Functional Probiotic Yoghurt with Spirulina

Rita Narayana and Asaram Kale

ABSTRACT
An attempt has been made in the present study to explore the potential of Spirulina, a cyanobacterium, photoautotrophic microorganism in initiating a stimulatory effect on the microflora of Probiotic yoghurt. Probiotic yoghurt was prepared by adding 1 percent inoculum of probiotic Bifidobacterium bifidum to yoghurt cultures viz., Streptococcus. salivarius ssp. thermophilus and Lactobacillus. delbrueckii ssp. bulgaricus. Spirulina enriched functional Probiotic yoghurt was prepared by using 1 gram of Spirulina per litre of mix. The pH and acidity of probiotic yoghurt and Spirulina enriched Probiotic yoghurt on 0 day was 4.31± 0.007, 0.96± 0.002 and 4.31± 0.009, 0.96± 0.005 respectively. On the 3rd it was 4.30 ± 0.003, 0.96 ± 0.004 and 4.31 ± 0.004, 0.96± 0.005 respectively. There was no significant difference in the pH and acidity of Probiotic and Spirulina enriched yoghurt between these 2 sampling periods. The pH and acidity of pro-biotic yoghurt and Spirulina enriched probiotic yoghurt on the 7th day was 4.28 ± 0.001, 1.11 ± 0.030 and 4.31 ± 0.004, 1.02 ± 0.023 respectively. Significant difference was noticed in pH and acidity in these two treatments on 7th day. The Spirulina enriched sample was less acidic than Probiotic yoghurt. There was virtually no difference in viable numbers of S. salivarius ssp. thermophilus, L. delbrueckii ssp. bulgaricus and Bifidobacterium bifidum on 0 and 3rd day. However the growth of the three lactic acid bacteria used was higher in Spirulina enriched yoghurt than in Probiotic yoghurt on the 7th day. The addition of cyanobacterial biomass to Bifidobacterium bifidum, S. salivarius ssp. thermophilus and L. delbrueckii ssp. bulgaricus had beneficial effect on their viability. No spoilage organism was detected at any sampling time, indicating the high degree of sanitation during processing and packaging products. Thus the abundance of bioactive substances in Spirulina is of great importance from a nutritional point of view as it provides new opportunity for the manufacture of functional dairy foods.

Key words: Functional yoghurt, Probiotic yoghurt, Spirulina.

INTRODUCTION
Lactic acid bacteria through its activity has bestowed many benefits to humans. These bacteria are widely used in foods and agricultural fermentations. In addition to their preservation, nutritional and therapeutic importance, these are attributed for the longevity of human life. Lactic acid bacteria are natural inhabitants of gastrointestinal tract. These microorganisms have a number of traits which make them particularly attractive as a “probiotic”. It has been recognized for centuries that cultures added to foods such as milk, yoghurt and cheese are beneficial to human health. In addition to good taste of fermented milks, they supply high quality proteins, are excellent source of calcium, phosphorus, potassium and increase the bioavailability of copper, zinc, manganese and phosphorus (Sherwood, 1990).

Yoghurt, a fermented traditional food in the Balkans and Middle East has achieved special prominence and economic importance in recent years due to its positive nutritional and health benefits (Ramasamy and Basak, 1991). The word “yoghurt” was derived from Turkish word “Jugurt” and it is known by a variety of names in different countries such as Dahi (India), Zabady (Egypt), Leban (Arab countries) and Yahourt (Yemen), Msk (Yugoslavia), skyr (Iceland) etc. (Tamime and Deeth, 1960). Presently, new and innovative types of yoghurt and yoghurt like products with probiotic cultures are marketed resulting in a phenomenal increase in the per capita consumption of yoghurt during the last few decades.

Bifidobacteria and certain Lactobacillus species have recently received attention as probiotic organisms maintaining a healthy equilibrium between the populations of beneficial and potentially harmful microorganisms in the gastrointestinal tract. They have been associated with health-promoting effects (Lankaputhra et al., 1996) and thus have been incorporated into a wide range of dairy foods marketed resulting in a phenomenal increase in the per capita consumption of yoghurt during the last few decades. Functional Probiotic Yoghurt with Spirulina

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Materials and Methods
Fresh cow's milk obtained from the Dairy Plant, Madras Veterinary College was separated using a cream separator.
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to obtain skim milk for the preparation of yoghurt (control) probiotic yoghurt and Spirulina enriched yoghurt. Skim milk powder testing 5% moisture and 95% solubility was purchased locally (SAGAR brand). Commercially available good quality cane sugar was used as the sweetening agent. Freeze dried DVS cultures containing yoghurt bacteria Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus salivarius ssp. thermophilus was obtained from Chr. Hansen, Denmark. Probiotic Bifidobacterium bifidum (NCDC-232) was obtained from National Collection of Dairy Cultures (NCDC), Division of Dairy Microbiology, National Dairy Research Institute, Karnal (Haryana). Spirulina from Parrys wellness products was used for the preparation of functional yoghurt.

