

Evidence-based formulation and overall acceptability of spirulina-enriched functional ice cream

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ABSTRACT

Introduction: Spirulina, a cyanobacterium recognized for its antioxidant, antiviral, and immunological properties, has been utilized for centuries as a natural source of protein and essential nutrients. Recent studies have highlighted its hypoglycemic and hypolipidemic effects, demonstrating its potential applications in managing non-communicable chronic diseases. However, the distinct flavor of spirulina can lead to low acceptability when incorporated into food products.

Objective: This research aimed to develop a proposal for a functional ice cream with spirulina containing no artificial flavors or colors based on scientific evidence due to the health benefits of *Spirulina platensis* and to evaluate its general acceptability in trained and untrained panels.

Methodology: For the collection of scientific evidence on the benefits and recommended dose of spirulina for the formulation of functional ice cream, a minireview was carried out in the indexed databases of Scopus, Embase and Pubmed. Only articles conducted in humans and with the consumption of spirulina powder were selected. For the analysis of general acceptability data, the spss and excel programs were used; for the evaluation of the nutritional profile, the Nutrisurvey software was used.

Results and discussions: Our findings indicate that the formulation was better received by health and nutrition professionals, while the trained panel reported a higher acceptability for the version containing less spirulina, primarily due to the aftertaste, as it is not a common ingredient.

Conclusion: New formulations with stronger flavors may be necessary to mask the aftertaste of spirulina, in order to produce an ice cream with functional properties, given that the spirulina content varies from 1 to 2 grams per serving.

KEYWORDS

Nutritional properties, consumer acceptance, fortified ice cream, antioxidants, spirulina.

INTRODUCTION

The prevalence of obesity is rising alarmingly in both developed and developing countries. It is projected that by 2030, 60% of women and 50% of men will be classified as overweight or obese. Additionally, by 2040, one in ten adults is expected to suffer from diabetes, resulting in nearly 642 million individuals living with this condition, with the diabetic population projected to increase by 60%. In response to this public health crisis, functional foods have emerged as vital components in the management of non-communicable chronic diseases. One such functional food is spirulina, a cyanobacterium renowned for its antioxidant, antiviral, and immunological properties. Spirulina has been shown to protect against heavy metal retention and provide antitoxic effects, with multiple studies indicating its efficacy in treating arsenic poisoning. Furthermore, it exhibits protective effects against certain cancers¹. The Food and Drug Administration (FDA) recognizes spirulina as "Generally Recognized as Safe" (GRAS) for human consumption².

Spirulina boasts an impressive absorption rate of 85% to 95% and contains approximately 65% protein, making it a

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complete source of all essential amino acids³. Research indicates that spirulina consumption is effective in managing glycemia and lipidemia in individuals with type 2 diabetes mellitus, leading to significant reductions in blood glucose levels, glycosylated hemoglobin (HbA1c), and blood lipid profiles, including decreases in triglycerides and low-density lipoproteins (LDL cholesterol) alongside increases in high-density lipoproteins (HDL cholesterol). Notably, studies have reported substantial decreases in triglycerides and total cholesterol following spirulina supplementation, with one study documenting a 10% reduction in total cholesterol and a 28% reduction in triglycerides among healthy volunteers, as well as a 15% increase in HDL cholesterol⁴. Additionally, spirulina has demonstrated the ability to lower total serum cholesterol, LDL cholesterol, and triglycerides in patients with hyperlipidemia and nephrotic syndrome.

Given its potential health benefits, spirulina may play a key role in dietary interventions aimed at improving lipid profiles in individuals with obesity and its associated comorbidities. Moreover, incorporating spirulina into foods designed for children and adolescents could foster increased consumption throughout adulthood. In this study, we aim to develop an artisanal ice cream formulation enriched with spirulina, free from artificial flavorings and colorings, to encourage its consumption. We assessed the overall acceptability of this product among both trained and untrained sensory panels.

HISTORY

Arthrospira and Spirulina were classified as one genus until their separation in 1989. Arthrospira includes over 50 species, with Arthrospira platensis, Arthrospira maxima, and Arthrospira fusiformis being the most studied. Spirulina has existed for over 3.5 billion years, initially thriving as anaerobic cyanobacterial filaments before evolving to use water in photosynthesis⁵. Its ability to fix carbon dioxide and produce organic matter is utilized in industrial food cultivation. The earliest recorded use dates back to 1520 when Hernán Cortés informed the Spanish monarchy about its production, and the Aztecs relied on it as a primary protein source. Its cultivation declined during Spanish colonial rule⁶. Spirulina has been a food source in Lake Chad, Africa, for centuries. Dangeard noted its consumption by the Kanembu tribe in 1940 and identified Arthrospira platensis in 1960. Commercial cultivation began in Mexico in the 1970s and is now widespread in countries like the United States, India, and China. The International Energy Agency (IEA) estimates global annual Spirulina production at around 10,000 tons of dry biomass, with China contributing nearly 50%⁷.

NUTRITIONAL ATTRIBUTES

Spirulina exhibits an exceptionally high absorption rate, ranging from 85% to 95%. It is rich in protein, comprising approximately 65% of its composition, and contains low levels

of sugars and carbohydrates. Notably, spirulina provides all the essential amino acids, including isoleucine (6%), leucine (9%), lysine (5%), methionine (2%), phenylalanine (5%), threonine (5%), tryptophan (2%), and others⁸. Additionally, spirulina contains proteins that are resistant to stress induced by high temperatures (35°-40°C) in the presence of light⁹.

Moreover, spirulina is a good source of lipids, containing nine types of fatty acids, including palmitic, linoleic, and linolenic acids. It also offers a rich mineral profile, with more than nine minerals present; potassium and sodium are the most abundant, followed by magnesium, phosphorus, calcium, and iron. Spirulina boasts over thirteen vitamins, predominantly from the B vitamin group, along with significant amounts of vitamin A, beta-carotene, choline, and folate. The U.S. Food and Drug Administration (FDA) has classified many dry Arthrospira products as "Generally Recognized as Safe" (GRAS) for human consumption². Doses ranging from 3 to 10 grams per day have been utilized in various clinical trials, and an intake of up to 30 grams daily is considered safe¹⁰. Additionally, the methionine content of spirulina, whether fresh or dehydrated, is comparable to that found in other meats such as chicken, beef, eggs, pork, and tuna⁸.

