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Research Article

Preparation and evaluation of value added functional flavoured milk using spirulina powder

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Background: The interest in spirulina lies due to its high level of protein and mineral content besides its excellent functionality and health promoting characteristics.

Methods: Spirulina powder was added to milk at different levels 0.3 and 0.5% respectively. Analysis of variance (ANOVA) with a least significant difference (LSD) test was applied for multiple sample comparisons to test for any significant differences ($p \leq 0.05$) in the mean values of all the groups.

Results: The average chemical composition of spirulina powder showed protein 68%, fat 6%, ash 9%, moisture 3%, carbohydrate (by difference) 14%, Iron 980 ppm and calcium 685 ppm. Gamma Linolenic acid (GLA) was found as a major fatty acid *i.e.*30mg/gm in spirulina powder.

Conclusion: An increase in protein content, essential fatty acid, iron and calcium and gamma linolenic acid at both the levels of spirulina powder addition in milk was observed when compared with control during the storage at refrigeration temperature over a period of 6 days. Separation of milk protein containing spirulina powder was carried out using SDS-PAGE. It showed that milk protein bands interact with spirulina proteins near 19 kDa.

Keywords: *flavoured milk**,** spirulina powder, storage study, sensory characteristics*

Introduction

When choosing what foods to eat, consumers prioritize both nutritional value and enjoyment. Research by (Yi et al., 2022) shows that roughly 69% of purchase decisions are influenced by nutrition. Fortification with beneficial ingredients can significantly impact consumer choice, with studies like (Xiao et al., 2021) indicating that most consumers are willing to switch to fortified products if the price increase is reasonable. This trend is particularly evident in the dairy industry, where rising health consciousness has driven demand for products enriched with additional nutrients. Spirulina's rise in popularity as a superfood has fueled this growth (Seyidoglu et al., 2017). Spirulina, a blue-green algae, is lauded for its exceptional nutrient profile. It boasts highly digestible protein with all essential amino acids, beta-carotene, gamma-linolenic acid (GLA), phycocyanin, a variety of vitamins and minerals, and other beneficial compounds (Fatima & Srivastava, 2017). These components are often missing from our modern diets, making spirulina a valuable addition for people of all ages and lifestyles. Beyond its impressive nutrient content, spirulina offers a range of potential health benefits. Studies like (Kalpana et al., 2017) suggest it may reduce recovery time and enhance energy levels in athletes. For those with digestive issues, spirulina may promote better nutrient absorption and elimination. Comparisons to other foods highlight spirulina's concentrated benefits: it boasts more calcium than whole milk (Janda-Milczarek et al., 2023), more iron than spinach and exceptional levels of beta-carotene and protein (Silva et al., 2017). Its antioxidant and anti-inflammatory properties are particularly noteworthy, exceeding those of commonly consumed vegetables (Mohiti et al., 2021). Spirulina is thirty-one times more potent than blueberries, sixty times more potent than spinach, and seven hundred times more potent than apples.

All known nutrients are present in spirulina, but the most significant phytonutrients that support healthy brain and nervous system function are gamma-linolenic acid and phycocyanin, a blue pigment. Our brains are largely composed of fat, with a significant portion being essential fatty acids (Calder, 2015). GLA, a polyunsaturated fatty acid, is crucial for proper brain function (Das, 2003). Studies like (Choopani et al., 2016) have shown spirulina to be a rich source of GLA, rivaling even breast milk (Sharoba, 2014). Moreover, this pigment is incredibly difficult to find in foods and plants. The blue pigment phycocyanin, a biliprotein that has been demonstrated to prevent the growth of cancer colonies, is abundant in spirulina (Prabakaran et al., 2020). Phycocyanin, the blue pigment in spirulina, has also generated interest for its potential role in reducing neuroinflammation and oxidative stress, both of which are linked to cognitive decline (Zhou et al., 2021; Sorrenti et al., 2021). The way spirulina functions in the brain may be linked to the chemical properties of its blue hue, specifically phycocyanin. Amino acids and phycocyanin aid in the development of neurons, which improves mental function. While more research is needed, the initial findings suggest a fascinating link between spirulina's unique color and its potential benefits for brain function (Trotta et al., 2022). Gamma-linolenic acid (GLA) concentration is yet another crucial component of spirulina powder. The human brain is composed of roughly 60% fat by dry weight, with a significant portion being essential fatty acids (Chang et al., 2009). These "healthy" fats are necessary for the proper function of nerve tissue in the brain (Calder, 2015). Among the most well-regarded for brain health is GLA, a polyunsaturated fatty acid (Calder, 2015). In fact, some studies have shown spirulina to be the second richest source of GLA after breast milk (Choopani et al., 2016). High levels of GLA are present in breast milk as newborns require rapid brain mass development for survival (Innis, 2008). A stronger, more developed brain allows infants to grasp language and understand their surroundings more quickly (Shahidi & Miraliakbari, 2004). Therefore, breast milk provides all the essential nutrients an infant needs to thrive (Dewey, 2001). Conversely, cow's milk believed to aid calf survival, contains very little GLA. The combination of GLA, a "good fat" promoting healing, and phycocyanin, which may improve brain function, makes spirulina a compelling dietary choice (Zhou et al., 2021).

Synthetic additives frequently compromise the biological safety of food since they can cause allergies and impede the absorption of some nutrients. Owing to these drawbacks, the current trend in food production is to create functional foods with natural, safe ingredients that enhance food's nutritional content while also providing the necessary functionality. In the Indian market, some dairy products with extra advantages have gained popularity. Many people are interested in functional foods since eating patterns are changing across the public. If vitamin supplements, proteins, vital fatty acids, and trace elements of natural origin were added to flavored milk, consumers would opt to take significantly fewer medications and synthetic vitamin and mineral supplements. Making functional milk drinks using spirulina powder is an easy approach to accomplish this goal. Though there is currently a small selection of tastes offered in India, the beverage industry that uses dairy components has made significant strides in production in recent years. Around the world, there are numerous varieties of syrups, sodas, and soft drinks that contain artificial flavors. The product's nutritional and medicinal benefits are the primary factors that contribute to its popularity and acceptability. At the moment, pulp-based drinks with artificial flavorings are available for purchase. A promising dietary supplement for raising the amount of health-promoting unsaturated fatty acids in cow's milk is spirulina. The addition of 0.3% (w/v) dried biomass of *S. platensis* to fermented milk can enhance the probiotic lactic ferments' ability to grow, produce acid, and survive. *S. plantensis* has an antifungal effect on molds and spoilage yeasts, as well as improving the nutritional and sensory qualities of the products. The phycocyanin extracted from spirulina can be used as a food coloring in ice cream. Because this pigment is light-sensitive, extra precautions need to be taken to keep it from fading. Spirulina can be pre-fermented with 0.3% yeast and 0.5% glucose to lessen off-flavors.

A spirulina milk drink with certain health benefits can be made by combining the spirulina solution with milk (De Oliveira et al., 2021). In extruded foods, spirulina was added at 5%, 10%, and 15% concentrations. Based on sensory analysis, the product with a 5% spirulina enrichment scored higher than those with a 10% and 15% enrichment. The nutritional composition of the spirulina and whey extruded products differed significantly from the control. After a month of microbiological analysis for bacteria and fungi, the created extruded items were deemed safe. Spirulina is a vital component of the human diet since it is a rich supply of essential amino acids, essential fatty acids, carotenoids, minerals, and vitamins. At 0.075%, 0.15%, 0.23%, and 0.3% concentrations, spirulina has been added to ice cream to replace 25%, 50%, 75%, and 100% of the stabilizer used in its manufacture. An improvement in the nutritional profile, overrun, and penetration value was noted with a higher spirulina content. It adds a natural green color to the ice cream as well. Similar sensory metrics were seen in ice cream that had 50% of the stabilizer substituted with spirulina as compared to the control group Malik et al., 2013. Flavored milk is becoming an integral part of the market milk industry as it is well accepted by consumers and consumed as a refreshing and nutritious milk beverage. Skimmed milk is a by-product of the dairy industry. It can be used for the production of flavored milk due to its unique nutritional value. The use of spirulina as a natural colorant in the production of flavored milk can be beneficial due to its high nutritional value. Flavored milk is a nutrient-rich beverage that contains higher levels of nutrients than unflavored milk. Flavored milk containing spirulina has an excellent nutritional profile and provides significant amounts of high-quality protein, calcium, iron, vitamins and other important nutrients.

