Original scientific paper - Izvorni znanstveni rad UDK: 637.146.34

Influence of *Spirulina platensis* powder on the microflora of yoghurt and acidophilus milk

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Received - Prispjelo: 08.09.2010.
Accepted - Prihvaćeno: 22.11.2010.

Summary

The main purpose of this research was to monitor the influence of the powdered *Cyanobacterium Spirulina platensis* addition to plain yoghurt and the yoghurt containing *Lactobacillus acidophilus* on survival of the microbiota 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 (0.5 or 1.0 (w/w) %) and without the addition of Spirulina powder was investigated. The yoghurts were prepared under hygienic laboratory conditions and their pH and acidity were controlled during the process. The samples of yoghurts were stored at 4 °C and investigated on days 1, 5, 10, 15, 20, 25 and 30. Viable counts of the lactic acid bacteria were above 6 cfu $g^{-1}$of all "spirulina powder" added samples whereas control yoghurt samples contained lower lactic acid bacteria count at the end of the storage period. Addition of 1 % *Spirulina platensis* powder into the yoghurts did not cause significant differences on the viable lactic acid bacteria ($p\leq0.05$). The results showed the positive effect of *S. platensis* powder on the survival of the lactic acid bacteria during storage of yoghurt ($P\leq0.05$). The sensory analysis was also performed for the yoghurt samples. Sensory scores of 0.5 % spirulina powder added yoghurt samples were better than 1 % spirulina powder added ones. It was determined that spirulina powder added yoghurt is a good medium of lactic acid bacteria during the 30 days of refrigerated storage.

Key words: *Spirulina*, *L. acidophilus*, yoghurt, probiotic yoghurt, acidophilus milk

Introduction

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 *Spirulina* is beneficial to health due to its chemical composition including compounds like essential amino acids, vitamins, natural pigments, and fatty acids, especially w-6 representatives such as g-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 B_{12}, Vitamin A, B_2, B_6, 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; Radulović et al., 2010).

Yoghurt and particularly probiotic yoghurts contribute to health by providing natural nutrients and by enrichment of the intestinal biota 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

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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. Adequate numbers of viable cells, namely the "therapeutic minimum", need to be consumed regularly to provide probiotic effect for consumers. Consumption should be more than 100 g per day of probiotic yogurt containing more than 10^6 cfu mL^{-1} (Lourens-Hattingh and Viljoen, 2001). The levels of probiotic bacteria available in these products have been reported between 10^5 to 10^8 cfu mL^{-1}, previously (Gueimonde et al., 2004).

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* addition and 0.3 % *S. platensis* powder (Akalin et al., 2009). However, there was limited number of researches of the *S. platensis* effects on the survival of Lactobacilli strains in yoghurt.

In this study, the aim was to investigate the effects of *S. platensis* powder on the survival of experimental microbiota in three yoghurt types during the storage (30 days at 4 °C). The investigated yoghurts and their microbiota were as follows: *L. acidophilus* in acidophilus milk (1), *L acidophilus*, *Lactobacillus delbrueckii ssp. bulgaricus* (*L. bulgaricus)* and *Streptococcus salivarius ssp. thermophilus (S. thermophilus*) in probiotic yoghurt (2) and *L. bulgaricus* and *S. thermophilus* in plain yoghurt (3).

Materials and methods

Starter cultures and ingredients

A commercial yogurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* (DELVO-D YOG CY 221 DSL, DSM Food Specialities, Moorebank, Australia) and *Lactobacillus acidophilus* (DELVO-PRO-LAFTI- L 10DSL, DSM Food Specialities, Moorebank, Australia) were used in a freeze dried direct vat set (DVS) form containing 10 E-11 and 10 E-10 cfu g^{-1}, respectively, and stored according to the producer's recommendations. Skim milk powder was provided by Sutas Dairy Products (Bursa, Turkey). *Spirulina platensis* was used in a powdered form (EGE RT Spirulina, EGE University, Bornova, Izmir, Turkey) and obtained from Ege University Science and Technology Research Center, Project Number: 99 BIL-06 (Bornova, Izmir, Turkey).

