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INFLUENCE OF *SPIRULINA PLATENSIS* POWDER ON VIABILITY OF *LACTOCOCCUS* STRAINS IN PROBIOTIC UF FETA CHEESE CONTAINING *MENTHA LONGIFOLIA* L.

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ABSTRACT

The objective of this research was to determine the effect of the powdered *Spirulina platensis* on viability of cheese starter cultures, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*, under refrigeration conditions. Retentate inoculated with the cheese starter cultures (1%w/v) and *L.acidophilus* (2%w/v) was enriched with *Mentha longifolia L.* (0.5 and 1%) and *Spirulina platensis* (0, 0.3, 0.5 and 0.8%). The samples of cheeses produced were stored at 4ºC for 45 days. The results showed the positive effect of *S. platensis* powder on the survival of Lactococcus strains during storage of cheese (P<0.05). It was found that the effect of 0.8% *S .platensis* powder addition on the viable counts was comparable to that of 0.3 and 0.5% addition. No differences were observed between cheeses manufactured with the different levels of *Mentha longifolia L.*

Keywords: *Spirulina platensis*, probiotic cheese, *Mentha longifolia* L., *Lactococci.*

INTRODUCTION

Spirulina is a filamentous cyanobacterium (Shimamatsu, 2004). It 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 (Guldas and Irikin, 2010). It has been claimed that consumption of *Spirulina* is beneficial to health due to its chemical composition including compounds like essential amino acids, vitamins, natural pigments and essential fatty acids, particularly gamma-linolenic acid, a precursor of the body's prostaglandins (Parada *et al*.,1998). The increasing medium salinity caused a significant increase in lipid content and linoleic and gamma-linolenic acids levels of *S. platensis* (Yilmaz *et al*., 2010) 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 (Guldas and Irkin, 2010; Henrikson, 1994). Consumers would need to ingest considerably less medicine and artificially produced vitamin and mineral supplements. A simple way of attaining this goal is the use of cyanobacteria in the manufacture of cultured dairy foods (Varga *et al.*, 2012). *Lactococci,* Gram-positive, non-spore forming, non-motile bacteria are known to produce lactic acid, flavor and texture in fermented dairy foods and prevent growth of organisms causing spoilage of foods. Two species viz., *Lactoccus lactis* subsp.*lactis* and *Lactoccus lactis* subsp. *cremoris* are the most common which used in preparation of fermented milks and cheese types products (Molnar *et al.*, 2005).

Since the long times, herbs and spices have been used in order to improve the flavor and organoleptic properties of foods and are applied in preventive and curative treatments in different diseases all over the world. Spices of the genus *Mentha* are wide spread and have been used for treatment of bronchitis, flatulence, anorexia, ulcerative colits and liver complaints due to their antiemetic, anti-inflammatory, carminative, diaphoretic, stimulant and analgesic activity (Džamić *et al.*, 2010; Nikšić *et al.*, 2012).

The effect of *S.platensis* on the viability of starter bacteria in fermented milks (de Caire *et al.*, 2000;Varga *et al.*, 2002; Gyenis *et al.*, 2005; Varga *et al.*, 2012), yogurt (Guldas and Irkin, 2010; Akalin *et al.*, 2009) and media (Bhowmik *et al.*, 2009; Parada *et al.*, 1998) has been investigated. However, the influence of the microalgae on the viability of starter bacteria in cheese, especially cheese containing *Mentha longifolia* has not been studied. The aim of this study was to investigate the effect of different concentrations of *S. platensis* powder on the survival of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* in eight cheese samples containing *Mentha longifolia* L. which were divided into two groups on basis of concentrations of *Mentha longifolia* L. and *S. platensis* added: 1- *Mentha longifolia* L. at the level of 0.5% and *Spirulina platensis* at the concentrations of 0, 0.3, 0.5 and 0.8% which were defined as Treatments M_1A_1, M_1A_2, M_1A_3 and M_1A_4, respectively; 2- *Mentha longifolia* L. at the level of 1% and *Spirulina platensis* at concentrations of 0, 0.3, 0.5 and 0.8% which were defined as Treatments M_2A_1, M_2A_2, M_2A_3 and M_2A_4, respectively. The viability of *Lactococci* was assessed on 1, 15, 30 and 45 days of storage at 4 ºC.

