International Journal of

Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 10 (1): 1-11, 2015
ISSN 1811-9743 / DOI: 10.3923/ijds.2015.1.11

Incorporation of *Spirulina platensis* into Probiotic Fermented Dairy Products

Anandi Kavimandan
Department of Biotechnology, Madhav Institute of Technology and Science, Gwalior, India

ABSTRACT

Today the human has become more cautious about their diet and health. Meanwhile pollutant has badly affected the human food stuff. The adulterated food is available for consumption which has very adverse effect on human health. Thus demand of healthy and pure food has increased. So, the world's attention has drawn on the area of probiotic. Probiotic has a good and healthy source of diet for human from centuries. On the other side, algaes are emerging as dietary supplements. Researcher thought about the combination of both in fermented dairy products as medium. Their effort was to enhance the functionality of food quality with addition of algae into it. In result of this combo, the viability of probiotic bacteria was also increased, acidity of food was also increased and their storage quality was also enhanced. There was more viability during storage to deliver more probiotics to human at time of their consumption. This study reviews the supplementation of *Spirulina platensis* on different fermented dairy products and its' effect on their physiochemical, microbiological and sensory attributes.

Key words: *Spirulina platensis*, probiotic, fermented food, lactic acid bacteria

INTRODUCTION

A probiotic is generally defined as a live microbial supplement that affects the host by improving its intestinal microbial balance (Fuller, 1989). There are many health benefits of regular intake of viable probiotic microorganism which includes lactose tolerance (Kim and Gilliland, 1983), antimicrobial (Yildirim and Johnson, 1998), anti-carcinogenic (El-Gawada *et al*., 2004), hypercholesterolemic (Kikuchi-Hayakawa *et al*., 2000; El-Gawada *et al*., 2005) and antimutagenic (Hsieh and Chou, 2006) and various other profitable effects.

Fermented dairy products are the carrier of these beneficial probiotic organisms. Which supply the probiotic in human gut in ample amount. Minimum amount of probiotic bacteria at the time of consumption is 10^5-10^6 CFU mL^{-1} (Samona and Robinson, 1994) but Schuller-Malyoth *et al*. (1968) reported that 10^6-10^8 CFU mL^{-1} is sufficient amount at the time of consumption.

Several reasons which affect the viability of probiotic counts in fermented milks are as follows: pH, titratable acidity, the presence of other microorganisms, temperature, oxygen content, nutrients and growth factors, food additives, application of new technologies such as microencapsulation and formulation of products (Shah, 2000; Gueimonde *et al*., 2004; Mortazavian *et al*., 2009; Cruz *et al*., 2010; Mohammadi and Mortazavian, 2011; Korbekandi *et al*., 2011).

Spirulina is a cyanobacterium, photoautotrophic microorganism which is widely distributed in nature and consumed by human as dietary supplement for decades because of its best known nutritional value.

The dried biomass of *S. platensis* nearly contains 3-7% moisture, 55-60% protein, 6-8% lipids, 12-20% carbohydrate, 7-10% ash, 8-10% fiber, 1-1.5% chlorophyll a and a wide range of vitamins (Belay, 1997; Cohen, 1997; Vonshak, 1997).

Spirulina platensis is especially rich in proteins. The proteins with the highest economic potential are the biliproteins (e.g., c-phycocyanin and allophycocyanin), which are water-soluble blue pigments. The protein fraction may have a phycocyanin content of up to 20% (Cohen, 1997). Fatty acid composition is largely influenced by environmental conditions. *Spirulina platensis* can be characterized by about 45-50% saturated and 50-55% unsaturated fatty acids. Up to 30% of fatty acids are gamma linolenic acid, a rare polyunsaturated fatty acid claimed to have medicinal properties. Beneficial *Spirulina* strains and an efficient processing procedure should yield biomass with at least 1% gamma-linolenic acid (Cohen, 1997; Vonshak, 1997).

Since the late 1970s, *S. platensis* has been marketed and consumed as a safe human food and has been approved for human nutrition by many governments, health agencies and associations of some 80 countries, including the United States and Hungary.

Spirulina have antiviral, anti-inflammatory and anti-tumor effects and it reduces the blood lipid profile, blood sugar, body weight and wound healing time. Henceforth, these microalgae are known as therapeutic and functional food (Fox, 1986; Dillon and Phan, 1993; Parada *et al.*, 1998; Kreitlow *et al.*, 1999; De Caire *et al.*, 2000; Merchant and Andre, 2001; Gyenis *et al.*, 2005).

Spirulina do not have cellulose in its cell wall. That's why it is an appropriate and important foodstuff for patients who have poor intestinal absorption and for geriatric patients (Richmond, 1984). A new high molecular weight polysaccharide with immunostimulatory activity has been isolated from *Spirulina* and is called "Immulina". This highly water-soluble polysaccharide represents the dry matter between 0.5 and 2.0% (w/w) (Pugh *et al.*, 2001).

