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Influence of *Spirulina platensis* powder on the starter culture viability in probiotic yoghurt containing spinach during cold storage

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ABSTRACT

Functional foods are among the topical issues in nutrition, nutritional foods, medicine and health fields. Considering the unique properties of Spirulina platensis, it can be used to enrich yogurt. The main purpose of this research was to monitor the influence of the powdered Cyanobacterium Spirulina platensis added to the yoghurt containing Lactobacillus acidophilus and spinach on the survival of yogurt starter culture during the refrigerated storage. The cell viability of yoghurt starter cultures (Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus) and Lactobacillus acidophilus under refrigeration conditions in yoghurts prepared with (0.3, 0.5 and 0.8 (w/w) %) the addition of Spirulina powder and with (10 and 13 (w/w) %) spinach was investigated. The yoghurts were prepared under hygienic laboratory conditions. The samples of yoghurts were stored at 4 °C and investigated on days 1, 7, 14 and 21. Viable counts of the lactic acid bacteria were above 6 log cfumL^{-1} of all "Spirulina powder" added at the end of the storage period. The results showed the positive effect of S. platensis powder on the survival of the lactic acid bacteria during the storage of probiotic yoghurt containing spinach ($P \leq 0.01$). It was determined that Spirulina powder added to yoghurt is a good medium for lactic acid bacteria during the 21 days of the refrigerated storage. According to final results, the probiotic yoghurt sample containing 0.5 % (w/w) Spirulina platensis and 10 %(w/w) spinach was selected as the preference treatment.

Key words: *Spirulina*, yoghurt starter culture, functhonal foods, spinach yoghurt.

INTRODUCTION

Because of the changing dietary habits of the general public, functional foods are products of interest to many people. 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. A simple way of attaining this goal is the use of cyanobacteria in the manufacture of cultured dairy foods (Varga *et al.*, 1999, Varga *et al.*, 2002). *Spirulina* has been used for many years as additive since it has high protein content and nutritional value. Cyanobacteria (blue-green algae) are photoautotrophic microorganisms which are widely distributed in nature. *Spirulina* is the best known genus of *Cyanobacteria* because of its unique nutritional properties (Caire *et al.*, 2000). It has been proved that consumption of S*pirulina* is beneficial to health due to its chemical composition including compounds like essential amino acids, vitamins, natural pigments, and fatty acids, especially ω-6 representatives such as α-linolenic (GLNA) acid, a precursor of the prostaglandin hormones in the body. In addition to high quality proteins, it contains high amounts of calcium, vitamin B12, Vitamin A, B2, B6, E, K and H, and many essential minerals and enzymes. *Spirulina* is also very rich in terms of iron content (Fox, 1986; Henrikson, 1994; Akalin *et al.*, 2009).

Yoghurt and particularly probiotic yoghurts contribute to health by providing natural nutrients and by enrichment of the intestinal microbiota with lactic acid bacteria and probiotic cultures. They provide several benefits for humans. Among them are better resistance to infections, stimulation of the immune system and better absorption of minerals and lactose (Hove *et al.*, 1999; Caire *et al.*, 2000). The probiotic activity of some strains with the ability to colonize the intestinal epithelium strengthening to stabilize the intestinal microflora, especially after antibiotic treatment, has been well described in previous researches (Piard and Desmazeaud, 1992; Gasson, 1993; Gardiner *et al.*, 2002; Kearney *et al.*, 2008).

Interest for probiotics has arisen in recent years especially in relation to the addition of *Bifidobacterium, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus reuteri* to the fermented dairy products such as yoghurt (Samaržija *et al.*, 2009). After ingestion, these probiotic cultures are believed to play a significant role in the intestinal system against some of the pathogenic microorganisms such as *Helicobacter pylori*, *Salmonella typhi* and *Yersinia enterocolitica* (Gardiner *et al.*, 2002; Jay *et al.*, 2005). To develop expected therapeutic benefits, a sufficient number of viable microorganisms must be assured throughout the entire shelf life of the product (Parada *et al.*, 1998).Throughout the shelf life, the product must have a certain number of viable probiotic microorganisms to provide therapeutic effects.