Materials and Methods

Milk: The cow's milk was collected from the Experimental Dairy, National Dairy Research Institute, Karnal.

Sugar: Sugar was purchased from local market, Karnal.

Flavour: Pineapple was purchased from local market, Karnal**.**

Colour: Apple green was purchased from local market, Karnal. (Colour only used for control flavoured milk and not used for spirulina flavoured milk).

Spirulina Powder: Spirulina powder was purchased from DXN shop, Shriram Tower, Nagpur, India. It was product of DXN Marketing, Malaysia.

Preparation of spirulina solution for incorporation into flavoured milk

A magnetic stirrer was used to mix the desired amount of spirulina powder in 15 milliliters of distilled water at 25˚C for 20 minutes. After being refrigerated for the entire night to hydrate the solution, it was exposed to 40 KHz radiation for 40 minutes. Using a magnetic stirrer, this was stirred for 15 minutes prior to being used in product processing.

Preparation of flavoured milk with spirulina powder

Flavoured milk without spirulina powder (Control) and flavoured milk with spirulina powder at 0.3 and 0.5 % were prepared by the method of Ramasamy & Shibu (1999). Spirulina powder solution was dispersed in milk during mix preparation before homogenization.

Proximate analysis

Spirulina powder and pasteurized flavoured milk with optimized levels of spirulina powder i.e., 0.3% and 0.5% and without spirulina powder were stored at 6- 8ºC for 7 days and samples were analyzed in 0, 2nd ,4th and 6th day of storage for the chemical composition. The titrable acidity by the method described by the American Public Health Association (1995), pH was measured using with a pH meter by the method described in IS:SP: 18 (Part XI,1981).The pH meter was first calibrated using standard buffers of pH 4.0 and 9.0 and then using buffer of pH 7.0 at 20.0 ± 0.1ºC. Viscosity by the method Roy & Sen (1994), total solids by Ranganna (1986), fat content determined by Gerber's method as described in the BIS (IS: 1224), protein by Kjeldhal method and separation of milk proteins using SDS-PAGE, moisture, iron and calcium by Atomic Absorption Spectroscopy (AAS) method. Fatty acids were determined by GC method and the extraction of fat was done by a modified Folch method (Iverson et al., 2001). Colour measurement by a hunter color lab and the values were expressed in the form of colour L* (Black/white), colour a*(redness/greenness) and colour b*(yellowishness/ blueness).

Organoleptic evaluation

A panel of seven expert judges evaluated the fresh and stored flavor-enhanced milk, containing varying amounts of spirulina, in order to assess the organoleptic properties of the two types of milk: the control and the spirulina-enriched flavor. A standard scorecard was used to assess the items' color, taste, odor, and general acceptability (Amerine et al., 1965).

Microbiological analysis

Ten milliliters (ml) of the samples were extracted, aseptically combined with nine milliliters of saline buffer, and shaken to homogenize them for microbiological investigation. The same diluents were used to make subsequent dilutions, and duplicate counting plates were always prepared by employing the proper dilutions (Harrigan et al., 1976). The pour plate method was used to count the total number of bacteria as well as yeasts, molds, and coliforms (Harrigan et al., 1998).

Statistical analysis

Data observed was expressed as mean values with standard errors. Analysis of variance (ANOVA) with a least significant difference (LSD) test was applied for multiple sample comparisons to test for any significant differences ($p \leq 0.05$) in the mean values of all the groups.

Results

Chemical and microbiological analysis of spirulina powder

The chemical composition of spirulina powder was determined and the results are presented in Table 1. The average values of protein, carbohydrate, fat, minerals and moisture in spirulina powder were found to be 68, 14, 6, 9, and 3%, respectively.

Table 1. Composition of spirulina powder[1]

Parameter	Average Values
Protein (%)	68.03 ± 0.21
Fat (%)	5.91 ± 0.15
Minerals (%)	9.1 ± 0.057
Moisture (%)	3.13 ± 0.18
Carbohydrate (%) (By difference)	14 ±0.0
Iron (ppm)	980 ± 0.17
Calcium (ppm)	685 ± 0.28

[1] Values represent average of 3 trials

Fatty acid profile of spirulina powder by GC

The fatty acids profile of spirulina powder is shown in **Figure 1**.

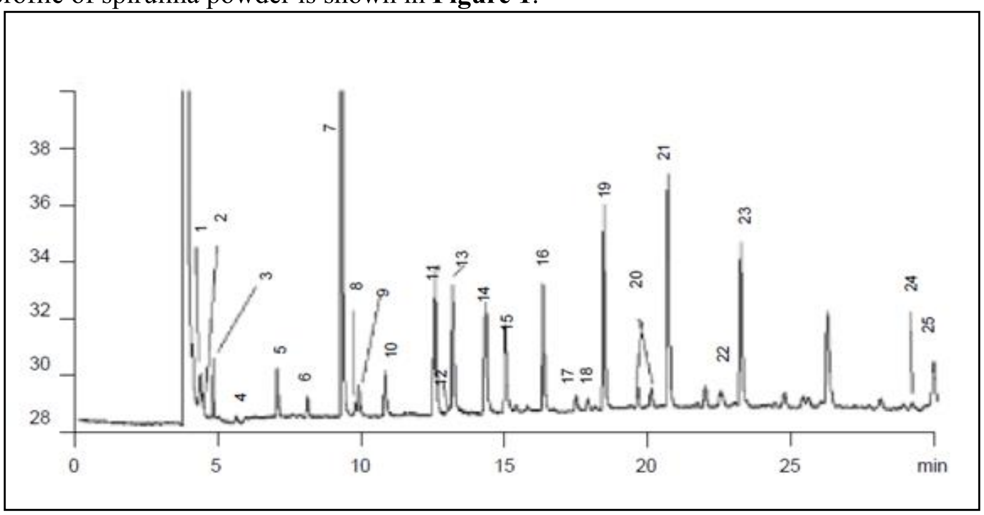

Figure 1. Fatty acid profile of spirulina powder by GC

1 is equivalent to capric acid (6:0), 2 to caprylic acid (8:0), and 3 to undecanoic acid (11:0). 5 = Myristic acid (14:0), 6 = Pentadecanoic acid (15:0), and 4 = Lauric acid (12:0). 8 = trans-palmitoleic acid (16:1t), 7 = palmitic acid (16:0). 9 = Acid palmitoleic (16:1), 10= Acid margaric (17:0), 11= trans-oleic acid (18:1t), 12= stearic acid (18:0), 13=(18:1) oleic acid 14 = 18:2 n-6 linoleic acid 15 = 18:3 n-6 gamma-linolenic acid 16 = n-3 stearidonic acid, 18:4 18 is gadoleic acid (20:1), 17 is arachidic acid (20:0), 20:2 n-6 = Eicosadienoic acid 21 = Arachidonic acid (20:4 n-6), 22 = Homogamma linoleic acid (20:3 n6), and 20 = Eicosatrienoic acid (20:3 n-3), Eicosapentaenoic acid (20:5 n-3), docosapentaenoic acid (22:5 n-3), and docosahexaenoic acid (22:6 n-3) are the values for 23 and 24, respectively.

Analysis of protein in spirulina powder by SDS-PAGE

Spirulina powder proteins were separated using SDS PAGE (figure 2). A major protein of 19 KDa was found during separation on SDS-PAGE.

