



RESEARCH ARTICLE

Nutritional and microstructural characterisation of spirulina-enriched ice cream

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Abstract

The fortification of ice cream with nutraceutical ingredients is gaining importance due to consumer demand for functional and health-promoting foods. Spirulina (*Arthrospira platensis*), a protein- and micronutrient-rich microalga, was incorporated into ice cream formulations at different levels to enhance nutritional and functional properties. The proximate analysis revealed that spirulina enrichment increased protein (8.06 % → 8.41 %) and iron content (0.00 % → 4.21 %) without altering fat retention. Physical attributes also improved, with higher overrun (93.2 % vs. 90.6 %), reduced whipping time (5.0 min vs. 6.5 min) and a lower freezing point (-3.8 °C vs. -3.4 °C), indicating a smoother texture and better aeration. SEM analysis confirmed significant microstructural modifications, showing a hierarchical porous network with air cell distribution, fibrous strands and interconnected micro-pores that favour creaminess and stability. These structural features support improved texture, melt resistance and functional efficiency. The findings establish spirulina-enriched ice cream as a novel functional dairy product with enhanced nutritional value, particularly as a natural source of protein and iron, while maintaining desirable technological and sensory properties. This study highlights spirulina's potential in addressing nutritional deficiencies and promoting sustainable food innovation.

Keywords: functional foods; ice cream fortification; iron supplementation; microstructural analysis; protein enrichment; scanning electron microscopy; sensory analysis

Introduction

The fortification of functional ice cream with nutraceutical ingredients has gained tremendous attention due to the growing concern of consumers towards well-being and health. Spirulina (*Arthrospira platensis*) is a protein-rich microalga with the reputation of being a sustainable protein-rich food possessing high essential amino acids, vitamins, minerals and bioactive pigments such as phycocyanin and β-carotene. Fortification of dairy foods with spirulina not only enhances the nutritional value but may even influence the structural and functional properties of the matrix. For consumer acceptability, the microstructural organisation and physical properties of fortified ice cream need to be analysed.

Physical attributes like overrun, melting resistance, freezing point, hardness and pH are key ice cream quality attributes. Overrun is an indicator of air incorporation on freezing that influences texture, creaminess and density directly. Hardness and melting characteristics are essential to sensory liking and storage stability, while pH and freezing point influence flavour intensity, microbial stability and ice crystal growth. Spirulina fortification could influence these parameters because of its protein-polysaccharide matrix and buffering capacity, thus changing texture, stability and consumer acceptability. To find out the structural alterations induced by spirulina addition, Scanning Electron Microscopy (SEM) examines microstructural properties such as air cell distribution, fat

destabilisation and ice crystal shape. Such properties have direct correlation with textural smoothness, creaminess and melt resistance. The combined analysis of SEM and physical characteristics gives a basic notion regarding the structural and functional basis of quality in fortified spirulina ice cream. The study examines a new variety of spirulina ice cream by using the SEM method with a high content of iron compared to other varieties.

Physical quality characteristics of ice cream are closely tied to acceptability by consumers. Overrun, the volume of air added during the freezing process, influences texture and density. Higher overrun results in a lighter product, with low overrun causing denser ice cream (1). Melting resistance is a critical property; a lower melting rate is defined as a stable emulsion and a more intense fat-protein network, which are preferable for sensory and storage quality (2). Hardness, as measured by textural analysis, is controlled by fat destabilisation and ice crystal distribution. Softness decreases consumer acceptability, with hardness preventing scooping (3, 4). Freezing point depression is induced by sugars, salts and stabilisers; the greater the freezing point depression, the smoother the texture because it prevents the growth of large ice crystals (5). pH also affects flavour stability and protein solubility and spirulina, through its inherent buffering, could affect acidity and thus microbial stability and sensory quality (6). Several studies on functional ice creams have shown that the addition of plant proteins, hydrocolloids, or microalgae impacts

overrun, melting resistance and hardness (7). Research indicates that plant protein enrichment enhanced viscosity and hardness, while showing that spirulina addition increased antioxidant capacity but had little influence on melting behaviour (8).

SEM ensures direct observation of microstructural structure, such as the size and distribution of ice crystals, fat globules and air cells. Finer and more even air bubble and ice crystal distribution leads to smoother texture and better melting stability (2). Stabilisers and proteins help in minimising ice recrystallisation upon storage, which can also be seen from SEM images (3). Experiments with the use of SEM in fortified ice creams have demonstrated that the addition of plant proteins or hydrocolloids leads to denser microstructures and reduced porosity. For example, the incorporation of spirulina into dairy systems enhanced the network structure due to high protein-polysaccharide content and textural stability was increased (6). SEM thus provides evidence for the visual effect of spirulina on the internal structure of fortified ice cream.

