International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(5): 510-515

www.veterinarypaper.com
Received: 24-07-2024
Accepted: 29-08-2024

Hency Rose
MTech Students,
Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Smitha J Lukose
Assistant Professor,
Department of Dairy Chemistry, Verghese Kurien Institute of Dairy and Food Technology, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Vinod Kumar Paswan
Assistant Professor,
Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Jude Felix
MTech Students,
Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Corresponding Author:
Hency Rose
MTech Students,
Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Comparative study on the physicochemical and antioxidant properties of Buttermilk fortified with *Spirulina plantensis* and commercially available buttermilk

Hency Rose, Smitha J Lukose, Vinod Kumar Paswan and Jude Felix

DOI: https://doi.org/10.22271/veterinary.2024.v9.i5h.1761

Abstract
A notable expansion in the assortment of dairy products, especially fermented products, has been observed recently in the food market. This may mainly be attributed to medical advice and the evolving preferences of individuals seeking to align their dietary choices with their lifestyle. Cultured buttermilk, a traditional fermented dairy beverage, emerges as a highly suitable product choice within this particular context. This research paper elucidates a comparative analysis between a newly developed cultured buttermilk, enriched with high protein microalgae *Spirulina platensis*, and a commercially available buttermilk sample. A comprehensive investigation was conducted to examine the physicochemical characteristics and antioxidant properties of buttermilk. The market sample exhibited notable levels of antioxidants and total phenolic content, which can be attributed to the inclusion of various spices and beneficial cultures. The profound impact of incorporating 0.25% *Spirulina* as a natural ingredient in cultured buttermilk is evident in its enhanced physicochemical properties and antioxidant content. Treatment with 0.25% *Spirulina* increased radical inhibition by 7% over the market sample. Commercial samples had statistically insignificant carotenoid concentrations, but treated samples had 7.83 mg/g. *Spirulina* increased chlorophyll concentration by 30% above the commercial sample. Total phenolic content was 4.2 mg GAE/100 gm in *Spirulina platensis*-enriched buttermilk and 1.2 mg in commercial buttermilk. This fortification technique not only improves the nutritional value of the buttermilk but also imparts functional health benefits.

Keywords: *Spirulina*, chlorophyll, carotenoid, c-phycocyanin, total phenolic content

1. Introduction
Fermentation has been utilized as a traditional method of food production, making it a natural and time-honored approach in human history. The study conducted by Adler *et al*. (2013) [2] has shed light on the current advancements in the realm of fermented foods, which have led to an upsurge in the utilization of functional ingredients during their manufacturing process. Milk and dairy products have consistently maintained a prominent role in the human diet throughout history (Erzen *et al*., 2014) [12]. Cultured buttermilk, referred to as "Chhash" in South Asian countries, is a widely recognised fermented dairy product that possesses therapeutic properties (Ghanshyambhai *et al*., 2015) [16]. In the Indian context, it is customary for people to consume cultured buttermilk as a post-meal beverage as it is believed to enhance digestion. Cultured buttermilk holds significant value in several Ayurvedic preparations owing to its positive impact on health (Devi *et al*., 2010) [11]. As per FSSAI standards, buttermilk is referred as the product that remains after the removal of milk fat by churning milk and cream to manufacture butter and milk fat products (FSSAI, 2011) [9]. The contemporary approach to produce cultured buttermilk involves introducing a combination of thermophilic and mesophilic homofermentative bacterial cultures into low-fat milk, which is subsequently subjected to fermentation and homogenization processes. The process of churning curd results in the separation of white butter, while the remaining liquid is commonly known as cultured buttermilk. In the context of commercial production of dahi or cultured buttermilk in India, cultured buttermilk entails the initial step of curd generation, followed by the homogenization

of the curd and its subsequent dilution with water.

