

Microencapsulated spirulina fortified yoghurt - An insight into physicochemical and sensory properties

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Abstract

Yoghurt is a widely consumed dairy product having good nutritional and functional properties. Incorporation of spirulina can enhance its health benefits due to its rich protein and antioxidant activity. However, direct addition affects sensory attributes, leading to reduced consumer acceptance. This study evaluates the impact of microencapsulated Spirulina-fortified yoghurt (ME-SP yoghurt) (treatment) on its physicochemical, microbiological and sensory properties, comparing it with plain yogurt as the negative control and Spirulina-incorporated yoghurt (without encapsulation) as the positive control. Results indicated that spirulina incorporation increased protein content in both positive control and treatment, but negatively influenced sensory acceptance in treatment due to colour and flavour changes. Microencapsulation effectively masked undesirable sensory characteristics while preserving nutritional benefits and an increase in protein content by 4.05%. More quantity of spirulina (1%) could be added to yoghurt when we used encapsulated spirulina than spirulina without encapsulation (0.5%). Textural analysis showed improved viscosity and stability in the microencapsulated sample than PC and NC. Microbiological analysis confirmed the probiotic viability in all samples within the acceptable range, based on the Food Safety and Standards Authority of India (FSSAI) and Codex Alimentarius standards for fermented dairy products. Sensory evaluation revealed that microencapsulation significantly enhanced flavour (7.9) compared to direct spirulina addition (6.96). This study concludes that microencapsulation is a viable technique to enhance the functional properties of spirulina-enriched yoghurt while maintaining its sensory appeal.

Introduction

Fermented foods such as yoghurt have a long-standing history of safe consumption and are widely recognised for their numerous health benefits. The safety of commercially available lactic acid bacteria (LAB) and probiotic strains used in yoghurt production has been well-established for individuals in good health, making yoghurt a reliable and beneficial dietary choice (Thakur *et al.*, 2016). As a popular dairy product, yoghurt is an excellent matrix for incorporating functional ingredients,

offering a versatile platform to enhance its nutritional profile and sensory attributes.

Spirulina, the nutrient-dense microalgae, has gained significant attention recently due to its exceptional nutritional profile, including high-quality protein (Becker, 2007), essential amino acids (Grosshagauer *et al.*, 2020), vitamins (Annapurna *et al.*, 1991), minerals (Janda-Milczarek *et al.*, 2023) and antioxidants (Bellahcen *et al.*, 2020). Incorporating spirulina into yoghurt presents an innovative approach to augmenting its nutritional value while providing a palatable and convenient

method of consumption. Incorporating spirulina into yoghurt can significantly enhance its nutritional profile, particularly by increasing protein and iron content. Spirulina is renowned for its high-quality protein, comprising about 60-70% of its dry weight and is rich in essential amino acids. Additionally, it contains substantial amounts of iron, contributing to its reputation as an iron-rich food (Podgorska-Kryszczuk, 2024). Previous studies demonstrated that spirulina supplementation in yoghurt promotes a synergistic effect with probiotics, potentially boosting the gut health benefits of yoghurt (Albuquerque *et al.*, 2024). Spirulina-fortified yoghurt offers a promising option for functional food development, particularly as a dietary supplement (Mesbah *et al.*, 2022). The addition of spirulina provides a cost-effective method for improving the nutritional profile of dairy products, which can appeal to health-conscious consumers. However, the direct addition of spirulina powder to yoghurt has posed challenges due to its strong taste, odour and distinct colour, which can affect consumer acceptance and overall product appeal. As a result, only a limited quantity of spirulina can be incorporated into yoghurt to maintain its acceptability. For instance, a study observed that adding 1% Spirulina powder to yoghurt resulted in a sensory acceptability score of 70%, whereas increasing the concentration to 2% reduced acceptability to 60%. This decline in sensory appeal with higher spirulina concentrations suggests that strong taste, odour, and colour limit the feasible inclusion levels in yoghurt formulations (Albuquerque *et al.*, 2024). While incorporating spirulina into yoghurt has been explored, there is limited research on the impact of microencapsulation on specific physicochemical properties, bioavailability and consumer acceptability. Existing studies have primarily focused on the nutritional enhancement of yoghurt with spirulina but have not extensively addressed how microencapsulation affects these critical factors. For instance, research has shown that microencapsulation can improve the thermal stability and antioxidant potential of spirulina, suggesting potential benefits for food applications (Guarienti *et al.*, 2021). Studies reported that microencapsulation helped to mask undesirable sensory attributes such as taste and colour in yoghurt containing spirulina and hence, larger quantities can be added to yoghurt. The sensory evaluation further indicated that encapsulation could enhance consumer satisfaction by preserving the nutritional and functional properties of spirulina while improving overall acceptability (Nourmohammadi *et al.*, 2020). Further studies are needed to optimise microencapsulation methods to enhance the functional and sensory qualities of spirulina-fortified yoghurt. Hence, an attempt has been made here to study the microencapsulation of spirulina and how it is enhancing the nutritional and functional properties of yoghurt.

This study aims to comprehensively analyse the physicochemical attributes of yoghurt enriched with microencapsulated spirulina powder (ME-SP). The investigation focuses on evaluating the impact of microencapsulation on key parameters such as sensory characteristics, texture, stability and overall consumer acceptance of the final product. The findings from this research will provide valuable insights into the potential of utilising microencapsulation techniques to develop innovative functional dairy products with enhanced physicochemical properties and improved consumer appeal.