It is concluded that combination of microalgae and probiotics can enhance growth and increase viability and acid production of probiotics in the products as well as in the gastrointestinal tract.

With reference to previous study (Shirota *et al.*, 1964; Stengel, 1970; Zielke *et al.*, 1978; Kurita *et al.*, 1979; Webb, 1982), the substances responsible for the stimulatory properties of this cyanobacterial biomass were identified as adenine, hypoxanthine and free amino acids due to their alkaline character (Gibson and Roberfroid, 1995; Parada *et al.*, 1998). On the other hand, microalgae present in fermented milks will affect the sensory properties of the final product.

EFFECT OF *S. PLATENSIS* ON DAIRY PRODUCTS AND PROBIOTIC

Guldas and Irkin (2010) studied the effect of *Spirulina platensis* dry biomass on probiotics of yoghurt and acidophilus milk. The main motive of study was to investigate the effect of dry *S. platensis* on plain yoghurt and *Lactobacillus acidophilus* containing yoghurt during refrigerated storage. All samples were prepared in high sterile condition in laboratory. The amount of *S. platensis* powder was taken 0.5 and 1% w/v, respectively. The pH and acidity of sample was controlled in 4°C of storage. Viability of samples was checked on 1, 5, 10, 15, 20, 25 and 30 days of storage. The viability of plain yoghurt was checked as *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* and the cell number of *L. acidophilus* in *L. acidophilus* containing yoghurt. Throughout the study in all investigation the viable count of all lactic acid bacteria were above 6 CFU g^{-1} in all *S. platensis* powder added samples. Control samples without *Spirulina* shown lower viability. There was no any significant difference observed in viable counts of samples of 1% w/v *Spirulina* powder concentrations ($p \leq 0.5$). In sensory analysis the 0.5% w/v concentration of samples score high than 1% w/v concentration of *Spirulina* containing samples. The study concluded that the viability of lactic acid bacteria was good during 30 days of storage of yoghurt.

In the study of all bacteria kept their viability as recommended compare to previous studies (Gueimonde *et al.*, 2004). But when compared with Akalin *et al.* (2009) study, the *L. bulgaricus* viability in yoghurt containing *Spirulina* was not high. The observations explain that there was not any significant difference in viability of *L. acidophilus* in both acidophilus milk and probiotic yoghurt ($p \leq 0.5$). There was a clear difference in viability of bacteria between *Spirulina* containing and non containing samples. Then, there was not any major difference between 0.5 and 1% w/v *Spirulina* powder enriched samples.

Slightly greenish color and algal flavor was investigated in yoghurt containing 1% w/v *S. platensis* powder. So, 0.5% w/v concentration of sample was accepted. It was assumed that with 1% w/v powder addition in fruit flavored yoghurt can not affect its sensory qualities.

Due to the presence of bioactive substance in *S. platensis* it possesses high nutritional values. Thus, it provides new opportunities for dairy products. Varga *et al.* (2002) investigated the influence of powder of *S. platensis* on bacteria of fermented milk. *Spirulina* enriched and non-enriched (control) fermented Acidophilus Bifidus Thermophilus (ABT) milk was produced using a starter culture having *Lactobacillus acidophilus*, *Bifidobacteria* and *Streptococcus thermophilus*. The sample was incubated at 40°C for 6 h. Then at pH 4.5-4.6, *S. platensis* powder was added. With reference to the study of Springer *et al.* (1998) they took 3 g L^{-1} *Spirulina* biomass to be optimum for sensory evolution and cost of products. Then cooled the sample at 25°C and filled into sterile and capped centrifuge tubes then cooled at 4°C for 24 h and then stored the half of samples at 15°C for 18 days and at 4°C for 42 days. Then all analyses were performed on 0, 3, 6, 9, 12, 15 and 18th day of storage at 15°C samples and at 4°C samples were analyzed on 0, 7, 14, 21, 28, 35 and 42 days of storage. The results predict that the powder of *S. platensis* showed a beneficial effect on the survival of ABT starter culture at both temperatures. The pH and titratable acidity values of 15°C samples showed a difference but the samples kept at 4°C showed stability in the reading of pH and titratable acidity. Counts of *L. acidophilus* were in the range of 10^7 CFU mL^{-1} at each day observation and *S. thermophilus* counts value were above than 10^9 CFU mL^{-1} in most cases in fact the counts of *Bifidobacteria* which are susceptible to acid were also good in samples. There was not any contamination detected during storage time which gave the evidence of highly sterile conditions. With above benefits the amino acid, essential vitamins and fatty acids present in milk were also improved due to cyanobacterial biomass addition.