The unique intrinsic chemical composition makes cultured buttermilk a widely recognised food. According to Smoczyński *et al*. (2012) [36], buttermilk comprises many water-soluble milk components such as milk proteins, lactose, and minerals, with significant amounts of milk fat globule membranes (MFGM). During the process of churning, the membranes undergo fragmentation, leading to the release of fragments into the milk plasma. Cell membrane constituents, such as polar lipids and milk fat globule membrane (MFGM) proteins, play a crucial role in promoting optimal physiological processes inside the human body (Garczewska-Murzyn, *et al*., 2022) [15]. As stated by Nirgude *et al*. (2013) [28], the inclusion of cultured buttermilk in one's diet has been found to contribute to improved digestion, enhanced immune system function, and decreased blood cholesterol levels. Cultured buttermilk is considered to be a valuable dietary source of various essential nutrients, including calcium, phosphorus, vitamin B2, vitamin B12, pantothenic acid (vitamin B5), zinc, potassium, protein, iodine, and molybdenum. Even though regarded as a nutritious and health-promoting beverage, it is important to note that it naturally lacks certain essential nutrients such as vitamin C, iron, and dietary fibre.

According to Kreitlow *et al*. (1999) [21], microalgae have the potential to serve as functional components in the production of high-quality foods that include enhanced nutritional value. *Spirulina platensis*, a planktonic photosynthetic cyanobacterium, is widely recognized as one of the most exceptional microalgae. *Spirulina* is becoming recognized as a comprehensive solution to address diverse needs, owing to its impressive nutritional makeup that lends itself to potential medicinal applications. It has a high amount of both micronutrients and macronutrients. The chemical makeup of the dry weight of the substance comprises proteins, carbohydrates, and several vitamins (including provitamin A, vitamin C, and vitamin E), as well as minerals including iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium, and zinc, which are present in the range of 60 to 70%. It is a good source of micronutrients deficient in milk iron and vitamin C. The utilisation of spirulina has become aligned with consumer consciousness regarding the significance of natural colourants, including chlorophyll, carotenoids, and C-phycocyanin, due to their nutritional, pharmacological, and health-related advantages (Bingula *et al*., 2016) [8].

The utilisation of this ingredient has been observed in a variety of food items, such as ayran, a traditional fermented dairy beverage, as well as kefir, yoghurt, soy-yogurt biscuits, ice cream, cheese, protein bars, and various other products (Rose *et al*., 2023) [32]. In a recent study conducted by Alizadeh *et al*. (2019) [3], it was observed that the incorporation of microalgae into fermented products resulted in a significant improvement in the survival rate of probiotics and overall nutritional effectiveness.

The primary objective of this current investigation was to assess the physicochemical properties and antioxidant attributes of both *Spirulina* fortified and commercially available buttermilk samples found within the market.

2. Materials and Methods

2.1. Materials

The double-toned milk with a fat content of 1.5% and solid non-fat content of 9.0% was purchased, pasteurized and stored at a temperature of 4 °C until they were needed for product preparation. The buttermilk sample obtained commercially was purchased from the Amul Milk outlet in Varanasi, India, and was stored at 4 °C until further analysis was performed. The freeze-dried direct vat-set (DVS) yoghurt culture (NCDC-167) was acquired from the National Collection for Dairy Culture, located in Karnal, India. This culture consists of a combination of thermophilic and mesophilic homofermentative bacterial strains. They were maintained at a temperature of -18 °C until they were utilised. The *Spirulina* sample was obtained from Heilen Biopharm Pvt. Ltd., an Indian company, and was afterwards stored in a refrigerated environment until its utilisation. The nutritional composition, antioxidant activity, and total phenolic content of the obtained *Spirulina* powder were assessed using a standardised technique.

