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Preparation and Evaluation of Value Added Functional Whey Based Dairy Drink Using Spirulina Powder

Srushty Patil* and Raman Seth

Department of Dairy Chemistry, National Dairy Research Institute, Karnal, Haryana, India

**Corresponding author*

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A B S T R A C T

Paneer whey was fortified with spirulina powder at two concentrations (0.3 and 0.5 %) for preparation of ready-to-serve (RTS) drink and evaluated for various physico-chemicals and sensory attributes during storage at 8°C. The study revealed that whey based spirulina drink containing 0.3% spirulina powder scored maximum for almost sensory quality attributes, i.e. appearance, color, flavor, odour and overall acceptability and showed protein content 0.76 %, essential fatty acid 8.023%,iron 5.16 ppm and calcium 137.37 ppm during the storage at refrigeration temperature over a period of 60 days.

Introduction

Dairy ingredients based beverage industry has made significant progress during the last several years in terms of production, with a limited range of flavors available in India. Many types of syrups, sherbets and soft drinks containing artificial fruit flavors are well known throughout the world. The basic factor considered is the nutritive and therapeutic values, which make the product popular and acceptable. At present fruit pulp based beverages generally contain synthetic flavors and are sold in the market. Whey supplemented with spirulina powder will be more beneficial to the consumer interms of availability of proteins, iron, essential fatty acids and calcium. Dairy drinks have high nutritional quality and increased energy value and will be useful in places where there are deficiencies of certain nutrients. Whey is the liquid obtained from curdling of milk with either acid coagulant or using rennet. It contains about half of the total solids of milk and is a source of precious nutrients like whey proteins, lactose, milk salts and most of water-soluble vitamins (Naik *et al.*, 2009). Whey possesses preventive and curative components and is especially used to treat a wide variety of ailments such as arthritis, anemia and liver complaints. In India, whey is obtained as a by-product in the preparation of channa, paneer,

cheese and casein (Cruz *et al.,* 2009). Many attempts have been reported on utilization of whey in the formulation of various dairy products, but still there is a lot of scope to explore the possibility of its utilization in beverage industries (Gagrani *et al.,* 1986).

Formulated different blends of whey beverages by using levels of mango pulp (8-12%) (Sikder *et al.,* 2001). Drink using spirulina powder and milk is currently receiving considerable attention.

Besides being delicious, these beverages are highly nutritious. The present study was conducted to develop delicious and nutritious beverages using the combination of spirulina powder and paneer whey.

Materials and Methods

Preparation of paneer whey

The standardized milk (4.5 % fat and 8.5 % SNF) were heated to 82°C and acidified by adding 2 % citric acid with continuous stirring, resulted in the coagulation of milk protein (casein). The liquid (whey) was separated by filtration through four fold muslin cloth. The filtered whey was centrifuged at 4°C to remove the residual fat. The whey obtained was stored at 4 ± 1°C and heated to 60°C before preparation of the beverage.

Preparation of spirulina solution for incorporation into whey beverage

Desired amount of spirulina powder was dissolved in 15ml distilled water at 25^{o}C and mixed with help of magnetic stirrer for 20 minutes. This solution was kept for hydration for overnight at refrigeration temperature and then irradiated at 40KHz for 40 min. Before using for product preparation this was remixed for 15 minutes with magnetic stirrer.

Preparation of whey based spirulina whey drink

The whey based spirulina whey drink was prepared by blending of whey and spirulina powder in different proportions like 0.3 and 0.5 % and also control whey drink without spirulina, respectively.

The recipe for preparation of 1 litre whey drink with addition of spirulina powder in varying proportions. The sugar 5 % were dissolved in whey by heating to 60ºC and then filtered through muslin cloth.

The spirulina powder was added to whey. Thus prepared whey drink was filtered and filled with previously sterilized glass bottles (200 ml) sealed airtight using crown corking. Then in bottle sterilization was done at 105ºC for 10 min and cooled to room temperature and stored at 8 ± 1°C for storage studies. Samples were drawn at a regular interval of 7 days and evaluated for various quality attributes.

Proximate analysis

The proximate analysis of whey, spirulina and whey drink was carried out for different chemical parameters. The total solids (TS), protein, ash, ascorbic acid, essential fatty acid, calcium and iron were estimated by the standard methods (Ranganna *et al.,* 1986)). The total acidity was calculated in terms of citric acid for whey drink by titrating against 0.1 N NaOH (AOAC, 1995).