Stock cultures of Lactobacillus delbrueckii ssp. bulgaricus were inoculated and propagated in MRS broth, Streptococcus salivarius ssp. thermophilus in M17 broth and Bifidobacterium bifidum in Bifidobacterium broth.

Preparation of yoghurt
Skimmed yoghurt was prepared as per the procedure outlined by Tamime and Robinson (1988).

Functional probiotic yoghurt
The same procedure was followed as for control yoghurt. In addition to yoghurt cultures 1 per cent of Probiotic Bifidobacterium bifidum was used.

Functional probiotic Spirulina enriched yoghurt
The procedure employed was the same as for functional probiotic yoghurt. In addition 1 gram of Spirulina per litre of yoghurt mix was used during inoculation.

Sensory evaluation
Sensory evaluation was carried out by using nine-point Hedonic scale. All the samples were appropriately coded before subjecting for sensory evaluation.

Chemical analysis
pH was estimated using a Jenway electronic pH meter. Acidity was estimated as per the procedure described in IS: SP: 18 (Part XI) - 1981.

Microbial analysis of Control Yoghurt, Probiotic and Spirulina enriched yoghurt
The presence of coliform organisms was estimated as per the procedure described in IS: 1479 (Part III) -1977. The yeast and mould counts in the samples were estimated as per the procedure described in IS: 1479 (Part III)-1977.

Enumeration of yoghurt bacteria in probiotic functional yoghurt
Enumeration of yoghurt cultures (L. bulgaricus, S. thermophilus and B. bifidum) in control, probiotic and spirulina functional yoghurt were performed on MRS, M17 and Bifidobacter agar respectively. Plates for enumeration of bifidobacteria were incubated at 37°C under aerobic condition. After 48 hours, the plates containing 30-300 colonies were counted for the cultures.

The probiotic functional yoghurt and spirulina enriched probiotic yoghurt were evaluated for sensory, chemical and microbial qualities on 0, 3rd, 7th days during storage at 4°C.

Statistical analysis
Six sets of trial were conducted and the data were subjected to statistical analysis as per the procedure stipulated by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Sensory characteristic of Probiotic and Spirulina enriched functional yoghurt
The Sensory scores of Yoghurt, Probiotic and Spirulina enriched functional yoghurt are presented in Table 1.

Organoleptic qualities revealed that Probiotic yoghurt and control yoghurt scored better in terms of taste due to the medicinal taste perceived on tasting Spirulina enriched yoghurt in initial trials. But this can be masked by adding fruit flavouring agents.

Chemical analysis of yoghurt, Probiotic Yoghurt and Spirulina enriched probiotic yoghurt
Table 2 shows the changes in acidity and pH of fermented milk. No post acidification changes occurred at this temperature. There was no significant difference in the pH and acidity of Probiotic Yoghurt and Spirulina enriched probiotic yoghurt. This coincides with the work of Varga et al., (2002) who found that the pH was stable during refrigerated storage at 4°C.

Table 3 presents the pH and acidity values of Probiotic Yoghurt and Spirulina enriched probiotic yoghurt on 3rd day. The results show no significant difference between the two treatments. This coincides with the work of Varga et al., (2002) and Dola Bhowmik et al., (2009).

Table 4 presents the pH and acidity values of Probiotic Yoghurt and Spirulina enriched probiotic yoghurt on the 7th day. There is a significant difference in the pH and acidity of the two treatments during the 7 day sampling period. The pH of the cyanobacterial samples was significantly higher than that of Probiotic control. This can be accounted for by the fact that the S. plantensis biomass is of alkaline character.

