did not significantly impact the overall panel acceptance of the products. Similarly, Morsy et al. [45] developed extruded foods incorporating *Spirulina* that were well-accepted in terms of appearance, color, porosity, flavor, texture, volume, hardness, and crispness in their study, demonstrating comparable acceptability to standard products. These sensory attributes were specific to the extruded food products evaluated by Morsy et al. [45] and were not the sensory attributes assessed in the present ice milk study. In the present study, the trained panelists evaluated flavor, body and texture, melting quality, and appearance. These findings suggest that while *Spirulina* can alter the color of food products, it does not necessarily diminish panel acceptance. Furthermore, incorporating microalgae biomass into ice cream aligns well with trained panelists' sensory evaluation, as many commercially available ice creams are already artificially colored, making naturally derived color an appealing alternative.

In summary, microalgae show promising applications in ice milk

formulations, where they can enhance nutritional value and serve as natural colorants, offering a potential alternative to synthetic additives. Recent studies have also demonstrated the successful incorporation of microalgae in various food systems, such as chicken filet enriched with lyophilized *Halimeda opuntia* extract [46], as well as meat products like pastirma and beef burgers using *Padina pavonica* extracts due to their antibacterial and antioxidant properties [47].

This study was conducted under controlled laboratory conditions and did not evaluate long-term storage stability of the ice milk. Additionally, the experimental scale and sample size were limited to laboratory-scale production, which may restrict direct extrapolation to industrial-scale applications.

4. Conclusions

This study investigated the enrichment of ice milk with *Chlorella vulgaris* and *Spirulina platensis*, highlighting their potential as functional dairy ingredients due to their rich bioactive composition. Overall, fortification with both microalgae significantly enhanced the antioxidant capacity of the final product and contributed to its natural green coloration. In addition, microalgae incorporation improved several quality attributes, including overrun, melting behavior, and overall sensory acceptance in terms of appearance, color, porosity, flavor, and texture. Between the two tested microalgae, *Spirulina platensis* showed superior functional performance, particularly in terms of antioxidant activity, total phenolics, and flavonoids. However, considering both functional and sensory outcomes, *Spirulina platensis* at 0.5–1.0% is recommended as the optimal range to achieve a balanced improvement in health-related properties and trained panelists acceptability. These findings support the use of microalgae as natural functional ingredients and potential substitutes for synthetic additives in dairy products.

CRediT authorship contribution statement

Wahid I. EL-Desoki: Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Mohamed A. Al-Soudy:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Hussin A. Ahmed:** Validation, Investigation, Formal analysis, Supervision. **Adel A. Tammam:** Resources, Investigation, Formal analysis, Validation, Supervision, Project administration, Visualization. **Oscar Zannou:** Writing – review & editing, Visualization, Validation, Software, Resources, Project administration, Funding acquisition, Data curation. **Reza Tahergorabi:** Visualization, Validation, Software, Resources, Project administration, Funding acquisition, Data curation. **Taha Mehany:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Funding acquisition, Data curation, Conceptualization, Methodology, Writing – original draft, Investigation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be available on reasonable request.

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