RESEARCH PAPER

Microencapsulation of Spirulina (*Limnospira platensis)* Biomass using the Spray-Drying Technique and its Application in Milk Chocolate

Hema Deupa[1], Ayushi Patel[2], Digvijay Verma[2], Pankaj Panwar[3], Zia Parveen[1], Priyanka Shankar[4]

[1] Department of Food and Nutrition, School of Home Science, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow – 226025, Uttar Pradesh, India.
[2] Department of Microbiology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow – 226025, Uttar Pradesh, India.
[3] Department of Food Grain and Oilseed Processing, ICAR–Central Institute of Post-Harvest Engineering and Technology (CIPHET), Ludhiana – 141004, Punjab, India.
[4] Assistant Professor, Department of Human Development and Family Studies, School of Home Science, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow – 226025, Uttar Pradesh, India.
Email: Priyanka.fn@bbau.ac.in
Corresponding Author:
Dr. Priyanka Shankar
Assistant Professor, Department of Human Development and Family Studies, School of Home Science, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow – 226025, Uttar Pradesh, India.
Email: Priyanka.fn@bbau.ac.in

ABSTRACT
The spirulina (*Limnospira platensis*) is known to be associated with undesirable, distinct flavor, odor, colour and non-homogeneous appearance, which limits overall consumer acceptability. The intervention of spray-drying technology with short-term exposure of thermosensitive components and a faster drying process has been attempted to address the undesirable characteristics of spirulina. The present study aims to microencapsulate spirulina particles using maltodextrin with the spray-drying technique to evaluate its impact on nutritional, functional and microbial properties of milk chocolate. The successful incorporation of microencapsulated spirulina into the milk chocolate not only significantly enhance its protein (8.12 g/ 100 g), minerals (ash) (4.28 g/ 100 g), moisture (3.01 g/ 100 g), lipid (36.63 g/ 100 g), antioxidant (17.9.3%), total phenolic (12.40 mg GAE/ 100 g) and flavonoid content (9.56 mg QE/ 100 g) but also considerably decreased carbohydrate (50.27 g/ 100 g) content along with stable water activity level (0.46aw) and improve its storage stability. Microbial analysis of this product reveals that consumers can safely consume milk chocolate up to sixteen days without using any preservatives. Absence of Escherichia coli, yeasts and moulds suggests that this product possess good hygienic quality of raw ingredients and inhibitory action of spirulina against pathogenic microorganisms throughout the period of 30 days. This study suggests that spray-dried microencapsulated spirulina with enriched nutritional, functional and antimicrobial properties can be effectively utilized in the formulation of milk chocolate. This approach may also be considered an effective solution for future applications in cakes, cookies and other bakery products.

Keywords: Microencapsulation, *Limnospira platensis*, Spirulina, Milk chocolate, Bioactive compounds, Antimicrobial activity
How to cite this article: Deupa H, Patel A, Verma D, Panwar P, Parveen Z, Shankar P.., Microencapsulation of Spirulina (*Limnospira platensis*) Biomass using the Spray-Drying Technique and its Application in Milk Chocolate. Int J Drug Deliv Technol. 2026;16(71s): 218-225. DOI: 10.25258/ijddt.16.71s.25
Source of support: Nil.
Conflict of interest: Nil.

INTRODUCTION

Chocolate has been a popular confectionery for thousands of years and has been recognized for its potential human benefits due to its antioxidant activity and flavonoid content derived from cocoa beans[1]. There are different types of chocolate depending on the usage of cocoa mass, cocoa butter, sugar and milk powder content in different proportions, i.e., dark, milk and white chocolate. Cocoa beans consist of many bioactive compounds like polyphenols (catechins, anthocyanidins and proanthocyanidins) along with vitamins, minerals (magnesium, potassium and iron) and dietary fibre, which contribute to health-promoting benefits[2].The rapidly increasing world population, which is projected to reach more than 10 billion by the end of 2026 posing serious challenges to the conventional food production system in meeting future nutritional demands. To ensure food security, affordability and sustainability, it is crucial to explore non-conventional nutritionally enriched food sources. Microalgae, lab-grown meat, insect-based protein and foods derived from cellular agriculture are examples of unconventional sources that offer sustainable ways to supply vital nutrients on limited arable land[3]. Spirulina

*Author for Correspondence: Priyanka.fn@bbau.ac.in

(*Limnospira platensis*), a cyanobacterium from the family *Oscillatoriaceae*, is one such unconventional source that provides an evolutionary linkage between algae and plants. Spirulina is rich in protein (60-70%), fatty acids, minerals, vitamins and amino acids[4,5]. The key pigments and bioactive metabolites content of spirulina confer immunomodulatory, anti-inflammatory, hypocholesterolemia, antioxidant and antidiabetic properties[6].