Fat content was determined by Gerber's method as described in the BIS (IS: 1224). Fatty acid profile was analyzed by gas liquid chromatography (GLC). Total protein was estimated by kjeldhal method and separation of protein was carried out by SDS-PAGE, Calcium and iron was done by atomic absorption spectroscopy (AAS).

Organoleptic evaluation

The fresh and stored whey drink prepared with varying levels of spirulina were served chilled for organoleptic evaluation and it was carried out by trained panel of seven judges for evaluation of the control and spirulina fortified whey drink.

The products was judged with standard score card for colour, flavour, odour, overall acceptability (Amerine *et al.*, 1965).

Microbiological analysis

Microbiological analysis were carried out by taking 10 ml of samples and aseptically mixed with 9 ml using saline buffer and homogenized by shaking. Subsequent dilutions were prepared with the same diluents and in all cases duplicate-counting plates were prepared by taking appropriate dilutions (Harrigan *et al.*, 1976). Total vplate count, yeast and mold and coliform count was carried out using the pour plate method (Harrigan *et al.*, 1998).

Statical 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 comparison to test for any significant differences ($P<0.05$) in the mean values of all the groups.

Results and Discussion

Proximate analysis of whey and spirulina powder

The data observed during to the various chemical analysis of whey and spirulina powder. It clearly indicates that whey and spirulina powder were as expected rich in protein respectively. Spirulina was found better in the average percentage value of 68, 14, 6, 9 and 3 % were recorded for protein, carbohydrate, fat, minerals and moisture respectively.

Sensory evaluation

The beverages prepared by blending of whey and spirulina powder in different combination were analyzed for various sensory attributes for their acceptance by giving score out of 100 by the panel of judges. The sensory scores obtained with respect to color, flavor, odour and overall acceptability. It was observed that whey drink prepared by addition of 0.3% spirulina was liked most by sensory panel members. The color, flavor and taste of 0.3% spirulina whey drink observed the highest organoleptic score other than the control whey drink and 0.5% spirulina whey drink respectively. The result revealed that an increase in level of spirulina powder lower down the sensory score of beverage. The storage study of spirulina whey drink revealed that all the characteristics i.e. appearance, colour, flavor, odour and overall acceptability of sensory evaluation were decreasing lower as the concentration of spirulina powder was increased to 0.5 %. This might be due to changes occurred during storage of whey drink.

Effect of storage on physico-chemical parameters of whey base spirulina whey drink

Effect of addition of spirulina powder on physico-chemical parameters of whey based spirulina whey drink and changes during storage were studied for improving nutritional quality and functional characteristic of spirulina whey drink.

The increase in total solid, protein, fat, viscosity was found to be significant, the rate

of increase in iron content from 1.76 ppm in control to 5.16 ppm in 0.3% spirulina powder and 6.33 ppm in 0.5% spirulina powder. The rate of increase in calcium content from 89.69 ppm in control to 137.73 ppm in 0.3% spirulina powder and 146.66 ppm in 0.5% spirulina powder. Increase in essential fatty acid such as gamma linolenic acid from 0.01% in control to 8.023 % in 0.3% spirulina powder and 8.023 % in 0.5% spirulina powder whey beverage was found to be statistically significant.

Fatty acids evaluation of whey drink

Fat samples were isolated from whey drink with or without spirulina powder and were analyzed by GLC using standard conditions and GLC profile of fatty acids are presented in figure respectively during storage at 6-8°C for whey drink. The chromatogram showed that there was a low concentration of gamma linolenic acid(GLA) in control as compared to spirulina powder (0.3 and 0.5%) containing whey drink. An increase in the gamma linolenic acid content in spirulina powder(0.3% and0.5%) in whey drink was observed as compared to control whey drink.

Separation of protein of whey drink

Whey drink with (0.3 and 0.5%) or without spirulina powder was stored at 6-8 °C. The samples were drawn at a interval of fifteen days and analysed for separation of protein by SDS-PAGE. Figure 4.86 shows the presence of different protein bands of spirulina powder, proteins bands of milk and proteins bands of whey drink.