Materials and methods

Ingredients

The necessary ingredients for yoghurt preparation, including skimmed milk powder, pasteurised milk and sugar, were obtained from a local market in Ernakulam. The essential mother culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was acquired from the Verghese Kurien Institute of Dairy and Food Technology, Mannuthy (Kerala Veterinary and Animal Sciences University). The algal strain of *Arthrospira platensis* was sourced from the algal culture laboratory at ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Kochi, Kerala and cultured under ambient conditions in the Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, under modified Zarrouk media as per methods outlined by Soni *et al.* (2019). The cultured spirulina biomass was then subjected to filtration using Whatman no. 1 filter paper and lyophilisation (LARK -Penguin Classic, India) at 45°C to produce spirulina powder, which was used to enrich the yoghurt.

Microencapsulation of spirulina

The microencapsulation process was carried out with certain modifications based on the methods described in previous studies (Allan-Wojtas *et al.*, 2008; Gbassi *et al.*, 2009; Nourmohammadi *et al.*, 2020). In summary, 4 ml of spirulina biomass (3%, w/v) in deionised water was mixed with 16 ml of sodium alginate solution (4%, w/v). The resulting mixture was mixed with 100 ml of sunflower oil containing 0.4% (v/v) Tween 80 and stirred for 30 min at 40 g using a magnetic stirrer. Following emulsion formation, 100 ml of calcium chloride solution (0.2 M, 4°C) was gradually added while continuously stirring at 200 rpm for 30 min. The formed microcapsules were incubated at room temperature with continuous stirring for 1 h to facilitate sufficient crosslinking with Ca²⁺ ions. Subsequently, the microcapsules were collected through centrifugation (Remi C-24 Plus, India) at 2500 rpm, 4°C, for 15 min.

The collected microcapsules were washed sequentially with isopropyl alcohol and deionised water and then filtered using Whatman No.1 filter paper. For secondary coating, a 6% (w/w) whey protein concentrate (WPC) solution was hydrated for 24 h at 4°C and adjusted to pH 7±0.1. Then 10 ml of WPC solution was added to 0.2 g of microcapsules and incubated at 37°C for 15 min of 2 h in an orbital shaker (Remi CIS 18 Plus, India). The WPC-coated microcapsules were then filtered, washed with distilled water, weighed and lyophilised for further analysis (Karadeniz *et al.*, 2018; Bellahcen *et al.*, 2020).

Microencapsulation efficiency

The microencapsulation efficiency of spirulina was determined using an indirect evaluation method, where the quantity of spirulina within the microcapsules was assessed by measuring its protein content (Machado *et al.*, 2014). The protein content of spirulina, sodium alginate and spirulina-loaded alginate microcapsules (excluding the WPC coating) was analysed using the micro-Kjeldahl method. The microcapsules were immersed in a 5% (w/v) sodium citrate solution at 45°C to facilitate protein measurement, allowing

the alginate capsules to dissolve. Once the microcapsules were disintegrated, the resulting mixture was dried in an oven, and the dried mass was accurately weighed within the 0.1 to 0.5 g range. The weighed sample was then transferred to a Kjeldahl flask for protein analysis. The microencapsulation efficiency was subsequently calculated using the equation:

$$\text{Encapsulation efficiency (EE)} = \frac{P}{P_0} \times 100$$

where P is the protein content of the microencapsulated microalgae and P₀ shows the protein content of the microalgae mass used in microencapsulation.

Release assessment of spirulina

To assess spirulina release from double-layer microcapsules, simulated gastrointestinal fluids were prepared as per the method outlined in a previous study (Mokarram *et al.*, 2009). A measured quantity of microcapsules was immersed in hydrochloric acid (pH 2) at 37°C for 3 h to simulate gastric fluid (SGF). The mixture was then filtered using Whatman No. 1 filter paper, and the collected residue was analysed for protein content. In the next phase, the intact microcapsules were transferred to a phosphate buffer (0.1 M, pH 7.4) and incubated at 37°C for 3 h to simulate intestinal fluid (SIF). The solution was again filtered, and the residues were used for protein measurement.

Morphology of the microcapsules

Scanning electron microscopy (SEM)

The morphology of double-layer microcapsules was examined using SEM with a Jeol 6390LA/Oxford XMX N system. The analysis was conducted at an accelerating voltage ranging from 0.5 to 20 kV, utilising a tungsten filament. Microcapsules were observed at magnifications of 250x, 500x, 750x, 1000x and 2000x, with an EDAX detector area of 30 mm². Lyophilised microcapsules were analysed using the freeze-fracture/cold block technique.

X-ray diffraction (XRD)

The XRD patterns were obtained using a Bruker D8 Advance X-ray diffractometer. The analysis was conducted with Cu K α radiation as the X-ray source, operating at 40 kV and 80 mA. The XRD diffractogram was recorded at room temperature over a 2 θ range of 10° to 80°C, with a scanning rate of 4°C per min.

Thermogravimetric analysis (TGA)

Before analysis, the sample was maintained at 25°C with a relative humidity of 53%. The thermal properties of the ME-SP derivative and pure spirulina powder were analysed using a TGA-DTA Perkin Elmer STA 6000 analyser. The derivative thermogravimetry (DTG) and differential thermal analysis (DTA) were conducted under a nitrogen flow of 250 ml min⁻¹, with the samples being heated from 40 to 750°C at a rate of 10°C per min. The DTG/DTA curves were used to determine the melting temperature (T_m), onset temperature (T_o) and enthalpy (ΔH) of the yoghurt samples.