The fortification of spirulina into food products is often limited by undesirable distinct flavor, odor and colour characteristics. The earthy odor arising from sulphur-containing aldehydes, ketones and alcohols compromises the overall acceptability of the final product. One of the studies reported by De Rijdt *et al.,*[7] showed that incorporating fresh and dried spirulina significantly increases protein content and antioxidant activity, regardless of biomass form. However, characteristic fishy, fungal and saline odor persisted in pasta and burgers. Elkot *et al.,*[8] enriched a whey-based sport beverage with spirulina and showed increased concentrations of antioxidants, phenolic compounds, vitamins and minerals. Ozbal *et al.,*[2] reported that the addition of 4% *Arthrospira* in white chocolate had a positive effect on nutritional and physicochemical parameters such as protein, lipids, mineral content, essential amino acids, PUFA, moisture and hardness. However, the overall acceptance of 4% *Arthrospira* in white chocolate remains lower (4.40). Barkallah *et al.,*[9] demonstrated that 0.25% spirulina significantly accelerated the fermentation process, nutritional and textural properties of yogurt. Although lower sensory characteristics were observed in the sample with higher microalgae concentrations (0.75 and 1%).

Effective utilization of spirulina in food products requires technological interventions. Recent studies have explored various techniques to mitigate undesirable properties and improve stability, among which spray-drying promotes faster drying and short-term exposure to heat. It is widely used for microencapsulation of thermosensitive components, viscosity, low water solubility and target delivery of bioactive ingredients[10,11]. Previous studies have demonstrated the impact of microencapsulation of spirulina into biscuits[12], chocolate milk[13], yogurt[14], ice cream[15] and milk beverages[16]. These findings suggest that microencapsulation significantly enhances the overall acceptability with increased nutritional, physicochemical and functional properties. Despite the fact that milk chocolate is consumed extensively across the globe, no systematic study has been performed on the impact of incorporating microencapsulated spirulina into milk chocolate and its effects on nutritional, physicochemical, functional and microbiological properties. Therefore, the present study was performed to evaluate the impact of microencapsulated spirulina incorporation on nutritional, functional and microbiological properties of milk chocolate. The results of this study indicate that our approach has the potential to facilitate the development of novel, non-conventional chocolate-based food products with promising application in future food innovations.

Materials and methods

Raw material

For the preparation of milk chocolate, cocoa butter, cocoa powder, milk powder, double-refined sugar and soya lecithin were procured from the local market. Spirulina powder was acquired from Parry Nutraceuticals Co., India and maltodextrin was procured from Boivencer HealthCare Pvt. Ltd, India.

Microencapsulation of Spirulina (*Limnospira platensis*)

The process of microencapsulation was done in accordance with Anarjan *et al.,*[17] and Tiepo *et al.,*[15]. Spirulina biomass (100 grams) was crushed using a motor pestle and added to maltodextrin solution (30% w/v, 90 grams of maltodextrin dissolved in 300 ml of distilled water) in a 1:3 ratio. The mixture was then stirred continuously using a magnetic stirrer for 45 minutes. A stable temperature (40°C) was maintained throughout the entire process. The mixture was atomized using a spray dryer with a capacity of 2-3 l/hour (SM Scientech, Kolkata, India) with an inlet temperature of 160°C and outlet at 108°C, air pressure 2 bar, flow rate 11mL/min and 2648 rpm. The dried microcapsules were stored at a temperature of 20°C in a closed, air-tight container until further use.

Preparation of milk chocolate samples

The composition of control and spirulina fortified chocolate (SFC)is presented in Table 1. Twenty percent of cocoa butter was melted using a microwave for 2 minutes. Then manual mixing of dry ingredients (cocoa powder, fine sugar and milk powder) was done at 25°C and the chocolate mass was pre-refined using a table top wet grinder at 50°C. After adding the remaining 80% of cocoa butter, vanilla essence and soy lecithin, the mixture was conched for 1 hour and 45 minutes. During the last 20 minutes of conching, microencapsulated spirulina particles were added to the chocolate liquor. The chocolate liquor was then tempered at 32-35°C using a double boiler, moulded at 24-27°C and cooled in a refrigerator. The solid chocolate bars were subsequently demoulded, wrapped in aluminium foil and stored at 12±5°C.