HEALTH BENEFITS

To elucidate the benefits associated with the consumption of spirulina powder, a mini-review of the literature from the past five years was conducted, concentrating on the advantages of spirulina intake in humans. The primary objective of this review was to develop a functional ice cream based on scientific evidence. A systematic search and selection of pertinent scientific articles were executed, as depicted in the PRISMA 2020 flowchart (Figure 1).

Spirulina has been shown to be effective in managing lipidemia in patients with type 2 diabetes mellitus and other comorbidities¹³. A systematic review of clinical trials indicated that the intake of Spirulina resulted in significant improvements in glycemic control and glycosylated hemoglobin levels, alongside reductions in lipid profiles, including triglycerides and low-density lipoprotein (LDL) cholesterol, as well as an increase in high-density lipoprotein (HDL) cholesterol¹⁸. Furthermore, the consumption of 2 grams of Spirulina daily for a duration of 8 weeks led to a decrease in triglycerides and cholesterol levels¹³, thereby significantly mitigating cardiovascular risk. Notably, a substantial reduction in both systolic and diastolic blood pressure was observed in both male and female participants following the consumption of 8 grams of Spirulina at 120 minutes¹⁷. Additionally, the intake of 6 grams of Spirulina over a 14-day period significantly increased hemoglobin levels¹¹. In patients with non-alcoholic fatty liver disease (NAFLD), significant improvements in fatty liver grade, oxidative stress markers, triglycerides, and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) were noted with the consumption of 2 grams of Spirulina for 8 weeks¹⁶. After 8 weeks of sup-

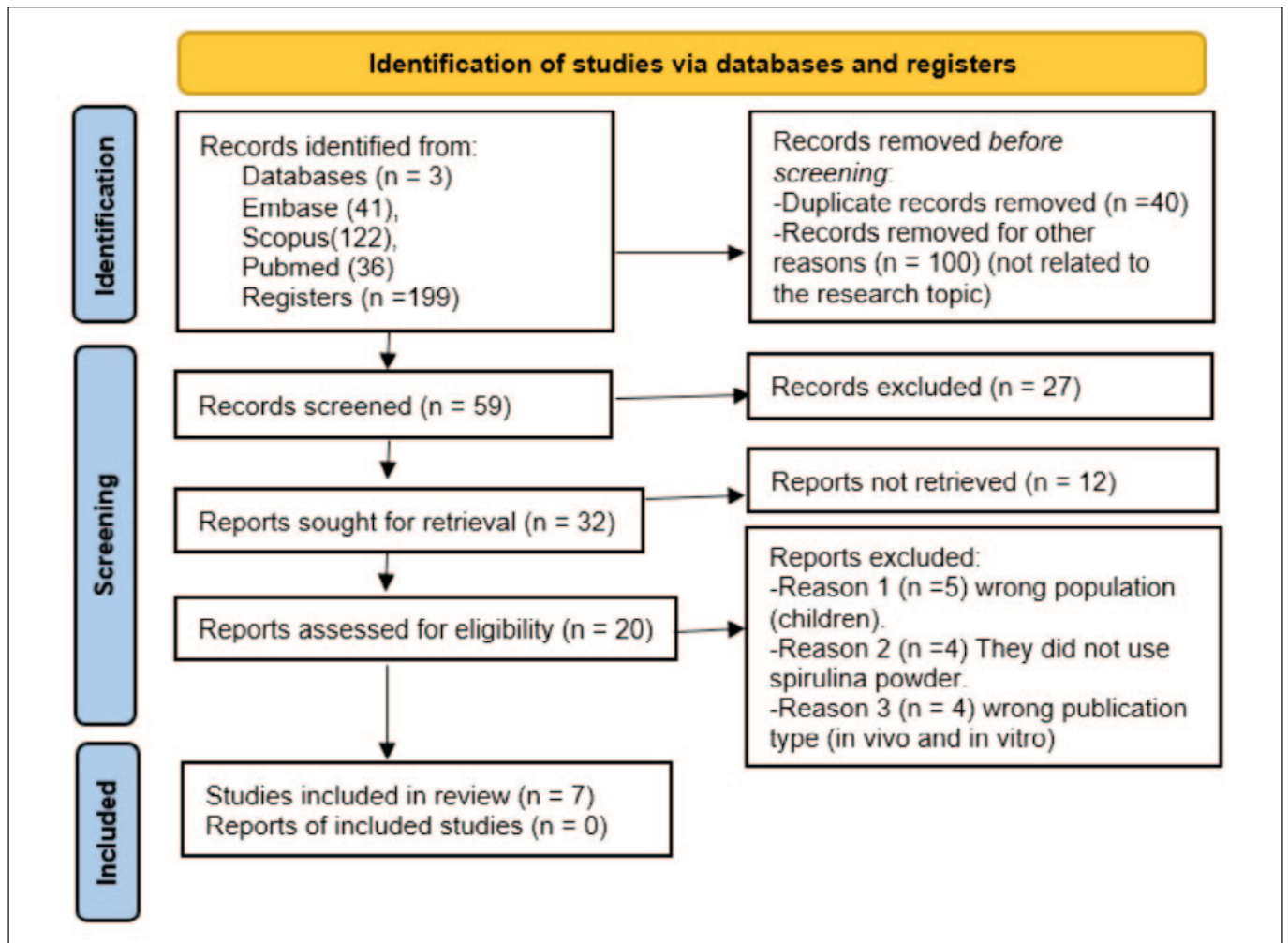


Figure 1. PRISMA 2020 flow diagram

plementation with 2 grams of Spirulina, participants with obesity exhibited significant upregulation of FNDC5 (Fibronectin type III domain-containing protein 5) and a reduction in the expression of NLRP3 (NLR family pyrin domain containing 3) and ACE2 genes (Angiotensin-converting enzyme 2)¹².

MECHANISMS OF ACTION

The mechanisms underlying the action of spirulina remain incompletely elucidated; however, existing studies suggest its metabolic effects. Notably, spirulina supplementation may offer potential benefits for weight management and the reduction of serum lipids. Its high amino acid content, which includes L-tyrosine and L-phenylalanine, could influence appetite and energy expenditure. Nevertheless, a systematic review did not find significant reductions in body measurements associated with spirulina consumption¹⁸. Spirulina has been shown to reduce hepatic lipid accumulation and improve lipid profiles by inhibiting low-density lipoprotein cholesterol (LDL-C) and increasing high-density lipoprotein cholesterol

(HDL-C) through the action of C-phycoyanin, which also plays a role in cholesterol metabolism¹⁸. Additionally, spirulina may enhance insulin production and sensitivity, potentially leading to lower blood glucose levels; however, no significant changes were observed in the participants studied¹⁸.