Materials and Methods

Materials

Raw cow milk, cream (35-40 % fat content), skim milk powder and granulated sugar were sourced from the local milk supplier in the Thanjavur district of Tamil Nadu. Stabiliser-emulsifier blends (EMJER sodium alginate agar agar powder) were used for consistency. Spirulina (*Arthrospira platensis*) powder was sourced from a certified nutraceutical supplier, "Green Spiro spirulina", Oddanchatram, Dindigul district of Tamil Nadu. All chemicals and reagents used were of analytical grade. Distilled water was used throughout the experiment.

Formulation of improved spirulina ice cream

Ice cream mix was made with standard recipes: milk, cream, skim milk powder, sugar and stabilizer-emulsifier blends. Spirulina powder was added at a 1.5 % level to the mix before pasteurisation. Pasteurisation was at 80 °C for 25 s, homogenization at 200 bar and ageing at 4 °C for 12 h. The aged mix was frozen in a batch ice cream freezer, with air being added. The product was hardened at -20 °C before further analysis. A plain sample without spirulina was also made for comparison.

Physical characteristics

Overrun

The overrun was determined by finding the difference in mass between the provided volume of the mixture and the resulting ice cream using the equation below:

$$\text{Overrun (\%)} = \frac{W_{\text{mix}} - W_{\text{icecream}}}{W_{\text{icecream}}} \times 100 \quad (\text{Eqn. 1})$$

Where, W_{mix} is the weight of unfrozen mix and W_{icecream} is the weight of ice cream of the same volume

Melting resistance

Resistance to melting was found by placing 50 g of ice cream on a wire mesh at room temperature (25 ± 2 °C) and measuring the volume of the liquid melted collected at specific time intervals until melting was complete.

Hardness

Hardness was measured on a texture analyser with a cylindrical probe (TA-XT2i, Stable Micro Systems, UK). The peak force (N) to penetrate the ice cream at -18 °C was noted.

pH evaluation

pH of the ice cream mixture was determined at 25 °C using a well-calibrated digital pH meter.

Freezing point

The freezing point was determined with a cryoscope (Lactoscope, high-performance model) according to AOAC-established procedures.

Scanning electron microscopy (SEM)

Ice cream samples were freeze-dried to remove moisture before scanning electron microscopy (SEM) analysis. The dried samples were fixed on aluminium stubs using double-sided conductive carbon tape and then gold-coated with a thin layer in a sputter coater (Quorum SC7620, UK). Microstructural analysis was performed using a Scanning electron microscope (JEOL JSM-6390LV, Japan) at an accelerating voltage of 15 kV. Various magnifications were used to take pictures to study the features of fat globules, protein network, air cell distribution and the shape of ice crystals.

Statistical Analysis

The physicochemical analysis of fortified ice cream with the parameters as follows:

Total Soluble Solids

The TSS was calculated by using the formula in Equation 2.

$$\text{TSS (\%)} = \frac{\text{weight of dried sample}}{\text{weight of actual sample}} \times 100 \quad (\text{Eqn. 2})$$

Fat

The determination of Fat in ice cream (Rose-Gottlieb method) is done with the following formula in Equation 3.

$$\text{Fat \%} = (\text{weight of extracted fat} / \text{weight of Ice cream}) \times 100 \quad (\text{Eqn. 3})$$

Titrateable acidity

$$\text{Acidity as formic acid (\%)} \text{ by weight } = \frac{0.23 \times V}{M} \quad (\text{Eqn. 4})$$

Where, V = Corrected volume of 0.05 N sodium hydroxide (mL), M = Weight of the sample (g)

pH

Standardise the pH meter using standard pH buffers. Determine the pH of the sample using a digital pH meter 7007 model (Digisun Electronics, India).

Carbohydrates

The estimation of carbohydrate by the phenol sulphuric acid method.

Ash content

The percentage of ash is calculated using the following formula.

$$\text{Ash (\%)} = \frac{100 \times w_1}{w_2} \quad (\text{Eqn. 5})$$

Result and Discussion

Chemical constituents

Table 1 presents the proximate composition of the control and fortified ice cream. The total soluble solids (TSS) showed a slight increase in the fortified sample (25.96 %) compared to the control (25.83 %), which provides an indication that the fortification process did not significantly affect the solids content. The protein content rose from 8.06 % to 8.41 %, which is attributed to the proteinaceous nature of spirulina powder. The fat content remained constant significantly (10.11 % vs. 10.12 %), which indicates that the fortification process did not exert a negative effect on fat retention during formulation.

A remarkable difference was noted in the iron composition, which increased significantly from 0.00 % in the control sample to 4.21 % in spirulina-fortified ice cream. This observation justifies the employment of spirulina as a natural iron supplement and, therefore, increases the nutritional value of the product. The carbohydrate level also increased slightly (from 20.15 % to 20.77 %), which can be attributed to the extra carbohydrates in the fortifying agent. Additionally, ash content increased slightly (from 0.48 % to 0.50 %), which is beneficial to the mineral enrichment of the product. Additionally, the pH decreased from 6.33 to 6.12, indicating a slight increase in acidity in the fortified product, as would be expected from the biochemical properties inherent in spirulina. This study confirms earlier findings that spirulina enrichment of milk products enhanced their nutrient content without harming the natural balance.