Table 1: Ingredients for control and spirulina fortified chocolate

Ingredients	Control Chocolate	Spirulina Fortified Chocolate
Cocoa butter	35%	35%
Fine sugar	32.83%	32.83%
Microencapsulated spirulina particles	-	2.51%
Milk powder	16.66%	16.66%
Cocoa mass	12.45%	12.45%
Soy lecithin	0.55%	0.55%

Characterization of milk chocolate

Proximate analysis

The AOAC method[18] was used to estimate the protein, fat, moisture, total ash and carbohydrate of the control and SFC samples.

Moisture content

Five grams of sample was weighed into a previously tared petri plate and then placed in a dry hot-air oven at 130±5°C for 2 hours, followed by cooling in a desiccator and re-weighing. The process of drying and cooling was repeated until a constant weight was obtained. The moisture content is calculated using the following equation (3):

$$Moisture(grams/100\ grams\ of\ sample) = \frac{(W1-W2)\times 100}{W1-W} \quad (3)$$

Ash content

Five grams of the dried sample was weighed into a tared crucible and charred over a low-flame burner. After that, the crucible was placed into a preheated muffle furnace at a temperature of 550°C for three to five hours. After cooling in a desiccator, the crucible was weighed. The ash content was calculated using equation (4):

$$Ash\ content\ (grams/100\ grams\ of\ sample) = \frac{Weight\ of\ the\ ash}{Weight\ of\ the\ sample} \times 100 \quad (4)$$

Protein content

Two grams of dried sample was weighed into a 500 mL capacity Kjeldahl flask and about 5 grams of digestive mixture (potassium sulphate and cupric sulphate) and 20 mL of concentrated sulphuric acid were added to the same sample. The mixture was digested for one and a half hours until the colour turned pale blue. Glass beads were added to avoid bumping during reaction. Further, thecontent of the flask was cooled, diluted with distilled water and made strongly alkaline by adding 40% (w/v) sodium hydroxide. The ammonia liberated was distilled into a receiver tank containing 25mL of N/10 sulphuric acid. The excess amount of acid in the receiver was back-titrated against N/10 sodium hydroxide solution using 2-3 drops of methyl red indicator. A blank reagent was similarly digested and distilled in a Kjeldahl unit. The crude protein value was calculated using the following equation (1):

$$Protein\ (grams/100\ grams\ of\ sample) = N\ (total\ Nitrogen) \times 6.25 \quad (1)$$

Fat content

Five grams of a moisture-free, dried sample were weighed and put into a cotton-plugged thimble. The flask was filled with petroleum ether and the thimble was set within the soxhlet apparatus. The extraction process was completed after three to four hours. The solvent was evaporated on a running water bath. All traces of petroleum ether were evaporated by placing the flask into a hot-air oven for 30 minutes. After cooling in a desiccator, the flask was weighed. The following equation (2) was used to determine the fat content:

$$Fat\ content\ grams/100 grams\ of\ sample = \frac{Weight\ of\ the\ extract\times 100}{Weight\ of\ the\ sample} \quad (2)$$

Carbohydrate content

The carbohydrate content of the sample was determined by calculating the difference from the sum of the values (per 100 grams) of moisture, protein, fat and ash content.

Bioactive compounds

Bioactive constituents such as the total phenolic content (TPC) and total flavonoid content (TFC) were determined using the Ferdous *et al.,*[19] & Santiago *et al.,*[20] method**.** To measure TPC, 20 µL of the sample extract was mixed with 40 mL of 10% (v/v) Folin-Ciocalteu reagent and 160 µL of sodium carbonate (700 mM) solution. The reaction mixture was incubated for 2 hours at room temperature to allow colour development. The absorbance was measured at 765 nm using a UV/Vis spectrophotometer (LMSP-UV1200) and the values were expressed in mg gallic acid equivalent (GAE) per 100 g of sample. The TFC was estimated by mixing 20 µL of sample extract with 20 µL of 10% (w/v) ammonium chloride, 1M sodium acetate and 180 µL of distilled water. The reaction mixture was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm using a UV/Vis spectrophotometer (LMSP-UV1200). The values were expressed in mg quercetin equivalent (QE) per 100 g of sample. Gallic acid and quercetin standard calibration curves were prepared at 200-3.125 µg/ mL.