Yoghurt preparation

Yogurts (set style) were made from cow's milk containing 3.4 (w/v) milk fat and 3.04 % (w/v) protein. Skim milk powder (3.5 % w/v) was added to raw milk at 38 °C. Raw milk was treated at 87 °C for 15 min, and then cooled to 45 °C (for plain and probiotic yoghurt) or 40 °C (for acidophilic yoghurt).

Three groups of product were prepared: (a) plain yoghurt contained only yoghurt starters, (b) probiotic yoghurt contained *L. acidophilus* in addition to yoghurt starter bacteria, and (c) acidophilus milk contained only *L. acidophilus*. The milk for each yoghurt type was divided into three aliquots: for control yoghurt which did not contain *S. platensis* powder, for yoghurt produced with 0.5 % (w/v) addition of *S. platensis* powder, and for yoghurt produced with 1.0 % (w/v) addition of *S. platensis* powder.

All cultures were used according to the manufacturer's instructions. Yoghurt starter cultures were poured into sterilized milk after the cooling to 45 °C and 40 °C and mixed thoroughly; starter culture mixtures were added in concentration of 0.1 % w/v. The mixtures were poured into 100 mL plastic cups and incubated at 40 °C and 42 °C until the pH 4.7 was reached. After fermentation, yoghurt samples were cooled and transferred to a refrigerator at 4 °C and stored at this temperature for 30 days.

Chemical analysis

Total solids, protein and the fat content were determined according to the Turkish Standard TS1330 (TSE, 2006). The pH value was recorded using a pH meter (Hanna Instruments, HI221 Microprocessor, Hanna Instruments Inc., Woonsocket, Rhode-Island).