Phycocyanin and other bioactive compounds derived from *Spirulina platensis* have been shown to effectively enhance glucose and lipid metabolism in obesity-related metabolic disorders through mechanisms such as AMP-activated protein kinase (AMPK) activation, Akt signaling, and anti-inflammatory pathways. Although there is significant preclinical evidence^{21,22} supporting these effects, robust studies in human populations remain limited²¹. Phycocyanin activates AMPK and Akt pathways, resulting in reduced hepatic gluconeogenesis and increased glycogen synthesis and glucose uptake in insulin-resistant models^{21,22}.

Furthermore, it enhances the translocation of glucose transporter type 4 (GLUT4) and provides protection to β -cells

through its antioxidant properties, thereby reducing oxidative stress and preserving insulin secretion²². Additionally, spirulina has been found to reduce lipogenesis by downregulating sterol regulatory element-binding protein 1c (SREBP-1c) and peroxisome proliferator-activated receptor gamma (PPAR γ), while promoting fatty acid oxidation via the activation of carnitine palmitoyltransferase 1 (CPT1)²³. It also inhibits pancreatic lipase activity, which reduces cholesterol absorption and improves lipid profiles²⁴. Moreover, spirulina suppresses nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling and upregulates antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), thereby reducing systemic inflammation and oxidative

stress²². Lastly, it modulates gut microbiota composition, leading to an increase in short-chain fatty acids (SCFAs) and enhanced insulin sensitivity²³.

The results presented in the table 1 indicate that a minimum consumption of 1 gram of spirulina yields health benefits. Furthermore, the majority of studies conducted over the past five years involving human subjects have utilized a minimum consumption of 2 grams. In light of the scientific evidence identified in the review, the homemade ice cream was formulated with servings containing 1 gram and 2 grams of spirulina, which will be assessed for general acceptability by both trained and untrained panels.

Table 1. Spirulina Studies in Humans

Reference	Country	Design	Population	Sample size	Doses	Duration	Comparison	Main Results
Y. Ali et al. 2023 ¹¹	United Kingdom	RDBPC crossover	Active cyclist Age 23 \pm 5 yrs	n=17 GE= 10 GP= 8	6g/day	14 days	Placebo	Significantly improved hemoglobin levels. No changes in performance parameters.
Armannia, F. et al 2023 ¹²	Iran	RDBPC	Obese subjects Age 44.83 \pm 3.04 yrs	n=24 GE=12 GP=12	2g/day	8 weeks	Placebo	Significant upregulation of FNDC5, reduction of NLRP3 and ACE2 genes expression. No changes in antropometric measures.
Rezaian M. et al 2023 ¹³	Iran	RDBPC	T2DM patients	n=46 GE= 23 GP= 23	2g/day as Spirulina sauce	8 weeks	Placebo	TG, TC and Waist circumference were significantly decreased, No significant changes for glycemic control.
Moradi, S. et al 2023 ¹⁴	Iran	RDBPC	Ulcerative colitis patients	n=80 GE= 40 GP= 40	1g/day	8 weeks	Placebo	Significantly increased serum iron levels in patients with ulcerative colitis. Non significant changes were observed in FOBT.
Far, Z. et al 2022 ¹⁵	Iran	Tripled Blind Placebo RCT	Patients with Hypertension	n=48 GE=24 GP=24	2g/day as Spirulina fortified dressing	8 weeks	Placebo	Non significant changes in anxiety levels based on the Holmes- Rahe questionnaire.
Mazloomi, S. et al 2022 ¹⁶	Iran	RDBPC	NAFLD patients	n=46 GE= 23 GP= 23	2g spirulina	8 weeks	Placebo	Significant improvement in fatty liver grade, oxidative stress markers, TG, and HOMA- IR. No significant change was found in BP and anthropometric measures.
Lympaki F, et al 2022 ¹⁷	Greece	Randomized crossover clinical trial	Healthy adults (Both male and female)	n=13	Trial 1: 2,5g spirulina cookie, Trial 2: 4, 6, 8 g spirulina	Postprandial effect for a Day	D-glucose, cookie without spirulina	Both 4 g and 8 g doses of spirulina reduced postprandial glucose levels at 120 minutes. Only the 8 g dose significantly reduced the AUC for glucose and systolic BP by 4% during the 90 to 120-minute interval

RDBPC (Randomized Double Blind Placebo Control trial), FNDC5 (Fibronectin type III domain-containing protein 5), NLRP3(NLR family pyrin domain containing 3), ACE2 (Angiotensin-converting enzyme 2), TG (Tryglicerides), TC (Total Cholesterol), FOBT (Fecal occult blood test), BP (Blood pressure), AUC (Area Under the Curve).

MATERIAL AND METHODS

In order to determine the recommended dosage of spirulina for the formulation of a functional ice cream, a scoping review was conducted. The review involved a comprehensive search of the SCOPUS, Embase, Web of Science, and PubMed/Medline databases, which was completed by July 20, 2023. The keywords employed in the search included "Spirulina," "Arthrospira platensis," "Health benefits," "Nutritional benefits," "Clinical trials," and "Consumption." All articles pertaining to human studies on the consumption of spirulina powder were included in this minireview. Based on

the scientific evidence identified in the minireview, two formulations of homemade ice cream were developed, each containing servings of 1 gram and 2 grams of spirulina, which will be evaluated for general acceptability by both trained and untrained panels.