It has been observed that *Spirulina platensis (S. platensis)* powder promotes the growth of lactic acid bacteria in synthetic media (Caire et al., 2000) and milk (Varga *et al.*, 2002; Akalin *et al*., 2009) and yoghurt made with *Bifidobacterium animalis* and 0.3 % *S. platensis* powder addition (Akalin *et al*., 2009). However, there was no research in related to effect *S. platensis* on the survival of yogurt starter culture in yoghurt containing spinach. In this research, the effect of micro algae; *Spirulina platensis* (0.3, 0.5 and 0.8 %) was examined on the viability of lactic acid bacteria in probiotic yogurt containing 10 and 13 % spinach.

MATERIALS AND METHODS

Starter cultures and ingredients:

- Cow's milk (Bargozideh company, Iran).
- A commercial yogurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* (Yc – 380 ,Chr. Hansen A/S, Hørsholm, Denmark) and *Lactobacillus acidophilus* (*La – 5*,Chr. Hansen A/S, Hørsholm, Denmark) were used in a freeze dried direct vat set (DVS), and stored according to the producer's recommendations.
- *Spirulina platensis* (Powdered form, Sina micro-algae Company, Iran).
- blanched chopped spinach.

Yoghurt preparation:

Yoghurts (set style) were made from cow's milk contains 3% (w/w) fat and 8 % (w/v) total solids.

Skim milk powder (2 % w/w), whey powder (1%) and 50% cream (3% w/w) were added to raw milk at 38 °C. Raw milk was treated at 90 - 95 °C for 5-10 min, and then cooled to 42 - 40 °C (for fermentation). All cultures were used according to the manufacturer's instructions. Milk was inoculated with yogurt culture and mixed thoroughly; immediately *Spirulina platensis* powder (0, 0.3, 0.5, 0.8 %(w/w)) was added to the milk and incubated at 40 °C. Fermentation continued up to the pH of 4.5 ± 0.02. After fermentation, yoghurt samples were cooled to 22 - 15 °C. Following blanched chopped spinach addition (10 and 13% (w/w)), the mixture was packed into 500-g plastic cups, transferred to a refrigerator at 4 °C and stored at this temperature for 21 days.

Microbiological analysis:

The samples were taken aseptically and the serial dilutions were prepared with 0.1 % sterile peptone water. M17 agar (Oxoid, UK) was used to enumerate *S. thermophilus*. The pH of the medium was 7.1. The inoculated plates were incubated at 37 °C for 48 h under aerobic conditions (*Varga*, 2006).

Acidified MRS agar (Oxoid, UK) with pH 5.4 was used for enumeration of *L. bulgaricus* (Varga , 2006). The plates were incubated at 37 °C for 72 h under anaerobic conditions.

Statistical analyses:
Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) procedure using SAS software (version 9.1; Statistical Analysis System Institute Inc., Cary, NC, USA). LSMEAN were used to compare the difference among mean values at the significant level of 0.01($p<0.01$). All experiments were replicated three times.

RESULTS AND DISCUSSION

Changes in Lactobacillus bulgaricus viability in probiotic yoghurt containing spinach during cold storage:
According to Fig.1 and 2, the presence of *S. platensis* powder caused a significant increase of viable counts of *L. bulgaricus* in the yoghurts ($p\leq0.01$). The presence of spinach did not cause the significant increase of viable counts of *L. bulgaricus* in the yoghurts. Increasing the storage time did not cause the significant increase of viable counts of *L.bulgaricus* in the yoghurts. Similar effects were also reported by Akalin *et al.* (2009) in yoghurt, Varga *et al.* (2002) in fermented milk, Fox (1986) and Metin and Irkin (2010).