Table 1: Sensory Scores of Probiotic and Spirulina enriched functional yoghurt (Mean ±SE)\(^\circ\)

| Sensory parameters        | Yoghurt | Probiotic | Spirulina |
|---------------------------|---------|-----------|-----------|
| Appearance                | 7.83 ± 0.15 | 7.83 ± 0.15 | 7.66 ± 0.19 |
| Flavour                   | 7.66 ± 0.19 | 7.83 ± 0.15 | 7.33 ± 0.19 |
| Body and texture          | 7.64 ± 0.19 | 7.81 ± 0.24 | 7.81 ± 0.0018 |
| Soursness                 | 7.66 ± 0.18 | 7.66 ± 0.19 | 7.52 ± 0.21 |
| Overall acceptability     | 7.69 ± 0.19 | 7.78 ± 0.00 | 7.58 ± 0.20 |

@ Average of six trials
Sensory scores were given by following the hedonic 9 point scale.
and it had a stimulatory effect on the viability of the probiotic organism. These findings were concurrent with the findings of Verga et al., (2002).

Table 5 shows L. delbrueckii ssp. bulgaricus count of \((10^7) 12.00 \pm 1.39\) and 9.33 \(\pm 1.54\) for probiotic and spirulina enriched yoghurt respectively, S. salivarius ssp. thermophilus count \((10^7)\) of 38.66 \(\pm 2.77\) and 36.16 \(\pm 1.99\) for probiotic and spirulina enriched yoghurt respectively and B. bifidium count \((10^7)\) of 4.00 \(\pm 1.06\) and 6.66 \(\pm 1.02\) for probiotic and spirulina enriched yoghurt respectively. This coincided with the work of Verga et al., (2002) who reported no loss of viability of lactobacilli and other lactic organisms on 0 day. No spoilage organisms were detected indicating the high degree of sanitation during processing and packaging of products.

Table 6 shows no significant difference in the counts of lactic acid bacteria in Probiotic Yoghurt and Spirulina enriched probiotic yoghurt, suggesting that these were growth of lactic acid bacteria. The count of lactic acid bacteria was more in Spirulina enriched probiotic yoghurt.

**Table 2**: Determination of pH and Acidity of yoghurt, Probiotic Yoghurt and Spirulina enriched probiotic yoghurt at 0 day. Mean ± SE of pH and Acidity at 0 day.

| Treatments                                      | pH       | Acidity  |
|-------------------------------------------------|----------|----------|
| Yoghurt                                         | 4.30±0.002 | 0.97±0.003 |
| Probiotic yoghurt                               | 4.31±0.007 | 0.96±0.002 |
| Spirulina enriched probiotic functional yoghurt | 4.31±0.009 | 0.96±0.005 |
| t value                                         | 0.286NS  |          |
| P value                                         | 0.780    |          |

@ Average of six trials

**Table 3**: Determination of pH and Acidity of Probiotic Yoghurt and Spirulina enriched probiotic yoghurt on 3rd day. Mean ± SE of pH and Acidity at 3rd day.

| Parameter       | Probiotic yoghurt | Spirulina yoghurt | t value | P value |
|-----------------|-------------------|-------------------|---------|---------|
| pH              | 4.30 ± 0.003      | 4.31 ± 0.004      | 1.672NS | 0.125   |
| Acidity         | 0.96 ± 0.004      | 0.96 ± 0.005      | 0.466NS | 0.651   |

@ Average of six trials

**Table 4**: Determination of pH and Acidity of Probiotic Yoghurt and Spirulina enriched probiotic yoghurt on 7th day. Mean ± SE of pH and Acidity at 7th day.

| Parameter       | Probiotic yoghurt | Spirulina yoghurt | t value | P value |
|-----------------|-------------------|-------------------|---------|---------|
| pH              | 4.29 ± 0.001      | 4.31 ± 0.004      | 2.863*  | 0.016   |
| Acidity         | 1.11 ± 0.030      | 1.02 ± 0.023      | 2.327*  | 0.042   |

@ Average of six trials

**Table 5**: Microbial evaluation of Probiotic Yoghurt and Spirulina enriched probiotic yoghurt at 0 day. Mean ± SE of Microbial count at 0 day.

| Microbial Quality (10^7) cfu/g | Probiotic Yoghurt | Spirulina enriched probiotic yoghurt | t value | P value |
|--------------------------------|-------------------|--------------------------------------|---------|---------|
| L. delbrueckii ssp. bulgaricus | 12.00 ± 1.39      | 9.33 ± 1.54                          | 1.284NS | 0.22    |
| S. salivarius ssp. thermophilus| 38.66 ± 2.77      | 36.16 ± 1.99                         | 0.737NS | 0.481   |
| B. bifidium                   | 4.00 ± 1.06       | 6.66 ± 1.02                          | 1.807NS | 0.100   |
| Coliform count                | Nil               | Nil                                  |         |         |
| Yeast and Mould count         | Nil               | Nil                                  |         |         |