Similar study was done by Gyenis *et al.* (2005). They studied the *Spirulina platensis* powder on acid production and growth of *Lactobacillus plantarum* and *Enterococcus faecium* starins. They used 3 g dm^{-3} *Spirulina* powder and thus solid content was ranging from 12-30%. The results showed the stimulation in acid production and growth rate of *L. plantarum* and *E. faecium* ($p<0.5$). Thus results showed that it was suitable for cost effective production of fermented feeds. And rapid production of acid also prevents the growth of undesirable microorganisms. In the present study, it was proved that *L. plantarum* was slightly poorer acidifier than *E. faecium* because the pH of products were between 5.15-5.34 and 4.62-5.10 in control and *Spirulina* supplemented samples after 22 h of fermentation at 30°C, respectively but cyanobactrerial biomass was significantly effective on *L. plantarum* in whole process at $p<0.5$. Thus it proved more productivity in shorter time.

Another study was done by Mocanu *et al.* (2013). The objective of the study was to investigate the effect of *S. platensis* biomass on microflora of fermented products. The fermented milks were produced using pasteurized milk, powder milk and Bb12 (*Bifidobacterium animalis* ssp. *lactis*) and La-5 (*Lactobacillus acidophilus*) starter cultures. About 0.5 and 1% *S. platensis* powder was added to products. The final products were stored at 5±1°C for 15 days. During the incubation and storage time the titratable acidity, pH, syneresis, water holding capacity, dynamic viscosity bacterial count were measured. Due to the lactose fermentation, titratable acidity was growing fast during the incubation period. The highest titratable acidity was obtained in 1% microalgae added and Bb12 inoculated samples. The result was 0.99 g lactic acid/mL product in Bb12 inoculated and 1% *Spirulina* added samples and in La-5 inoculated and 1% *Spirulina* added 0.936 g lactic acid/mL product and the lowest 0.783 g lactic acid/mL was in control of La-5 inoculated samples at the 15th day of storage.

In microalgae added products, the water holding capacity was reduced to 13.39% at the end of storage period and maximum viable counts 3.3×10^{7} CFU mL^{-1} product was found in Bb12 containing 1% microalgae added sample. The rheological analysis of product did not show any significant effect on *Spirulina* added products. No difference in the shear stress and dynamic viscosity of fluid.

In order to promote the functionality of dairy products the *Spirulina* was used abundantly. In the study of Beheshtipour *et al*. (2012); the impact of *Arthospira platensis* on probiotic yoghurt was determined. Three concentrations of *A. platensis* were taken (0.25, 0.50 and 1%) and a control sample was taken (without supplementation of cyanobacteria). They evaluated the effect on different parameters e.g., pH, titratable acidity and redox potential and viability of bacteria during fermentation and 28 days of storage at 5°C. The probiotic bacteria composition of yoghurt was *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. The supplementation of *A. platensis* significantly increases the bacterial counts of *L. acidophilus* and *Bifidobacteria* at the end of fermentation and during storage period. The *A. platensis* treated samples showed slower pH decline, faster acidity increase, longer incubation time and greater final acidity than control. The 1% enrichment of algal biomass in simple showed better results in both probiotic microorganism inoculated samples. It was found that 0.5% enriched sample also gave more than 10^{7} CFU mL^{-1} of bacterial count until the end of storage. All samples were run in triplicates, the lactic acid and acetic acid quantity was also measured at the end of 28 days of storage. The 0.5% addition of microalgae biomass gave more satisfactory organoleptic evaluation results than 1% addition. Thus it was more cost effective. Assessment of lactic acid and acetic acid was done by HPLC. The 1% microalgae addition showed lowest acceptability and unpleasant flavor. The cause of this low value is due to oxidation of polyunsaturated fatty acid; due to the presence of minerals as pro-oxidant. The color change was observed form greenish to bluish and graininess caused by insoluble algae particles. So, it has lowest score for mouthfeel. No significant difference was obtained between the treatments containing 0.25 and 0.50% microalgae ($p<0.05$) powder.

THERAPEUTIC BENEFITS

Therapeutic benefits of probiotic microorganism played a crucial role in modern diet of human being. So, to intensify this quality, now researcher used microalgae addition in fermented dairy products. Thus, Bhowmik *et al*. (2009) performed an experiment to see the stimulation in growth of three lactic acid bacteria *L. acidophilus* MTCC447; *L. casesi* MTCC1423 and *S. thermophilus* MTCC1938 on addition of *S. platensis* biomass at various amount e.g., 1 , 5 and 10 mg mL^{-1}. They promoted the growth up to 171.67 and 185.84%, respectively at pH 6.2. The maximum growth was observed at 10 mg mL^{-1} concentration of *S. platensis* up to 10 h at 145.90, 171.67 and 185.84% in *L. casei*, *L. acidophilus* and *S. thermophilus*, respectively.

Gibson and Roberfroid (1995) predicted that the bifidobacterial count were 0.2 log cycle higher in *Spirulina* that supplemented products than in control during storage. The one reason behind this was the presence of *Spirulina* that stimulate the acid production in samples. So, a pH value was lower in that sample. Similar results were also obtained in previous studies and minimum growth was observed in *S. thermophilus* in 10 mg mL^{-1} enriched *Spirulina* samples compared to other lactic acid bacteria.