Changes in Streptococcus thermophilus viability in probiotic yoghurt containing spinach during cold storage:
According to Fig. 3 and 4, the presence of *S. platensis* powder caused a significant increase of viable counts of *S. thermophilus* in the yoghurts ($p\leq0.01$). The presence of spinach did not cause the significant increase of viable counts of *S. thermophilus* in the yoghurts. Increasing the storage time caused the increase of viable counts of *S. thermophilus* in the yoghurts during the twenty-one days. Similar effects were also reported by Akalin *et al*. (2009) in yoghurt, Varga *et al*. (2002) in fermented milk, Fox (1986) and Metin and Irkin (2010).

The viability of *S. thermophilus* was higher than of *L. bulgaricus* (Fig.1,2,3 and 4) at the end of the storage period.,as reported similarly by previous researchers.

In general, the viability of the bacteria in all yoghurts increased when *S. platensis* powder was added during the storage period. Yoghurt starter bacteria need nutrients to grow and survive (Kearney *et al.*, 2008). *S. platensis* powder may represent a unique source of nutrients for these bacteria since it contains significant concentrations of amino acids, precursors of nucleic acids, vitamins, mineral and etc., among them also derivation of vitamin B which is a well known promoter for the probiotic bacteria (Fox, 1986). From the survival curves, it can be seen that *S. platensis* powder added into the all yoghurt resulted in a better growth of all added bacteria. It was probably caused by the nutritive properties of *S. platensis* designated by Akalin et al. (2009). Better growth and survival can be explained with the presence of nitrogenous substances such as free amino acids, peptone etc. derived from *S. platensis* biomass (Varga *et al.*, 1999; Varga *et al*., 2002; Molnar *et al*., 2005; Akalin *et al*., 2009).

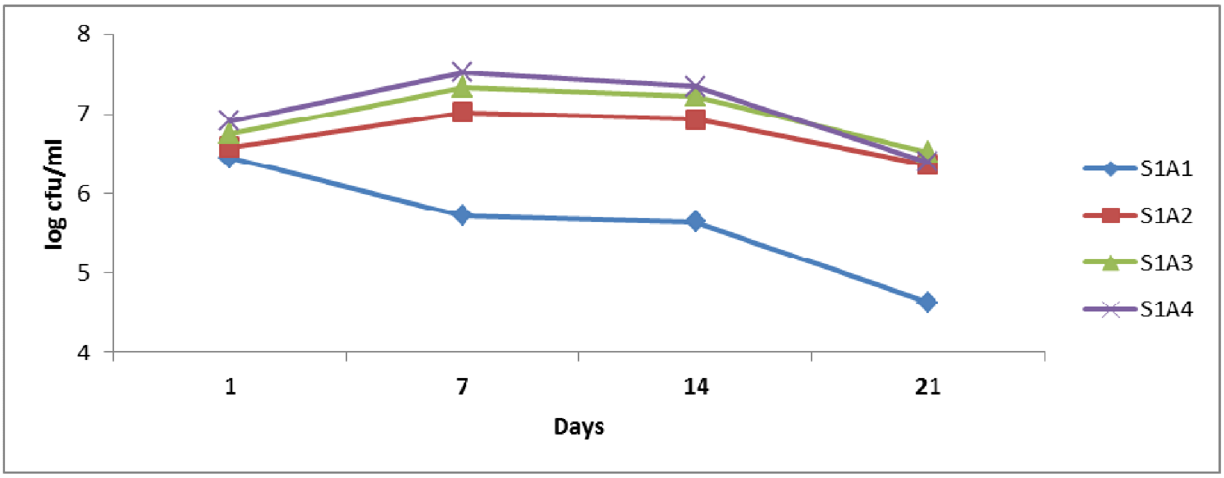

Figure 1. Changes in the viable counts of *L. bulgaricus* (log cfumL^{-1}) in probiotic yoghurt containing *spirulina* (S_1A_1:0% , S_1A_2 :0.3% , S_1A_3 :0.5% , S_1A_4 : 0.8%) and 10% spinach during the storage