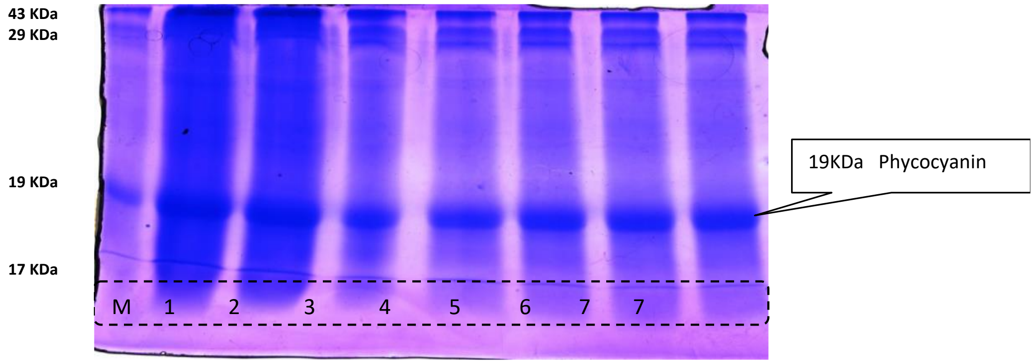

Figure 2. Protein separation of Spirulina powder analyzed by SDS-PAGE

Lane (1-7) – Spirulina powder sample
Lane M – SDS Marker (17-43 KDa)

Chemical analysis of flavoured milk containing spirulina powder during storage

Pasteurized flavoured milk with and without spirulina powder was stored at refrigeration temperature (6-8ºc) to study the shelf life. The samples were drawn on 0th, 2th , 4th , 6th day and were analyzed for chemical composition and microbiological quality (Table 2). A detailed study was conducted on the physico-chemical, microbiological and sensory attributes of flavoured milk containing spirulina powder and flavoured milk (control).

Table 2. Chemical composition of flavoured milk

Parameters	Control	0.3% spirulina powder	0.5% spirulina powder
Fat (%)	3.0 ± 0.01	3.3 ± 0.01	3.4 ± 0.01
Total protein (%)	3.4 ± 0.21	3.6 ± 0.24	3.7 ± 0.27
Ash (%)	0.70 ± 0.18	0.72 ± 0.03	0.73 ± 0.02
Total solids (%)	13.20 ± 0.25	13.80 ± 0.13	13.95 ± 0.04
Moisture (%)	86.8 ± 0.12	86.2 ± 0.26	86.05 ± 0.24
Acidity (% LA)	0.14 ± 0.13	0.13 ± 0.22	0.12 ± 0.12
pH	6.66 ± 0.11	6.62 ± 0.25	6.63 ± 0.25

Sensory evaluation

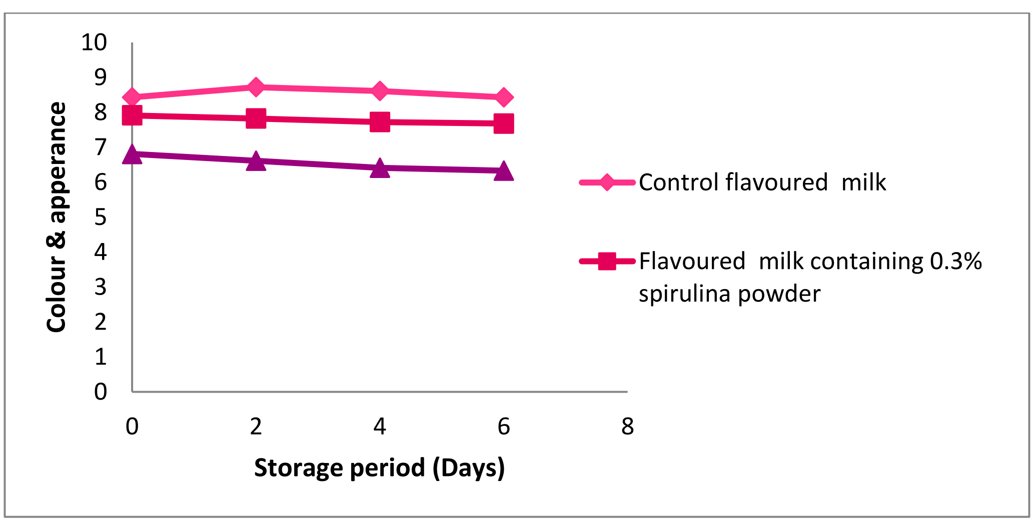

Figure 3. Effect of spirulina powder on the colour and appearance of pasteurized flavoured milk during storage at 6-8°C

The sensory evaluation of control and spirulina powder containing pasteurized flavoured milk during storage at 6 – 8 ºc for 6 days was carried out at an interval of two days to study changes in the sensory attributes. Sensory evaluation was carried out by a team of 7 selected panelists within the institute on the basis of 100 point composite scorecard. Various sensory score attributes *viz.,* color, appearance, odour, flavor, body and consistency of control and spirulina powder flavoured milk samples over the entire storage periods. It revealed that control as well as the spirulina powder pasteurized flavoured milk samples retained their color, appearance, odour, flavor, body and consistency up to 6 days of storage showed in the figure 3 to 6.

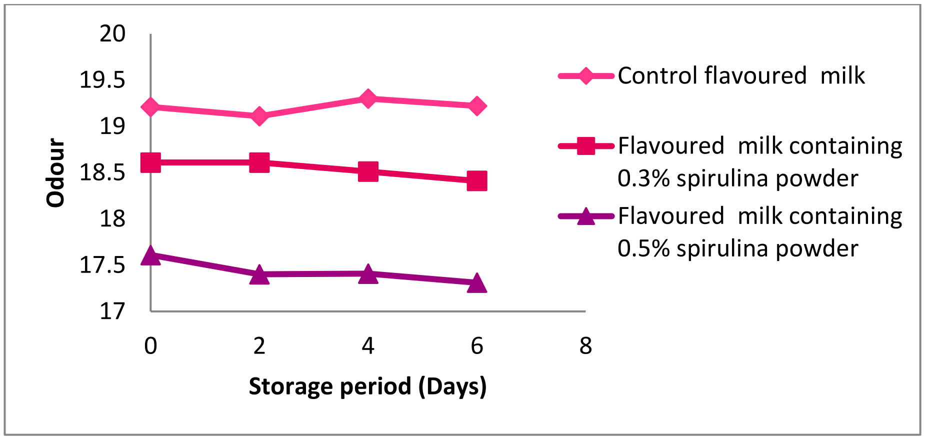

Figure 4. Effect of spirulina powder on the odour of pasteurized flavoured milk during storage at 6-8°C

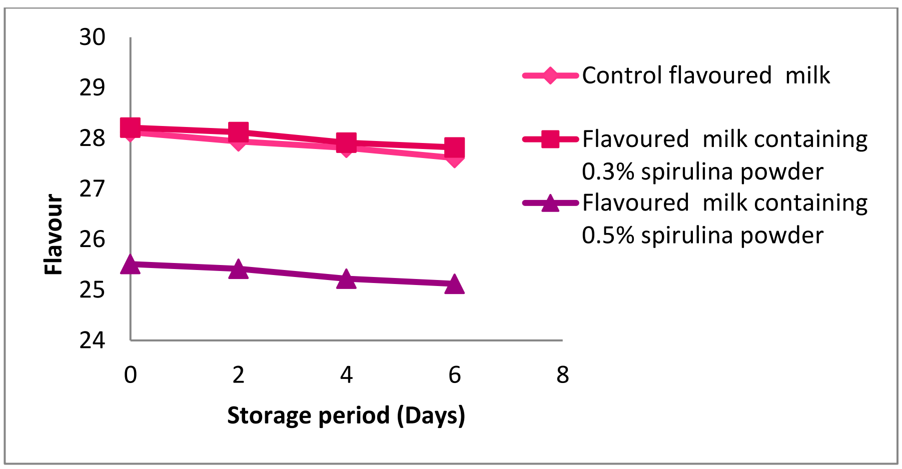

Figure 5. Effect of spirulina powder on the flavour of pasteurized flavoured milk during storage at 6-8°C