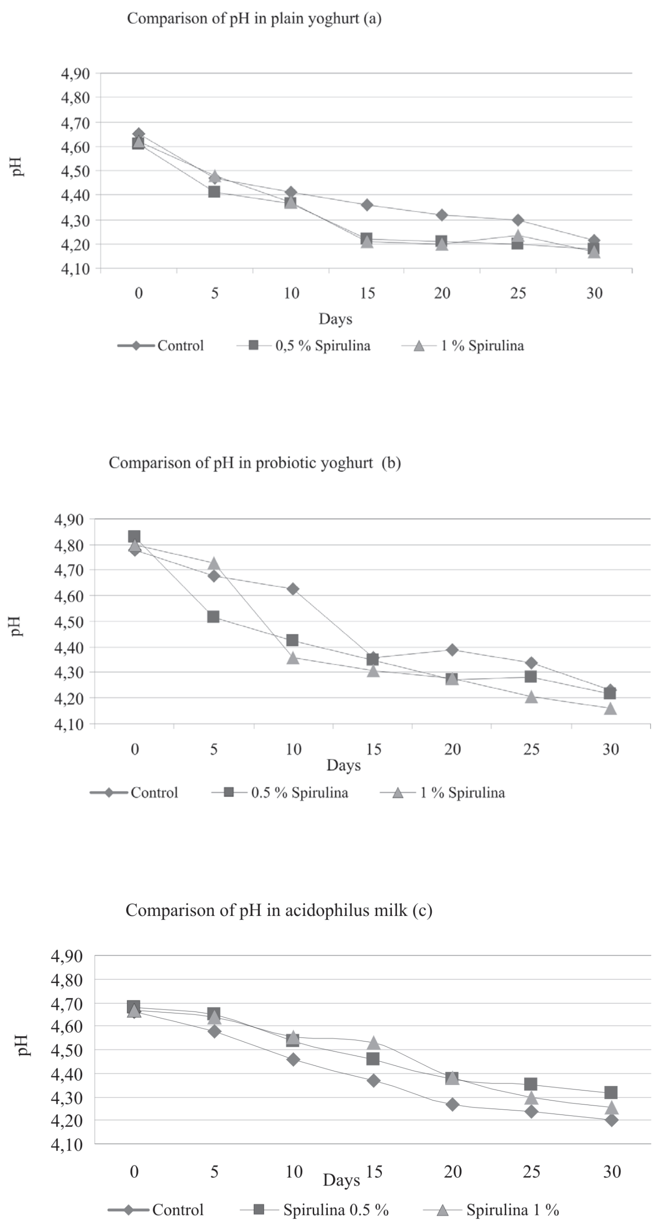

Figure 1. Comparison of pH values in plain yoghurt (a), probiotic yoghurt (b) and acidophilus milk (c) regarding to *Spiriluna platensis* powder content, N=3

Microbiological analysis

Samples were taken aseptically by using a laminar flow cabinet (Nüve Basic Laboratory Equpment, MN090-120, Class-II, Nüve Inc., Ankara, Turkey) 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. MRS- Sorbitol agar was used for determination of *L. acidophilus* after incubation at 37 °C for 3 days under anaerobic conditions (Tharmaraj and Shah, 2003).

Sensory analysis

The sensory characteristics were evaluated according to Metin (Metin, 2006). A panel composed of 5 experienced members from Balikesir university was used to determine the yoghurts' score for external appearance (color), flavor, taste and texture with a point scale from 0 to 5 (0 means spoiled sample and unacceptable for human consumption and 5 means very good).

Statistical analyses

Statistical analyses were done by using SPSS 15.0 software for Windows, SPSS Inc., Chicago, Illinois, USA. One-way analysis of variance (ANOVA) test were done for determination of the mean differences. The level of significance between means was determined by the Tukey HSD test.

Results and discussion

Moisture, protein and fat content of yoghurts were determined as 88.15±0.2 %, 4.0±0.2 % and 3.8±0.4 % for the three batches intended for three types of yoghurts, respectively.

According to Figure 1a, b and c, the increase in the *S. platensis* powder content caused slight decrease in the pH values of the experimental yoghurt samples (p≤0.01). pH values of the three yoghurt types were decreased to approximately 4.20 during the storage period. This was probably caused from the addition of powdered *S. platensis* which promoted the growth of experimental bacteria.

Similar findings related to this decrease in yoghurts caused by *S. platensis* powder, were also notified by Varga et al. in the *Spirulina* added yoghurts (Varga and Szigeti, 1998; Varga et al., 2002).

Both yoghurt starter bacteria and probiotic bacteria such as *L. acidophilus* 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 derivates of vitamin B which is a well known promoter for the probiotic bacteria (Fox, 1986). From the survival curves (Figures 2 and 3), it can be seen that *S. platensis* powder addition into the all yoghurt types resulted in better growth of all added bacteria. It was probably caused by the nutritive properties of *S. platensis* designated by Akalin et al. (2009).

In general, the viability of the bacteria in all yoghurts increased when *S. platensis* powder was added during the storage period. However, the difference among higher (1 %) and lower (0.5 %) addition was not seen always observed. (Figures 2 and 3). Most probiotic bacteria are more sensible to environmental factors and have higher nutritional requirements than the other bacteria (Gardiner et al., 2002). For this reason, the second expectation was that the nutritive properties of *S. platensis* powder might have especially positive influence on *L. acidophilus* tested.

As seen from Fig. 2b and 3b, the presence of *S. platensis* powder did not cause the significant increase of viable counts of S. *thermophilus* in the yoghurts (p≤0.05). The viable counts increased maximum 0.5 log cfu g^{-1} in plain yoghurt compared to control sample. After 1 % of *Spirulina* powder addition, the viable counts were 6.5 and 7.7 log cfu g^{-1} in plain yoghurt and probiotic yoghurt at the end of the storage, respectively (Figures 1b and 2b). The viable counts of *S. thermophilus* were slightly lower than others' findings which were about 8 log cfu g^{-1} in algal yoghurts (Fox, 1986; Varga and Szigeti, 1998). Caire et al. (2000) also reported that *S. platensis* powder stimulated the growth of *S. thermophilus* in milk. It was reported by Varga et al. (2002) that the viable counts of *S. thermophilus* in ABT fermented milks containing *S. platensis* powder were higher than in the control samples at the end of the refrigerated storage.