MATERIALS AND METHODS

Starter culture and Additives

Chr. Hansen R 704 cheese starter culture (*Lactococcus lactis* ssp. *lactis* and *Lactoccus lactis* ssp. *cremoris*, Chr. Hansen's Lab, Horsholm, Denmark) was used as starter culture in the manufacture of cheese. *L.acidophilus La-5* was obtained from Chr.Hansens dairy cultures, Denmark. Cultures were used in a freeze dried direct vat set (DVS). *Spirulina platensis* was used in a powdered obtained from Gheshm Sina Microalgae Co., Tehran, Iran. *Menthe longifolia L.* was used in a powdered obtained from Goar, Markazi, Iran.

Cheese production

Cow's milk with 3.42% fat, 8.12% total solid and 3.01% protein was pasteurized at 72ºC for 15 seconds and then ultrafiltrated at 55ºC. The retentate obtained was homogenized at 100 bar and 50°C and pasteurized at 77ºC for 1 min. After cooling to 35ºC, retentate was inoculated with R 704 starter culture (1%w/v) and *L.acidophilus* (2%w/v) and divided into 2 groups on basis of concentrations of *Mentha longifolia* L. and *S. platensis* added: 1- *Mentha longifolia* L. at level of 0.5% and *S. platensis* at levels of 0, 0.3, 0.5 and 0.8% which were defined as Treatments M_1A_1, M_1A_2, M_1A_3 and M_1A_4, respectively; 2- *Mentha longifolia* L. at level of 1% and *S. platensis* at levels of 0, 0.3, 0.5 and 0.8% which were defined as Treatments M_2A_1, M_2A_2, M_2A_3 and M_2A_4, respectively. The lots containing 0.5% (M_1A_1) and 1% *Mentha longifolia* L. (M_2A_1) without *S. platensis* were considered as the controls. Rennet (30 mg 100 L^{-1}) was added to each lot. Retentate mixed was immediately poured into 300-g containers and let it coagulate for 30 min. Dry salt (3%) was distributed on the surface of coagulum. the containers were sealed with aluminum cover, incubated at 30ºC for 48 h and stored under refrigeration for up to 45 days.

Sampling

Cheese samples were taken for bacterial enumeration on 1, 15, 30 and 45 days of storage.

Microbiological analysis

From each cheese sample, 10 g cheese was transferred into a sterile bag under aseptic conditions and homogenized in 90 mL of sterile trisodium citrate solution (2g 100 mL^{-1}) for 2 min using a Labblender stomacher. Serial dilutions were prepared by adding 1mL to 9 mL sterile peptone water (0.1 g 100 mL^{-1}). Samples were tested for counts of starter lactococci using standard methods (Vanderzant and Splittoesser, 1992). Starter lactococci were enumerated on M17 agar (Oxoid) and incubated aerobically for 48 h at 37ºC.

Statistical analysis

Each treatment was performed in three replications. The experiment was subjected to Randomized Complete Block Design and analyzed using SAS 9.1 software. A significance level of $p<0.05$ was used.