2.2. Preparation of cultured buttermilk

The cultured buttermilk sample with Spirulina enriched wwas prepared having the similar composition to that of the market sample. The pasteurised double toned milk with a fat content of 1.5% and a solid non-fat content of 9.0% was subjected to a temperature of 42 °C using a table-top stirring hot plate. A concentration of 0.25% (w/w) *Spirulina* biomass was incorporated into milk, and afterwards, spices such as ginger, chilies and curry leaves in the form of juice was prepared freshly were added at a concentration of 0.4% (w/w) to the *Spirulina*-milk mix. The 0.4% (w/w) was chosen after the intial sensory evaluation done with the various level of spices concentration in which the specific flavour of *Spirulina* was not detectable. Subsequently, the process of pasteurisation was conducted at a temperature of 90 °C for a duration of 10 minutes within a water bath. Subsequently, the process involves the introduction of yoghurt culture (NCDC-167) through inoculation, followed by thorough mixing of the culture inside the milk. The milk was put into beakers with pre-sterilized lids. The specimens were subjected to incubation at a temperature of 42 °C within a controlled incubator for a duration of 7 hours. Curd setting was seen following a fermentation period of seven hours. Following the curd setting process, agitation or breaking was conducted for a duration of 90 seconds using a laboratory blender operating at a speed of 10,000 revolutions per minute (rpm). Subsequently, pasteurised cooled water was added in curd-to-water ratio of 2:1. and homogenization was performed. The experiment involved the implementation of a two-stage homogenization process utilising a laboratory homogenizer. The first stage was conducted at a pressure of 100 kg/cm^2, followed by the second step at a pressure of 20 kg/cm^2.The *Spirulina* fortified spiced buttermilk sample was named as-SSBM (*Spirulina* Spiced Buttermilk) and the market sample was named as CSBS (Commercial Spiced Buttermilk Sample)

2.3. Physico-Chemical Analysis

The determination of total solids in the buttermilk samples was conducted by drying the samples in an oven at a temperature of 105 °C for an extended period of time, until a consistent weight was achieved. The ash content was determined using the process of igniting samples in an electric furnace, which effectively eliminated all organic materials at a temperature of 550 °C. The protein concentration was assessed using the Kjeldahl method, employing a nitrogen conversion factor of 6.38. The Gerber method was employed to ascertain the fat content of the sample, following the guidelines outlined in the International Standard (IS: 1224). The method employed for the quantification of calcium in this

study was adapted from the work of Sehgal (2020) [33]. The approach outlined by Cunniff (1998) [10] was employed to determine the total carbohydrates and energy content (Kcal/100 mL) of the sample. The experimental procedures were conducted in triplicate, ensuring the reliability and reproducibility of the obtained results.

2.4. Antioxidant Analysis

2.4.1. DPPH Inhibition Activity

The experimental procedure outlined by Kang and Saltveit (2002), in their study was employed to assess the free radical scavenging potential of the samples under investigation. This assessment was conducted by utilising the popular 1,1-diphenyl-2-picrylhydrazil (DPPH), which is widely recognised for its ability to measure such activity. The evaluation of the DPPH radical scavenging activity was conducted at a wavelength of 517 nm, and the findings were expressed as the percentage of inhibition achieved. The experimental procedures were conducted in triplicate.

The results were expressed as:

$$\%\text{DPPH scavenging activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

2.4.2. Analysis of Total Phenolic Content

The estimation of the total phenolic content was conducted using the Folin–Ciocalteu method, as originally described by Singleton and Rossi in 1965 [35], with slight modifications implemented. In this experimental procedure, a precise volume of 0.5 mL of the sample extract was carefully measured and transferred into a test tube. Subsequently, a measured quantity of 2.5 mL of diluted Folin–Ciocalteu (FC) Reagent was added to the test tube, ensuring accurate pipetting techniques were employed. To complete the reaction mixture, an additional 1 mL of 7.5% Na_2CO_3 solution was introduced. The experimental setup involved the utilisation of a test tube, which was meticulously shielded with a layer of aluminium foil. Subsequently, the test tube was subjected to an incubation period in a light-deprived environment, maintaining the ambient temperature of the room. This incubation duration lasted for a span of 1 hour. The absorbance at a wavelength of 760 nm was quantified using the UV-1800 spectrophotometer, a commonly employed tool in food research for analysing various compounds and their optical properties. A standard calibration curve was generated using varying concentrations of gallic acid ranging from 0 to 800 mg/L. The quantification of the total phenolic content was performed by expressing it in milligrammes of Gallic acid equivalents (GAE) per 100 grammes. The regression equation (y = 0.0769x + 0.0041, R2 = 0.998) was derived from the standard curve, which was constructed by plotting absorbance values against Gallic acid concentrations (mg/100 mg).

2.4.3. Analysis of chlorophylls and carotenoids content

The determination of chlorophyll and carotenoid content in the samples followed the methods described by Kumar *et al.* (2010) [22] and Lichtenthaler and Wellburn (1983) [23].