Similar study was done by Fadaei *et al.* (2013) but differ from others by the addition of spinach in *Spirulina* added yoghurt. They saw the effect on *L. bulgaricus* and *S. thermophilus* counts of products. They took 0.3, 0.5 and 0.8% w/w *Spirulina* concentration and added it in to yoghurt samples. They used spinach in 10 and 13% w/w amount in products enriched with *Spirulina*. The yoghurt samples were stored at 4°C and evaluated on 1, 7, 14 and 21 days of storage period. Viable counts of all lactic acid bacteria were above than 6 log CFU mL^{-1} in all *Spirulina* supplemented products until the end of storage ($p \leq 0.01$). It was determined that samples containing 0.5% w/w *Spirulina* and 10% spinach were selected as most significant. The viability of *S. thermophilus* was higher than *L. bulgaricus* at the end of storage. Yoghurt with 0.8% *Spirulina* had maximum effect on increasing the viable numbers of bacteria. But thus caused increase in product cost. With this addition of 0.5% *Spirulina* maintained the number according to the standards of International Dairy Federation during storage. There was no major difference in viability of probiotic bacteria on addition of two concentration of spinach but it was effective in sensory evaluation of samples.

In the studies of Varga *et al.* (2012a), they worked on the impact of *Arthospira platensis* on growth and acid production (pH) of many strains of *Lactococcus* and *Leuconostoc* in milk. By making of *Spirulina* containing cultured milk and investigated its effect on viability of lactococci in refrigeration period. Use of 0.3% *Spirulina* biomass was significant for many mesophilic LAB strains. In organoleptic evaluation of products the maximum score was achieved in mixed cultures of *Lactococcus lactis* ssp. *lactis* NCAIM B.2128 and *L. lactis* ssp. *cremoris* ATCC 19257 enriched with sucrose at 10% and *S. platensis* biomass at 0.3% and strawberry-kiwifruit flavor at 1.5%; during the first two weeks of refrigerated storage at 4°C, the *Spirulina* dry matter increases the counts of *Lactococci* in fermented milk products. As described by the hungry food regulations, LAB cultures should contain minimum 10^7 CFU g^{-1} over entire shelf life of products and *Lactococci* counts were high in both the products. Enumeration of probiotic bacteria was done by the pour plate technique in 0, 6 and 12 h of interval.

Different health authorities in the world are looking forward that the probiotic products should deliver at least 10^6-10^7 CFU g^{-1} to large intestine at the time of consumption (Glasen, 1992; Krishnakumar and Gordon, 2001). Various researchers reported that *Bifidobacterial* number is often low in fermented dairy products (Klaver *et al.*, 1993; Hughes and Hoover, 1995; Lankaputhra *et al.*, 1996; Adhikari *et al.*, 2000). Varga *et al.* (2012b) studied the effect of oligofructose, insulin, honey and powder *S. platensis* on probiotic bacteria present in the milk both during fermentation and refrigeration specially in *Bifidobacterium* spp.; in above all natural substrates, the *S. platensis* have an antifungal effect on spoilage of yeast and molds during storage. There counts were very less as compared to control. About 0.3% cyanobacterial biomass has stimulatory effect on pH of *L. acidophilus* La-5 in milk as well as *Bifidobacterium animalis* ssp.

Lactis Bb-12 in milk compared to control and the mixed culture of both was also effective in pH depletion with same proportion of algal biomass. With these, having significant change in viable counts in milk compared to control.

EFFECT OF AQUEOUS SUSPENSION OF *SPIRULINA PLATENSIS*

De Caire *et al.* (2000) were studied the effect of aqueous suspension of *Spirulina platensis* 945 dry biomass extracted at pH 6.8 and 5.5 and its effect on four lactic acid bacteria *S. thermophilus* TH4, *L. delbruekii* ssp. *bulgaricus* YL1, *L. lactis* ssp. *lactis* C2 and *L. acidophilus* LO1 present in milk. *Spriulina platensis* grown in zarrouk media were filtered at stationary phase and washed with acid until its pH reached at 7. Then dried the biomass at 30°C. Two suspension of *Spirulina* were prepared. One with distilled water at pH 6.8 and other in phosphate 0.1 M pH 5.5 and kept in refrigerator for 24 h. They were then added in reconstituted skim milk 10% w/v and heated at 100°C for 20 min. The TH4, YL1 and C2 were cultured at 37°C in milk with suspension of *S. platensis* to a final concentration of 3 mg mL^{-1} at pH 6.8 and C2 and LO1 at pH 5.5 with 6 mg mL^{-1} and control were without *Spirulina*. Samples were taken at 0, 2, 4, 8, 10 and 20 h of intervals to investigate the live bacterial counts. Increment in C2 and LO1 growth in 6 mg mL^{-1} samples at pH 5.5 were observed by 22.3% after 4 h and 22.8% after 8 h, respectively. For 3 mg mL^{-1} of sample at pH 6.8, 13.42% for C2, 9.29% for YL1 and 8.22% for TH4 compared to control and after 8 h it was 3.46, 9.73 and 7.76%, respectively.