RESULTS AND DISCUSSION

The population of *Lactococcus lactis* ssp. *Lactis* and *Lactococcus lactis* ssp. *cremoris* in the probiotic cheeses during the storage period are shown in Figs. 1 and 2. During the storage at 4ºC, the *Lactococci* counts were significantly higher ($p < 0.05$) in the cyanobacterial cheeses than in controls. Control cheese samples contained lower *Lactococcus* strains during the storage. M_1A_4 and M_2A_4 showed the highest viability during the storage period in comparison to the other treatments tested in every group. In the first group, *Lactococcus* count in Treatment M_1A_4 was recorded the highest value (9.07 ± 0.08 Log_{10} cfu ml^{-1}) on day 1, then a down ward tendency was observed in all treatments during storage time (Fig.1). The reduction might be due to the low growth ability of *Lactococci* under acidic conditions (Kasimoğlu *et al.*, 2004). After 45 days of storage at 4°C, the *Lactococci* counts were found to be within the range of 7.67 ± 0.02, 7.56 ± 0.09, 7.55 ± 0.04 and 7.28 ± 0.07 Log_{10} cfu ml^{-1} in Treatments M_1A_4, M_1A_3, M_1A_2 and M_1A_1, respectively. In the second group, after first day (9.04 ± 0.04, 8.94 ± 0.03, 8.87 ± 0.03 and 8.36 ± 0.08 Log_{10} cfu ml^{-1}), 15 days (8.71 ± 0.01, 8.60 ± 0.03, 8.43 ± 0.05 and 8.12 ± 0.03 Log_{10} cfu ml^{-1}), 30 days (7.77 ± 0.01, 7.72 ± 0.02, 7.64 ± 0.04 and 7.42± Log_{10} 0.02 cfu ml^{-1}) and 45 days (7.67 ± 0.02, 7.58± 0.02, 7.47 ± 0.03 and 7.30 ± 0.05 Log_{10} cfu ml^{-1}) of storage, similar trend was observed in Treatments M_2A_4, M_2A_3, M_2A_2 and M_2A_1, respectively. Hence, higher the level of *S.platensis* in cheese was directly affected *Lactococcal* viability during storage time. As shown in Figs.1 and 2, a gradual decrease was observed in viable *Lactococcus* counts during storage time. These observations are in line with those of Varga *et al.* (2012), who reported that the *Lactococcus* count was significantly higher (P<0.05) in the mesophilic fermented milk enriched with 3% *Spirulina* than in the control product. They observed an increase in *Lactococci* counts of both products during the first week of storage;

However, viability declined slowly thereafter. After 42 days of storage, the *Lactococci* count in the fermented milk was reprted to be 7.43± 0.05 Log_{10} cfu $ml^{-1}$by them. This value was comparable to that obtained in the present study after 45 days of storage at 4°C.

The present study clearly showed that *S. platensis* led to Lactococcal improved viability in cheese. It was in concordance with the findings of Parada *et al.* (1998), who demonstrated extracellular products of *S. platensis* promote *lactococcus lactis* growth in vitro, and de Caire *et al.* (2000), who found that the addition of dry *S. platensis* to milk (6mg ml^{-1}) stimulated growth of *Lactococcus lactis* by 27%. Molnar *et al.* (2005) also reported that acid production of all the screened *Lactococcus* strains could be stimulated by *S. platensis* biomass. This effect can be attributed to the presence of free amino acids, peptone, adenine and hypoxanthine in the algal biomass (Akalin *et al.*, 2009; Guldas and Irkin, 2010).

There was no difference in population between the groups ($p > 0.05$). Hence, no differences were observed between cheeses manufactured with the different levels of *Mentha longifolia* L.