Table 1. Physico-chemical analysis of fortified ice cream

S. No.	Chemical composition	Control (%)	Fortified Ice cream (%)
1	Total soluble solids	25.83	25.96
2	Protein	8.06	8.41
3	Fat	10.11	10.12
4	Iron	0.00	04.21
5	Carbohydrate	20.15	20.77
6	Ash	0.48	0.50
7	pH	6.33	6.12

Physical features

Table 2 shows the physical properties of ice cream samples. The fortified ice cream (93.2 %) contained more overrun than the control (90.6 %), indicating enhanced air incorporation and aeration properties. This improvement may be attributed to the emulsifying and stabilising properties of spirulina proteins that enhance the stability of foam during whipping. Whipping time fell substantially from 6.5 min under control to 5.0 minutes for fortified ice cream, indicating improved whipping efficiency and reduced time to achieve the desired texture. This characteristic is useful in industrial processes as it shortens processing time. The freezing point of fortified ice cream (-3.8 °C) was lower than that of the control (-3.4 °C) because of the increase in soluble solids by spirulina fortification. Lower freezing point increases smoothness and flavour of ice cream by reducing the size of ice crystals. Parallel enhancements in physical qualities have been documented on fortification of milk products with natural supplements rich in minerals and proteins, in addition to the technological advantage of fortification, indicating nutritional enhancement.

Table 2. Physical parameter

S. No.	Parameters	Over run (%)	Whipping (min)	Freezing point (°C)
1	Control	90.6	6.5	-3.4
2	Fortified Ice cream	93.2	5.0	-3.8

Overall impact of fortification

The findings show that spirulina addition was very effective in enhancing the nutritional quality of ice cream, particularly in protein and iron content, without affecting the good physical and sensory characteristics. The minimal pH reduction and the depression of the freezing point did not impair the stability of the product; instead, they provided a higher creaminess and higher functionality. Thus, spirulina-enriched ice cream can be deemed a functional dairy product with improved nutritional quality and acceptable processing and quality characteristics.

Scanning electron microscope (SEM) analysis

The morphology of the sample surface was studied by Model: EVO 18 - Scanning Electron Microscopy (SEM) at various magnifications ranging from 200X to 3000X, to have an idea about its microstructural properties, porosity and surface pattern. The lower magnification micrographs of 200X and 300X (Fig. 1-2) clearly showed the presence of pores of irregular shapes distributed randomly on the surface. The pores varied in size and geometry, with interconnected pores, thus suggesting a heterogeneous porous structure. The peripheries of the pores' mouths were smooth, except for a few that had rough and fractured peripheries. Such heterogeneity in pores is a manifestation of non-uniform structural consolidation that happens in sample formation. The presence of macro-pores adds bulk porosity, which is beneficial in increasing permeability-related properties, aeration and diffusion, depending on the application.

Fibril and branched structures were observed at 500X and 800X magnifications traversing the surface (Fig. 3-4). These fibril structures were twisted or ruptured strands, reflecting a likelihood of either incomplete coalescence of structural units or failure of an integrated network. These fibrous residues may be sites of reinforcement within the matrix, which may contribute to the structural stability. However, the presence of microcracks and ruptured fibrils can also reflect poor interfacial bonding, which can impact the mechanical stability as a whole. At 2500X and 3000X magnifications, the micrographs indicated the presence of extensively developed micro-porosity with a high density of cavities ranging from nano- to micro-scale, which were randomly distributed over the surface (Fig. 5-6). These pores were irregular in shape and closely distributed, resulting in a highly rough surface morphology. The hierarchical porous structure observed at this magnification suggests high surface area, which may have a strong impact on functional properties, including adsorption, liquid interactions and encapsulation efficiency. In addition, the presence of interconnecting microchannels suggests a potential for high mass transfer and diffusion. In summary, the SEM analysis identifies that the sample features a multi-scale porous architecture, with large surface pores, fibrous intermediate structures and intricate micro-porosity exposed at higher resolutions. Hierarchical porosity can confer desirable properties, including light-weight construction, increased surface reactivity and improved functional effectiveness. Nonetheless, the inhomogeneous pore arrangement and ruptured fibrils also signify structural inhomogeneity and this could influence consistency and mechanical integrity.