Each sample was subjected to centrifugation at a speed of 5000 revolutions per minute (rpm) for a duration of 10 minutes, with a total volume of 1 mL for each sample. The solid material was solubilized in 1 mL of ethanol and afterwards subjected to sonication at a temperature of 65 °C for a duration of 30 minutes. Following the sonication process, the solutions underwent centrifugation at a speed of 10,000 revolutions per minute for a duration of 5 minutes. The quantification of pigment content was conducted through the measurement of absorbance (A) in the supernatant at wavelengths of 666 nm, 653 nm, and 470 nm. The pigment content was then determined using the following mathematical equations:

$$\text{Chlorophyll}_a \left(\frac{\text{mg}}{\text{L}}\right) = 15.65 \times A_{666} - 7.340 \times A_{653}$$

$$\text{Chlorophyll}_b \left(\frac{mg}{L}\right) = 27.05 \times A_{653} - 11.21 \times A_{666}$$

$$\text{Total Chlorophylls} \left(\frac{mg}{L}\right) = \text{Chlorophyll}_a + \text{Chlorophyll}_b$$

$$\text{Carotenoids} \left(\frac{mg}{L}\right) = (1000 \times A_{470} - 2.860 \times [\text{Chlorophyll}_a] - 85.9 \times [\text{Chlorophyll}_b])/245$$

2.3.4. Analysis of C-Phycocyanin

The determination of C-Phycocyanin content was conducted in accordance with the methodology outlined by Pan-utai and Iamtham (2019) [29]. The experimental procedure involved subjecting each sample to centrifugation at a speed of 5000 revolutions per minute (rpm) for a duration of 10 minutes. The volume of each sample used in this process was precisely measured to be 1 millilitre (mL). The pellet was reconstituted by suspending it in 1 mL of distilled water, followed by subjecting it to ultrasonic treatment for a duration of 30 minutes. The supernatant was obtained through the process of centrifugation at a speed of 10,000 revolutions per minute for a duration of 5 minutes. Subsequently, the optical density of the collected supernatant was determined at wavelengths of 615 and 652 nm. The estimation of C-Phycocyanin content was determined through the utilisation of the subsequent equation.

$$\text{C} - \text{phycocyanin} \left(\frac{\text{mg}}{\text{mL}}\right) = (OD_{615} - 0.474 \times OD_{652})/5.34$$

3. Result and Discussion

3.1. Physicochemical Analysis

The chemical compositions of the *Spirulina*-enriched buttermilk and the commercial sample were found to be comparable except for calcium as demonstrated in Table 1. The experimental findings indicate that the incorporation of *Spirulina* into buttermilk resulted in 0.33% and 7% increase in the levels of protein and calcium as compared to the market sample. The observed phenomenon can be attributed to the elevated protein concentration present in *Spirulina*, as indicated by the findings of Lupatini *et al.* (2017) [24]. The experimental group that received *Spirulina* supplementation exhibited the most notable elevation in calcium concentration (82.05 mg/100 g) against the market sample (75mg/100 g), which was statistically significant (p <0.5).

One notable limitation observed in fermented products, particularly in the case of kefir as highlighted in a recent study conducted by Ustun-Aytekin *et al.* (2022) [37], is a

reduction in calcium content compared to that of milk. On the contrary, the research findings also indicated that kefir enriched with Spirulina demonstrated higher amounts of calcium in comparison to regular kefir. Therefore, it is possible to produce a calcium-rich product through the enrichment of spirulina, even after the fermentation process. This intervention may prove advantageous for those experiencing calcium deficiency and osteoporosis, as it fulfils their daily calcium requirements (800-1000mg/day-RDA, ICMR, 2020) [1]. In the present scenario, the incorporation of spirulina can contribute to the maintenance of a suitable calcium balance within the dietary intake.

The property of viscosity characterises the degree of resistance exhibited by a fluid when subjected to flow. It directly influences the sense of touch associated with cultured buttermilk to a considerable extent. The occurrence of elevated viscosity in cultured buttermilk can also lead to diminished phase separation due to its impact on the probability of whey separation from the network when the viscosity of the buttermilk is increased. The observed increase in viscosity (1.81 cps) of cultured buttermilk upon addition of modest doses of *Spirulina* powder may be attributed to the binding abilities or stabilising effects between the serum and aqueous phase of the powder. These results exhibit similarity with the findings reported by Mudgil *et al.* (2016) [26]. Although the treated product exhibits a reduction of 0.18% in fat content compared to the market sample, it is important to note that the viscosity of the treated product has increased by 0.03%. This increase in viscosity can be attributed to the presence of 0.25% *Spirulina* in the treated product. According to the findings, *Spirulina* has been identified as a potential fat substitute in low-fat food products.