Antioxidant analysis

The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described by Santiago *et al.,*[20] with modifications. A volume of 100 µL of the sample extract was transferred into a test tube and 3 mL of methanol was added. Subsequently, 150 µL of DPPH solution (3.30 mmol/L) was added to the test sample and incubated in the dark at room temperature for 30 minutes. The absorbance of the resulting solution was measured at 515 nm using a UV/Vis spectrophotometer (LMSP-UV1200). For the control sample, 150 µL of DPPH solution was mixed with 3 mL methanol, incubated for 30 minutes in the dark at room temperature and the absorbance was determined at 515 nm. The antioxidant activity was computed using the following equation (5):

$$Antioxidant\ activity\ (\%) = \frac{Absorbance_{control} - Absorbanc_{sample}}{Absorbance_{control}} \times 100 \quad (5)$$

Water activity

Water activity was determined with one gram of sample evenly distributed on a plastic tray and the readings were recorded after reaching the moisture equilibrium at 25ºC using a water activity (A_w) meter (Novasina, Labstart-aw, Switzerland).

Microbial analysis

Microbial analysis was performed for 30 days for all the samples. The total aerobic plate count was determined

according to International Organization for Standardization (ISO) 4833-1:2013. *Escherichia coli* count was determined according to ISO 16649-2:2001, where appropriately diluted samples were spread on suitable medium in Petri dishes and incubated for 24 h at 37ºC. Yeast and molds were analysed according to the ISO 21527-2:2008, where the samples were spread into Potato-Dextrose Agar (PDA) medium followed by incubation for 5 days at 25-30ºC. The logarithmic number of colony-forming units per gram of material (log CFU g^{-1}) was used to express the microbial counts[21].

Statistical analysis

All experimental analyses were performed in triplicate. Results are represented as the mean ± standard deviation. For normally distributed data, an independent-sample t-test($p<0.05$) was applied. All statistical tests were carried out using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2021.

Result

Table 2 shows results obtained from the proximate composition of the control and spirulina fortified chocolate (SFC). The incorporation of microencapsulated spirulina particles significantly increased the moisture (3.01 ± 0.96 %), ash (4.28 ± 0.23 %), protein (8.12 ± 0.92 %), total lipid (36.63 ± 0.77 %) and water activity level (0.46 ± 0.01 %) of the SFC sample compared with the control sample. A significant decrease was observed in the carbohydrate content (50.27 ± 2.27 %) of the SFC sample after incorporation of microencapsulated spirulina particles.

Table 2: Proximate composition of control and spirulina fortified chocolate

Parameters	Control Chocolate	Spirulina Fortified chocolate	T-value	P-value
Composition				
Moisture (%)	0.53 ± 0.25	3.01 ± 0.96	-4.330	0.01*
Ash (%)	2.53 ± 0.07	4.28 ± 0.23	-12.608	<0.01*
Protein (%)	5.76 ± 0.31	8.12 ± 0.92	-4.192	0.01*
Total lipids (%)	34.34 ± 1.16	36.63 ± 0.77	-2.816	0.04*
Carbohydrate (%)	56.82 ± 0.72	50.27 ± 2.27	4.759	<0.01*
Water activity (Aw)	0.43 ± 0.01	0.46 ± 0.01	-3.674	0.02*

All experiments were executed in triplicate and the values for each sample were presented as mean ± standard deviation; *Significant at $p<0.05$

Table 3 shows the bioactive composition and antioxidant activity of control and SFC sample. The addition of microencapsulated spirulina particles significantly increased the total phenolic (12.40 ± 1.00 mg GAE/ 100 g), total flavonoid content (9.56 ± 0.94 mg QE/ 100 g) and antioxidant activity (17.93 ± 0.55 %) of the SFC sample when compared to the control sample.

Table 3: Bioactive composition and antioxidant activity of control and spirulina fortified chocolate

Parameter	Control Chocolate	Spirulina Fortified chocolate	T-value	P-value
Bioactive compounds				
Total phenolic content (mg GAE/ 100 g)	8.70 ± 1.00	12.40 ± 1.00	-5.867	0.01*
Total flavonoid content (mg QE/ 100 g)	4.77 ± 1.05	9.56 ± 0.94	-4.532	<0.01*
Antioxidant				
DPPH (%)	15.34 ± 0.53	17.93 ± 0.55	-5.789	<0.01*

All experiments were executed in triplicate and the values for each sample were presented as mean ± standard deviation; *Significant at $p<0.05$, GAE- gallic acid equivalent, QE- quercetin, DPPH- 2,2-diphenyl-1-picrylhydrazyl

Table 4: Number of colonies formed in the plate over a period of 30 days (at 10^{-5} Dilution factor)