IMPACT OF MICROALGAE ON BACTERIAL STRAINS

Varga (1999) developed a way to use *S. platensis* biomass enriched with trace elements for manufacturing of fermented milks; to stimulate the acid production and growth rate of LAB. The effect of 3 g L^{-1} of *Spirulina* with trace elements on pure and mixed culture of *S. salivaricus* ssp. *thermophilus* CH1, *L. delbrueckii* ssp. *bulgaricus* CH2, *L. acidophilus* La-5 and *Bifidobacteria bifidum* Bb12 in milk medium was observed.

The components of cyanobacterial biomass responsible were iodine, zinc, selenium, vitamin (B complex, C, A, E) and nitrogenous compounds (peptone, adenine, hypoxanthine) were tested. In the experiment the rate of inoculation was 1% (v/v) and for *Bifidobacteria bifidum* Bb12 6% (v/v). The *S. thermophilus* and *L. bulgaricus* were inoculated at 42.5°C where as *L. acidophilus* and *Bifidobacteria bifidum* at 37.5°C. The pH was checked by 1 h of interval. It was concluded that the microalgae have a positive impact on four strains of starter culture with variation.

The effect of *S. platensis* on *S. thermophilus* during 2-5 h of fermentation was due to the presence of trace element and nitrogenous components with vitamin addition, have good acid production in it but have greater effect on *L. bulgaricus*. In case of *L. acidophilus* the peptone and vitamin were most effective than any other substances; vitamin E and selenium inhibited the acid production but in *Bifidobacteria bifidum* only peptone enhances acid production to a satisfactory level. In case of mixed cultures of above strains the rate of inoculation was between 0.1 and 6% v/v with respect to a single strain. The mixed culture of *S. thermophilus* and *L. bulgaricus* was incubated at 42.5°C and *L. acidophilus* and *Bifidobacteria bifidum* at 37.5°C.

In the combination of *S. thermophilus* and *L. bulgaricus*, acid production was increased but growth unaffected. In combination of *S. thermophilus* and *L. bulgaricus* both production and growth was enhanced. In case of *S. thremophilus* and *Bifidobacteria bifidum* both pH and growth kinetics was inhibited.

When *L. bulgaricus* or *L. acidophilus* mixed with *Bifidobacteria bifidum*, then there was an increase in growth of rod shaped *Lactobacilli*. When the effect was seen on combination of *S. thermophilus*, *L. acidophilus* and *Bifidobacteria bifidum* then the growth of *Bifidobacteria bifidum* enhanced remarkably.

Then in storage period of 30 days at 4°C and 15 days at 15°C the growth of rod shaped bacteria increased in sample than coccus shaped. At 4°C of storage viability was high over 10^8 CFU g^{-1} of yoghurt samples and pH was above 4 in entire storage time.

Yeast and mold counts were in both control and concentration samples over 10^1 CFU g^{-1} by 6th day and 10^5 CFU g^{-1} by 15th day at 15°C; in one month of storage at 4°C, lower counts of yeast and molds than control. This was due to antifungal property of *S. platensis*. *Enterococci* and coliforms were not found in any sample.

The growth of microorganisms was also depending on interaction between rod and cocci. Excessive acid production prevents undesirable microorganism in products.

In the study of Asvanyi-Molnar *et al*. (2009), the objective was to the study changes in acid production of mesophilic lactic acid bacteria grown in milk. Milk samples enriched with *Spirulina* at different concentration (0, 0.3, 0.5 and 0.8%) were inoculated at the rate of 1% with the mesophilic LAB strains to be tested. The pH was investigated at regular intervals. Result of the study showed that *Spirulina* concentration were effective to increase the acidity of lactococci (*L. lactis* ssp. *lactis* Ha-2 and *L. lactis* ssp. *cremoris* W-24). The cyanobacterial biomass used 0.1-0.8% was significantly increase ($p<0.05$) the acidity development by lactococci between 6th and 12th h of the fermentation process. Optimum organoleptic properties were achieved in the product formulation prepared with the mixed culture of *L. lactis* ssp. *lactis* NCAIM B.2128 and *L. lactis* ssp. *cremoris* ATCC 19257 and supplemented with sucrose at 10%, *Spirulina* biomass at 0.3% and strawberry-kiwifruit puree at 1.5%.

Parada *et al*. (1998) demonstrated the stimulatory effect of *Spirulina* addition on the growth of coccus shaped starter bacteria in their studies. This happened due to the presence of extracellular products in late lag phase of *Spirulina*. So it was suggested that the extracellular products got from late log phase of *Spirulina* culture were stimulated the LAB viability. In this investigation, the filtrate of *Spirulina* was added to deman rogosa and sharpe agar with this the bacterial growth of all strains were enhance. A similar effect was observed using an enrichment media. Zarrouk media addition same as culture filtrate did not change the growth observed in media without extracellular products. So to get to know the media composition, chemical analysis were performed 7.3, 1.7 and 2% before the growth of *S. platensis* and 11.5, 8.9 and 1.3% after late log phase. Change in the record showed that *Spirulina* acted as photoautotropic microorganism that consumed nitrogen from the culture media and liberated exopolysaccharide and other compounds that could be responsible for the stimulatory effect on LAB.