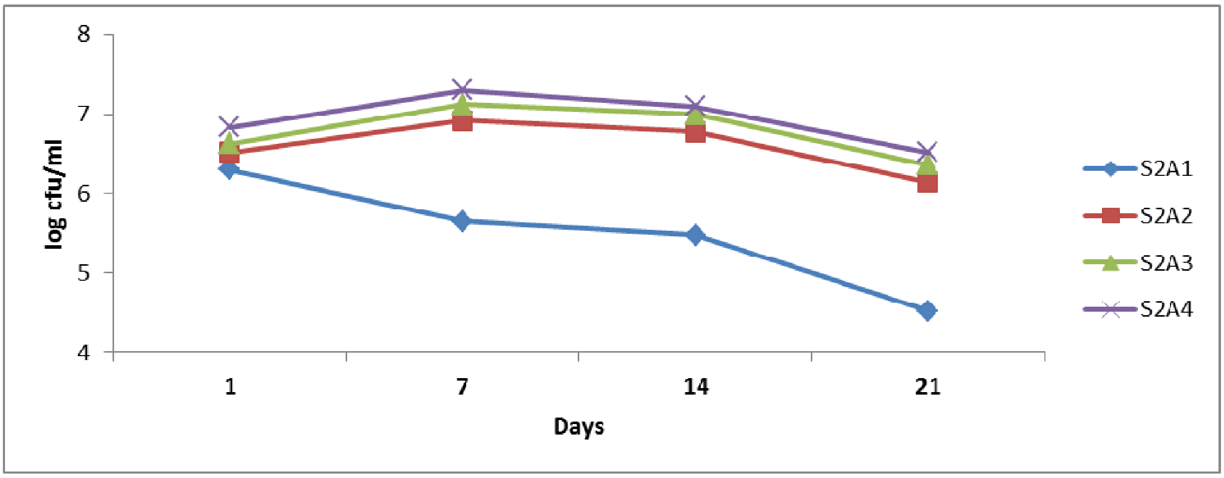

Figure 2. Changes in the viable counts of *L. bulgaricus* (log cfumL^{-1}) in probiotic yoghurt containing *spirulina* (S_2A_1:0% , S_2A_2 :0.3% , S_2A_3 :0.5% , S_2A_4 : 0.8%) and 13% spinach during the storage

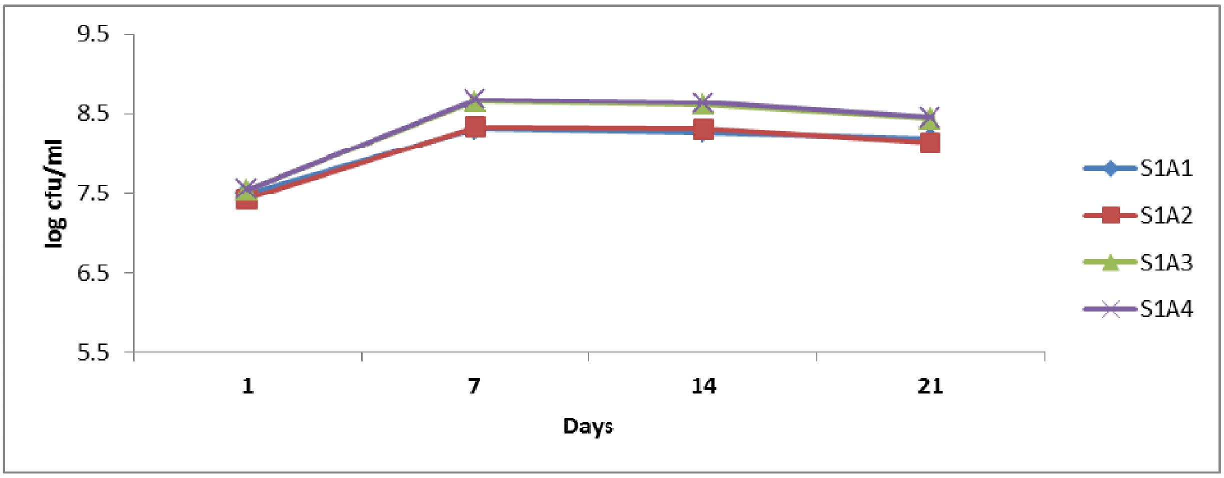

Figure 3. Changes in the viable counts of *S. thermophilus* (log cfumL^{-1}) in probiotic yoghurt containing *spirulina* (S_1A_1:0% , S_1A_2 :0.3% , S_1A_3 :0.5% , S_1A_4 : 0.8%) and 10% spinach during the storage