Storage day	Control Chocolate	Spirulina Fortified chocolate	Control Chocolate	Spirulina Fortified chocolate	Control Chocolate	Spirulina Fortified chocolate
	Total plate count		***Escherichia coli***		**Yeast and Moulds**	
Day 01	Abs	Abs	Abs	Abs	Abs	Abs
Day 08	4 x 10^6 CFU/g	3 x 10^6 CFU/g	Abs	Abs	Abs	Abs
Day 16	2.1 x 10^7 CFU/g	7 x 10^6 CFU/g	Abs	Abs	Abs	Abs
Day 24	5.5 x 10^8 CFU/g	2.2 x 10^7 CFU/g	Abs	Abs	Abs	Abs
Day 30	1.24 x10^8 CFU/g	4.5 x 10^7 CFU/g	Abs	Abs	Abs	Abs

Where, Abs- Absence of microbial growth

The total colonies formed in the control and SFC sample are presented in **Table 4**. The result exhibited absence of microbial growth on day 1, indicating good hygiene practices were followedin chocolate manufacturing. As storage time progressed, a gradual increase in total plate count was observed for both the control and SFC sample. The control sample showed a higher total plate count compared to the SFC sample from day 08 to day 30. The control sample had a higher microbial count of 1.24 x 10^8 CFU/g, whereas the SFC sample showed a lower count of 4.5 x 10^7 CFU/g on day 30. The reduced microbial growth in the SFC sample suggests that fortification of microencapsulated spirulina in the SFC sample was effective in inhibiting microbial growth during storage. No pathogenic microorganisms such as *Escherichia coli*, yeast and moulds were detected over a period of 30 days, indicating microbial safety and improved shelf life under proper storage conditions.

Discussion

The present study was performed to analyze the impact of microencapsulated spirulina particles on nutritional, functional and microbial properties of milk chocolate. The enrichment of microencapsulated spirulina particles significantly increased the protein, moisture, ash and lipid content, while decreasing the carbohydrate content in SFC sample comparison compared to the control sample. These findings were inconsistent with the previous literature. A study performed by De Oliveira *et al.,*[13] reported that fortification of chocolate milk with 5.0% (F1) and 8.75% (F2) of microencapsulated spirulina LEB-18 resulted into significant increase average protein (F1= 12.00g, F2= 13.79g), moisture (F1= 4.02g, F2= 4.21g), ash (F1= 3.09g/ 100g, F2= 2.76g), lipid (F1= 3.74g, F2= 3.66g) and carbohydrate (F1= 77.15g, F2= 75.58g) content. Similarly, Da Silva *et al.,*[12] also reported that the fortification of 20%microencapsulated spirulina biomass improves the nutritional characteristics, such as protein (10.8 g), ash (4.39 g) and decreases carbohydrate content (83.3 g) in biscuit products in comparison with their control sample. These findings suggested that increasing the concentration of microencapsulated spirulina *sp.* in functional food significantly improved the nutritional value of the final product.

The phenolic compounds present in microalgae such as spirulina biomass are a potential source of antioxidant activity, among other biological roles. Phenolic compounds such as simple phenols, flavonoids, phenylpropanoids, phenolic acids, tannins and lignans are produced as secondary metabolites and are considered an important class of natural antioxidants[22]. The addition of microencapsulated spirulina particles significantly improved the total phenolic content (12.40 mg GAE/ 100 g) in SFC sample in comparison with the control sample. These findings were in accordance with the previous literature. A study conducted on chocolate milk formulations with 5.0% (F1) and 8.75% (F2) of microencapsulated spirulina LEB-18 showed a consistent increase in total phenolic content (F1= 602.31 mg GAE/ 100 g, F2= 683.98 mg/ 100 g) compared with their control sample[13]. Similarly, previous studies on cookies fortified with 6% of *A. platensis* (0.90 mg GAE/ 100 g), 6% of *P. tricornutum* (0.62 mg GAE/ 100 g) and5% A. *platensis biomass* (1.4 to 2.3 mg GAE/100 g) also reported a significant increase in total phenolic content compared with their respective control sample[23,24], although the concentrations were lower than those observed in the present study. Moreover, in the present study, microencapsulated spirulina significantly increased the total flavonoid content (9.56 mg QE/ 100 g) of SFC sample in comparison with the control sample. In accord with the present study, Koli *et al.,*[25] reported a significant increase in the total flavonoid content of 10, 15 and 20% *Spirulina sp.* CCC 540 fortified pasta (ranging from 95.63 to 118.46 pimol QE/ 100 g) in comparison with their respective control sample. Similarly, Maryam *et al.,*[26] study also stated a significant increase in total flavonoid content of algae candies *A. platensis* and *C. vulgaris* (0.5 mg CAE/ 100 g) in comparison with the respective control sample, though their concentrations were lower. Thus, retention of phenols and flavonoid compounds in SFC sample suggested that the

*Author for Correspondence: amrita.bharti@sharda.ac.in

resilience of spirulina against high temperature, light and oxygen even after microencapsulation favours the preservation of bioactive compounds.