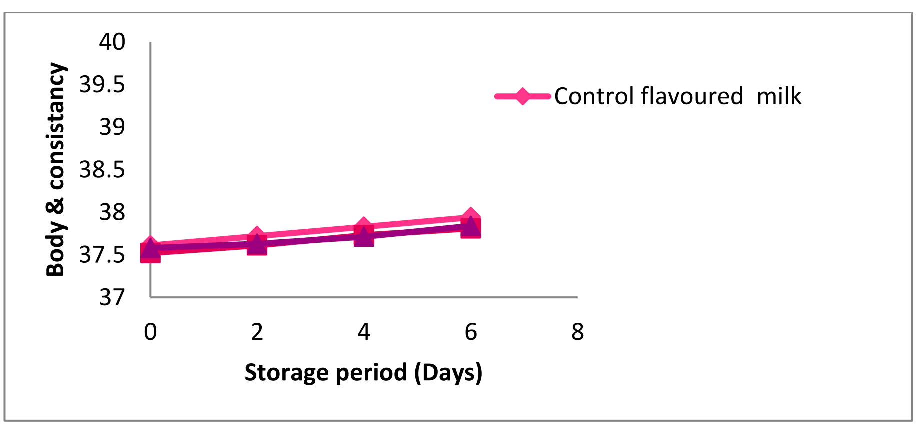

Figure 6. Effect of spirulina powder on the body and consistency of pasteurized flavoured milk during storage at 6-8°C

Effect of storage on physico-chemical parameters of spirulina flavoured milk

a) Titrable acidity

The changes in titratable acidity of control as well as spirulina powder (0.3 and 0.5%) containing pasteurized flavored milk samples during storage as showed in figure 7.

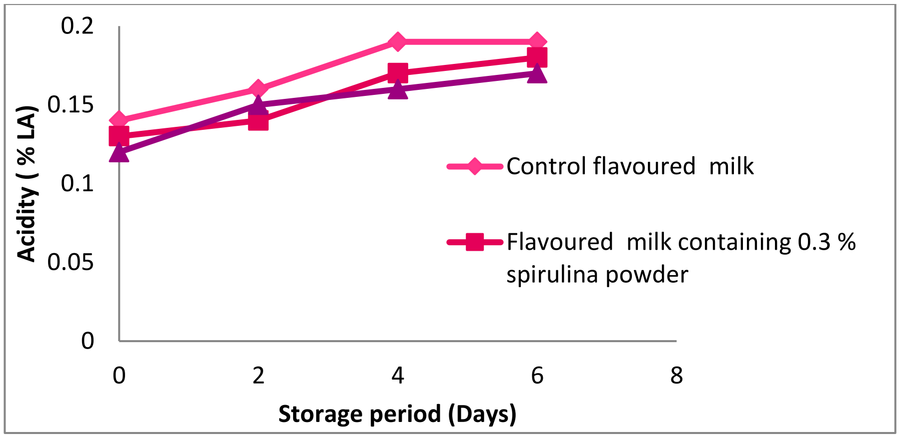

Figure 7. Effect of spirulina powder on the titratable acidity (% lactic acid) content of pasteurized flavoured milk during storage at 6-8°C

b) pH

The pH of pasteurized and sterilized flavoured milk with or without spirulina powder is showed in figure 8.

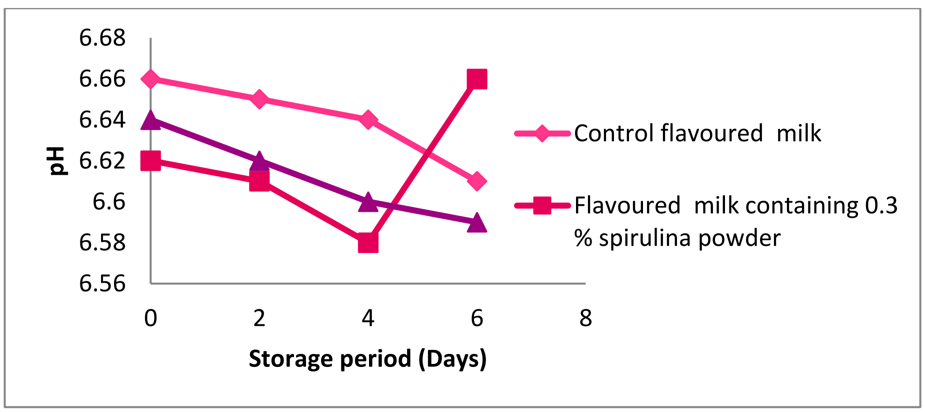

Figure 8. Effect of spirulina powder on the pH content of pasteurized flavoured milk during storage at 6 - 8°C

c) Viscosity

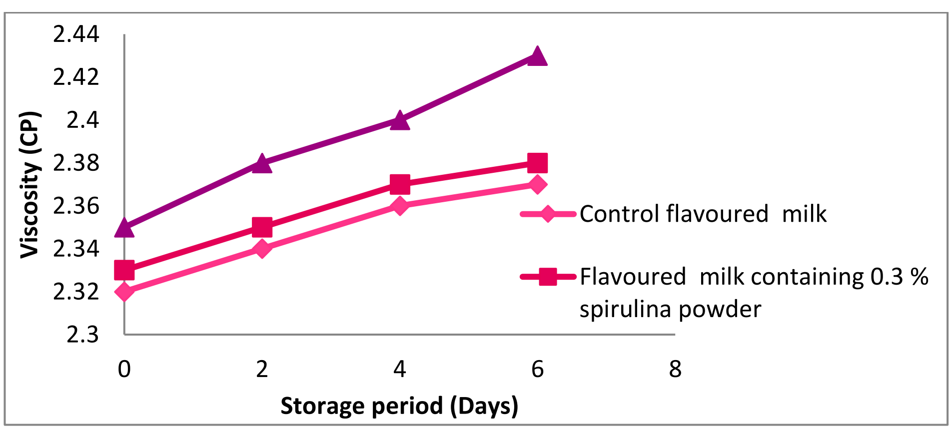

Figure 9. Effect of spirulina powder on the viscosity of pasteurized flavoured milk during storage at 6-8°C

Viscosity is an important functional property in controlling the consistency of various liquid foods and is also useful in evaluating the thickening property of proteins. The viscosities of pasteurized flavoured milk with (0.3 and 0.5%) (2.33 to 2.38 and 2.35 to 2.43 CP) and without spirulina powder (2.32 to 2.37 CP) are shown in figure 9.

d) Colour Measurement

The colour of pasteurized flavoured milk was determined by a hunter color lab and the values were expressed in the form of colour L* (Black/white), colour a*(redness/greenness) and colour b*(yellowishness/ blueness). Figures 10 to 12 indicated an overall decreasing trend in lightness, higher L* values in control pasteurized flavoured milk as compared with spirulina powder containing pasteurized flavoured milk.