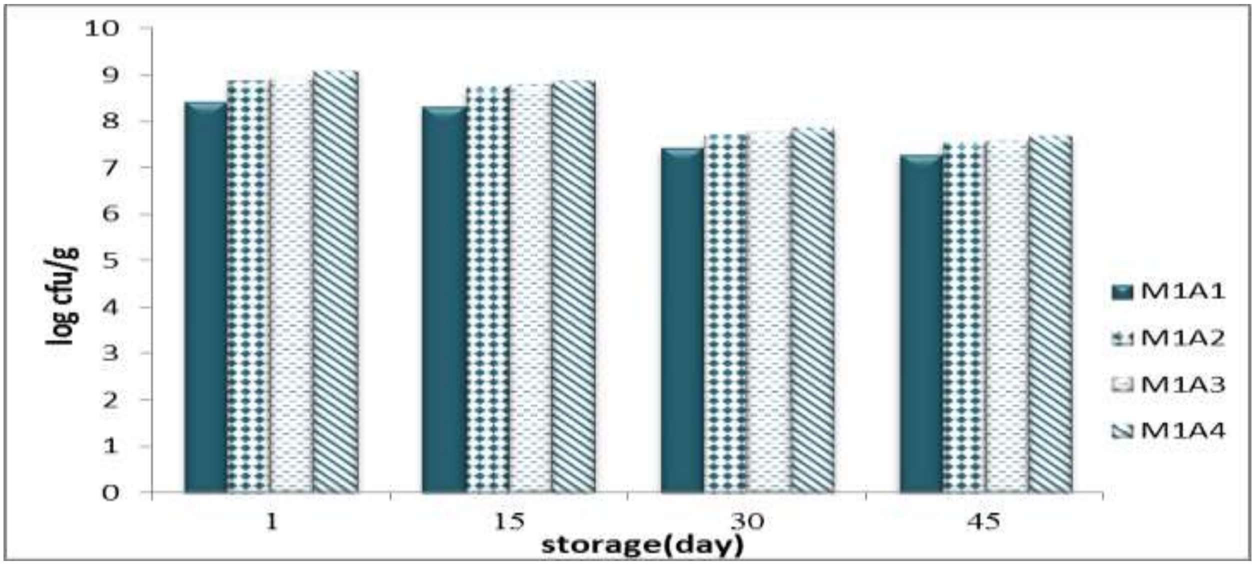

Fig.1. Changes of the viable counts of starter *lactococci* in probiotic Iranian white cheese containing different concentrations of *Spirulina platensis* and 0/5% *Mentha longifolia L.* during cold storage (M_1A_1:0%, M_1A_2:03% , M_1A_3:05%, M_1A_4:08%). LSD $_{0.05}$ = 0.0818

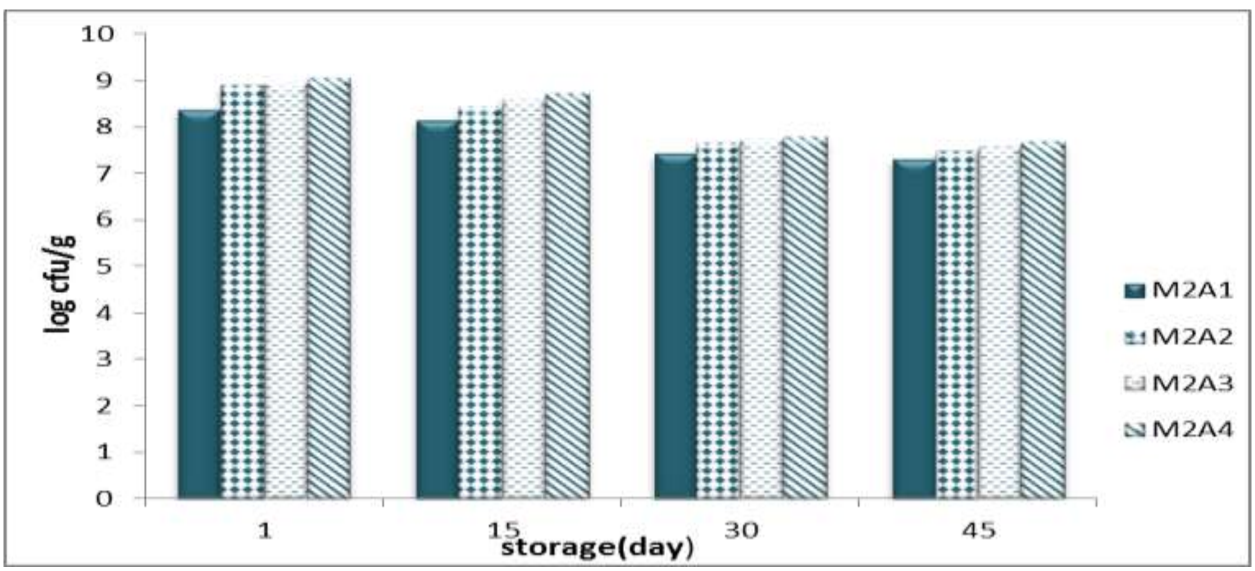

Fig.2. Changes of the viable counts of starter *lactococci* in probiotic Iranian white cheese containing different concentrations of *Spirulina platensis* and 1% *Mentha longifolia L.* during cold storage. (M_2A_1:0% , M_2A_2:03%, M_2A_3:05%, M_2A_4 :08%). LSD $_{0.05}$ = 0.0647

Conclusion

The results suggested that *Spirulina platensis* stimulated the growth of *Lactococci* in probiotic cheese, which resulted in improved viability of these organisms.

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