Table 1: Comparative study on physicochemical characteristics of *Spirulina* fortified sample and market sample

Parameters	SSBM	AMSBM
Protein (g/100 g on dry weight basis)	1.83±0.01 ns	1.5±0.01 ns
Fat (g/100 g on dry weight basis)	1.32±0.01 ns	1.5±0.01 ns
Total Solids (g/100 g on dry weight basis)	9.88±0.01 ns	9.74±0.01 ns
Ash (g/100 g on dry weight basis)	0.74±0.01 ns	0.73±0.01 ns
Calcium (mg/100 g on dry weight basis)	82±0.05 s	75±0.05 s
Viscosity (cP)	1.81±0.01cP ns	1.78±0.01cP ns

Results are expressed as the mean ± standard deviation (n = 3). The values are significantly different between treatment means at $p < 0.5$. ns- non significant, s- significant

3.2. Antioxidant properties

Considerable focus has been directed towards the potential contribution of enriched meals, which incorporate bioactive chemicals derived predominantly from microalgae, to the treatment of a variety of human diseases. Microalgae possess a substantial quantity of free radical scavengers, hence elucidating their notable antioxidant capacity. Therefore, the antioxidant capability of *Spirulina* was assessed by measuring its chlorophyll and carotenoid levels, total phenolic content, and DPPH-radical scavenging activity.

3.2.1. DPPH radical scavenging activity

According to the data shown in Table 2, it was observed that SSBM demonstrated a significant level of antioxidant activity, specifically in relation to its ability to scavenge DPPH radicals. The CSBS exhibits a DPPH inhibitory activity of 41.97%. This could potentially be attributed to the inclusion of spices abundant in antioxidants and the existence of beneficial culture within the sample. The inclusion of 0.25% *Spirulina* in the treated sample resulted in a significant enhancement in radical inhibition capacity, with a notable rise of 48.22%. According to the findings of Fradinho *et al.* (2020) [14], an increased proportion of microalgae leads to a greater level of antioxidant activity. Previous research has demonstrated that the inclusion of *Chlorella vulgaris* and *Dunaliella* sp. in fortified yoghurts leads to an augmentation of the antioxidant activity of the yoghurt. The inclusion of *Spirulina* in one's dietary intake has the potential to raise the concentrations of chlorophylls, carotenoids, and phycocyanin, thereby leading to an elevation in the capacity to scavenge free radicals.

3.2.2. Chlorophyll content

In comparison to the CSBS with a chlorophyll content of 0.33mg/g, the inclusion of *Spirulina* resulted in a substantial improvement in chlorophyll content (30.95mg/g). Additionally, the incorporation of *Spirulina* also led to enhanced DPPH inhibitory activities ($p<0.5$). The outcomes of this study were consistent with the results reported by Barkallah (2017) [7], who conducted a study examining the impact of *Spirulina*-fortified yoghurt. The chlorophyll content of *Spirulina* fortified yoghurt, expressed on a dry weight basis, was determined to be 27.6 mg/g at a concentration of 0.25%. Ismaiel *et al.* (2016) [18] reported that an increase in the capacity to scavenge free radicals can be attributed to an elevation in the concentration of chlorophyll. The study conducted by Marzorati *et al.* (2020) [25] focused on the investigation of chlorophyll extraction techniques utilising supercritical CO_2 and water. The findings indicate that the dried *Spirulina* powder had a total chlorophyll content of 9.1 mg/g. The differences in chlorophyll content yield can be attributed to variations in extraction methodologies.

3.2.3. Carotenoid content

Carotenoids, a group of naturally occurring pigments, have been identified as a prominent class of compounds in various biological systems. According to the research conducted by Ambarasan *et al.* (2011), it has been found that *Spirulina* exhibits a significant presence of carotenoids, with a concentration of up to 4000 mg/kg. Among these carotenoids, β-carotene has been identified as the predominant compound. Carotenoids are known to possess a diverse range of beneficial properties, including antioxidant, anti-aging, and anti-inflammatory activities.