The difference in the viable counts of the samples with *S. platensis* powder between initial and last day of storage was maximum 1.6 and 1.0 log cfu g^{-1} in plain and probiotic yoghurts, respectively. Similar effects were also reported by Akalin et al. (2009) in yoghurt and Varga et al. (2002) in fermented milk.

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

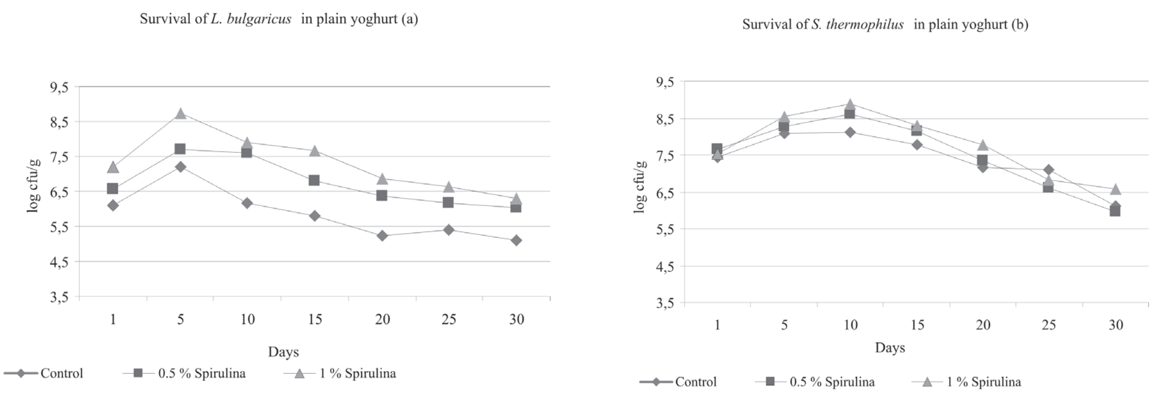

Figure 2. Changes in the viable counts of *L. bulgaricus* (a) and *S. thermophilus* (b) in plain yoghurt during the storage (log cfu g^{-1})