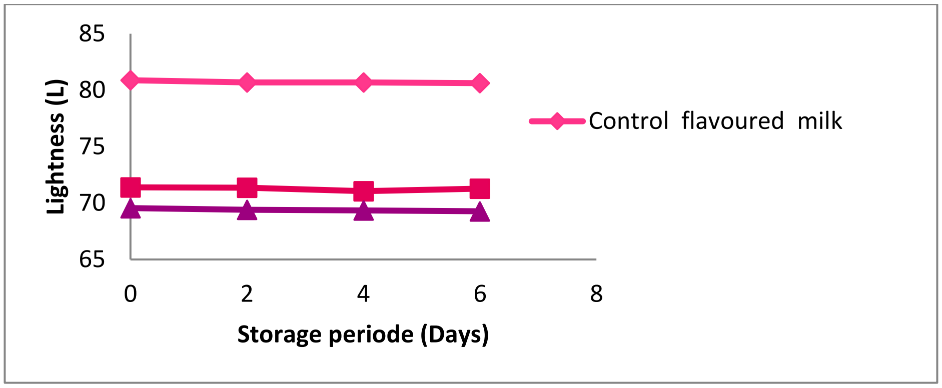

Figure 10. Effect of spirulina powder on the colour parameter of as lightness of pasteurized flavoured milk during storage at 6 – 8 ºC

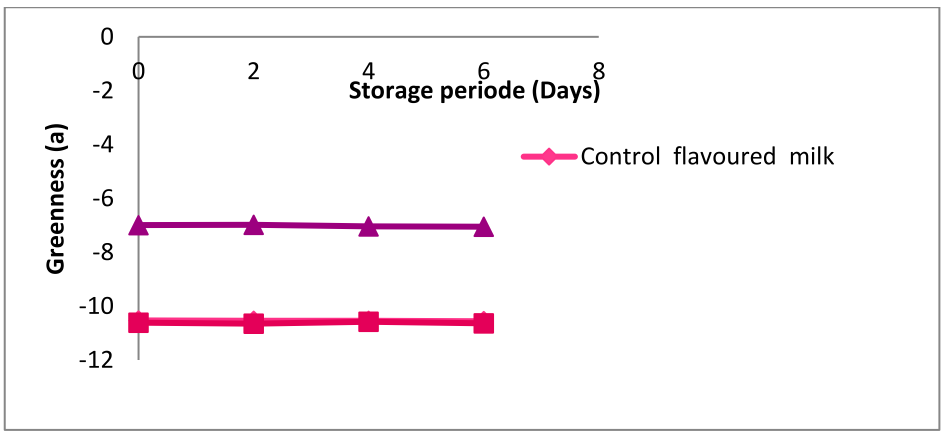

Figure 11. Effect of spirulina powder on the colour parameter of as greenness of pasteurized flavoured milk during storage at 6 – 8 ºC

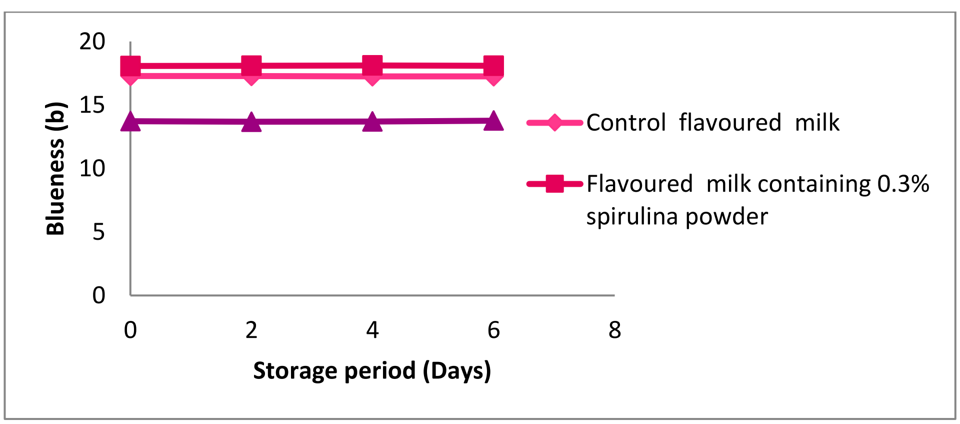

Figure 12. Effect of spirulina powder on the colour parameter of as blueness of pasteurized flavoured milk during storage at 6 – 8 ºC

Microbiological analysis

a) Total Plate counts

The changes in total plate counts during storage are presented in figure 13.

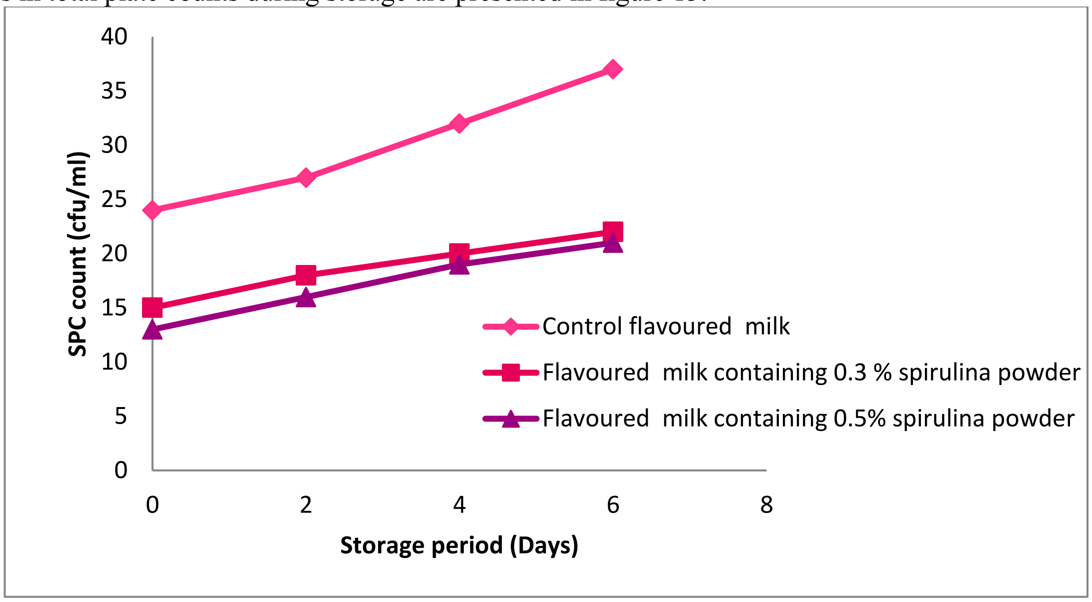

Figure 13. Effect of spirulina powder on the total plate count of pasteurized flavoured milk during storage at 6 – 8 ºC

b) Yeast and mold counts

No growth of yeast and molds was observed in spirulina powder (0.3 and 0.5%) containing pasteurized flavored milk throughout the storage period at 6 - 8°C and 37°C.

c) Coliform counts

No growth of coliforms was observed in spirulina powder containing pasteurized flavoured milk throughout the storage period at 6 - 8°C and 37°C even when the agar plates were incubated for a longer period (72 hours).

GC of fat isolated from pasteurized flavoured milk containing spirulina powder during storage at 6-8°C

Fat samples were isolated from flavoured milk with or without spirulina powder and were analyzed by GC using standard conditions and GC profile of fatty acids are presented in figures 14 to 19 respectively during 0th to 6th day of storage at 6 - 8°C for pasteurized flavoured milk.