The carotenoid content of the treated sample was measured to be 7.83 mg/g, whereas in the commercial sample, it was found to be 0.7 mg/g. According to Marzorati *et al.* (2019), it has been observed that carotenoids possess the ability to effectively quench singlet oxygen and scavenge radicals. This unique characteristic of carotenoids leads to the termination of oxidation chain reactions. In the study conducted by Barkallah *et al.* (2017) [7], it was observed that the carotenoid content in 0.25% *Spirulin*a treated sample exhibited a value of 10.86 mg/g on a dry weight basis. The researchers made a noteworthy observation, reporting that the dried *Spirulina* powder exhibited a total carotenoid content amounting to 3.5 mg/g. The variability in carotenoid content yield can be attributed to the diverse extraction methodologies employed. The incorporation of carotenoids in *Spirulina*-fortified buttermilk renders it a cost-effective beverage with potential health benefits. The presence of carotenoids as colour pigments in various organisms is not only visually appealing but also serves a functional role as antioxidant compounds. The degradation of pigment quality in buttermilk is observed to occur over the course of storage.

3.2.4. C-Phycocyanin content

C-Phycocyanin levels were detected in SSBM, with 0.028 mg/g representing a statistically significant difference (p <0.5). This substance was not present in the CSBS. Barkallah *et al*. (2017) [7] observed a similar result in their study of the effect of yoghurt fortified with *Spirulina*. They reported that 0.25% *Spirulina* fortified sample contained 0.29 mg/g of C-Phycocyanin based on its dry weight. C-Phycocyanin with a purity of 0.7 is regarded food grade and between 0.7 to 3.9 is called reactive grade, and greater than 3.9 is termed analytical grade (Rito-Palomares *et al*., 2001) [31]. The purity of Spirulina added buttermilk was 1.32% which is above 0.7%. Marzorati *et al*. (2019) reported the yield of C-Phycocyanin using the water extraction method including electrocoagulation, dialysis, and protein salting-out procedure yielded- 250 mg/g of C-Phycocyanin with a very high purity of 2.2.

3.2.5. Total Phenolic Content (TPC)

A notable difference was discerned between the commercial sample and the samples treated with *Spirulina* in terms of total phenolic content (p<0.5). The incorporation of *Spirulina platensis* in the cultured buttermilk resulted in a measured value of 4.2 mg GAE/100 gm (GAE- Gallic Acid Equivalents) whereas the commercially available sample exhibited a lower value of 1.2 mg GAE/100 gm. The observed total phenolic content (TPC) in the commercial sample could potentially be attributed to the presence of phenolic compounds. Alshuniaber *et al*. (2021) [4] examined the biomass of Spirulina and found that it contains a significant amount of phenolic compounds. These chemicals have a crucial role in various biological processes, including redox mechanisms, reducing reactions, and oxygen quenching. Nevertheless, the capacity of these phenolic compounds to serve as a natural and sustainable reservoir of food preservatives for future utilisation is noteworthy. In a study conducted by Niccolai *et al*. (2019) [27], the fermentation of *Spirulina* biomass was found to lead to an elevation in phenol concentration.

Table 2: Comparative study on antioxidant and TPC of *Spirulina* fortified sample and Commercial sample

Parameters	SSBM	AMSBM
DPPH activity (%)	48.22±0.03	41.97±0.03
Chlorophyll content (mg/gm)	30.83±0.02	0.2±0.02
Carotenoid content (mg/gm)	7.83±0.03	0.7±0.01
C-Phycocyanin Content (mg/gm)	0.027±0.01	0
Total Phenolic content mg GAE/100 gm	4.2±0.01	1.26±0.01

Results are expressed as the mean ± standard deviation (n = 3). The values are significantly different between treatment means at p < 0.5.