Sensory evaluation

The sensory evaluation results presented in Table 3 and Fig. 7 show that the different groups of samples were generally well accepted across all attributes, with scores ranging from 7.5 to 9 on a 9-point

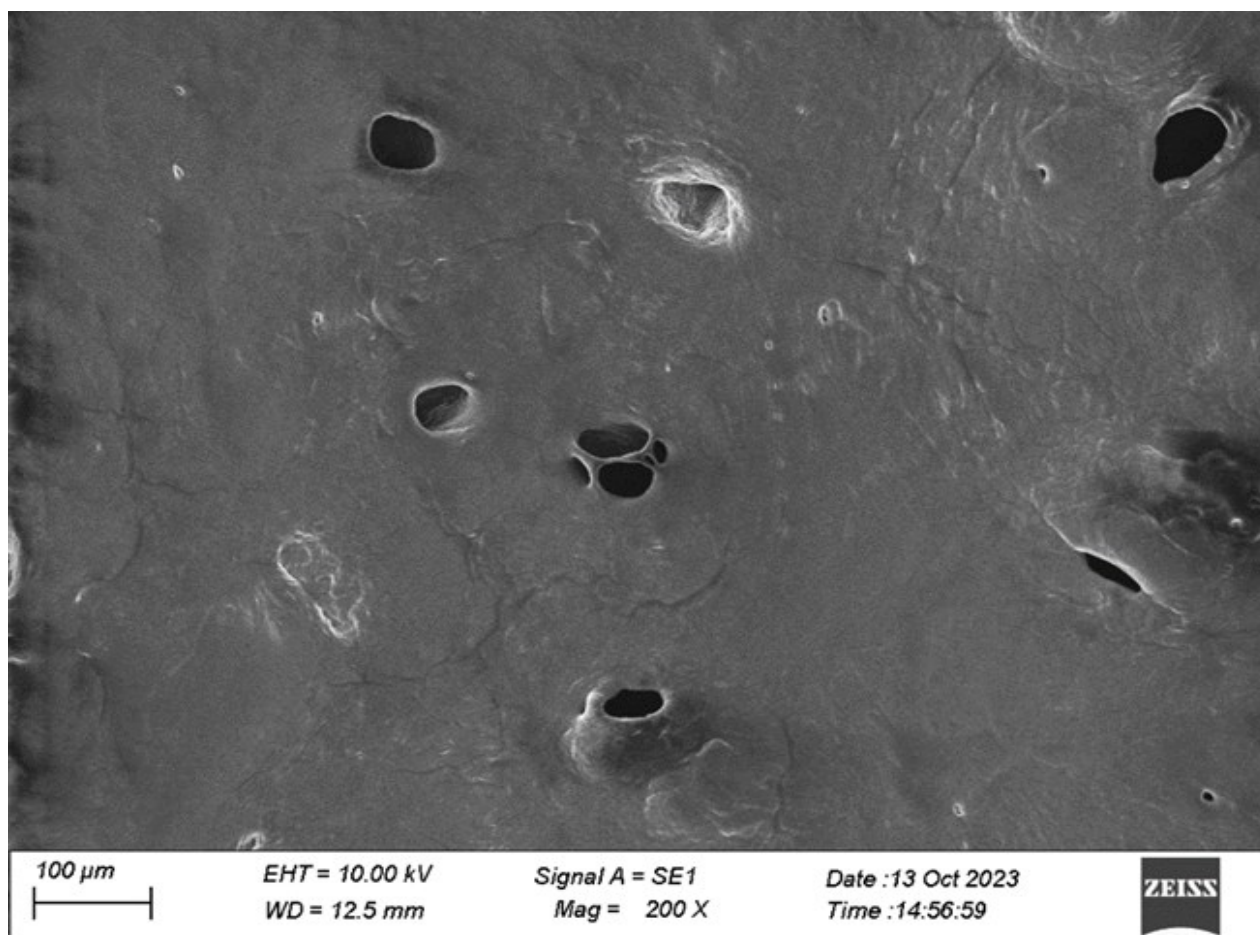


Fig. 1. SEM showing overall porosity and surface morphology of spirulina ice cream with 200X magnification, 100 μm scale.