The ice cream formulations were created using an artisanal recipe based on an anglaise sauce infused with fresh mint. Specifically, two samples were produced: Sample 1, which contained 12 grams of dried spirulina, and Sample 2, which contained 24 grams. The quantities of each formulation are detailed in Table 2 and 3. The nutritional composition of both

Table 2. Spirulina – enriched ice cream formulation

Ingredients	Sample 1 with 12 g Spirulina		Sample 2 with 24 g of Spirulina	
	g	%	g	%
Dried spirulina	12	0.83	24	1.64
Fresh mint	60	0	60	0
Stabilizer	5	0.35	5	0.34
Sugar	120	8.29	120	8.22
Powdered milk	80	5.53	80	5.48
Milk cream	150	10.37	150	10.28
Egg yolks	80	5.53	80	5.48
Fresh milk	1000	69.11	1000	68.54

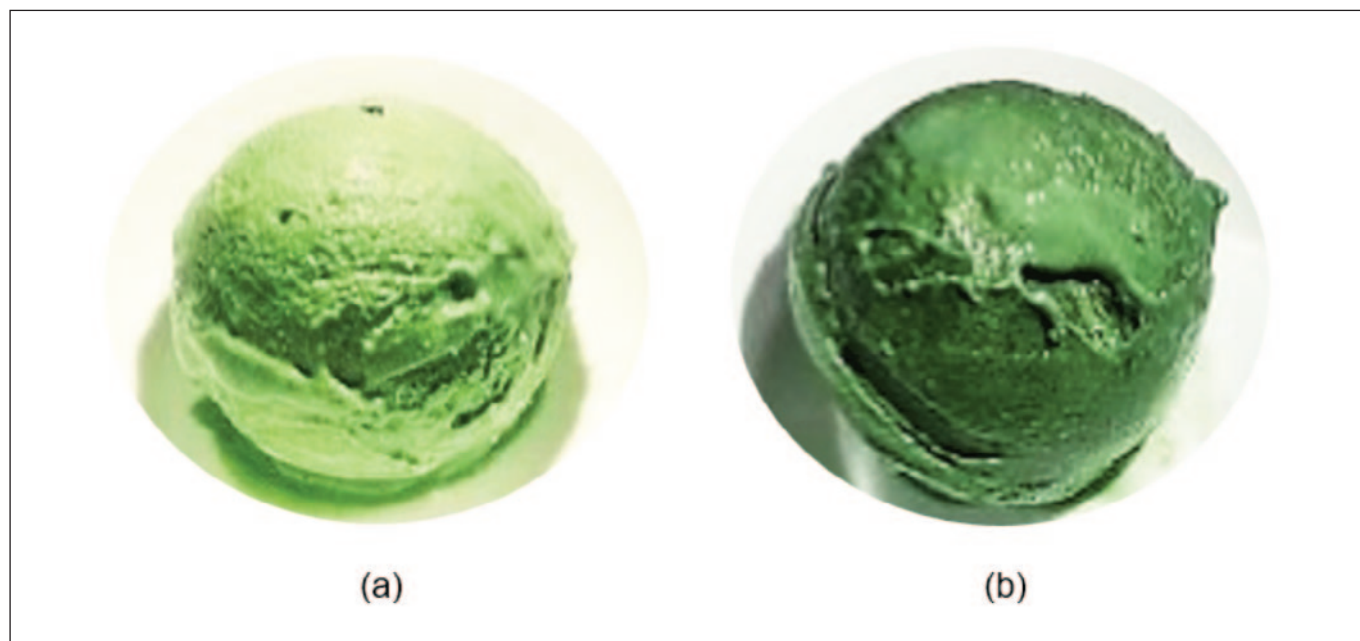


Figure 2. (a) Recipe of the Spirulina ice cream (Sample 1 with 12 g); (b) Spirulina ice cream of sample 1 (With 24 g of spirulina)

Table 3. Spirulina-enriched ice cream nutritional Composition per serving (90g)

Nutrients	Ice Cream Sample 1 (formulation with 12 g of spirulina)		Ice Cream Sample 2 (formulation with 24 g of spirulina)	
	g	%(DV)	g	%(DV)
Calories	106 kcal	-	108 kcal	-
Carbohydrates (g)	11.57	4.00	11.73	4.05
Fat (mg)	4.83	7.00	4.88	7.05
Protein (g)	4.12	6.84	4.48	7.47
Dietary fiber (g)	0.27	0.89	0.30	1.00
Iron (mg)	0.52	3.47	0.7	4.68
Magnesium (mg)	14.23	4.58	15.464	5.00
Calcium (mg)	120.58	12.05	121.34	12.16
Potassium (mg)	161.72	4.63	170.32	4.84
Sodium (mg)	52.65	2.63	59.26	2.95
Vitamin C (mg)	1.56	1.58	1.63	1.63
Folic acid (ug)	14.89	3.74	15.49	3.89
Vitamin B6 (mg)	0.06	4.89	0.06	5.11
Vitamin B2 (mg)	0.22	18.11	0.24	20.00
Vitamin B1 (mg)	0.06	6.37	0.08	7.89
Vitamin E (mg)	0.02	0.16	0.02	0.16
Carotene (mg)	0.02	-	0.02	-
Vitamin A (ug)	68.14	8.53	68.32	8.53
Cholesterol(mg)	57.84	-	57.84	-
PUFA (mg)	0.39	3.95	0.41	4.05

samples was analyzed using nutritional software (Nutrisurvey). Furthermore, a general sensory acceptability evaluation was conducted with two panels: a trained panel (n=23), which included chefs (n=5), professors (n=8), and graduated gastronomy students (n=10), and a non-trained panel (n=22), comprising administrators (n=5) and nutritionists (n=17). The sensory evaluation utilized a five-point scale: "I like it very much," "I like it moderately," "I neither like nor dislike," "I dislike moderately," and "I dislike it very much." For the analysis of general acceptability data, the SPSS and Excel programs were employed, while the evaluation of the nutritional profile was conducted using Nutrisurvey software.

RESULTS AND DISCUSSION

All the details regarding the formulations and nutritional profiles of the ice cream recipes for Sample 1 (containing 12 g of spirulina) and Sample 2 (containing 24 g of spirulina) are presented in detail in table 2 and 3 below.

A comparative analysis of the nutritional profiles of our functional ice cream formulation, which incorporates spirulina, and a commercially available vanilla ice cream with analogous characteristics reveals notable differences. Our formulation contains 50% fewer calories, includes dietary fiber, exhibits a lower total carbohydrate content, and has

300% less total fat. Furthermore, it offers a greater quantity of micronutrients in comparison to conventional commercial ice creams.

The hedonic scale assessment of ice cream with 12 g and 24 g of spirulina showed no significant difference in overall acceptability. The 12 g sample scored 3.91 out of 5, while the 24 g sample scored 3.84. The lower acceptance of the higher concentration may stem from limited exposure to spirulina. Our artisanal recipe excluded artificial colors and flavors as the study of Ramón-García did²⁵, making it harder for non-experts to identify the spirulina flavor. Acceptability decreased with increased spirulina content, yet overall scores were higher than expected, indicating a need for prior exposure. Acceptability levels were nearly identical, though slightly more individuals preferred the lower concentration. These results align with other evaluations noting an after-taste in higher spirulina samples. Notably, a significant portion of the population was unfamiliar with spirulina, and the acceptability of spirulina ice cream containing 12 and 24 grams was predominantly attributed to the group of nutritionists. This may be due to their knowledge and familiarity with health-beneficial products. Additionally, the graduates demonstrated a high level of acceptability, even

surpassing that of the chefs who participated in the tasting; this could be attributed to the age of the graduates, as younger individuals exhibited a greater openness to new flavors and a willingness to experiment with them.