4. Conclusion

This study revealed that the incorporation of *Spirulina* into buttermilk resulted in a greater array of health benefits compared to the commercially available counterpart. Due to its affordability, *Spirulina* exhibits potential for cost-effective processing methods. The treated product was optimised to contain 0.25 percent *Spirulina* based on the sensory evaluation, which revealed that the off-flavor was not detectable and that the mouthfeel and flavour profiles were comparable to those of the control sample. The addition of 1% microalgae concentration was found to have an unsuitable sensory property due to insoluble *Spirulina* particles that caused graininess and a change in oral texture. It was demonstrated that a higher concentration of *Spirulina* decreases the solubility, making it less desirable. However, it is noteworthy that the incorporation of spices has been implemented as a strategic approach to enhance the overall acceptability of the product. However, high amount of *Spirulina* can be incorporated to impart antioxidant properties provided encapsulation techniques has been done to inherent taste attributes and enhancing the bioavailability of the product.

5. References

1. ICMR - NIN. A brief note on nutrient requirements for Indians, the recommended dietary allowances (RDA) and the estimated average requirements (EAR). c2020.
2. Adler P, Bolten CJ, Dohnt K, Hansen CE, Wittmann C. Core fluxome and metafluxome of lactic acid bacteria under simulated cocoa pulp fermentation conditions. Appl Environ Microbiol. 2013;79(18):5670-5681.
3. Alizadeh Khaledabad M, Ghasempour Z, Moghaddas Kia E, Rezazad Bari M, Zarrin R. Probiotic yoghurt functionalised with microalgae and Zedo gum: chemical, microbiological, rheological and sensory characteristics. Int J Dairy Technol. 2020;73(1):67-75.
4. Alshuniaber MA, Krishnamoorthy R, AlQhtani WH. Antimicrobial activity of polyphenolic compounds from Spirulina against food-borne bacterial pathogens. Saudi J Biol Sci. 2021;28(1):459-464.
5. Anbarasan V, Kumar VK, Kumar PS, Venkatachalam T. *In vitro* evaluation of antioxidant activity of blue green algae Spirulina platensis. Int J Pharm Sci Res. 2011;2(10):2616.
6. Aneja RP, Mathur BN, Chandan RC, Banerjee AK. Technology of Indian milk products: handbook on process technology modernization for professionals, entrepreneurs and scientists. Dairy India Yearbook; c2002.
7. 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;84:323-330.
8. Bingula R, Dupuis C, Pichon C, Berthon JY, Filaire M, Pigeon L, *et al*. Study of the effects of betaine and/or C-phycocyanin on the growth of lung cancer A549 cells *in vitro* and *in vivo*. J Oncol; c2016.
9. Food Safety and Standards Authority of India. Compendium chapter 2 food product standards. c2020. Available from: https://www.fssai.gov.in/upload/uploadfiles/files/Compendium_Food_Additives_Regulations_08_09_2020-compressed.pdf
10. Cunniff PA. Official methods of analysis of AOAC International. 16th ed. Arlington, MA: Association of Official Analysis Chemists; c1998.
11. Devi MCA. Role of milk & milk products in traditional medicinal systems. Indian Dairyman. 2010;62(3):114-119.
12. Erzen N, Kac M, Pravst I. Perceived healthfulness of dairy products and their imitations: nutrition expert's perspective. Agro Food Industry Hi Tech. 2014;25:24-27.
13. Fernández-Rojas B, Medina-Campos ON, Hernández-Pando R, Negrette-Guzmán M, Huerta-Yepez S, Pedraza-Chaverri J. C-phycocyanin prevents cisplatin-induced nephrotoxicity through inhibition of oxidative stress. Food Funct. 2014;5(3):480-490.