Preparation of yoghurt

As per the method outlined in a previous study (Nourmohammadi *et al.*, 2020), plain yoghurt was prepared by heating pasteurised milk to 40°C using a thermometer to monitor the temperature. Skim milk powder (1%) and sugar (8-9%) were then added with continuous stirring (Nourmohammadi *et al.*, 2020). The mixture was heated to 90°C with constant stirring and simmered for 10 min. After boiling, the milk was cooled to 30-35°C at room temperature. The cooled milk was then poured into centrifuge tubes for analysis or into bowls for consumption. A 1% yoghurt culture was added and gently mixed to ensure even distribution. Spirulina was fortified by adding 1% microencapsulated spirulina to the plain yoghurt while mixing gently (67% spirulina [w/w]). The mixture was incubated at 42°C overnight to allow fermentation. After incubation, control yoghurt, yoghurt with 0.3% (w/v) spirulina powder and ME-SP yoghurt were prepared and stored at 4°C for 15 days.

Proximate analysis

The moisture, protein, fat, and ash content of yoghurt formulations were determined following the AOAC (2005) guidelines. Moisture content was determined gravimetrically using a hot air oven (Rotek Instruments-RHO-24HNS-1813) by heating at 105°C. Protein estimation was performed by assessing total nitrogen through Kjeldahl digestion and distillation using a unit from Borosil India (100KID000006). Fat content was determined using the Soxhlet apparatus (Rotek Instruments-RHMS-60-1807) with petroleum ether and total ash was determined through combustion (at 550°C) using a Muffle furnace (Rotek Instruments RMFH-4-1804).

Determination of physicochemical properties of yoghurt

Estimation of pH

Yoghurt samples underwent pH analysis using a digital pH meter (Ins Mask Model number: IS12BL), by the procedure outlined by AOAC (2005). Before usage, the pH meter was calibrated with buffer solutions of pH 4.00 and pH 7.00. To assess the pH of the sample, it was placed in a beaker and thoroughly mixed. The pH meter electrode was immersed in the sample and the resulting pH value was recorded.

Estimation of total solids

Total solids were determined using the methods described by AOAC (2005). A 5 g sample of yoghurt was taken in a pre-weighed petri dish. Then, it was heated by placing it in a water bath for 15 min. It was kept in a hot air-drying oven for 3 h at 100°C. Then, it was cooled by placing it in a desiccator for half an hour. The dish was reweighed and the percentage of total solid residues was determined using the equation below.

$$\text{Total solids (\%)} = \frac{\text{Dry weight}}{\text{Weight of the sample}} \times 100$$

Estimation of syneresis

Syneresis refers to the liquid, similar to whey, that appears on the surface of yoghurt when it is left at room temperature and separates

(Walstra, 1993). To measure syneresis in yoghurt, a 450 mL specimen of set-type yoghurt was placed at room temperature for 2 h. The liquid whey separated was siphoned off from the sample, collected in a graduated cylinder and its volume was measured.

Viscosity

The viscosity measurements of the samples were performed by a digital Viscometer (RDVD II, Brookfield, USA) using Spindle No. LV-2 with 0.5 rpm speed for 1 min. The sample temperature was set at 15°C and its volume was 100 ml. Viscosity was reported in centipoise.

Microbiological analysis

Coliforms, yeast and mold testing were done adhering to the Standard Methods for the Examination of Milk Products (Marth, 1978), employing Violet Red Bile Agar (VRB) for coliforms and acidified Potato Dextrose Agar (PDA) for yeast and mold detection.

Sensory evaluation

The sensory evaluation of Plain yoghurt, spirulina incorporated and ME-SP fortified yoghurts (stored at 4°C) was conducted by 20 panellists aged 20-50 years, 7 days after production. Each panellist received 3 yoghurt samples to evaluate and comment on the sensory characteristics. The panellists were asked to evaluate the taste, colour and appearance, texture, flavour and overall acceptability of the final product depending on a nine-point hedonic scale, ranging from 9 (like highly liked) to 1 (highly disliked) for each organoleptic characteristic (Zamri *et al.*, 2023).

Statistical analysis

The characteristics of yoghurt were assessed with values recorded three times and presented as both mean±standard deviation. Subsequently, one-way analysis of variance (ANOVA) was applied and groups with marginal means exceeding 5% underwent Tukey's test. All statistical analyses were performed using SAS version 9.3 (SAS Institute, 2012).

Results

Microencapsulation efficiency and release study

The microencapsulation efficiency of alginate-based capsules coated with whey protein concentrate (WPC) was found to be 47.8±1.2%. This efficiency suggests that the encapsulation technique effectively retained a substantial portion of the bioactive components while allowing some diffusion losses during the encapsulation process. The observed ME falls within the expected range for alginate-based microcapsules, where efficiency is influenced by factors such as polymer concentration, cross-linking conditions and coating uniformity. The incorporation of WPC as a secondary coating likely contributed to structural stability and protection of encapsulated spirulina, though minor losses may have occurred due to permeability variations in the alginate matrix. Optimising the polymer-to-core ratio and cross-linking conditions may enhance encapsulation efficiency in future formulations.

The release study indicated that 85-90% of spirulina remained encapsulated in simulated gastric fluid (pH 1.2) after 2 h, confirming the protective effect of the encapsulation system under acidic conditions. Upon transition to simulated intestinal fluid (pH 6.8), a gradual release began within 30 min, with near-complete release (95-100%) achieved within 2 h. The encapsulation of spirulina with a combination of alginate and whey protein concentrate (WPC) also improved the stability of spirulina (retaining 90% of bioactive compounds after 7 days of storage at 4°C) and enhanced its functionality in yoghurt.