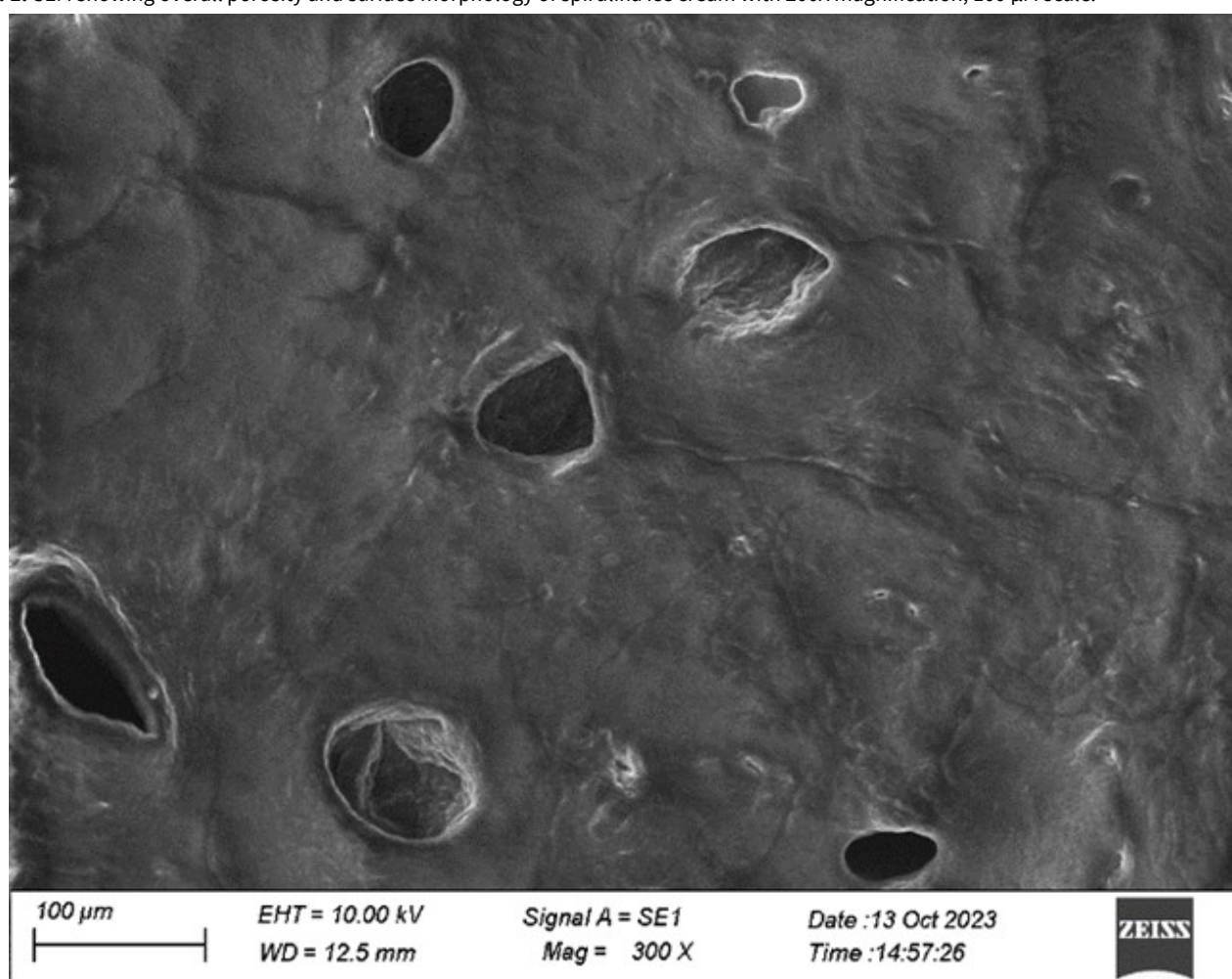


Fig. 2. SEM showing the pore wall texture of spirulina ice cream with 300X magnification, 100 μm scale.