The antioxidant activity in the present study was used to predict the oxidative stability of the SFC sample. There was increased antioxidant activity in the SFC sample with 17.93 mg AAE/g in comparison with the control sample. The findings were consistent with previous literature[12,13]. Previous studies have shown that *Arthrospira platensis* contains a diverse range of biologically active phytochemicals, including phenolic compounds, flavonoids, carotenoids, sterols, vitamins and anthraquinones, which effectively scavenge free radicals and inhibit oxidative reactions. Villano *et al.,*[27] reported that lower EC50 values contribute to higher antioxidant activity, indicating a sample with high antioxidant ingredients requires a lower concentration to neutralize free radicals. Thus, the enhanced antioxidant activity observed in the recent study showed a greater free radical-scavenging capacity of microencapsulated spirulina particles in the SFC sample than in the control sample.

Water activity provides crucial information regarding the chemical, physical and microbiological stability of a food product. This parameter is most frequently used to regulate the raw ingredients in the chocolate manufacturing stage. The chocolate's stability in storage is also impacted by the amount of water content. The fat content makes it challenging to absorb moisture in the chocolate product. However, the amorphous nature of sugar should also be considered, as it affects the water activity by increasing viscosity, unpleasant gummy texture and accelerated moisture absorption[28]. In the present study, there was a significant increase in the water activity (0.46_{aw}) of the SFC sample; however, the observed values remained within the ideal range for chocolate. The water activity of chocolate typically remained below 0.60, which is considered ideal for extending shelf life, inhibiting microbial growth, eliminating the need for preservatives and ensuring the product safety for human consumption.

As per the norms, the aerobic CFU/g of the sample for chocolates, which is less than $<10^5$ is considered satisfactory, between 10^5-$<10^6$ is considered acceptable and greater than $>10^7$ is considered unsatisfactory[29]. In accord to this, the consumers can safely ingest the microencapsulated spirulina fortified milk chocolate up to the 16th day. In contrast to the present study, a chocolate fortified with *Centella asiatica, Abelmoschus esculentus* and *Psidium guajava* reported a shelf life of 30th days after production[30]. The absence of pathogenic microorganisms like *E. coli*, yeasts and moulds in the SFC sample through 30-days of the storage period indicated good hygienic quality of raw ingredients, low water activity and antimicrobial potential of spirulina against *E. coli*, fungi and yeasts. The results of the present study were in accord with the previous studies conducted on *Aspergillus niger*, *Aspergillus fumigatus, Aspergillus flavus, Candida albicans, Candida tropicalis and Candida glabrata,* which are all susceptible to the antibacterial and antifungal effects of spirulina[31,32].

Conclusion

Spray drying microencapsulation is an effective strategy used to mask off flavour, strong odor and protect heat-sensitive bioactive compounds against thermal degradation with the help of a coating material. Therefore, the present study aimed to microencapsulate spirulina with maltodextrin using the spray-drying technique and evaluate its impact on nutritional, functional and microbial properties of milk chocolate. The results demonstrated that microencapsulated spirulina is a promising food ingredient owing to high protein, lipid, minerals (ash), antioxidant, phenolic and flavonoid content. Microencapsulation enabled the successful incorporation of spirulina into milk chocolate while preserving the nutritional and functional attributes with minimal losses. Furthermore, microbial analysis indicated that the microencapsulated spirulina fortified milk chocolate remained microbially safe for consumption up to 16th days without any use of chemical preservatives. The enhanced nutritional and antioxidant potential and acceptable microbial stability suggest that microencapsulated spirulina serves as a promising functional ingredient for the development of functional milk chocolate. Moreover, spray-drying technology offers considerable potential for application in other bakery and confectionery products, contributing to the development of nutritionally enriched food products with added health benefits...