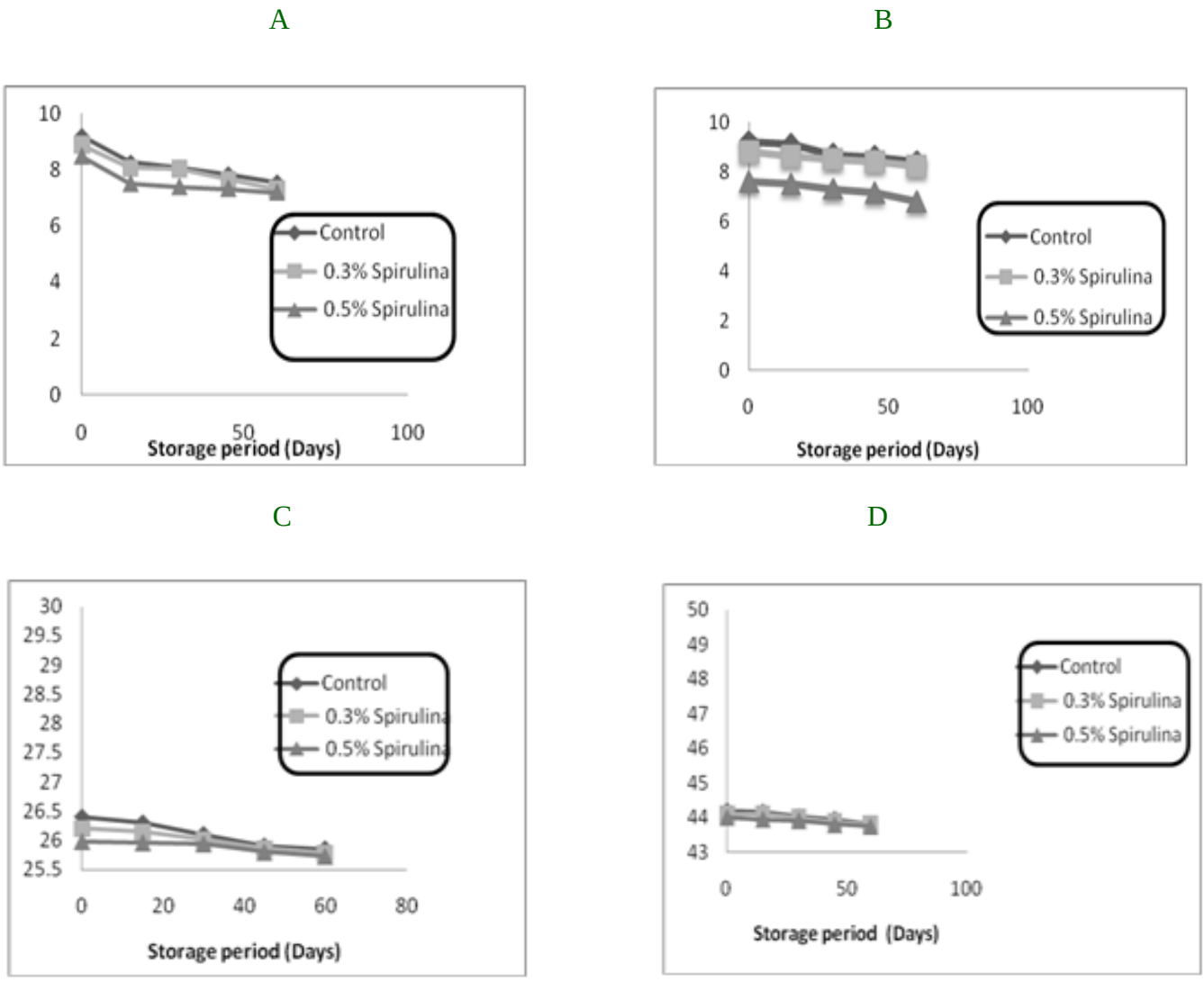
Lane 1 shows the standard molecular marker (6.5 KDa to 45 KDa). Lane 2 shows the different protein present in milk. It was observed from the figure that, milk contain low molecular weight protein as the fractionated bands were present in the molecular range (2.5 to 20 KDa).

Lane 3 shows the different protein present in control whey drink. It was observed from the figure that, control whey drink contain higher molecular weight protein as the fractionated bands were present in the molecular range(14 to 44 KDa). Lane 4 shows the different protein present in spirulina powder. It was observed from the figure that, spirulina powder contain low molecular weight protein as the fractionated bands were present in the molecular range (19 to 40 KDa). A major band appeared in spirulina powder which on comparison with standard molecular marker showed molecular weight about 19 – 20 KDa This protein was identified as phycocyannin.

Lane 5 and 6 shows the different protein present in 0.3 % containing spirulina powder in milk and whey drink. It was obsered from the figure that, it contain both spirulina powder protein and milk protein of low molecular weight of bands were present in the molecular range (1.5 to 19 KDa) and along with some interaction of milk proteins and spirulina powder proteins which appeared as single band on SDS- PAGE.