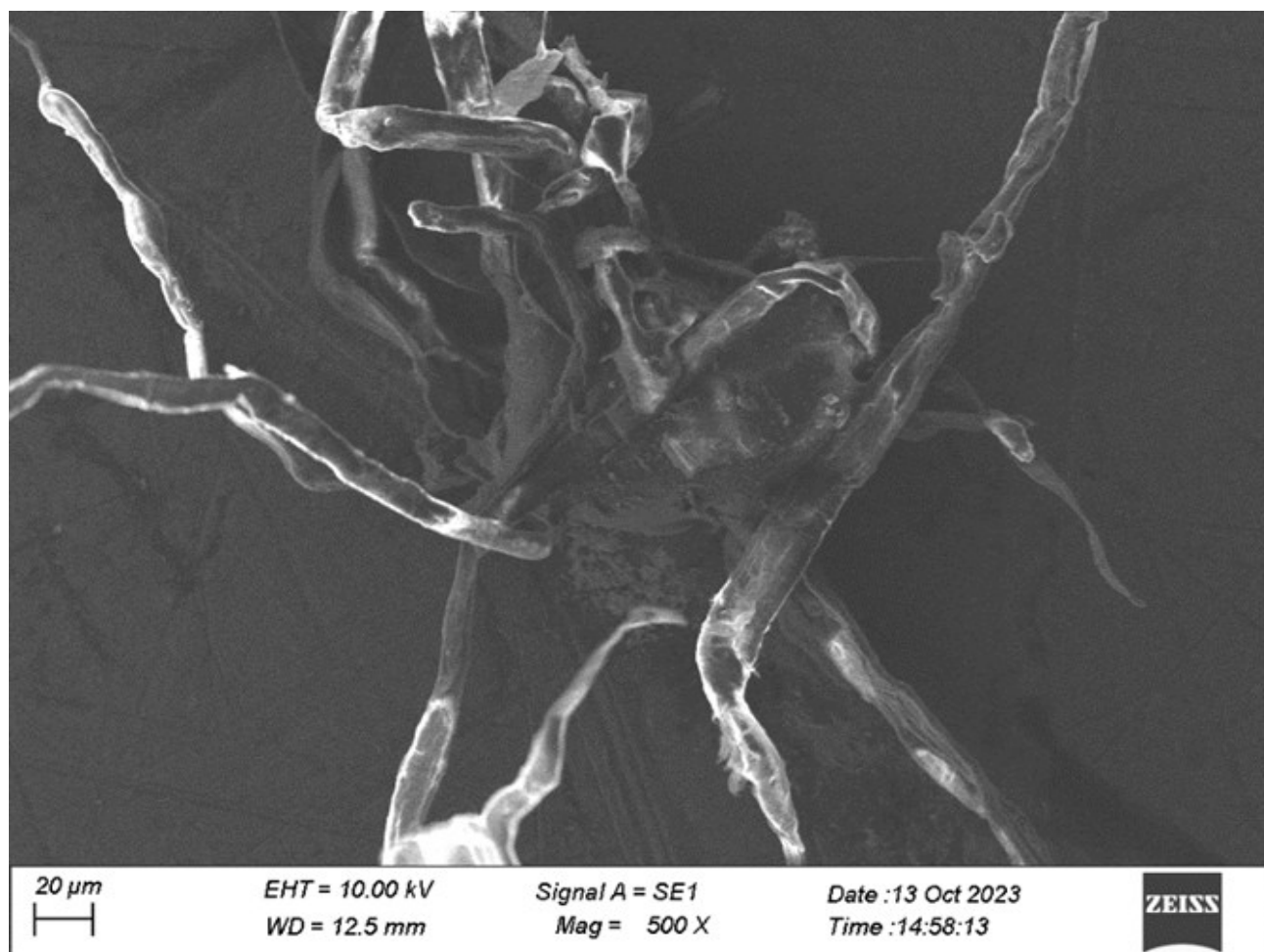


Fig. 3. SEM showing network-like formations of spirulina ice cream with 500X magnification, 20 μm scale.



Fig. 4. SEM showing broken fibrils, microstructures, or debris of spirulina ice cream with 800X magnification, 20 μm scale.