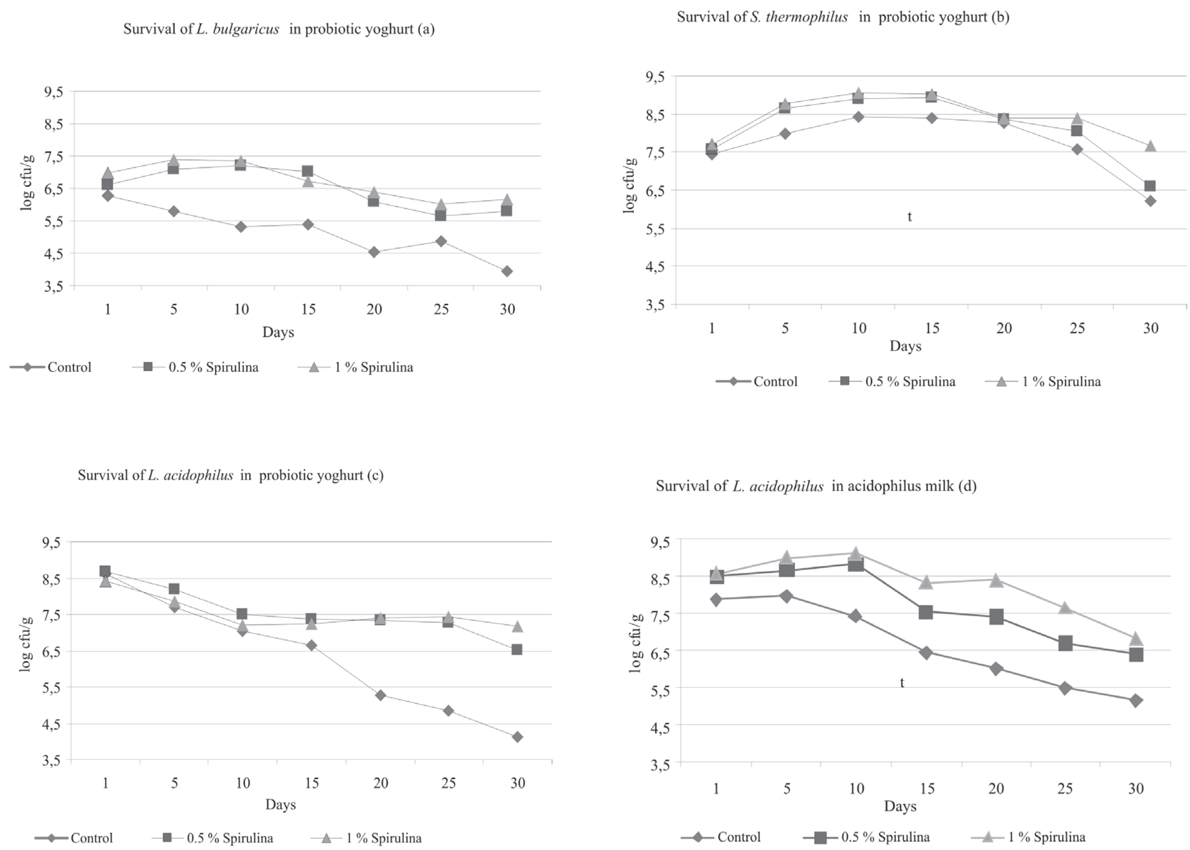

Figure 3. Changes in the viable counts of *L. bulgaricus* (a), *S. thermophilus* (b) and *L. acidophilus* (c) in probiotic yoghurt and *L. acidophilus* in acidophilus milk (d) during the storage (log cfu g^{-1})

the level of *S. thermophilus* was 1.5 log cfu g^{-1} higher than *L. bulgaricus* in probiotic yoghurt (Vinderola et al., 2000; Akalin et al., 2009).

As seen from Figure 2 a and 3 a, the viable cell counts of *L. bulgaricus* changed more significantly when the content of *S. platensis* powder was increased ($p \leq 0.05$). If we consider the end of the storage, after *Spirulina* powder addition in concentrations of 0.5 and 1 %, the presence of *L. bulgaricus* increased 1 and 1.3 log cfu g^{-1} according to control sample in plain yoghurt, respectively (Figure 2a). This increase was about 1.8 and 2.2 log cfu g^{-1} in the probiotic yoghurt, respectively (Figure 3a). 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).

In the present study, the counts of *L. bulgaricus* in the samples *Spirulina* powder added were above 6 log cfu g^{-1} in plain yoghurt and 5.80 log cfu g^{-1} in probiotic yoghurt. The *L. bacillus* counts in yoghurts were mentioned as above 7 log cfu g^{-1} by Varga and Szigeti (1998) and below 6 log cfu g^{-1} by Akalin et al. (2009). In contrary to the study of Akalin et al. (2009), *L. bulgaricus* counts were not higher than the counts of other bacteria. The increase in the survival rate of *L. bulgaricus* together with L. acidophilus in probiotic yoghurt can be attributed to the competitiveness among the bacteria as already observed previously (Varga et al., 2002; Donkor et al., 2006).

L. acidophilus count increased 2.4 and 3.1 log cfu g^{-1} in the samples with 0.5 and 1.0 % *Spirulina* powder at the end of storage period, respectively. The evaluation of the growth curves of *L. acidophilus* indicated that the survival of *L. acidophilus* was higher than the others during the whole storage period. *L. acidophilus* showed higher counts when compared to *L. bulgaricus* and *S. thermophilus*. Similar result related with *L. acidophilus* was also found in reconstituted sweet whey by Matijević et al. (2009). They found that *L. acidophilus* better grew during fermentation in comparison with *Bifidobacterium animalis* subsp. *lactis* regardless to the added amount of lactulose.