Sensory tests on various products containing spirulina were conducted. Gershwin (2008) describes a study in which tests were performed using 1 g, 2.5 g, and 5 g of spirulina per recipe in cookies, yielding general acceptability scores of 3.00, 2.66, and 2.41, respectively, on a scale of 5 points. Notably, as the concentration of spirulina increased, the acceptability decreased²⁶. To minimize bias, we did not disclose the specific products used or the functional benefits of spirulina prior to the tests, as knowledge of health benefits or nutritional composition may influence acceptability. After the tests, participants were informed about the composition of the samples and their associated health benefits. We then inquired about their knowledge, previous consumption, potential acceptance of these products, and other related items. Very few studies have been conducted on the incorporation of spirulina into ice cream formulations. A recent study developed a functional ice cream using *Spirulina platensis*, in which both fat and sugar content were reduced²⁷. All recipes featured a 50% reduction in fat and a

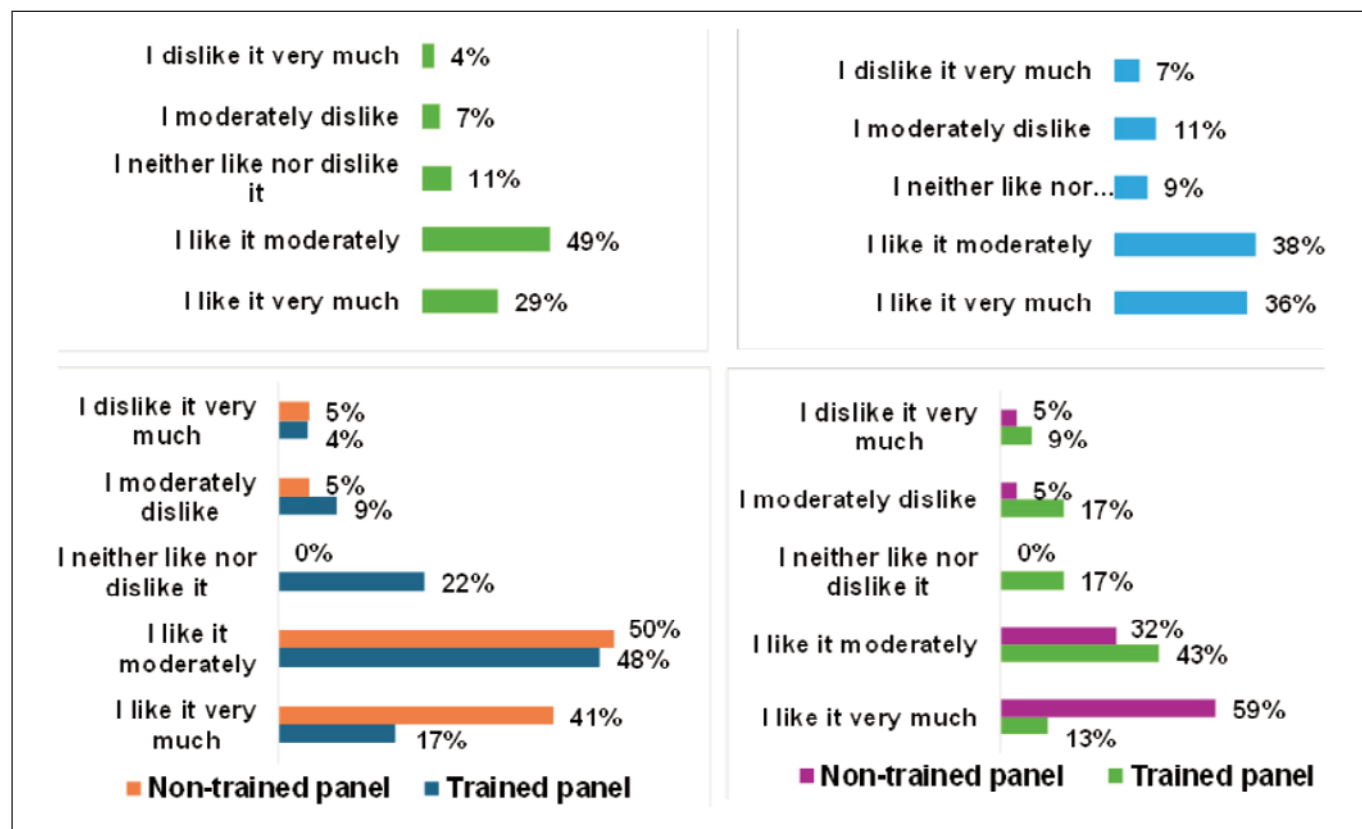


Figure 3. (a) Hedonic scale of mint ice cream with spirulina 12 g (Sample 1) in general (b), by panel; (c) Hedonic scale of mint ice cream with spirulina 24 g (Sample 2) in general and (d) by panel