REFERENCE

1. Faccinetto-Beltran P, Gomez-Fernandez AR, Santacruz A, Jacobo-Velázquez DA. Chocolate as carrier to deliver bioactive ingredients: Current advances and future perspectives. Foods. 2021 Sep 1;10(9):2065. Available from: https://doi.org/10.3390/foods10092065
2. Ozbal B, Celekli A, Gun D, Bozkurt H. Effect of Arthrospira platensis incorporation on nutritional and sensory attributes of white chocolate. International Journal of Gastronomy and Food Science. 2022 May 18;28:100544. Available from: https://doi.org/10.1016/j.ijgfs.2022.100544
3. Chaudhary S, Singh R, Upadhyay A, Kaur BP, Talwar B. Enhancing the applicability of Spirulina platensis in food systems: the role of double coated encapsulation. Food Research International. 2026 Mar 18;233(Pt 1):119012. Available from: https://doi.org/10.1016/j.foodres.2026.119012
4. Almeida LMR, Da Silva Cruz LF, Machado BAS, Nunes IL, Costa JAV, De Souza Ferreira E, et al. Effect of the addition of Spirulina sp. biomass on the development and characterization of functional food. Algal Research. 2021 Jun 30;58:102387. Available from: https://doi.org/10.1016/j.algal.2021.102387
5. Lafarga T, Fernandez-Sevilla JM, Gonzalez-Lopez C,

Acien-Fernandez FG. Spirulina for the food and functional food industries. Food Research International. 2020 May 26;137:109356. Available from: https://doi.org/10.1016/j.foodres.2020.109356

6. Sharma R, Rana A. Algal Elixirs: Unraveling the multifaceted impact of Spirulina in human health. Food Bioscience. 2024 Oct 30;62:105365. Available from: https://doi.org/10.1016/j.fbio.2024.105365
7. De Rijdt M, Verhaegen J, Dohogne D, Brostaux Y, Haubruge E, Goffin D. Impact of spirulina biomass physical form on texture, cooking properties, and sensory profile of enriched pasta and meat analogues. Applied Food Research. 2025 Sep 5;5(2):101328. Available from: https://doi.org/10.1016/j.afres.2025.101328
8. Elkot WF, Elmahdy A, El-Sawah TH, Alghamdia OA, Alhag SK, Al-Shahari EA, et al. Development and characterization of a novel flavored functional fermented whey-based sports beverage fortified with Spirulina platensis. International Journal of Biological Macromolecules. 2023 Dec 28;258(Pt 2):128999. Available from: https://doi.org/10.1016/j.ijbiomac.2023.128999
9. Barkallah M, Dammak M, Louati I, Hentati F, Hadrich B, Mechichi T, et al. Effect of Spirulina platensis fortification on physicochemical, textural, antioxidant and sensory properties of yogurt during fermentation and storage. LWT. 2017 May 30;84:323–30. Available from: https://doi.org/10.1016/j.lwt.2017.05.071
10. Silva EK, De Barros Fernandes RV, Borges SV, Botrel DA, Queiroz F. Water adsorption in rosemary essential oil microparticles: Kinetics, thermodynamics and storage conditions. Journal of Food Engineering. 2014 May 14;140:39–45. Available from: https://doi.org/10.1016/j.jfoodeng.2014.05.003
11. Deupa, H, Shankar, P, Panwar P. Microencapsulation of Spirulina using Spray-Drying Technique: Impact on Morphological and Structural Characteristics. Journal of Microencapsulation. Forthcoming / In press
12. Da Silva SP, Valle AFD, Perrone D. Microencapsulated Spirulina maxima biomass as an ingredient for the production of nutritionally enriched and sensorially well-accepted vegan biscuits. LWT. 2021 Jan 29;142:110997. Available from: https://doi.org/10.1016/j.lwt.2021.110997
13. De Oliveira TTB, Reis IMD, De Souza MB, Da Silva Bispo E, Maciel LF, Druzian JI, et al. Microencapsulation of Spirulina sp. LEB-18 and its incorporation in chocolate milk: Properties and functional potential. LWT. 2021 May 9;148:111674. Available from: https://doi.org/10.1016/j.lwt.2021.111674
14. Da Silva SC, Fernandes IP, Barros L, Fernandes A, Alves MJ, Calhelha RC, et al. Spray-dried Spirulina platensis as an effective ingredient to improve yogurt formulations: Testing different encapsulating solutions. Journal of Functional Foods. 2019 Jun 19;60:103427. Available from: https://doi.org/10.1016/j.jff.2019.103427
15. Tiepo CB, Gottardo FM, Mortari LM, Bertol CD, Reinehr CO, Colla LM. Addition of Spirulina platensis in handmade ice cream: Phisicochemical and sensory effects/Adicao de Spirulina platensis em sorvete caseiro: Efeitos fisico-quimicos e sensoriais. Braz. J. Dev. 2021 Sep;7(9):88106-23.
16. Mohammed DM, El-Messery TM, Baranenko DA, Hashim MA, Tyutkov N, Marrez DA, et al. Effect of Spirulina maxima microcapsules to mitigate testicular toxicity induced by cadmium in rats: Optimization of in vitro release behavior in the milk beverage. Journal of Functional Foods. 2023 Dec 14;112:105938. Available from: https://doi.org/10.1016/j.jff.2023.105938
17. Anarjan N, Tan CP. Developing a three component stabilizer system for producing astaxanthin nanodispersions. Food Hydrocolloids. 2013 Jan 1;30(1):437-47.
18. AOAC. Official Method 996.06 of 19th edn, Fat (total saturated and unsaturated) in foods. Hydrolytic extraction gas chromatographic method, Gaithersburg, MD, 2012.
19. Ferdous UT, Nurdin A, Ismail S, Yusof ZNB. Evaluation of the antioxidant and cytotoxic activities of crude extracts from marine Chlorella sp. Biocatalysis and Agricultural Biotechnology. 2022 Nov 21;47:102551. Available from: https://doi.org/10.1016/j.bcab.2022.102551
20. Santiago NG, Lima DOM, Botelho J, Da Silva VF, Santos RA, De Vargas FM Junior, et al. Bacupari Flour (Garcinia brasiliensis) Improves the Nutritional Profile and Antioxidant Potential of Nile Tilapia Burgers. Applied Sciences. 2026 May 21;16(10):5138. Available from: https://doi.org/10.3390/app16105138
21. Nguyen VL, Tran MT, Nguyen-Thi TD, Nguyen MA, Le MT, Nguyen TM, et al. Valorization of cocoa (Theobroma cacao L.) pod husks as a fruit pulp substitute in mango jam formulations: effects on jam qualities during storage and sensory discrimination. Sustainable Food Technology. 2024 Dec 26;3(1):333–42. Available from: https://doi.org/10.1039/d4fb00331d
22. El-Baky HH, El Baz FK, El-Baroty GS. Production of phenolic compounds from Spirulina maxima microalgae and its protective effects. African Journal