Varga and Szigeti (1998) found minimum 8 log CFU mL^{-1} for viable counts of *S. thermophilus* in both natural and algal yoghurt during storage at 4°C. The survival rate of *S. thermophilus* was better than that of both *L. bulgaricus* and *B. animalis*. The viable counts of *S. thermophilus* were higher by 2-3 log orders than those for *L. bulgaricus* in yoghurt samples.

Molnar *et al*. (2005) reported that the addition of *S. platensis* caused a decrement in pH of yoghurt sample. The cause of this decline was the effect of *Spirulina* on *L. bulgaricus*. Higher viable counts were found in 1st day storage of microalgae supplemented yoghurt. They also studied the effect of *Spirulina* biomass on single strain of mesophilic lactic acid bacteria used in concentration of 3 g dm^{-3}. It significantly stimulate the acid production of ($p<0.05$) probiotic bacteria during the first 2 weeks of storage at 4±2°C, with increase in bacterial counts; due to its alkaline nature and buffering capacity; during fermentation and 1st week of storage the viability percentage was declined.

Prakash and Pooja (2011) reported the preparation of low fat and high protein frozen yoghurt supplemented with papaya pulp and *Spirulina*. The aim of the study was to obtain better quality frozen yoghurt with optimum concentration of *Spirulina*. It was found that 2-8% of *Spirulina* with 100% papaya pulp were more acceptable. The frozen yoghurt with 6% *Spirulina* and 10% papaya pulp was found best having score 8.6 in sensory characteristics among all treatments. It was found that higher level of *Spirulina* badly affect the organoleptic characteristics of frozen yoghurt.

CONCLUSION

Thus we can consume algae supplemented probiotic food in same cost. The counts were sufficient at the time of consumption and there was a very less chance of contamination. So, it is easy to intake two sources in same time in same cost and takes benefit of both. As we know that the *S. platensis* rich in vitamin and amino acid content and Lactic Acid Bacteria (LAB) are consumed for good gut health.

Algae and LAB combination gives a new opportunity for manufacturer related to sensory evaluation. Combination of these two with other dietary sources e.g., fruits and vegetables can make new healthy and tasty food. It gives new opportunity in the taste, color, flavors, texture and quality, some more fermented food and with addition of traditional fermented food in same cost. In fact, co-culturing of algae with microorganism responsible for fermentation other than LAB e.g., *Saccharomyces cerevisiae* and *Aspergillus niger* can work together on sensory attributes to make more desirable food stuff for consumers in health benefit.

REFERENCES

Adhikari, K., A. Mustapha, I.U. Grun and L. Fernando, 2000. Viability of microencapsulated bifidobacteria in set yogurt during refrigerated storage. J. Dairy Sci., 83: 1946-1951.

Akalin, A.S., G. Unal and M.C. Dalay, 2009. Influence of *Spirulina platensis* biomass on microbiological viability in traditional and probiotic yogurts during refrigerated storage. Ital. J. Food Sci., 21: 357-364.

Asvanyi-Molnar, N., Z. Sipos-Kozma, A. Toth, B. Asvanyi and L. Varga, 2009. Development of functional dairy food enriched in spirulina (*Arthrospira platensis*). Tejgazdasag, 69: 15-22.

Beheshtipour, H., A.M. Mortazavian, P. Haratian and K.K. Darani, 2012. Erratum to: Effects of *Chlorella vulgaris* and *Arthrospira platensis* addition on viability of probiotic bacteria in yogurt and its biochemical properties. Eur. J. Food Res. Technol., 235: 1213-1213.

Belay, A., 1997. Mass Culture of *Spirulina* Outdoors the Earthrise Farms Experience. In: *Spirulina platensis (Arthrospira)* Physiology Cell Biology and Biotechnology, Vonshak, A. (Ed.). Taylor and Francis, London, pp: 131-158.

Bhowmik, D., J. Dubey and S. Mehra, 2009. Probiotic efficiency of *Spirulina platensis*-Stimulating growth of lactic acid bacteria. World J. Dairy Food Sci., 4: 160-163.

Cohen, Z., 1997. The Chemicals of *Spirulina*. In: *Spirulina platensis* (Arthrospira): Physiology, Cell-Biology and Biotechnology, Vonshak, A. (Ed.). Taylor and Francis Ltd., UK., pp: 175-204.

Cruz, A.G., R.S. Cadena, E.H.M. Walter, A.M. Mortazavian, D. Granato, J.A.F. Faria and H.M.A. Bolini, 2010. Sensory analysis: Relevance for prebiotic, probiotic and synbiotic product development. Compr. Rev. Food Sci. Food Saf., 9: 358-373.