14. Fradinho P, Niccolai A, Soares R, Rodolfi L, Biondi N, Tredici MR, *et al*. Effect of *Arthrospira platensis* (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. Algal Res. 2020;45:101743.
15. Garczewska-Murzyn A, Smoczyński M, Kotowska N, Kiełczewska K. Effect of buttermilk and skimmed milk powder on the properties of low-fat yoghurt. J Food Sci Technol. 2022;59(6):2160-2167.
16. Ghanshyambhai MR, Balakrishnan S, Aparnathi KD. Standardization of the method for utilization of paneer whey in cultured buttermilk. J Food Sci Technol. 2015;52:2788-2796.
17. Indian Standards Institution. IS: 1224 (Part-I); Determination of fat by Gerber method. New Delhi, India; c1981.
18. Ismaiel MMS, El-Ayouty YM, Piercey-Normore M. Role of pH on antioxidants production by Spirulina (Arthrospira) platensis. Braz J Microbiol. 2016;47:298-304.
19. Kang HM, Saltveit ME. Antioxidant capacity of lettuce leaf tissue increases after wounding. J Agric Food Chem. 2002;50(26):7536-7541.
20. Kim SK, editor. Handbook of marine microalgae: biotechnology advances. Academic Press; c2015.
21. Kreitlow S, Mundt S, Lindequist U. Cyanobacteria - A potential source of new biologically active substances. In: Progress in Industrial Microbiology. Vol. 35. Elsevier; c1999. p. 61-63.
22. Kumar P, Ramakritinan CM, Kumaraguru AK. Solvent extraction and spectrophotometric determination of pigments of some algal species from the shore of Puthumadam, southeast coast of India. Int J Oceans Oceanogr. 2010;4(1):29-34.
23. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents.
24. Lupatini AL, Colla LM, Canan C, Colla E. Potential application of microalga Spirulina platensis as a protein source. J Sci Food Agric. 2017;97(3):724-732.
25. Marzorati S, Parolini M, Colombo G, Idà A, Schievano A, Verotta L, *et al*. Supercritical CO_2 and green extraction methods for added value products: carotenoids, chlorophylls and phycocyanin from Spirulina microalgae.
26. Mudgil D, Barak S, Darji P. Development and characterization of functional cultured buttermilk utilizing Aloe vera juice. Food Biosci. 2016;15:105-109.
27. Niccolai A, Shannon E, Abu-Ghannam N, Biondi N, Rodolfi L, Tredici MR, *et al*. Lactic acid fermentation of *Arthrospira platensis* (*spirulina*) biomass for probiotic-based products. J Appl Phycol. 2019;31:1077-1083.
28. Nirgude R, Binorkar SV, Parlikar GR, Kirte MC, Savant DP. Therapeutic and nutritional values of takra (buttermilk). Int Res J Pharm. 2013;4(2):29-31.
29. Pan-utai W, Iamtham S. Extraction, purification and antioxidant activity of phycobiliprotein from *Arthrospira platensis*. Process Biochem. 2019;82:189-98.
30. Rao AV, Rao LG. Carotenoids and human health. Pharmacol Res. 2007;55(3):207-216.
31. Rito-Palomares M, Nunez L, Amador D. Practical application of aqueous two-phase systems for the development of a prototype process for c-phycocyanin recovery from Spirulina maxima. J Chem Technol Biotechnol. 2001;76(12):1273-1280.
32. Rose H, Bakshi S, Kanetkar P, Lukose SJ, Felix J, Yadav SP, *et al*. Development and characterization of cultured buttermilk fortified with Spirulina plantensis and its physico-chemical and functional characteristics. Dairy. 2023;4(2):271-284.
33. Sehgal S. A laboratory manual of food analysis. Wiley: New Delhi, India; c2020.
34. Seo YJ, Kim KJ, Choi J, Koh EJ, Lee BY. Spirulina maxima extract reduces obesity through suppression of adipogenesis and activation of browning in 3T3-L1 cells and high-fat diet-induced obese mice. Nutrients. 2018;10(6):712.
35. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144-148.
36. Smoczyński M, Staniewski B, Kiełczewska K. Composition and structure of the bovine milk fat globule membrane—some nutritional and technological implications. Food Rev Int. 2012;28(2):188-202.
37. Üstün-Aytekin Ö, Çoban I, Aktaş B. Nutritional value, sensory properties, and antioxidant activity of a traditional kefir produced with *Arthrospira platensis*. J Food Process Preserv, 2022, 46(3).
38. Wu Q, Liu L, Miron A, Klímová B, Wan D, Kuča K. The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: An overview. Arch Toxicol. 2016;90:1817-1840.

How to Cite This Article
Rose H, Lukose SJ, Paswan VK, Felix J. Comparative study on the physicochemical and antioxidant properties of Buttermilk fortified with *Spirulina plantensis* and commercially available buttermilk. International Journal of Veterinary Sciences and Animal Husbandry. 2024;9(5):510-515.