Similar trade was observed in spirulina powder protein and whey protein of low molecular weight of bands were present in the molecular range (18 to 19 KDa) and along with some interaction of whey proteins and spirulina powder proteins which appeared as single band on SDS- PAGE. During storage of whey drink at 6-8 °C, separation of protein by SDS-PAGE at interval of 0, 15^{th}, 30^{th}, 45 th and 60^{th} day showed no difference in protein bands.

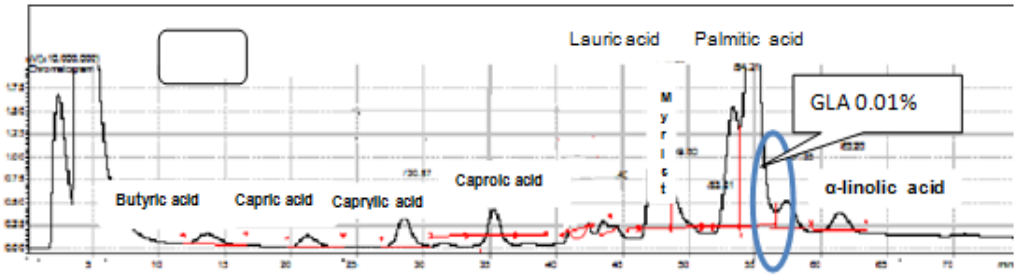
Fig.1 Sensory evaluation of whey drink containing with and without spirulina powder during storage at 6-8°C Whereas, 0^{th} to 60^{th} = Storage period in days



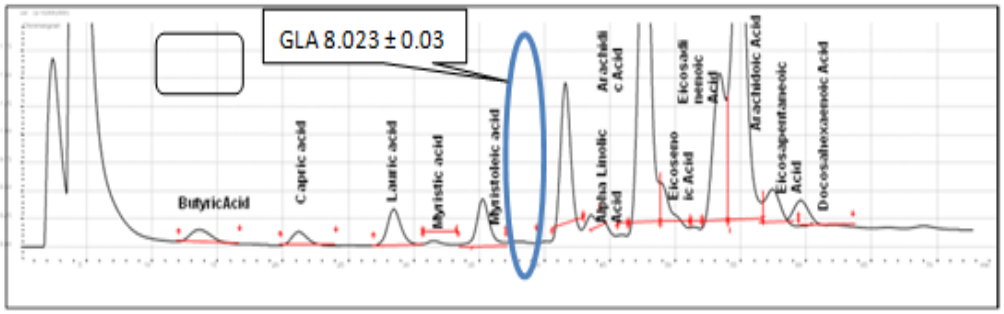
A = Colour & appearance, B = Odour, C = Flavour, D = Overall acceptability
Control - Whey drink sample without spirulina
0.3% spirulina - Whey drink sample with 0.3% spirulina
0.5% spirulina - Whey drink sample with 0.5% spirulina

Fig.2 Fatty acid profile of whey drink containing with and without spirulina powder during storage at 6-8°C

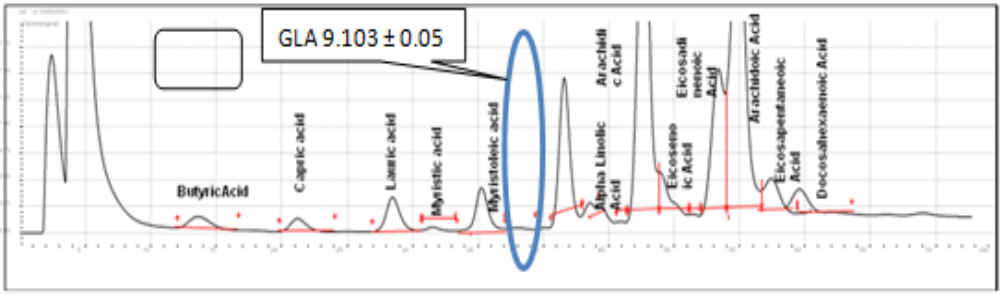
A



B



C



A = Control whey drink (0.01% gamma linolenic acid) ,
B = Whey drink containing 0.3% spirulina powder (8.023 ± 0.03 gamma linolenic acid),
C = Whey drink containing 0.5% spirulina powder (9.103 ± 0.05 gamma linolenic acid)

Fig.3 Stability of spirulina powder protein in whey drink during storage as analyzed over SDS-PAGE