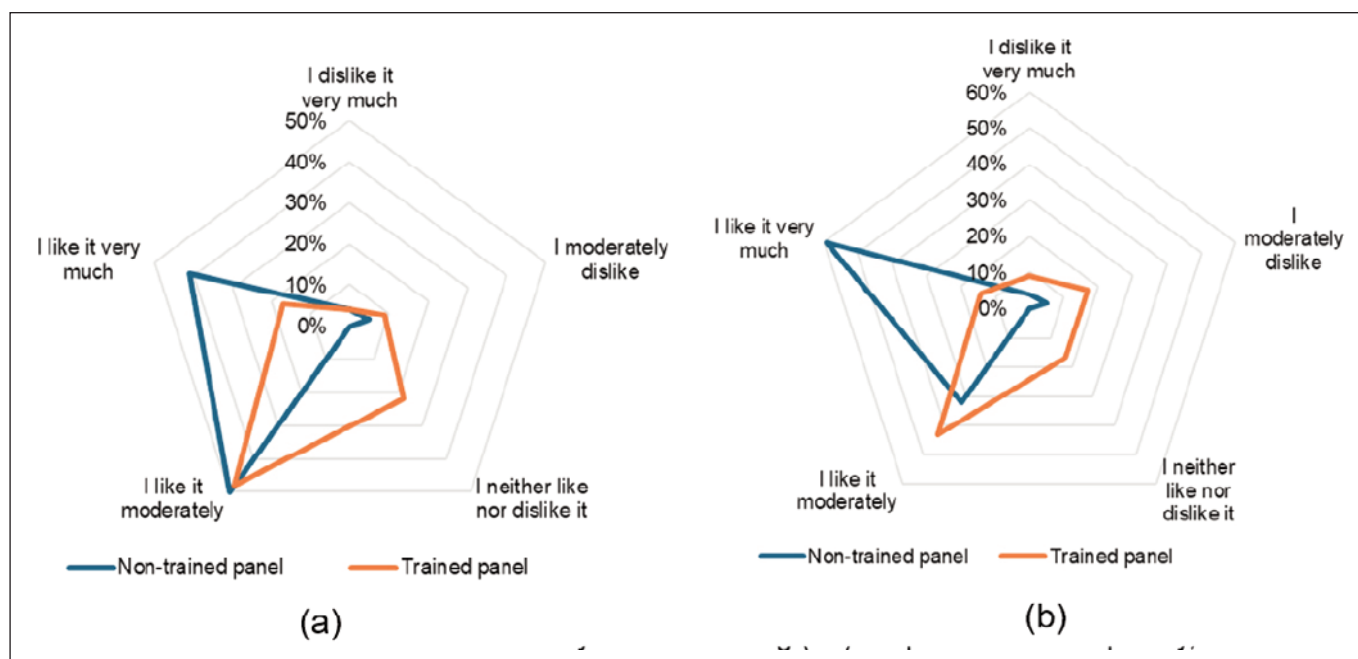


Figure 4. General sensory acceptability of spirulina enriched mint ice cream : (a) with 12 g spirulina (Sample 1), (b) with 24 g spirulina (Sample 2)

25% reduction in sugar. Additionally, spirulina, inulin, and emulsifiers were incorporated into some formulations. The total amount of spirulina powder added varied from 7.3 to 8.6 grams per recipe, which is lower than the quantity used in this research, ranging from 12 to 24 grams per recipe. The primary ingredients are similar to those used in this formulation, including powdered milk, stabilizers such as carboxymethyl cellulose, sugar, and cream. Another study added spirulina powder to an enriched vanilla ice cream formulation, developing four samples (T1, T2, T3, and T4) with 1.25 g, 2.5 g, 3.75 g, and 3.75 g of spirulina powder added to 825 g of vanilla ice cream²⁸.

A sensory acceptability evaluation was performed on two samples: Sample 1 with 12 g of spirulina powder in mint-infused ice cream and Sample 2 with 24 g of spirulina. Using a 5-point scale, results revealed that 7% of participants strongly disliked Sample 1, while 4% strongly disliked Sample 2. Additionally, 11% moderately disliked both samples, with 11% neutral toward Sample 1 and 8% neutral toward Sample 2. Moderate liking was reported by 49% for Sample 1 and 38% for Sample 2. Finally, 29% liked Sample 1 very much, compared to 36% for Sample 2. The average acceptability rating was 3.91 (78.22%) for Sample 1 and 76.89% for Sample 2.

When comparing our results with those presented in the study by Jadhav²⁸, which examined four samples of vanilla ice cream infused with spirulina, it is crucial to note that none of the samples contained the quantities of spirulina utilized

in our research. Specifically, we incorporated 12 g of spirulina in Sample 1, whereas the sample with the highest spirulina content in Jadhav's study contained only 5 g (T4). The second formulation evaluated in that study included 3.75 g (T3) of spirulina. Consequently, our Sample 1 contained 220% more spirulina than T3 and 38% more than T4. In contrast, Sample 2 contained 540% more spirulina than T3 and 176% more than T4.

Despite the significantly lower amounts of spirulina in the ice cream samples T3 and T4 from the Jadhav study, their acceptability ratings were notably high, with T3 at 87.33% and T4 at 89.22%. In contrast, our results indicated that Sample 1 received a rating of 78.22%, while Sample 2 received a rating of 76.89%. In a separate study conducted by da Silva Faresin²⁷, four formulations of ice cream containing spirulina were evaluated, each containing between 10% and 15% more spirulina than our Sample 1. Comparatively, our Sample 2 contained 70%, 75%, 80%, and 73% more spirulina than samples IC6, IC7, IC10, and IC11, respectively. Despite the varying percentages of spirulina—and a greater difference with Sample 2—the samples from the da Silva Faresin study achieved acceptability ratings of 71.00% (IC6), 71.56% (IC7), 74.11% (IC10), and 72.78% (IC11). These ratings are quite close to those obtained in our study: 78.22% for Sample 1 and 76.89% for Sample 2.

Unlike the sugar and fat-reduced recipe, which contains additional ingredients that may diminish certain sensory charac-

teristics of spirulina powder, such as Chantilly and flavoring agents, the overall impression from the nine-point evaluation of ice cream formulations with spirulina powder ranged from $71\% \pm 19.37\%$ to $74.11\% \pm 18.44\%$. In contrast, the overall acceptability of our samples varied from 73.33% to 78%, despite having a higher spirulina content. This suggests that mint, as an aromatic herb, may be more effective in masking the taste and aftertaste of spirulina compared to traditional flavoring agents.

Notably, the amount of spirulina in each recipe significantly influences its protein content. Our formulations contain 5.41 g (12 g of spirulina per recipe) and 5.84 g (24 g of spirulina per recipe) of protein per 100 g portion. In contrast, the fat- and sugar-reduced ice cream contains between 2.52 g and 2.85 g of protein per 100 g. Additionally, a handmade spirulina ice cream formulation with 0.6% to 1.2% spirulina powder contains between 3.48 ± 0.07 g and 3.54 ± 0.14 g of protein per portion²⁹. Therefore, our formulations provide a higher protein content due to their spirulina content, making them an excellent alternative for increasing protein intake in children and teenagers.

The trained and untrained panelists were analyzed separately. For sample 1, which consisted of 12 grams of spirulina, a higher percentage of untrained panelists (41%) indicated that they liked it a lot, compared to only 17% of the trained panelists. However, the responses for the "I like it moderately" option were quite similar, with untrained panelists at 50% and trained panelists at 48%. In sample 2, which comprised 24 grams of spirulina, the untrained panelists again demonstrated a higher percentage (59%) of those who expressed a strong preference for the product, while only 14% of the trained panelists reported the same. Similarly, in the "I like it moderately" category, the untrained panelists accounted for 32%, while the trained panelists were slightly higher at 43%.