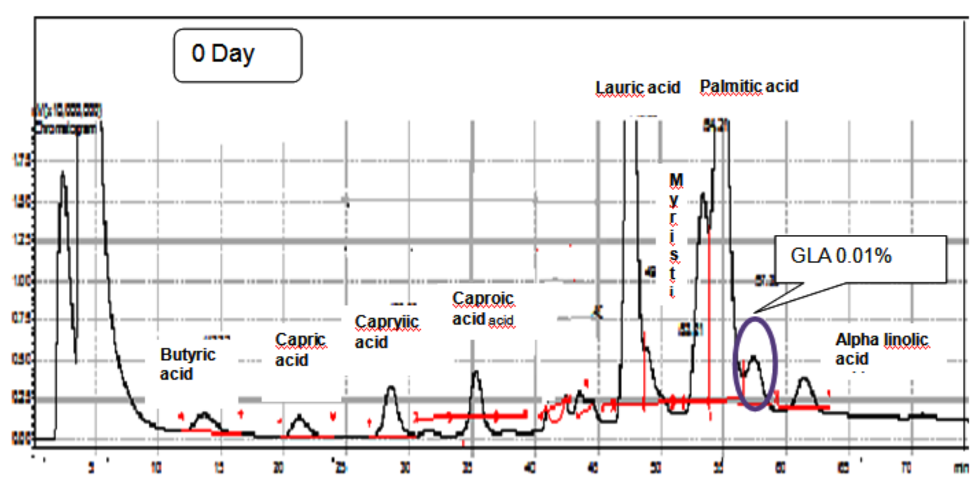

Figure 14. GC of fat isolated from 0 day sample of control pasteurized flavoured milk without spirulina powder

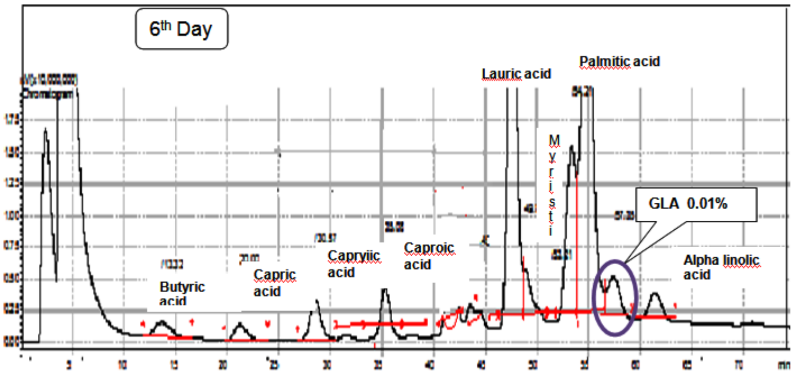

Figure 15. GC of fat isolated from 6 th sample of control pasteurized flavoured milk without spirulina powder

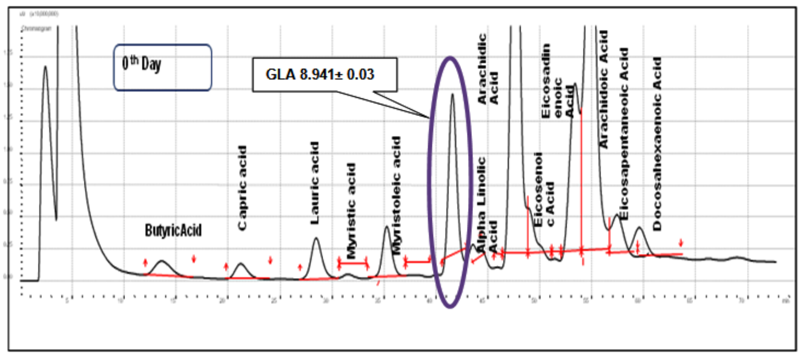

Figure 16. GC of fat isolated from 0 day sample of pasteurized flavoured milk with 0.3% spirulina powder

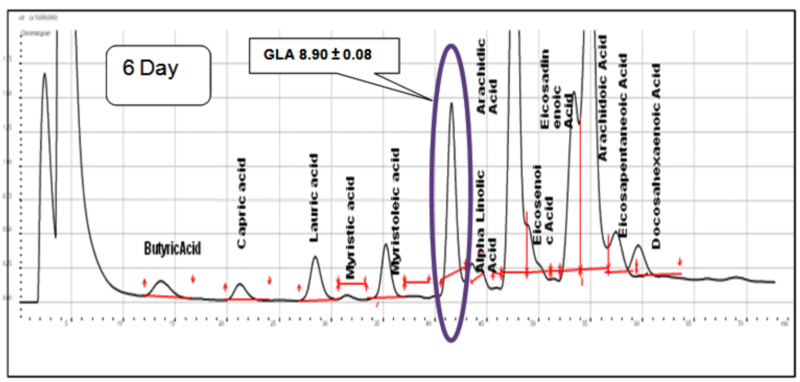

Figure 17. GC of fat isolated from 6 day sample of pasteurized flavoured milk with 0.3% spirulina powder

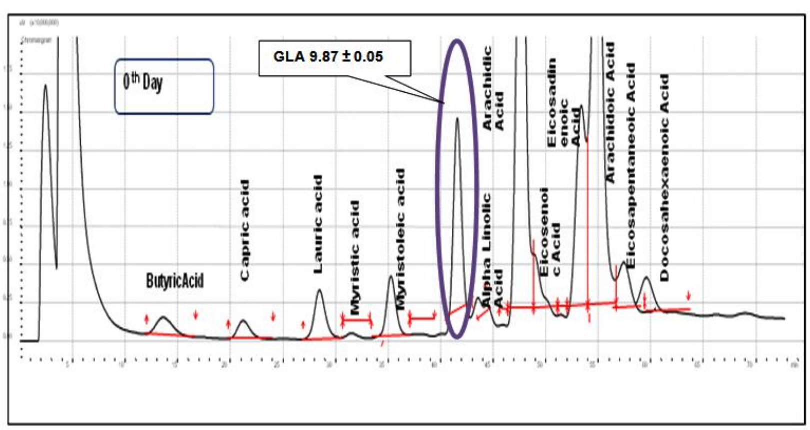

Figure 18. GC of fat isolated from 0 day sample of pasteurized flavoured milk with 0.5 % spirulina powder

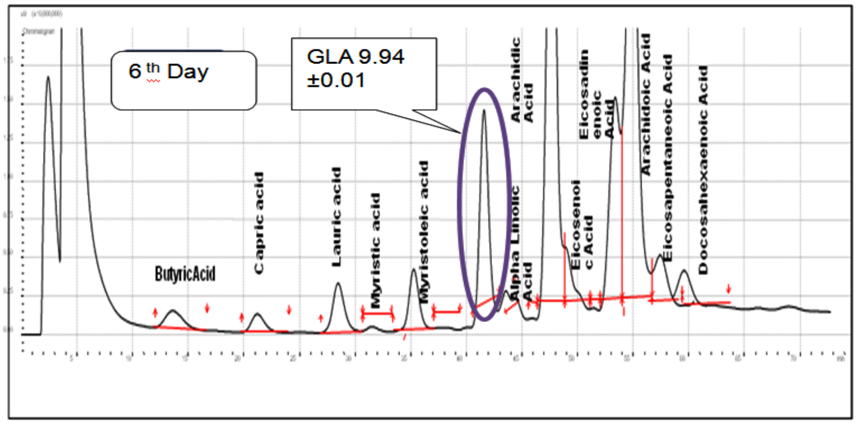

Figure 19. GC of fat isolated from 6 th day sample of pasteurized flavoured milk with 0.5 % spirulina powder