De Caire, G.Z., J.L. Parada, M.C. Zaccaro and M.M.S. de Cano, 2000. Effect of *Spirulina platensis* biomass on the growth of lactic acid bacteria in milk. World J. Microbiol. Biotechnol., 16: 563-565.

Dillon, J.C. and P.A. Phan, 1993. Spirulina as a source of proteins in human nutrition. Bulletin l'Institut Oceanographique, 12: 103-107.

El-Gawad, A.I.A., E.M. El-Sayed, S.A. Hafez, H.M. El-Zeini and F.A. Saleh, 2004. Inhibitory effect of yoghurt and soya yoghurt containing bifidobacteria on the proliferation of Ehrlich ascites tumour cells *in vitro* and *in vivo* in a mouse tumour model. Br. J. Nutr., 92: 81-86.

El-Gawada, A.I.A., E.M. El-Sayeda, S.A. Hafez, H.M. El-Zeinia and F.A. Saleh, 2005. The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. Int. Dairy J., 15: 37-44.

Fadaei, V., F. Mohamadi-Alasti and K. Khosravi-Darani, 2013. Influence of *Spirulina platensis* powder on the starter culture viability in probiotic yoghurt containing spinach during cold storage. Eur. J. Exp. Biol., 3: 389-393.

Fox, R.D., 1986. Algaculture: La Spirulina, un espoir pour le monde de la faim. Edisude, France.

Fuller, R., 1989. Probiotics in man and animals. J. Applied Microbiol., 66: 365-378.

Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr., 125: 1401-1412.

Glasen, H., 1992. Living bacteria in yoghurt and other fermented milk products. Eur. Dairy Mag., 1: 6-15.

Gueimonde, M., S. Delgado, B. Mayo, P. Ruas-Madiedo, A. Margolles and C.G. de los Reyes-Gavilan, 2004. Viability and diversity of probiotic *Lactobacillus* and *Bifidobacterium* populations included in commercial fermented milks. Food Res. Int., 37: 839-850.

Guldas, M. and R. Irkin, 2010. Influence of *Spirulina platensis* powder on the microflora of yoghurt and acidophilus milk. Mljekarstvo, 60: 237-243.

Gyenis, B., J. Szigeti, N. Molnar and L. Varga, 2005. Use of dried microalgal biomasses to stimulate acid production and growth of *Lactobacillus plantarum* and *Enterococcus faecium* in milk. Acta Agraria Kaposvariensis, 9: 53-59.

Hsieh, M.L. and C.C. Chou, 2006. Mutagenicity and antimutagenic effect of soymilk fermented with lactic acid bacteria and bifidobacteria. Int. J. Food Microbiol., 111: 43-47.

Hughes, D.B. and D.G. Hoover, 1995. Viability and enzymatic activity of bifidobacteria in milk. J. Diary Sci., 78: 268-276.

Kikuchi-Hayakawa, H., N. Onodera-Masuoka, M. Kano, S. Matsubara, E. Yasuda and F. Ishikawa, 2000. Effect of soymilk and Bifidobacterium-fermented soymilk on plasma and liver lipids in ovariectomized Syrian hamsters. J. Nutr. Sci. Vitaminol., 46: 105-108.

Kim, H.S. and S.E. Gilliland, 1983. *Lactobacillus acidophilus* as a dietary adjunct for milk to aid lactose digestion in humans. J. Dairy Sci., 66: 959-966.

Klaver, F.A., F. Kingma and A.H. Weerkamp, 1993. Growth and survival of bifidobacteria in milk. Neth. Milk Dairy J., 47: 151-164.

Korbekandi, H., A.M. Mortazavian and S. Iravani, 2011. Technology and Stability of Probiotics in Fermented Milks. In: Probiotic and Prebiotic Foods: Technology, Stability and Benefits to Human Health, Shah, N.P., A.G. da Cruz and J.D.A.F. Faria (Eds.). Chapter 7, Nova Science Publishers, New York, USA., ISBN-13: 9781616688424, pp: 131-169.

Kreitlow, S., S. Mundt and U. Lindequist, 1999. Cyanobacteria-a potential source of new biologically active substances. J. Biotech., 70: 61-63.

Krishnakumar, V. and I.R. Gordon, 2001. Probiotics: Challenges and opportunities. Dairy Ind. Int., 66: 38-40.

Kurita, H., O. Tajima and T. Fukimbara, 1979. Isolation and identification of nucleosides in *Chlorella* extract. J. Agric. Chem. Soc., 53: 131-133.

Lankaputhra, W.E.V., N.P. Shah and M.L. Britz, 1996. Survival of bifidobacteria during refrigerated storage in presence of acid and hydrogen peroxide. Milchwissenschaft, 17: 65-70.

Merchant, R.E. and C.A. Andre, 2001. A review of recent clinical trials of the nutritional supplement *Chlorella pyrenoidosa* in the treatment of fibromyalgia, hypertension and ulcerative colitis. Altern. Ther. Health Med., 7: 79-91.