Another study³⁰ developed a handmade ice cream that incorporated 1% Spirulina biomass relative to the total mass of the ice cream. In contrast, other research formulated ice creams with 0.6% and 1.2% Spirulina²⁹, which fall between the percentages used in this study: 0.83% and 1.64% for each formulation, respectively. However, the carbohydrate content was higher due to the inclusion of condensed milk, resulting in an overall sensory acceptability that was lower than our findings. The average sensory evaluation scores for aroma, color, texture, and flavor of the ice cream samples were converted to percentages, yielding 76.33% ($\pm 9.67\%$) for the ice cream with 0.6% Spirulina powder and 70.56% ($\pm 12\%$) for the ice cream with 1.2%. As indicated by our results, an increase in Spirulina powder correlates with a decrease in overall acceptability. Since inulin does not negatively impact palatability, it may be beneficial to include it in future formulations to enhance fiber

content and reduce sugar levels. Consequently, Spirulina could be incorporated into the development of ice cream and other functional products.

CONCLUSIONS

Due to its antioxidant, hypolipidemic, and hypoglycemic properties, spirulina can be beneficial for obesity-related metabolic disorders. Notably, spirulina supplementation has been shown to significantly reduce total cholesterol and triglyceride levels. In terms of carbohydrate metabolism, the consumption of spirulina enhances glycosylated hemoglobin, improves insulin resistance, and positively impacts glucose response, thereby supporting the treatment of metabolic syndrome. This improvement in glucose metabolism is attributed to its phycocyanin content, which activates the expression of glucokinase and insulin signaling pathways in the pancreas and liver. As a result, spirulina enhances pancreatic and liver function and increases the synthesis of hepatic glycogen, leading to lower blood glucose levels.

Despite its numerous benefits, there have been very few studies on the incorporation of spirulina into ice cream formulations. Interestingly, higher concentrations of spirulina powder in formulations tend to correlate with lower overall acceptability. Therefore, more research is needed to explore the incorporation of spirulina into various ice cream formulations, particularly those that are easy to consume, to promote the development of functional food products containing spirulina.

Additionally, no existing studies have utilized aromatic herbs to mitigate the unique taste of spirulina. This highlights the need for further research into alternative formulations to achieve optimal sensory acceptability and to demonstrate the potential health benefits of this product. The nutritional profile of spirulina, which includes vitamins, minerals, and amino acids, positions it as a promising ingredient for developing functional products aimed at improving nutritional status and health, as well as managing chronic diseases related to obesity.

REFERENCES

1. M Misbahuddin MA. Effect of spirulina on the levels of zinc, vitamin E and linoleic acid in the palm skin extracts of people with prolonged exposure to arsenic. *Bangladesh J Pharmacol*. 2013;8(1):DOI: 10.3329/bjpv.v8i1.13694.
2. US Food and Drug Administration. GRAS exemption claim for *Spirulina platensis* as an ingredient in foods claims. 2011; Available from: <https://www.plantpills.co.uk/pdf/GRN000394.pdf>
3. Ponce López E. Superfood for a world in crisis: Spirulina at low cost. *Idesia*. 2013;31(1):135-139. DOI:10.4067/S0718-34292013000100016.