@ Average of six trials

**Table 6**: Microbial evaluation of Probiotic Yoghurt and Spirulina enriched probiotic yoghurt on 3rd day. Mean ± SE of Microbial count on 3rd day.

| Microbial Quality (10^7) cfu/g | Probiotic Yoghurt | Spirulina enriched probiotic yoghurt | t value | P value |
|--------------------------------|-------------------|--------------------------------------|---------|---------|
| L. delbrueckii ssp. bulgaricus | 9.66 ± 1.28       | 10.16 ± 0.872                        | 0.322NS | 0.753   |
| S. salivarius ssp. thermophilus| 26.00 ± 2.26      | 26.50 ± 0.92                         | 0.204NS | 0.842   |
| B. bifidium                    | 8.33 ± 0.95       | 11.00 ± 0.96                         | 1.963NS | 0.077   |
| Coliform count                 | Nil               | Nil                                  |         |         |
| Yeast and Mould count          | Nil               | Nil                                  |         |         |

@ Average of six trials
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**Table 7:** Microbial evaluation of Probiotic Yoghurt and Spirulina enriched probiotic yoghurt on 7th day. Mean ± SE of Microbial count on 7th day.

| Microbial Quality (10^6) cfu/g | Probiotic Yoghurt | Spirulina enriched probiotic yoghurt | t value | P value |
|-------------------------------|-------------------|-------------------------------------|---------|---------|
| L. delbrueckii ssp. bulgaricus | 25.16 ± 1.83      | 37.50 ± 2.32                       | 4.170*  | 0.001   |
| S. salivarius ssp. thermophilus | 25.50 ± 1.74      | 32.00 ± 1.46                       | 2.855*  | 0.017   |
| B. bifidum                   | 3.00 ± 0.93       | 7.00 ± 1.46                        | 2.309*  | 0.043   |
| Coliform count               | Nil               | Nil                                 |         |         |
| Yeast and Mould count        | Nil               | Nil                                 |         |         |

@ Average of six trials

suggesting that Spirulina would have had a stimulatory effect on lactic cultures as reported by Dola Bhowmik et al., (2009).

Table 7 shows that on the 7th day of *L. delbrueckii* ssp. bulgaricus, *S. salivarius* ssp. thermophilus, *B. bifidum* in Probiotic Yoghurt were 25.16 ± 1.83 x (10^6)cfu/g, 25.50 ± 1.74 x (10^6)cfu/g, 3.00 ± 0.93 x (10^6)cfu/g respectively and in Spirulina enriched probiotic yoghurt the count of *L. delbrueckii* ssp. bulgaricus, *S. salivarius* ssp. thermophilus, *B. bifidum* were 37.50 ± 2.32 x (10^6)cfu/g, 32.00 ± 1.46 x (10^6)cfu/g, 7.00 ± 1.46 x (10^6)cfu/g respectively.

In accordance to Median and Jordano (1995) the initial counts of *Bifidobacterium* spp. declined from 4.00 ± 1.06 x 10^6 cfu/g on 0 day to 3.00 ± 0.93 x (10^6) cfu/g on the 7th day in Probiotic yoghurt and from 6.66 ± 1.02 x (10^6)cfu/g to 7.00 ± 1.46 x 10^6 cfu/g in Spirulina enriched functional probiotic yoghurt. Loss of viability of bifidobacteria during storage was more pronounced than was that of other lactic acid bacteria. This suggested that Spirulina biomass has a stimulatory effect even on the growth of lactic acid bacteria. This may be due to addition of extra cellular products obtained from a late log phase culture of Spirulina which promoted the growth of lactic cultures as reported by Parade et al., (1998).

**CONCLUSION**

A number of health benefits have been claimed by the consumption of foods containing probiotics which are live microorganism actively enhancing the health of consumers by improving the balance of microflora in the gut. Bifidobacteria and certain Lactobacillus species have recently received attention as probiotic organisms maintaining a healthy equilibrium between the population of beneficial and potentially harmful microorganisms in the gastrointestinal tract. Consumers would need to ingest considerably less medicine and artificially produced vitamin and mineral supplements if fermented milks were enriched with vitamins, proteins, essential fatty acids and trace elements of natural origin. The addition of cyanobacterial bio mass to *Bifidobacterium bifidum, S. salivarius* ssp. thermophilus and *L. delbrueckii* ssp. bulgaricus was of beneficial effect on their viability. No spoilage organism was detected at any sampling time, indicating the high degree of sanitation during processing and packaging products.

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