Microcapsules morphology

Scanning electron microscopy (SEM)

The SEM images of the ME-SP yoghurt (Fig. 1) revealed crucial insights into surface morphology, structural integrity and encapsulation efficiency. At lower magnifications (x250 and x500), the microcapsules appeared as irregularly shaped particles with

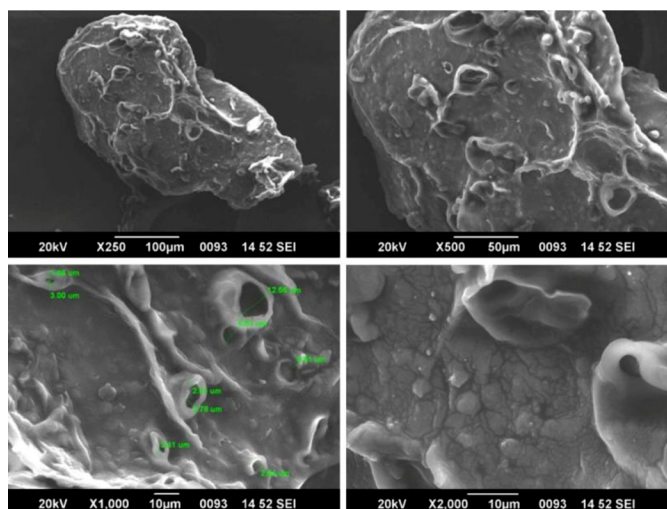


Fig. 1. SEM image of microencapsulated spirulina (ME-SP)

rough and porous surfaces, indicating successful encapsulation of spirulina within the polymer matrix. Higher magnifications (x1000 and x2000) revealed microscale pores ranging from 3.60 to 7.01 μm in diameter, likely formed due to drying processes and interactions between encapsulating agents.

X-ray diffraction (XRD)

The XRD analysis of ME-SP revealed a broad peak around $2\theta = 20\text{--}22^\circ\text{C}$, which is a characteristic feature of amorphous materials (Fig. 2). This observation suggests that the encapsulation process successfully altered the crystalline nature of spirulina's bioactive compounds, leading to a more disordered molecular arrangement.

Thermogravimetric analysis (TGA)

The TGA analysis of ME-SP was conducted using Differential Thermal Analysis (DTA) and Differential Scanning Calorimetry (DSC), revealing multiple stages of thermal degradation. An initial weight loss occurred between 50°C and 150°C , attributed to moisture evaporation in DTA. A major endothermic peak at 261.8°C indicated the decomposition of proteins, carbohydrates and pigments, signifying moderate thermal stability. A significant exothermic peak at 399.7°C corresponded to the oxidative breakdown of lipids and polysaccharides, followed by a gradual weight decline beyond 500°C , indicating residual organic matter combustion. In addition, the onset temperature ($T_o = 261.8^\circ\text{C}$), melting temperature ($T_m = 399.7^\circ\text{C}$) and enthalpy change ($\Delta H \approx 15.7 \text{ J g}^{-1}$) were determined from the DTG/DTA curves for the microencapsulated spirulina, further confirming the enhanced thermal stability compared to lyophilised spirulina ($T_o = 363.2^\circ\text{C}$, $T_m = 363.2^\circ\text{C}$, $\Delta H \approx 15.6 \text{ J g}^{-1}$). The delayed onset of decomposition and higher thermal transition stability in the ME-SP sample demonstrate the protective role of encapsulation.

Compared to the lyophilised spirulina powder, microencapsulation significantly improved thermal stability. The lyophilised spirulina exhibited an initial moisture loss of approximately 5% between 30 and 150°C , followed by a substantial weight reduction ($\sim 20\%$) between 150 and 300°C due to the degradation of volatile organic compounds. The DTG curve of lyophilised spirulina showed a peak at 250°C , indicating the highest decomposition rate during this phase, whereas microencapsulated spirulina displayed a delayed primary decomposition event at 261.8°C . Additionally, the exothermic peak of lyophilised spirulina occurred around 270°C , whereas microencapsulated spirulina showed a later degradation at 399.7°C , suggesting enhanced structural stability. These differences

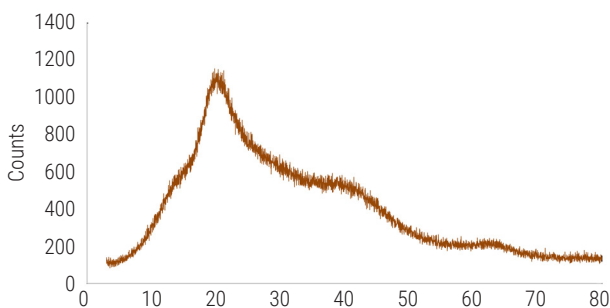


Fig. 2. XRD patterns of microencapsulated spirulina (ME-SP)

highlight that microencapsulation provides an additional protective matrix, delaying thermal degradation and ensuring greater stability for applications in high-temperature food processing. Thermal stability testing confirmed the protective role of microencapsulation, as it delayed the decomposition of spirulina's bioactive compounds compared to non-encapsulated spirulina.

Thermal stability testing confirmed the protective role of microencapsulation, as it delayed the decomposition of spirulina's bioactive compounds compared to non-encapsulated spirulina. Differential thermogravimetric (DTG) analysis confirmed sequential degradation, with major weight loss events at 250.3 , 376.2 and 470.2°C , demonstrating that encapsulation provides enhanced protection against thermal decomposition. The thermal analysis (DTA and DTG) revealed distinct differences between lyophilised spirulina and microencapsulated spirulina (ME-SP) (Fig 3a, b). The DTA curves showed endothermic and exothermic transitions corresponding to moisture loss, decomposition and structural transformations, while the DTG profiles indicated weight-loss events at different temperature ranges, reflecting moisture evaporation, protein/pigment degradation and decomposition of encapsulating material. Compared to lyophilised spirulina, the ME-SP displayed shifts in thermal events, reduced peak intensities and broader degradation peaks, suggesting that the sodium alginate-WPC encapsulating matrix enhanced thermal stability, delayed decomposition and protected spirulina bioactives.