4. Tan E, Faller E. Lipid Lowering Effects of Herbal Supplements: A Review. *Res J Pharm Technol*. 2022;15(1):270-278. DOI:10.52711/0974-360X.2022.00044.
5. Papapanagiotou G, Gkelis S. Taxonomic revision of commercially used *Arthrospira* (Cyanobacteria) strains: a polyphasic approach. *Eur J Phycol* [Internet]. 2019;54(4):595-608. Available from: <https://doi.org/10.1080/09670262.2019.1624832>
6. Sotiroudis T. Health aspects of *Spirulina* (*Arthrospira*) microalga food supplement. *Serbian Chem Soc*. 2013;78(3):395-405. DOI:10.2298/JSC121020152S.
7. Thevarajah B, Nishshanka GKSH, Premaratne M, Nimarshana PH V, Nagarajan D, Chang J-S, et al. Large-scale production of *Spirulina*-based proteins and c-phycoerythrin: A biorefinery approach. *Biochem Eng J*. 2022;185:108541. DOI:10.1016/j.bej.2022.108541.
8. Montevecchi G, Santunione G, Licciardello F, Köker Ö, Masino F, Antonelli A. Enrichment of wheat flour with *Spirulina*. Evaluation of thermal damage to essential amino acids during bread preparation. *Food Res Int*. 2022;157:111357. DOI:10.1016/j.foodres.2022.111357.
9. Hongsthong A, Sirijuntarut M, Yutthanasirikul R, Senachak J, Kurdrud P, Cheevadhanarak S, et al. Subcellular proteomic characterization of the high-temperature stress response of the cyanobacterium *spirulina platensis*. *Proteome Sci*. 2009 Aug 2;7:33. DOI:10.1186/1477-5956-7-33.
10. Sotiroudis T, Chemical GS-J of the S, 2013 U. Health aspects of *Spirulina* (*Arthrospira*) microalga food supplement. *J SERBIAN Chem Soc*. 2013;78(3):395-405. DOI:10.2298/JSC121020152S.
11. Ali Y, Aubeeluck R, Gurney T. Fourteen-Days *Spirulina* Supplementation Increases Hemoglobin, but Does Not Provide Ergogenic Benefit in Recreationally Active Cyclists: A Double-Blinded Randomized Crossover Trial. *J Diet Suppl* [Internet]. 2023; Available from: <https://doi.org/10.1080/19390211.2023.2263564>
12. Armannia F, Ghazalian F, Shadnough M, Keyvani H, Gholami M. *Spirulina* for Protection Against COVID-19 via Regulating ACE2, FNDC5, and NLRP3: A Triple-Blind Randomized Placebo-Controlled Trial in Obese Adults. *J Cell Mol Anesth* [Internet]. 2023;8(2):105-15. Available from: <https://doi.org/10.22037/jcma.v8i2.39585>
13. Rezaiani M, Sasani N, Kazemi A, Mohsenpour MA, Babajafari S, Mazloomi SM, et al. The effect of spirulina sauce on glycemic index, lipid profile, and oxidative stress in type 2 diabetic patients: A randomized double-blind clinical trial. *Food Sci Nutr* [Internet]. 2023;11(9):5199-208. Available from: <https://doi.org/10.1002/fsn3.3479>
14. Moradi S, Foshati S, Poorbaferani F, Talebi S, Bagheri R, Amirian P, et al. The effects of spirulina supplementation on serum iron and ferritin, anemia parameters, and fecal occult blood in adults with ulcerative colitis: A randomized, double-blinded, placebo-controlled trial. *Clin Nutr ESPEN* [Internet]. 2023;57:755-63. Available from: <https://doi.org/10.1016/j.clnesp.2023.08.019>
15. Far ZG, Babajafari S, Kojuri J, Nouri M, Ashrafi-Dehkordi E, Mazloomi SM. The Effect of *Spirulina* on Anxiety in Patients with Hypertension: A Randomized Triple-Blind Placebo-Controlled Clinical Trial. *J Nutr Food Secur* [Internet]. 2022;7(2):181-8. Available from: <https://doi.org/10.18502/jnfs.v7i2.9331>
16. Mazloomi SM, Samadi M, Davarpanah H, Babajafari S, Clark CCT, Ghaemfar Z, et al. The effect of *Spirulina* sauce, as a functional food, on cardiometabolic risk factors, oxidative stress biomarkers, glycemic profile, and liver enzymes in nonalcoholic fatty liver disease patients: A randomized double-blinded clinical trial. *Food Sci Nutr* [Internet]. 2022;10(2):317-28. Available from: <https://doi.org/10.1002/fsn3.2368>
17. Lympaki F, Giannoglou M, Magriplis E, Bothou DL, Andreou V, Dimitriadis GD, et al. Short-Term Effects of *Spirulina* Consumption on Glycemic Responses and Blood Pressure in Healthy Young Adults: Results from Two Randomized Clinical Trials. *Metabolites* [Internet]. 2022;12(12). Available from: <https://doi.org/10.3390/metabo12121180>
18. Bohórquez-Medina SL, Bohórquez-Medina AL, Benites Zapata VA, Ignacio-Cconchoy FL, Toro-Huamanchumo CJ, Bendezu-Quispe G, et al. Impact of spirulina supplementation on obesity-related metabolic disorders: A systematic review and meta-analysis of randomized controlled trials. *NFS J*. 2021;25:21-30. DOI:10.1016/j.nfs.2021.09.003.
19. Ren Z, Xie Z, Cao D, Gong M, Yang L, Zhou Z, et al. C-Phycocyanin inhibits hepatic gluconeogenesis and increases glycogen synthesis via activating Akt and AMPK in insulin resistance hepatocytes. *Food Funct* [Internet]. 2018;9(5):2829-39. Available from: <http://dx.doi.org/10.1039/C8FO00257F>
20. Hao S, Li F, Li Q, Yang Q, Zhang W. Phycocyanin Protects against High Glucose High Fat Diet Induced Diabetes in Mice and Participates in AKT and AMPK Signaling [Internet]. Vol. 11, *Foods*. 2022. Available from: <https://doi.org/10.3390/foods11203183>
21. Szulinska M, Gibas-Dorna M, Miller-Kasprzak E, Suliburska J, Miczke A, Walczak-Galezewska M, et al. *Spirulina maxima* improves insulin sensitivity, lipid profile, and total antioxidant status in obese patients with well-treated hypertension: a randomized double-blind placebo-controlled study. *Eur Rev Med Pharmacol Sci* [Internet]. 2017;21(10):2473-81. Available from: <https://europepmc.org/article/med/28617537>
22. Ou Y, Lin L, Yang X, Pan Q, Cheng X. Antidiabetic potential of phycocyanin: Effects on KKAY mice. *Pharm Biol* [Internet]. 2013 May 1;51(5):539-44. Available from: <https://doi.org/10.3109/13880209.2012.747545>
23. Hua P, Yu Z, Xiong Y, Liu B, Zhao L. Regulatory efficacy of spirulina platensis protease hydrolyzate on lipid metabolism and gut microbiota in high-fat diet-fed rats. *Int J Mol Sci* [Internet]. 2018;19(12). Available from: <https://doi.org/10.3390/ijms19124023>
24. Nagaoka S, Shimizu K, Kaneko H, Shibayama F, Morikawa K, Kanamaru Y, et al. A Novel Protein C-Phycocyanin Plays a Crucial Role in the Hypocholesterolemic Action of *Spirulina platensis* Concentrate in Rats. *J Nutr* [Internet]. 2005;135(10):2425-30. Available from: <https://doi.org/10.1093/jn/135.10.2425>
25. RAMÓN GARCÍA DF, Méndez AS, Inga EM, CHANCASANAMPA HÁ, Norabuena E, Gonzáles T, et al. Impacto del helado dietético con

- yacón (*Smallanthus sonchifolius*) en la hipoglicemia y aceptabilidad. *Nutr Clínica y Dietética Hosp* [Internet]. 2022;42(2). Available from: <https://doi.org/10.12873/422garcia>
26. Borowitzka MA, ME Gershwin, A. Belay (eds). *Spirulina in human nutrition and health*. *J Appl Phycol*. 2009;21:747-748. DOI:10.1007/s10811-009-9467-0.
27. da Silva Faresin L, Devos RJB, Reinehr CO, Colla LM. Development of ice cream with reduction of sugar and fat by the addition of inulin, *Spirulina platensis* or phycocyanin. *Int J Gastron Food Sci*. 2022;27:100445. DOI:10.1016/j.ijgfs.2021.100445.
28. Jadhav RR, Chawla V. Enrichment of Vanilla Ice-cream with *Spirulina* Powder. *Int J Res Eng Sci Manag* [Internet]. 2022;5(3):1-4. ISSN:2581-5792. Available from: <https://journal.ijresm.com/index.php/ijresm/article/view/1807>
29. Winarni Agustini T, Farid Ma'ruf W, Widayat W, Suzery M, Hadiyanto H, Benjakul S. Application Of *Spirulina Platensis* On Ice Cream And Soft Cheese With Respect To Their Nutritional And Sensory Perspectives. *J Teknol*. 2016;78(4-2):245-251. DOI:10.11113/jt.v78.8216.
30. Tiepo CBV, Gottardo FM, Mortari LM, Bertol CD, Reinehr CO, Colla LM. Addition of *Spirulina platensis* in handmade ice cream: Physicochemical and sensory effects/Adição de *Spirulina platensis* em sorvete caseiro: Efeitos físico-químicos e sensoriais. *Braz J Dev*. 2022;7(3):88106-88123. DOI:10.1016/j.ijgfs.2021.100445.