The similar relationship between plain yoghurt and probiotic yoghurt was also observed for the viable counts of other two yoghurt bacteria investigated. This can be explained by the fact that the growth of probiotic bacteria populations in the fermented functional foods are much lower in the presence of the starters than when the probiotics are grown alone (Champagne and Mollgaard, 2008). According to the taste panel, the highest scores were given by the panelists to the probiotic yoghurt. *Spirulina* content did not influence the taste and the score of the yoghurts, significantly ($p \leq 0.05$). However, the scores of acidophilic yoghurt were also high. There was no excessive sour taste reported in acidophilic yoghurt.

Conclusion

All investigated bacteria kept their viable numbers in the recommended levels, compared to the previous studies (Gueimonde et al., 2004). The viability of *L. bulgaricus* in the yoghurts with *S. platensis* powder addition was not as high as found, previously (Akalin et al., 2009). This observation may be explained with the differences among the strains of probiotic bacteria (*Bifidobacterium animalis*) used in both studies. There was no significant difference between the acidophilus milk and the probiotic yoghurt in terms of viable *L. acidophilus* counts ($p \leq 0.05$).

From the comparison of the viable counts between control samples and the samples in which *S. platensis* powder was added, it was obvious that the viability of the bacteria was increased when *S. platensis* powder was added. However, it was found that the effect of 1 % *S. platensis* powder addition on the viable counts was similar to that of 0,5 % addition. Considering sensory properties, the slightly greenish color and algal flavor was assigned by the panelists to the yoghurt in which 1 % of *S. platensis* powder was added. Therefore, 0.5 % of *S. platensis* powder addition would be more acceptable than 1 %. However, it can be speculated that in fruit yoghurt or fruit flavored yoghurts, 1 % of *S. platensis* powder might also be used without negatively affecting the organoleptic properties.

Utjecaj praška Spiruline platensis na mikrofloru jogurta i acidofilnog mlijeka

Sažetak

Glavni cilj ovog istraživanja bio je ispitivanje utjecaja dodatka praškastog *Cyanobacterium Spirulina platensis* u jogurt te jogurt obogaćen s *Lactobacillus acidophilus* na preživljavanje mikroorganizama tijekom čuvanja u hladnjaku. Ispitivano je preživljavanje starter kulture jogurta (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*) te *Lactobacillus acidophilus* u uzorcima pripremljenim uz dodatak 0,5 % ili 1 % (w/w) te bez dodatka spiruline, nakon čuvanja u hladnjaku. Uzorci su pripremljeni u higijenskim laboratorijskim uvjetima te je tijekom postupka kontrolirana njihova pH vrijednost i kiselost. Uzorci jogurta čuvani su na 4 °C te su ispitivanja izvršena nakon 1, 5, 10, 15, 20, 25 i 30 dana čuvanja u hladnjaku. Broj živih stanica bakterija mliječne kiseline bio je iznad 6 cfu/g za sve uzorke s dodanim praškom *Spiruline platensis*, dok su kontrolni uzorci jogurta sadržavali manje bakterija mliječne kiseline na kraju vremena skladištenja. Dodatak 1 % *Spiruline platensis* u uzorke jogurta nije dao značajne razlike u broju živih bakterija mliječne kiseline ($p \leq 0,05$). Rezultati pokazuju pozitivan učinak dodatka praška *Spiruline platensis* na preživljavanje bakterija mliječne kiseline tijekom razdoblja skladištenja jogurta. Provedena je i senzorska analiza pripremljenih uzoraka. Senzorske ocjene uzoraka pripremljenih uz dodatak 0,5 % *Spiruline platensis* bile su bolje od uzoraka uz dodatak od 1 %. Zaključeno je da su uzorci kojima je dodan prašak dobar medij za bakterije mliječne kiseline tijekom 30 dana hladnog skladištenja.

Ključne riječi: *Spirulina*, *L. acidophilus*, jogurt, probiotički jogurt, acidofilno mlijeko

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