of Biotechnology. 2009;8(24).

23. Batista AP, Niccolai A, Fradinho P, Fragoso S, Bursic I, Rodolfi L, et al. Microalgae biomass as an alternative ingredient in cookies: Sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. Algal Research. 2017 Jul 24;26:161–71. Available from: https://doi.org/10.1016/j.algal.2017.07.017
24. Egea BC, Campos AL, De Carvalho-Eliane JC, Danesi DG. Antioxidant and nutritional potential of cookies enriched with Spirulina platensis and sources of fibre. J Food Nutr Res. 2014;53(2):171-9.
25. Koli DK, Rudra SG, Pabbi S, Kalwani M. An assessment of the nutritional, physicochemical and sensory attributes of pasta enriched with fresh Spirulina wet biomass. Journal of pharmacognosy and phytochemistry. 2021;10(1):1174-9.
26. Maryam S, Oztop MH, Doğdu S, Marangoz MA, Zeshan Z, Hayat MQ, et al. Spirulina and chlorella derived hard candies as functional food. Journal of Functional Foods. 2024 Nov 20;123:106565. Available from: https://doi.org/10.1016/j.jff.2024.106565
27. Villano D, Fernandez-Pachon, Moya ML, Troncoso AM, Garcia-Parrilla MC. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta. 2006 May 5;71(1):230–5. Available from: https://doi.org/10.1016/j.talanta.2006.03.050
28. Beckett ST. The science of chocolate. 2008. Available from: https://doi.org/10.1039/9781847558053
29. Gilbert RJ, De Louvois J, Donovan T, Little C, Nye K, Ribeiro CD, Richards J, Roberts D, Bolton FJ. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. Communicable disease and public health. 2000 Sep 1;3(3):163-7.
30. Raja K, Narayanan SNG, Thiruvengadam S, Romauld I. The influence of chocolate fortification with Centella asiatica, Abelmoschus esculentus, and Psidium guajava on the content of biologically active substances. Journal of Food Processing and Preservation. 2021 Jan 23;45(4). Available from: https://doi.org/10.1111/jfpp.15271
31. Abdel-Moneim AME, El-Saadony MT, Shehata AM, Saad AM, Aldhumri SA, Ouda SM, et al. Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi Journal of Biological Sciences. 2021 Sep 17;29(2):1197–209. Available from: https://doi.org/10.1016/j.sjbs.2021.09.046
32. Ismail HA, El-Sawah TH, Ayyash M, Adhikari B, Elkot WF. Functionalization of Ricotta cheese with powder of spirulina platensis: physicochemical, sensory, and microbiological properties. International Journal of Food Properties. 2023 Jul 27;26(1):1968–83. Available from: https://doi.org/10.1080/10942912.2023.2238916.