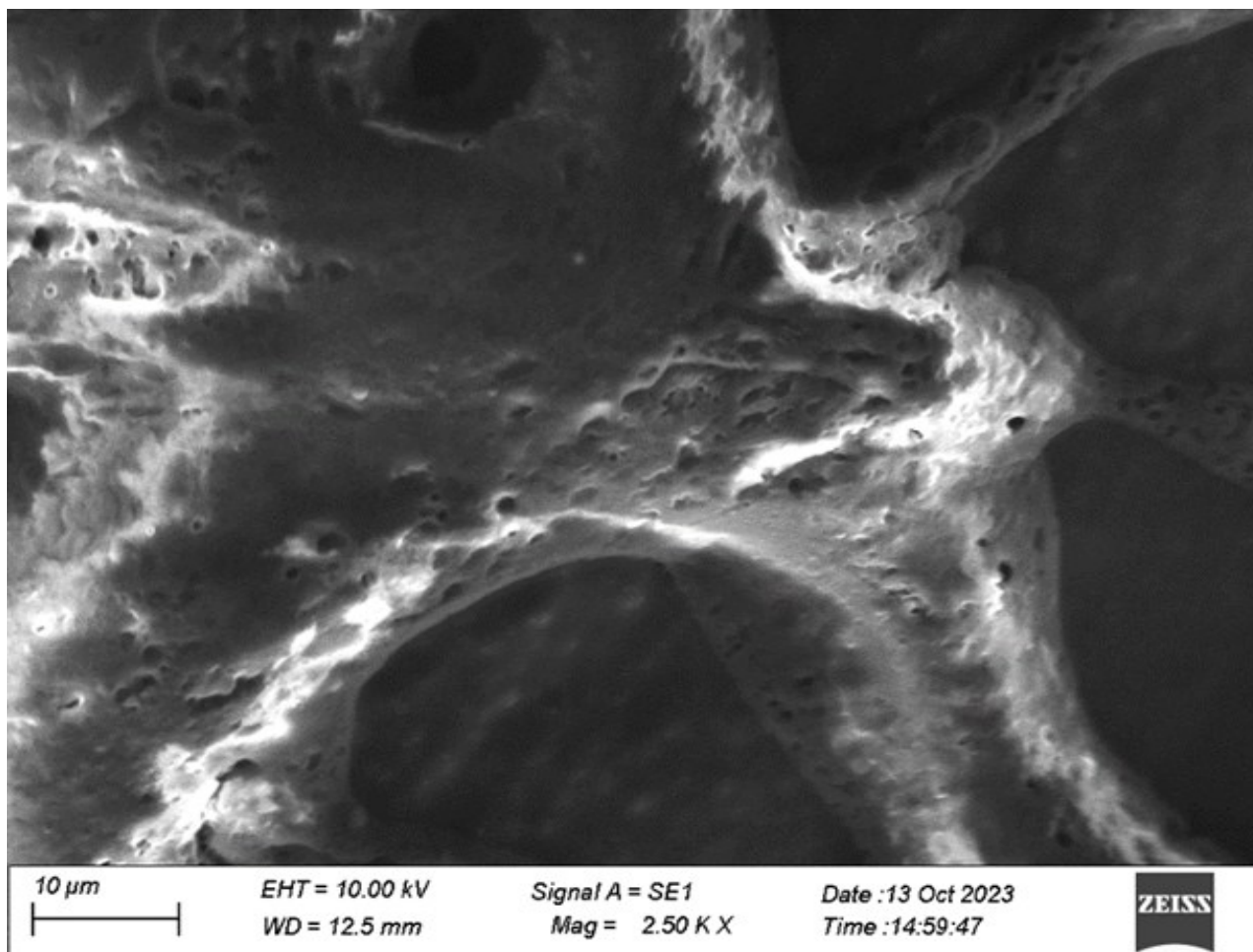


Fig. 5. SEM showing porous network or micro-channel system of spirulina ice cream with 2.50KX magnification, 10 μm scale.

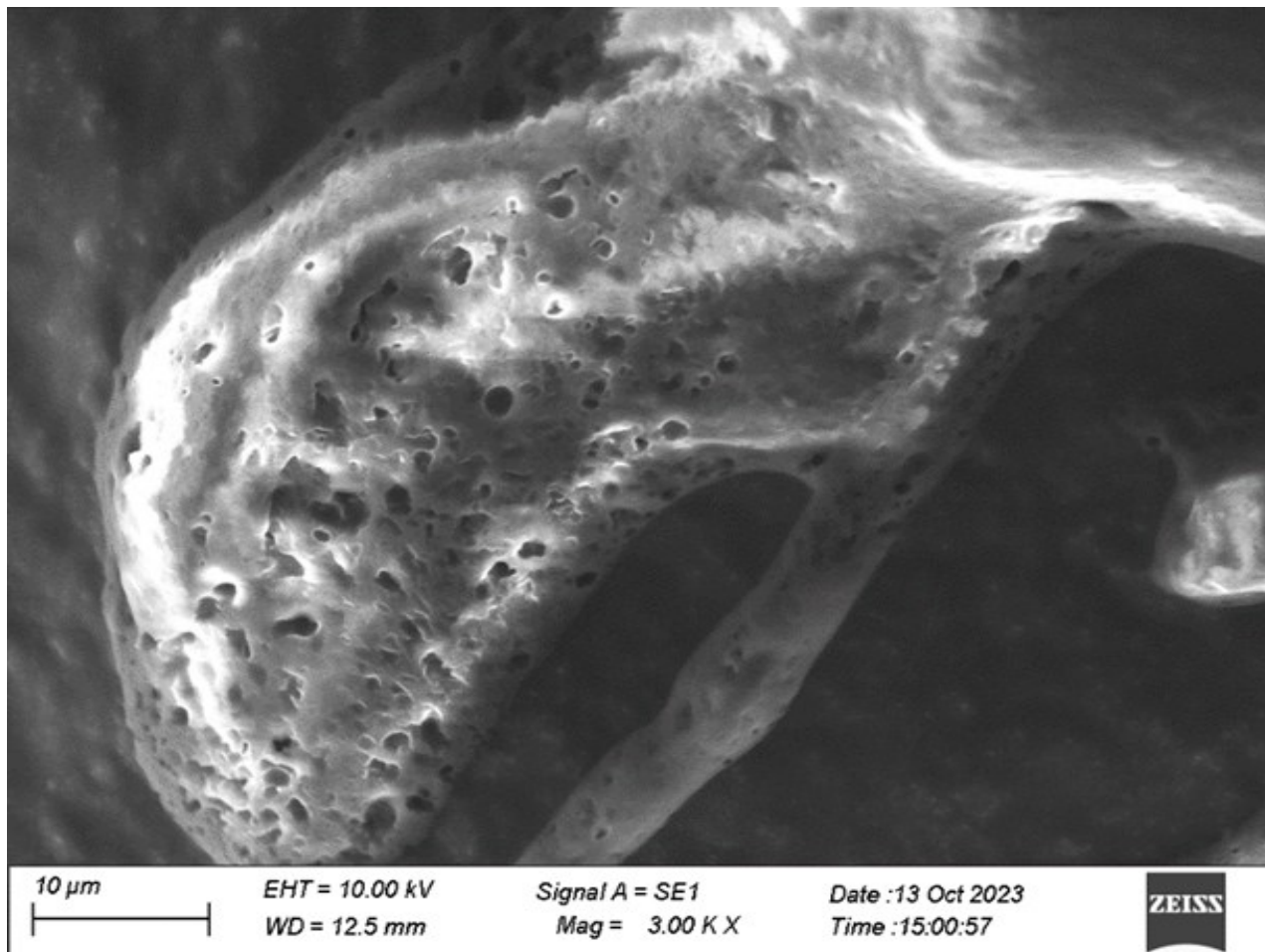
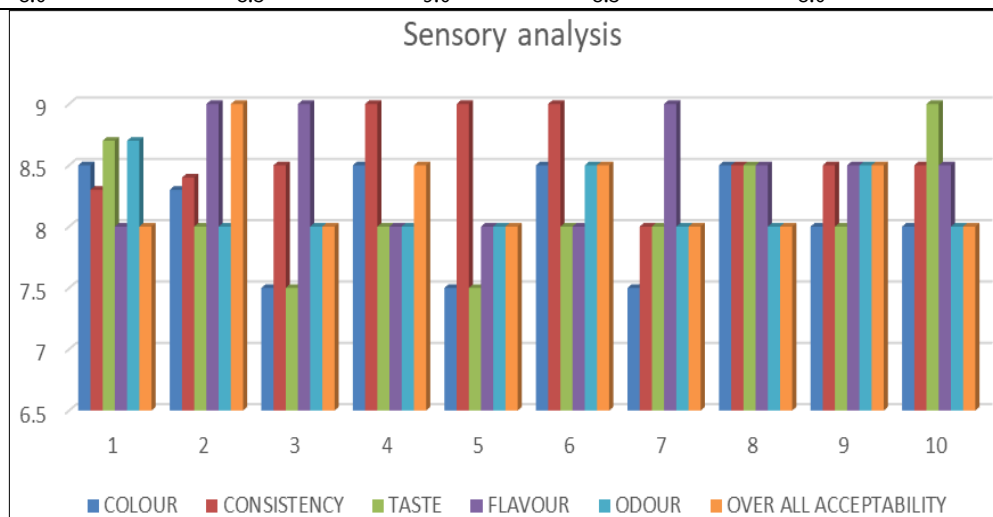