Viscosity

The viscosity analysis of yoghurt samples- plain yoghurt (NC), spirulina-incorporated yoghurt (PC) and ME-SP yoghurt (T) showed significant differences under increasing shear stress. The control yoghurt exhibited the lowest viscosity, with minor increases under shear stress, indicating its shear-thinning behaviour. The spirulina-incorporated yoghurt showed a notable viscosity increase, likely due to the water-binding capacity and structural reinforcement of spirulina's proteins and polysaccharides. The ME-SP yoghurt exhibited the highest viscosity across the shear stress range, surpassing both control and spirulina-incorporated yoghurt (Fig. 4). This indicates that microencapsulation enhanced, viscosity development of the yoghurt.

Proximate composition

The proximate composition analysis of plain yoghurt (NC), spirulina-incorporated yoghurt (PC) and ME-SP yoghurt (T) (Table 1) showed significant variations in protein and fat content. The protein content was significantly higher in both the Positive control (PC) and Treatment (T) groups compared to the Negative control (NC) (PC: 4.14%, T: 4.05% vs. NC: 1.22%), indicating that the treatments increased protein levels. In contrast, fat content was significantly lower in the PC (2.51%) and T (2.74%) groups compared to NC (3.39%), suggesting a reduction in fat due to the treatments. No significant differences were observed in moisture (NC: 78.99%, PC: 73.98%, T: 71.87%) and ash content (NC: 0.43%, PC: 0.73%, T: 0.8%) among the groups. These results highlight that the treatments affected protein and fat content but had no impact on moisture or ash levels. Regarding fat content, the treatment group (T) ($2.74 \pm 0.22\%$) exhibited a moderate increase compared to the PC group ($2.51 \pm 0.23\%$). However, it remained lower than the

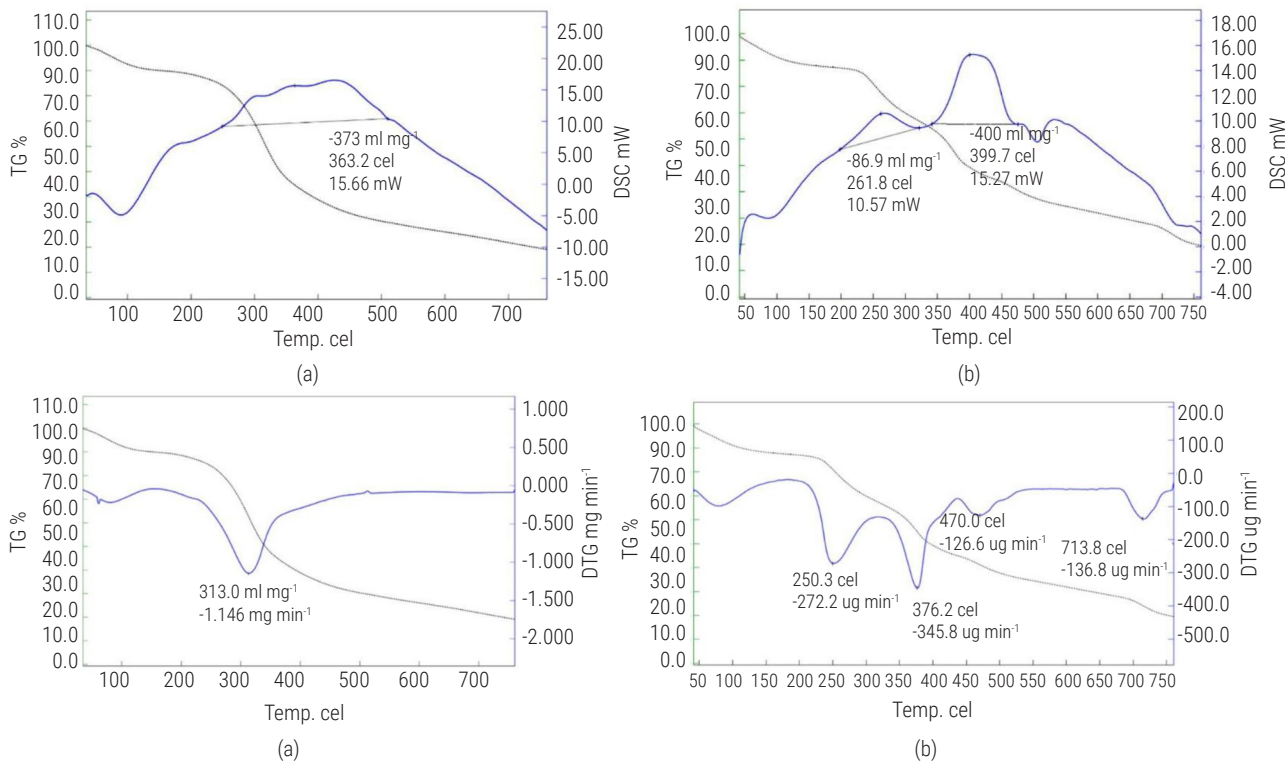


Fig. 3. DTA graph of (a) Lyophilised spirulina, (b) microencapsulated spirulina (ME-SP). DTG graph of (c) Lyophilised spirulina (d) microencapsulated spirulina (ME-SP)

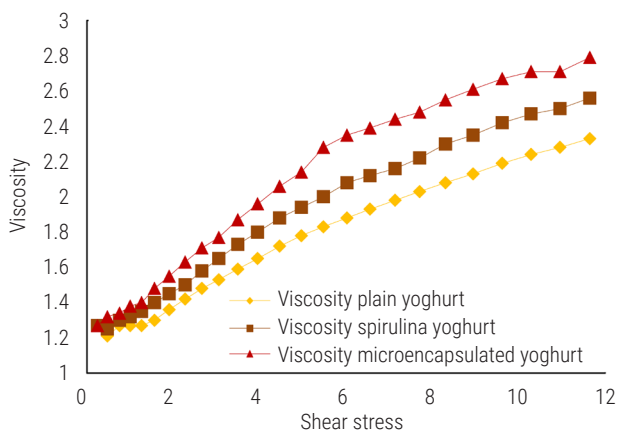


Fig. 4. Shear stress v/s Viscosity graph of yoghurt combinations

NC group (3.39±0.41%), suggesting that microencapsulation helps to regulate the lipid profile while maintaining stability. This trend suggests that microencapsulation may influence lipid interactions within the yoghurt matrix.