Separation of protein in flavoured milk during storage by using SDS-PAGE

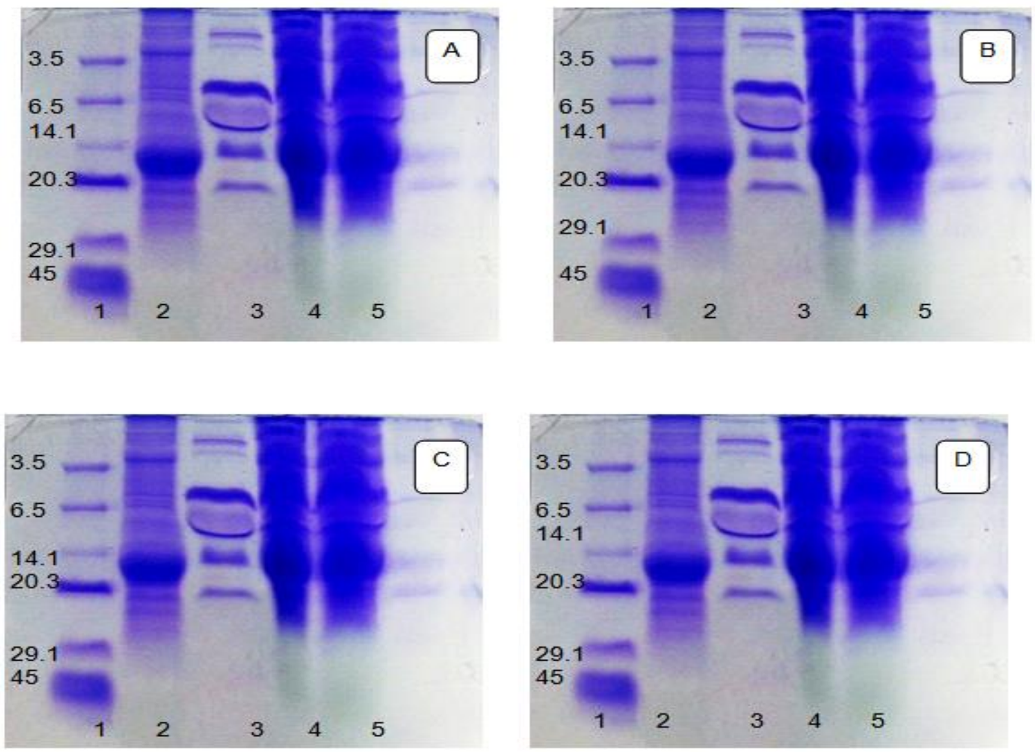

Figure 20. Protein separation of pasteurized flavoured milk during storage at 6-8°C

1- Molecular marker 4 - 0.3% spirulina flvavoured milk
2– Spirulina powder sample 5 - 0.5% spirulina flvavoured milk
Flavoured Milk- control
A-0 Day , B-2nd Day, C-4th Day, D-6th Day Sample of pasteurized flavoured milk

Pasteurized flavoured milk with (0.3 and 0.5%) or without spirulina powder was stored at 6-8 °C. The samples were drawn at an interval of two days and analyzed for separation of protein by SDS-PAGE. Figure 20 shows the presence of different protein bands of spirulina powder and protein bands of pasteurized flavoured milk.

Analysis of iron and calcium content in flavoured milk during storage at 6-8°C using an Atomic Absorption Spectrophotometer (AAS)

The changes in iron and calcium content in control as well as spirulina (0.3 and 0.5%) containing in pasteurized flavoured milk stored 6-8°C samples stored at 37°C were studied using AAS. It was observed from the figure 21 to 22.

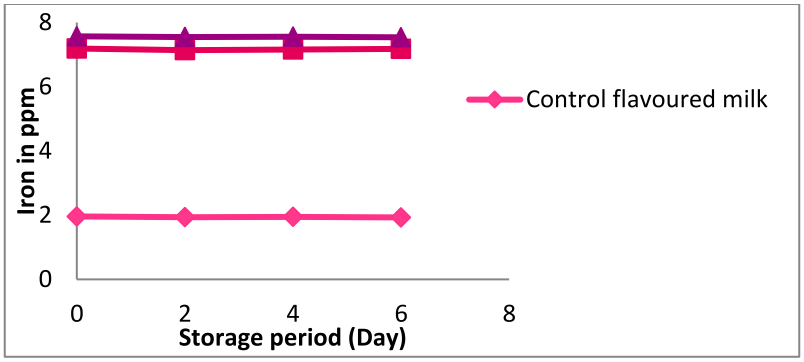

Figure 21. Effect of spirulina powder on the Iron content (ppm) of pasteurized flavoured milk during storage at 6 – 8 ºC

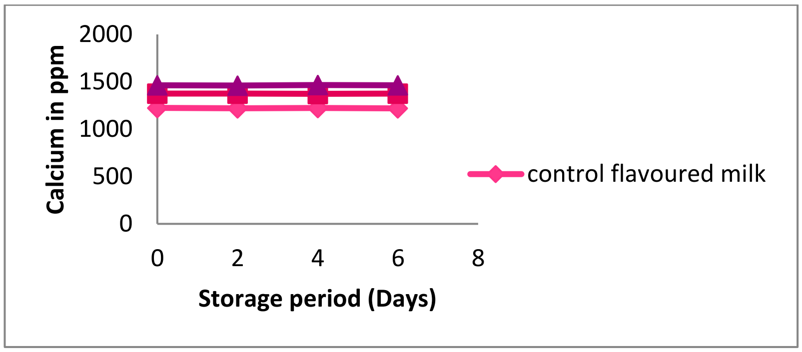

Figure 22. Effect of spirulina powder on the calcium content (ppm) of pasteurized flavoured milk during storage at 6-8ºC

Discussion

Fatty acid profile of spirulina powder by GC

Figure 1 showed that the saturated fatty acids (SFA) with short (C4:0-C8:0) and long (C10:0-C18:0) chains were present in spirulina powder. Significant amounts of ω-3 and ω-6 polyunsaturated fatty acids (PUFA), such as gamma linolenic acid (GLA) (C18:3) and linolenic acid (LA) (C18:2), were present. Sánchez et al. (2003) reported similar observations. Clinical and epidemiological research on GLA demonstrated its positive effects on human health by lowering the risk of cardiovascular disorders (Ackman, 1988; Chamorro et al., 1996; Leaf & Weber, 1988; Tokusoglu & Unal, 2003).

Analysis of protein in spirulina powder by SDS-PAGE

This protein was identified in earlier research as phycocyanin, a significant protein found in spirulina powder (Freindenreich et al., 1978, MacColl, 1983). These results align with previous observations made by Patel et al. (2006).

Chemical analysis of flavoured milk containing spirulina powder during storage

Effect of spirulina powder concentration on proximate composition of flavoured milk

Table 2 shows the proximate composition of a control sample and formulations containing different concentrations of spirulina powder (0.3% and 0.5%). Proximate composition refers to the analysis of basic components like fat, protein, ash, moisture, and total solids content. The data reveal an increasing trend in fat content (3.0% to 3.4%) with increasing spirulina powder concentration. This suggests that spirulina powder itself may be a source of fat. Similarly, total protein content also increased (3.4% to 3.7%) with increasing spirulina concentration, indicating that spirulina is a good source of protein, which aligns with well-established knowledge about this superfood. Ash content, representing the mineral content, showed a slight increase (0.70% to 0.73%) with increasing spirulina concentration. This suggests that spirulina powder contributes some minerals to the overall composition. Total solids content, which reflects the non-water portion of the sample, also increased (13.20% to 13.95%) with increasing spirulina concentration, as expected due to the addition of the powder itself. As a consequence, moisture content (water content) showed a slight decrease (86.8% to 86.05%) with increasing spirulina concentration.

Sensory evaluation

Following six days of preservation, every one of these characteristics showed a noteworthy decline ($p \leq 0.05$) in both the control and pasteurized milk samples flavored with spirulina powder.