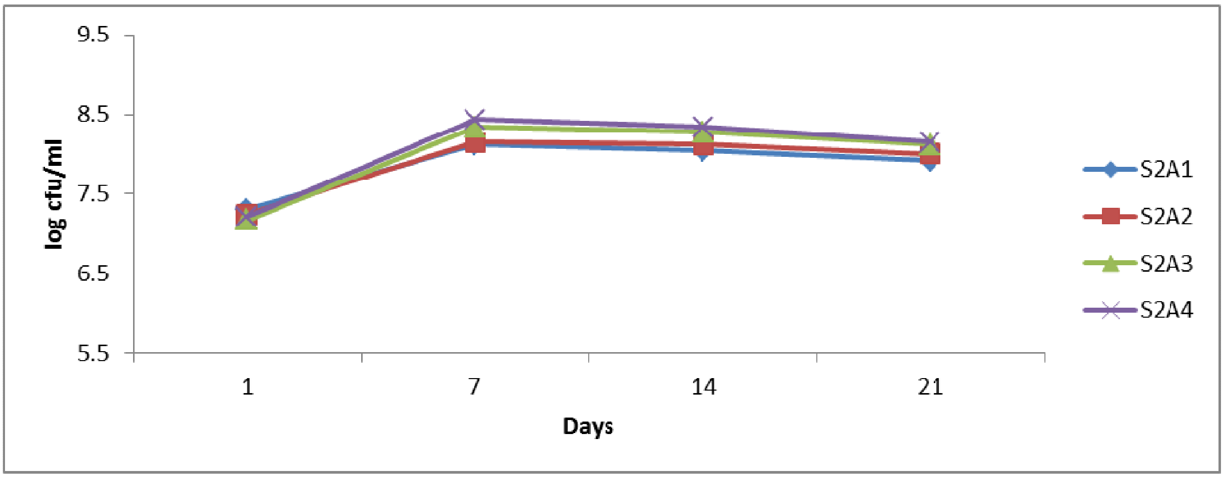

Figure 4. Changes in the viable counts of *S. thermophilus* (log cfumL^{-1}) in probiotic yoghurt containing *spirulina* (S_2A_1:0% , S_2A_2 :0.3% , S_2A_3 :0.5% , S_2A_4 : 0.8%) and 13% spinach during the storage

In addition to the above benefits, the cyanobacterial biomass increased the essential amino acid and vitamin contents of cow's milk and also improved its fatty acid composition. The abundance of bioactive components in *S. platensis* is of great importance from a nutritional point of view because the cyanobacterial biomass provides a new opportunity for the manufacture of functional dairy foods (Varga *et al*., 2002).

According to the taste panel (not reported data), the highest scores were given by the panelists to the probiotic yoghurt containing 0.5 % *spirulina platensis* and 10 % spinach.

CONCLUSION

The results of this research demonstrated that the *S. platensis* biomass positively influenced the yoghurt starter bacteria viability in the probiotic yoghurt containing spinach during the storage period. Yoghurt with 0.8 % *spirulina* had the maximum effect on increasing the viability of yoghurt starter bacteria; but, caused the increase of production costs. Yoghurt with 0.5 % *Spirulina* maintained the number of probiotic bacteria at International Dairy Federation (IDF) standard level during storage. Between the two concentrations of spinach, there was no difference in yoghurt starter bacteria viability; but only, it was effective in the sensory evaluation of the product ,so that the concentration of 10% spinach had higher scores. So, the probiotic yoghurt sample containing 0.5 % (w/w) *Spirulina platensis* and 10 %(w/w) spinach was selected as the preference treatment.

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