Table 1. Proximate composition of yoghurt samples

Groups	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
Negative control (NC)	1.22±0.03 ^a	3.39±0.4 ^a	78.99±0.14 ^a	0.43±0.03 ^a
Positive control (PC)	4.14±0.12 ^b	2.51±0.23 ^b	73.98±0.18 ^a	0.73±0.04 ^a
Treatment (T)	4.05±0.1 ^b	2.74±0.22 ^b	71.87±1.87 ^a	0.8±2.1 ^a

Values are expressed as mean ± SD (n=3). Superscripts a and b indicate significant differences among the treatments.

Physicochemical characteristics

pH: The treatment (T) group (4.9±0.27) exhibited a higher pH than the NC group (4.65±0.14) and the PC group (3.51±0.25), indicating a moderately acidic effect due to microencapsulation. This increase in pH suggests that microencapsulation moderates the acidity typically associated with spirulina addition (Table 2). It is observed that coating *Lactobacillus acidophilus* microcapsules with whey protein resulted in yogurt with pH levels comparable to control samples, indicating reduced acidity (Mousa et al., 2014).

Total solids: The total solids (non-fat) content of the treatment (T) group (21.33±0.2%) was slightly lower than the PC group (22.39±0.28%) but higher than the NC group (18.90±0.28%) without any significant difference (Table 2). The total solids (fat) in the treatment (T) group (20.45±0.43%) were similar to the NC group (21.82±0.31%) but slightly lower than the PC group (23.40±0.33%). This indicates that microencapsulation does not significantly alter the total solids content, suggesting that the encapsulation process preserves the yogurt's structural integrity.

Syneresis: Syneresis was statistically significantly lower in both the Positive control (PC) (11.96%) and Treatment (T) (10.11%)

groups compared to the Negative control (NC) (19.45%) (Table 2), indicating reduced water separation in the treated groups.

Microbiological quality

Microbiological analysis confirmed the absence of mold, yeast and coliform bacteria in all yoghurt samples throughout the 4-week storage period at 4°C (Table 3). Total plate count (TPC) remained below 10^3 CFU g⁻¹, while no detectable levels of yeast and mold (<10 CFU g⁻¹) or coliforms (<1 CFU g⁻¹) were observed in any sample. These results indicate that the yoghurt was processed under good hygienic and sanitary conditions, ensuring microbiological safety and stability during refrigerated storage.

Sensory evaluation

The sensory evaluation (Fig. 5) shows that the ME-SP yoghurt (T) had the highest scores across all parameters, indicating superior consumer acceptability. It had the best appearance (7.7), likely due to improved colour uniformity, and the highest aroma score (7.5), suggesting better masking of spirulina's natural odour. Flavour (7.9) matched the control but was significantly better than spirulina-incorporated yoghurt (6.96), highlighting the effectiveness of encapsulation in reducing undesirable tastes. The treatment group also had the best texture (7.9), contributing to its highest overall acceptability (7.52). These findings confirm that microencapsulation enhances sensory properties, making spirulina-enriched yoghurt more appealing.

Discussion

The encapsulation efficiency values observed were similar to those reported in previous studies (Khalil *et al.*, 1998; Allan-Wojtas *et al.*, 2008), which suggests that this combination offers moderate protection for spirulina, ensuring stability while allowing for controlled release properties. A previous study reported an encapsulation efficiency of $44.54 \pm 0.06\%$ for alginate-WPC microcapsules encapsulating spirulina (Nourmohammadi *et al.*, 2020). The release study indicated that spirulina remained encapsulated in simulated gastric fluid, confirming the protective effect of the microcapsule system against acidic conditions. A controlled release pattern was observed in simulated intestinal fluid, with $95.4 \pm 2.3\%$ of the encapsulated spirulina being released within 120 min. These results

align with those reported by Nourmohammadi *et al.* (2020), where complete release occurred in intestinal conditions. Similarly, it was noted that a gradual release of encapsulated spirulina in simulated gastrointestinal fluids suggested effective pH-dependent release properties (Zamri *et al.*, 2023).

The encapsulation efficiency values also confirm the effectiveness of the alginate-WPC matrix in preserving bioactive components under varying environmental conditions (Abd El-Salam and El-Shibiny, 2015; Gonzalez-Reza *et al.*, 2018). The present study surpasses the encapsulation efficiency reported in previous studies (Abd El-Salam and El-Shibiny, 2015; Gonzalez-Reza *et al.*, 2018), likely due to optimised formulation parameters that enhanced retention. Additionally, although certain studies report higher microencapsulation values, such as 82.3% in microcapsules using 3% sodium alginate and 1% spirulina (Zamri *et al.*, 2023), it is important to note that such high efficiencies often come at the cost of increased viscosity and compromised release kinetics, which may limit the bioavailability of the encapsulated compound. Furthermore, an encapsulation system using an alginate-hydroxyethyl cellulose composite achieved a 75% efficiency (Guerrero *et al.*, 2020). However, such systems often involve additional processing steps or require synthetic additives, whereas the current formulation maintains a natural, food-grade profile without compromising encapsulation performance. Studies on *Lactobacillus bulgaricus* microencapsulation have reported microencapsulation values close