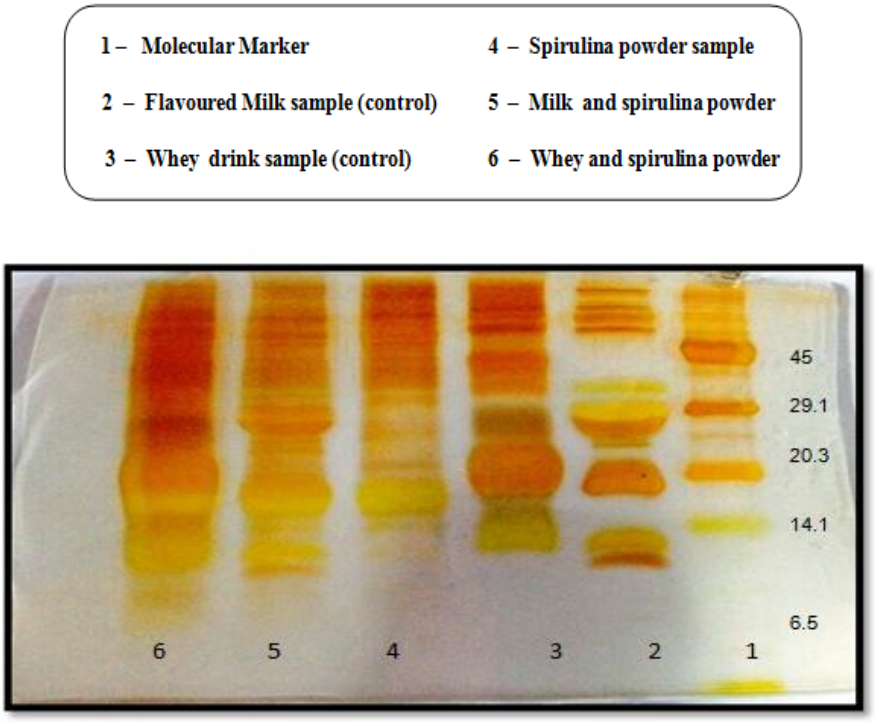
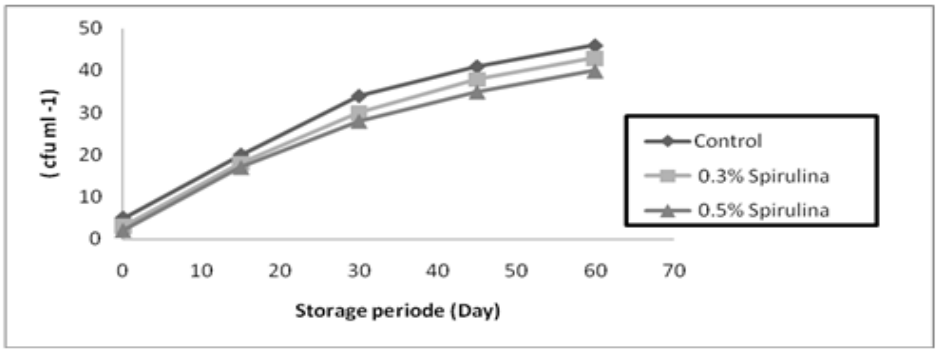


Fig.4 Total plate count (cfu/ml) of whey drink containing with and without spirulina powder during storage at 6-8°C Whereas, 0^{th} to 60^{th} = Storage period in days



Control - Whey drink sample without spirulina
0.3% spirulina - Whey drink sample with 0.3% spirulina
0.5% spirulina - Whey drink sample with 0.5% spirulina

Microbial analysis

The beverage samples were analysis periodically for total plate count and yeast and mold count. The data obtained with respect to microbial load are summarized in figure4. Total plat e counts of fresh beverage samples i.e. control, 0.3% and 0.5% spirulina was 5.0 x 10^{-4} CFU mL-1,3.0 x 10^{-4} CFU mL-1 and 2.0 x 10^{-4} CFU mL-1 respectively.

As the storage period proceed the total plate count was increase but more count was observed in control as compared with 0.3 and 0.5 % spirulina whey drink. No coliform, yeast and mold was observed during storage.

The whey can be found successful for the development of whey based spirulina whey drink with optimum sensory characteristics. The nutritious drink with better storage life could be developed by addition of spirulina powder up to certain extent. Spirulina based whey drink have excellent colour, flavour, odour, high in protein, essential fatty acid, calcium and iron were estimated to be high which means that spirulina powder covered unplesent taste of whey very successfully. An increase of both functional and nutrition concentration might improve the characteristics of the addition of spirulina powder in whey.

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