Fig. 6. SEM showing micro-pores and rough morphology of spirulina ice cream with 3.0 KX magnification, 10 μm scale.

Table 3. Data for sensory analysis conducted

Group	Colour	Consistency	Taste	Flavour	Appearance	Overall acceptability
1	8.5	8.3	8.7	8.0	8.7	8.0
2	8.3	8.4	8	9.0	8.0	9.0
3	7.5	8.5	7.5	9.0	8.0	8.0
4	8.5	9.0	8.0	8.0	8.0	8.5
5	7.5	9.0	7.5	8.0	8.0	8.0
6	8.5	9.0	8.0	8.0	8.5	8.5
7	7.5	8.0	8.0	9.0	8.0	8.0
8	8.5	8.5	8.5	8.5	8.0	8.0
9	8.0	8.5	8.0	8.5	8.5	8.5
10	8.0	8.5	9.0	8.5	8.0	8.0

**Fig. 7.** Graph showing the sensory analysis of fortified ice cream.

hedonic scale. Colour scores varied between 7.5 and 8.5, indicating good visual appeal. Consistency ratings were uniformly high (8.0-9.0), suggesting desirable texture and uniformity. Taste and flavour scores ranged from 7.5 to 9, reflecting favourable organoleptic qualities with minor variations among samples. Appearance scores (8.0–8.7) demonstrated overall visual uniformity and appeal. The overall acceptability ranged from 8.0 to 9.0, confirming that all samples were well liked, with Groups 2, 4, 6, 8 and 9 showing slightly higher acceptability (8.5–9.0). Overall, the results indicate that the samples maintained good sensory quality and consumer acceptance across all evaluated attributes. The minor variations in sensory attributes may be attributed to differences in ingredient ratios or processing conditions affecting flavour perception and texture stability. In general, the high scores across all attributes suggest that the formulations were well-developed, with no adverse sensory effects and that the products were palatable and visually appealing to consumers.

Conclusion

Spirulina fortification of ice cream significantly enhanced its nutritional profile, particularly protein and iron content, while maintaining desirable fat content and overall balance. Physical properties such as overrun, whipping efficiency and freezing point improved, which are favourable for texture and industrial production. Microstructural analysis demonstrated a multi-scale porous architecture with fibrous structures, supporting enhanced aeration and creaminess. These results establish spirulina-fortified ice cream as a functional dairy product, offering improved nutritional quality and acceptable technological characteristics. The incorporation of spirulina presents opportunities for addressing nutritional deficiencies, especially iron deficiency, while promoting sustainable food development.

Authors' contributions

SA conceptualised, formulated the manuscript and analysed the data. MA guided the research by formulating the research concept and approving the final manuscript. LR helped in summarising and revising the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

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