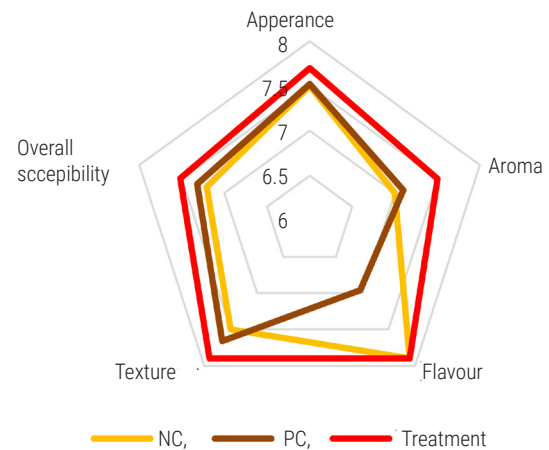


Fig. 5. Sensory score of yoghurt combinations

Table 2. Physicochemical characteristics of yoghurt

Groups	Syneresis (%)	Total solids-non-fat (%)	Total solids-fat (%)	pH
Negative control (NC)	19.45±0.25 ^a	18.90±0.28 ^a	21.82±0.31 ^a	4.65±0.14 ^a
Positive control (PC)	11.96±0.7 ^b	22.39±0.28 ^a	23.40±0.33 ^a	3.51±0.25 ^a
Treatment (T)	10.11±0.9 ^b	21.33±0.2 ^a	20.45±0.43 ^a	4.9±0.27 ^a

Values are expressed as mean±SD (n=3). Superscripts a and b indicate significant differences among the treatments.

Table 3. Microbiology analysis

Parameter	NC (Control yoghurt)	PC (Spirulina yoghurt)	Treatment (Microencapsulated spirulina yoghurt)
Total plate count (TPC) (CFU g ⁻¹)	<10 ³	<10 ³	<10 ³
Yeast and mold (CFU g ⁻¹)	<10	<10	<10
Coliforms (CFU g ⁻¹)	<1	<1	<1

to 100% (Chen *et al.*, 2014), but these results pertain to bacterial encapsulation, which differs fundamentally in particle interaction and stability compared to spirulina encapsulation.

The release behaviour demonstrates that the microcapsules effectively protect spirulina in gastric environments and deliver their contents in the targeted intestinal environment, consistent with previous reports (Allan-Wojtas *et al.*, 2008; McClements, 2015; Chai *et al.*, 2018; Grgic *et al.*, 2020). The efficiency observed in the present study reflects a well-optimised balance between encapsulation stability, structural integrity and release characteristics. Unlike some high-ME formulations that may suffer from excessive rigidity, potentially affecting the controlled release of bioactives, the present system ensures effective entrapment while maintaining a suitable matrix for gradual diffusion. This positions the current encapsulation approach as a superior alternative, particularly for food applications where maintaining bioactive functionality during storage and digestion is crucial. Future optimisations, such as fine-tuning crosslinking conditions and exploring additional stabilisers, could further enhance microencapsulation without compromising functionality. Nonetheless, the findings of this study highlight the potential of the current system for improving the stability and controlled release of spirulina, making it a strong candidate for functional food formulations. A crucial aspect of encapsulation is its ability to protect bioactive compounds, particularly in gastric environments and release them in the intestines where they can exert their beneficial effects (de Vos *et al.*, 2010; Goncalves *et al.*, 2021; Bauer-Estrada *et al.*, 2023). This aligns with previous findings, which demonstrated that encapsulation protects bioactive ingredients from gastric degradation and enables their targeted delivery. The release of spirulina in the intestinal environment also facilitates its prebiotic potential by supporting the growth of beneficial gut microbiota, particularly *Lactobacillus* populations (Bortolini *et al.*, 2022; Zhang *et al.*, 2022; Iatrou *et al.*, 2023). The controlled release profile observed here aligns with earlier reports that alginate microcapsules dissolve in alkaline intestinal conditions due to ion-exchange interactions between calcium and phosphate ions (Burgain *et al.*, 2011). Furthermore, the incorporation of WPC likely enhanced the mechanical strength of the microcapsules while enabling the gradual disintegration required for efficient intestinal delivery (de Vos *et al.*, 2010).

The SEM analysis indicates that the microencapsulation process resulted in the formation of porous, irregularly shaped microcapsules with varying surface roughness. These characteristics could influence the controlled release behaviour and stability of the encapsulated spirulina under different environmental conditions. The SEM analysis further corroborated the structural characteristics of the microcapsules, revealing porous, irregularly shaped surfaces. These structural features are key to the controlled release behaviour and stability of the encapsulated spirulina, as the pores may allow for the diffusion of moisture and air, which could affect the stability of bioactive compounds (Rajam *et al.*, 2012; Mahdavi *et al.*, 2016). The porous nature of the capsules may thus influence the release kinetics, which is important for functional applications (Trojer *et al.*, 2015; Nixon, 2019).

The absence of sharp crystalline peaks in the XRD pattern indicates that the encapsulation matrix, composed of sodium alginate and WPC, effectively masked the native crystallinity of spirulina components. The formation of this amorphous structure is likely

due to the strong interactions between bioactive molecules and the encapsulating agents, which disrupt the ordered crystalline lattice (Liu *et al.*, 2023). XRD patterns of spirulina reported in previous studies often show small but discernible crystalline features (notably at $\sim 2\theta \approx 10^\circ$ and $\approx 20^\circ$), which have been attributed to ordered protein/phycoerythrin structural motifs in raw biomass (Lemos *et al.*, 2020).