Effect of storage on physico-chemical parameters of spirulina flavoured milk

a) Titratable acidity

The acidity of control (0.14 to 0.19 %LA) as well as spirulina powder (0.3 and 0.5%) containing pasteurized flavoured milk samples (0.13 to 0.18 %LA and 0.12 to 0.17 %LA) during storage. However, non-significant differences ($p \geq 0.05$) were observed in titrable acidity between control and spirulina powder (0.3 and 0.5%) containing pasteurized flavoured milk throughout the storage period. Similar trends for titratable acidity in ice cream containing 0.3 % spirulina powder were reported by Malik et al., 2013.

b) pH

There was a significant difference ($p \leq 0.05$) decreasing in pH of pasteurized flavoured milk samples containing spirulina powder (0.3 and 0.5%) 6.62 to 6.66 and 6.64 to 6.59) and control (6.66 to 6.61). There was no significant difference ($p \geq 0.05$) was observed between control and spirulina powder (0.3 and 0.5 %) containing flavoured milk during storage. Similar trends in pH of ice cream containing 0.3 % spirulina powder were reported by Malik et al.,2013.

c) Viscosity

In the present study, it was observed that the addition of spirulina powder increased the viscosity of pasteurized flavoured milk. This may be due to high water capacity of spirulina protein (1.45 gm of water per gm of protein) (Boukhari et al., 2018). Statistical analysis of the data also revealed that there was a significant difference ($p \leq 0.05$) in increasing viscosity in both control as well as in spirulina powder (0.3 and 0.5%) flavoured milk but non- significant difference was observed between control and spirulina powder (0.3 and 0.5%) flavoured milk during entire storage period. An increase in the viscosity of pasteurized flavoured milk may be due denaturation of proteins take place during thermal processing (de Wit & Swinkels, 1980).

d) Colour Measurement

There was a decrease in greenness (more negative a* values) and no significant change in blueness (more positive b* values) during storage of (control) flavoured milk with or without spirulina powder. The application of color during the first processing stage and heating of the mixture to 90˚C to attain a uniform dispersion of color may have caused color fading because of specific chemical alterations during control storage that altered these color parameters. Over the course of the storage period, it was found that the control samples were lighter than the spirulina powder including pasteurized flavoured milk. Pasteurized milk with flavoring is naturally green in spirulina powder, so there is no color fading or chemical change during storage.

Microbiological analysis

a) Total Plate counts

It was revealed that there was a significant difference ($p \leq 0.05$) increasing in total plate counts of control pasteurized flavoured milk samples throughout the storage period. Total plate counts of spirulina powder (0.3 and 0.5%) containing pasteurized flavoured milk after 6 days (19 and 21 cfu/ml) were lower count was observed as compared with control (37 cfu/ml). There was no significant difference ($p \geq 0.05$) observed between control and spirulina powder (0.3 and 0.5%) containing flavoured milk during storage. Our findings are also in accordance with earlier work carried out by Malik et al.,2013 observed that the preventive effect of spirulina powder in ice cream, yoghurt, and acidophilus milk lead to lower microbial counts. Spirulina powder contains antimicrobial activity due to which there is less growth of other bacteria in the flavoured milk.

b) Yeast and mold counts

In heat treatments, i.e pasteurization of flavoured milk there was a complete destruction of microflora such as yeast and molds.

c) Coliform counts

Indicating proper pasteurization of the products and no post pasteurization contamination in the products was observed. During pasteurization of flavoured milk was a complete destruction of microflora such as coliform.

GC of fat isolated from pasteurized flavoured milk containing spirulina powder during storage at 6-8°C

The chromatogram showed in figure 14 to 19 that there was a low concentration of gamma linolenic acid (GLA) in control 0^{th} day (0.01%) and 6^{th} day (0.01%) as compared to spirulina powder (0.3 and 0.5%) containing flavoured milk. An increase in the gamma linolenic acid content in spirulina powder 0.3% flavoured milk (0^{th} day 8.941% and 6^{th} day 8.90%) 0.5% (0^{th} day 9.87% and 6^{th} day 9.94%) in flavoured milk was observed as compared to control flavoured milk.

Separation of protein in flavoured milk during storage by using SDS-PAGE

The conventional molecular protein marker (3.5 KDa to 45 KDa) is depicted in Figure 20, Lane 1. The various proteins found in spirulina powder are displayed in Lane 2. Because the fractionated bands in the molecular range (3.5 to 20 KDa) were present, it was possible to determine from the Figure that spirulina powder contained low molecular weight protein. Spirulina powder had a prominent band that, when compared to a conventional molecular marker, revealed a molecular weight of roughly 19–20 KDa. The distinct proteins found in the flavor-controlled milk are displayed in Lane 3. Additionally, figure 20 showed that the flavor-infused control milk had a low molecular weight due to the presence of several bands in the 2.5–20 KDa molecular range. The proteins found in 0.3 and 0.5% spirulina powder including flavor-infused milk are displayed in Lanes 4 and 5, along with some evidence of the interaction between the spirulina and milk proteins, which was seen as a single band on the SDS-PAGE. Protein bands separated by SDS-PAGE at intervals of 0, 2, 4, and 6 days during the storage of flavor-infused milk at 6–8 °C revealed no variation.

Analysis of Iron and Calcium content in flavoured milk during storage at 6-8°C using Atomic Absorption Spectrophotometer (AAS)

In Figure 21 to 22, there was a significant ($p \leq 0.05$) increase in iron content in spirulina powder containing pasteurized flavoured milk 0.3% (7.19 to 7.18 ppm) and 0.5% (7.57 to 7.54 ppm) samples when compared with control (1.96 to 1.93 ppm) during storage. It was observed from figure 21 and 22 that, there was a significant ($p \leq 0.05$) increase in calcium content in spirulina powder containing pasteurized flavoured milk 0.3% (1373.73 to 1374.73 ppm) and 0.5% (1462.62 to 1463.62 ppm) samples when compared with control (1224.24 to 1219.24 ppm) during storage. However, non-significant differences ($p \geq 0.05$) were observed in iron and calcium content between control and spirulina powder containing pasteurized flavoured milk throughout the storage period.

Conclusion

A chemical composition analysis was performed on powdered spirulina. Spirulina powder utilized in this study had an average molecular makeup of 68% protein, 14% carbohydrate, 6% fat, 9% minerals, and 3% moisture. In summary, the dairy industry can benefit greatly from the use of spirulina powder to improve the nutritional and functional value of milk and milk products. Flavoured milk and whey drink containing spirulina powder possessed desirable colour and appearance, flavour, overall acceptability, body and consistency upto 6 days for pasteurized flavoured milk. Spirulina powder containing milk beverage fetched a slightly lower sensory scores ($p \leq 0.05$) than the control during entire storage periods. There was a significant increase ($p \leq 0.05$) in acidity of control as well as flavoured milk containing spirulina powder during storage. The titratable acidity was more in control samples than the corresponding flavoured milk containing spirulina powder. There was a significant decrease ($p \leq 0.05$) in pH of control as well as flavoured milk containing spirulina powder during storage. The viscosity of flavoured milk containing spirulina powder was significantly more ($p \leq 0.05$) than the corresponding control without spirulina powder. Decrease in lightness, greenness, blueness in control as well as flavoured milk containing spirulina powder was observed in the all samples during storage. Flavoured milk containing spirulina powder showed less total plate counts when compared with control. These counts increased gradually in flavoured milk containing spirulina powder during storage. Fatty acid analysis of flavoured milk containing spirulina powder showed higher amount of gamma linolenic acid as an essential fatty acid when compared with control.

During storage there was no change in concentration of gamma linolenic acid in flavoured milk containing spirulina powder. SDS–PAGE separation of protein in flavoured milk and whey drink containing spirulina powder showed a 19 KDa molecular weight spirulina protein present in flavoured milk. This protein band interacts with the protein of milk. Atomic absorption spectroscopy (AAS) showed that there was the increase in iron and calcium content in flavoured milk containing spirulina powder when compared with the control sample. During storage, there is no change in the iron and calcium content in flavoured milk containing spirulina powder as well as in control sample. The results obtained in the present investigation showed the successful incorporation of spirulina powder during the preparation of flavoured milk. The addition spirulina powder in flavoured milk increased the protein content, essential fatty acids i.e. gamma linolenic acid, iron and calcium. On the basis of sensory evaluation of flavoured milk and whey drink, it was found that spirulina powder at the both level of 0.3% and 0.5 % can be added in flavoured milk but 0.3% level of spirulina powder gave the better result in terms of sensory evaluation when compared with 0.5% level of spirulina powder. Thus, from the present study it can be concluded that spirulina powder offers great potential for use in the dairy industry in order to enhance the functional and nutritive value of milk and milk products.

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Conflict of interest

The author declares no conflict of interest. The manuscript has not been submitted for publication in any other journal.

Ethics approval

Not applicable.

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