Mocanu, D.G., E. Botez, V.O. Nistor, G.D. Andronoiu and G. Vlasceanu, 2013. Influence of *Spirulina platensis* biomass over some starter culture of lactic bacteria. J. Agroaliment. Process. Technol., 19: 474-479.

Mohammadi, R. and A.M. Mortazavian, 2011. Review article: Technological aspects of prebiotics in probiotic fermented milks. Food Rev. Int., 27: 192-212.

Molnar, N., B. Gyenis and L. Varga, 2005. Influence of a powdered *Spirulina platensis* biomass on acid production of lactococci in milk. Milchwissenschaft, 60: 380-382.

Mortazavian, A.M., K. Rezaei and S. Sohrabvandi, 2009. Application of advanced instrumental methods for yogurt analysis. Crit. Rev. Food Sci. Nutr., 49: 153-163.

Parada, J.L., G.Z. de Caire, M.C.Z. de Mule and M.M.S. de Cano, 1998. Lactic acid bacteria growth promoters from *Spirulina platensis*. Int. J. Food Microbiol., 45: 225-228.

Prakash, D.R. and K. Pooja, 2011. Preparation of low fat and high protein frozen yoghurt enriched with papaya pulp and *Spirulina*. Trends Biosci., 4: 182-184.

Pugh, N., S.A. Ross, H.N. ElSohly, M.A. ElSohly and D.S. Pasco, 2001. Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos*-aquae and *Chlorella pyrenoidosa*. Planta Med., 67: 737-742.

Richmond, A., 1984. Spirulina. In: Micro-algal Biotechnology, Borowitzka, M.A. and L.J. Borowitzka (Eds.). University Press, Cambridge, UK., pp: 85-121.

Samona, A. and R.K. Robinson, 1994. Effect of yogurt cultures on the survival of bifidobacteria in fermented milks. J. Soc. Dairy Technol., 47: 58-60.

Schuller-Malyoth, R., A. Ruppert and F. Muller, 1968. The microorganisms of the bifidus group (*Lactobacillus bifidus*). I. Historical review, nutritional, physiological and therapeutic aspects, morphology, culture procedures and taxonomy. Milchwissenschaft, 23: 356-360.

Shah, N.P., 2000. Probiotic bacteria: Selective enumeration and survival in dairy foods. J. Dairy Sci., 83: 894-907.

Shirota, M., N. Nagamatsu and Y. Takechi, 1964. Method for cultivating lactobacilli. U.S. Patent No. 3,123,538.

Springer, M., O. Pulz, J. Szigeti, V. Ordog and L. Varga, 1998. Verfahren zur Herstellung von biologisch hochwertigen Sauermilcherzeugnissen. European Patent No. DE 19654614 A1. http://www.google.com.bz/patents/DE19654614A1?cl=zh.

Stengel, E., 1970. Anlagentypen und Verfahren der technischen Algenmassenproduktion. Berichte der Deutschen Botanischen Gesellschaft, 83: 589-606.

Varga, L. and J. Szigeti, 1998. Microbial changes in natural and algal yoghurts during storage. Acta Alimentaria, 27: 127-135.

Varga, L., 1999. Effect of a cyanobacterial biomass enriched with trace elements on thermophilic dairy starter cultures. Ph.D. Thesis, Pannon Agricultural University, Hungary.

Varga, L., J. Szigeti, R. Kovacs, S. Buti and T. Foldes, 2002. Influence of a *Spirulina platensis* biomass on the microflora of fermented ABT milks during storage (R1). J. Dairy Sci., 85: 1031-1038.

Varga, L., N.M. Asvanyi and J. Szigeti, 2012a. Manufacturing technology for a *Spirulina*-enriched mesophilic fermented milk. Proceedings of the International Scientific Conference on Sustainable Development and Ecological Footprint, March 26-27, 2012, Sopron, Hungary.

Varga, L., J. Sule and J. Szigeti, 2012b. Stimulation of probiotic lactobacilli and bifidobacteria in cultured dairy foods. Proceedings of the International Scientific Conference on Sustainable Development and Ecological Footprint, March 26-27, 2012, Sopron, Hungary.

Vonshak, A., 1997. Appendices. In: *Spirulina platensis* (Arthrospira) Physiology, Cell-Biology and Biotechnology, Vonshak, A. (Ed.). Taylor and Francis Ltd., London, UK., pp: 213-226.

Webb, L.E., 1982. Detection by Warburg manometry of compounds stimulatory to lactic acid bacteria. J. Dairy Res., 49: 479-486.

Yildirim, Z. and M.G. Johnson, 1998. Characterization and antimicrobial spectrum of bifidocin B, a bacteriocin produced by *Bifidobacterium bifidum* NCFB 1454. J. Food Prot., 61: 47-51.

Zielke, H., H. Kneifel, L.E. Webb and C.J. Soeder, 1978. Stimulation of lactobacilli by an aqueous extract of the green alga *Scenedesmus acutus* 276-3a. Eur. J. Applied Microbiol. Biotechnol., 6: 79-86.