The XRD analysis showed that the encapsulation process effectively reduced the crystallinity of spirulina's bioactive compounds, which may enhance stability and dissolution rates (Marjanovic *et al.*, 2024). This reduction in crystallinity results from interactions between spirulina and the encapsulating agents, leading to an amorphous network that enhances the encapsulation efficiency. A study demonstrated that encapsulating phenolic extracts from *Spirulina* sp. LEB-18 using alginate particles achieved a high encapsulation efficiency of 88.97% (Machado *et al.*, 2022). In this study, the resulting particles were amorphous, providing protection to the phenolic compounds under acidic conditions and facilitating their release in intestinal environments, thereby improving bio-accessibility (Machado *et al.*, 2022). This structural modification is beneficial for controlled release and could improve the shelf life and efficacy of spirulina in functional foods.

Previous studies on microencapsulated spirulina using alginate matrices revealed that encapsulation enhances the stability of bioactive compounds, protecting the antioxidant potential of spirulina during cooking with a 37.8% retention rate and producing microparticles with low water solubility, making them suitable for products requiring cooking in water (Bortolini *et al.*, 2022). The encapsulated spirulina showed enhanced heat resistance, which is important for its use in functional foods requiring high-temperature processing which is investigated by the thermal properties of microencapsulated powders using different wall materials (Pan-Utai and Iamtham, 2020). The thermal analysis provided insights into how various encapsulating agents affect the stability of spirulina's bioactive compounds under thermal conditions. These findings collectively suggest that encapsulation, particularly using agents like alginate, can enhance the thermal stability of spirulina's bioactive compounds, offering protection against thermal decomposition.

Studies have shown that adding spirulina to yogurt improves its textural properties, likely due to the higher protein content and the formation of a three-dimensional network that entraps water, thereby increasing viscosity (Malik *et al.*, 2013). The higher viscosity observed in ME-SP yoghurt can be attributed not only to spirulina's contribution but also to the structuring effects of the encapsulating materials (sodium alginate and WPC), which reinforced the yoghurt matrix. Research indicates that microencapsulation of spirulina affects the physicochemical properties of yogurt, including viscosity, by altering the interaction between spirulina and yogurt components (Nourmohammadi *et al.*, 2020).

The rheological behaviour of spirulina-incorporated yoghurt showed shear-thinning characteristics, typical of bioactive fortification in yoghurt (Beal *et al.*, 1999). Thus, the microencapsulation of spirulina in yoghurt resulted in a thicker texture and improved viscosity, demonstrating that encapsulation can enhance both stability and textural quality. Studies have demonstrated that incorporating microencapsulated spirulina into yoghurt leads to improved

physicochemical properties. For instance, research observed that yogurt with microencapsulated spirulina exhibited a higher pH, reduced syneresis (the separation of liquid from the yogurt) and a consistent increase in viscosity during storage. These changes are attributed to the protective effects of microencapsulation, which shields spirulina's bioactive compounds from interactions that could negatively impact the yogurt's texture and stability (Nourmohammadi *et al.*, 2020). Microencapsulation contributed to the reduction of syneresis, as seen in the lower water separation in the ME-SP yoghurt compared to the NC group. This outcome supports earlier findings that microencapsulation improves the water-holding capacity and stability of dairy products (Rani *et al.*, 2012). Additionally, studies have emphasised that increasing total solids in yoghurt formulations reduces syneresis and enhances texture (Afzaal *et al.*, 2022; Arab *et al.*, 2023), which aligns with the current observations.

The slightly lower total solids content in the ME-SP group could be attributed to the encapsulation matrix, which adds bulk without directly contributing to measurable solids. Similar trends have been noted in other studies using encapsulated bioactives. Importantly, this indicates that the encapsulation process did not significantly compromise the yoghurt's structural integrity. The higher pH observed in the ME-SP yoghurt suggests that microencapsulation may buffer the acidity associated with spirulina addition. This finding corresponds with earlier research, where encapsulation of *L. acidophilus* or bioactives with whey protein resulted in yoghurts with pH values closer to control samples, thus reducing acidity (Mousa *et al.*, 2014; Malik *et al.*, 2013; Barkallah *et al.*, 2017). The buffering effect of the encapsulation system could therefore enhance product stability and improve consumer acceptance.

Overall, these results demonstrate that microencapsulation helps preserve yoghurt quality by maintaining desirable pH, texture and water-holding properties, while minimising undesirable syneresis. This study demonstrates that microencapsulation effectively enhances the functional properties of spirulina-enriched yogurt. By improving spirulina's interaction with the yoghurt matrix, microencapsulation enhances viscosity, reduces syneresis and stabilises the product. It also regulates the release and preserves the functionality of bioactive compounds, aligning with prior research on dairy applications. A key advantage is the ability to incorporate higher spirulina concentrations without compromising texture or sensory appeal. The study successfully integrated 0.3% (w/v) spirulina powder and 1% microencapsulated spirulina, boosting the yoghurt's protein, vitamin and antioxidant content while preserving its bioactive properties during fermentation and storage. ME-SP yoghurt offers superior nutrition, increasing protein levels while maintaining a balanced fat profile. This makes it a functional dairy product supporting muscle health, immune function and overall well-being without altering texture or mouthfeel. Microencapsulation further enhances bioavailability, ensuring controlled release and efficient absorption.

Although certain elements of microencapsulation research have been previously explored, the present work extends the field by introducing thermal (TGA/DTA) and crystallographic (XRD) analyses to demonstrate the stabilising role of the alginate-WPC system. Moreover, by assessing simulated gastrointestinal release and integrating the microcapsules directly into yoghurt, this study provides both mechanistic insights and practical application,

highlighting a clear advancement toward real-world food systems. Overall, this study highlights microencapsulation as a transformative strategy for functional yoghurt development. It optimises spirulina's stability, bioavailability and sensory properties, positioning ME-SP yoghurt as a promising next-generation functional dairy product with significant health benefits.

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