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Change in Probiotic Microflora and Physico-chemical Characteristics of Rose Hip Marmalade Bio-yoghurts During Refrigerated Storage

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Yoghurt, produced by adding rose hip marmaleda and *L. acidophilus*, was stored for 15 d at 4 ± 1 °C and changes in selected microbiological and physicochemical parameters were monitored during storage. Ascorbic acid and pH values of yoghurt samples decreased during storage. There were minimal differences in the aerobic mesophilic bacteria counts among yoghurt types. *S. thermophilus* counts of yoghurt samples similarly decreased with concentration of ascorbic acid ($p < 0.05$) and *L. bulgaricus* count ($p < 0.01$). The alteration in *L. bulgaricus* counts of yoghurt samples was similar to *S. thermophilus*. *L. acidophilus L. bulgaricus* and *S. thermophilus* counts ranged in 7.21, 7.19, 5.41 and 2.01, 9.83, 6.97 log cfu g^{-1}, respectively. Fruit, storage period and ascorbic acid importantly affected on the viable counts of *L. acidophilus* ($p < 0.01$). Coliform, *S. aureus*, yeast and mould were not determined any yoghurt samples during the storage period of 15 d. Probiotic culture count decreased under the level of 10^6 cfu/g after 6 d.

Key Words: Yoghurt, Probiotic, Rose hip, *Lactobacillus acidophilus*.

INTRODUCTION

Yoghurt is a dairy product which is naturally produced with fermentation by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*[1-3]. In this paper, *Streptococcus salivarius* subsp. *thermophilus*, as *S. thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus as *L. bulgaricus* is designated. It has become a very popular staple food and its consumption is still increasinga[4]. The healthy properties of yoghurt, to a great extent arise from the action of viable yoghurt bacteria and their metabolites[5]. Currently, a variety of different flavouring ingredients such as fruits, natural flavours and/or synthetic flavours are used in commercial yoghurt production. This situation contributes to increase in the yoghurt consumption and the diversity of presentation of the product. Fruit is one of the most preferable ingredients in yoghurt production. The fruits generally preferred in yoghurt production are apricot, black cherry, blackcurrant, mandarin, peach, pineapple, raspberry and strawberry. However, rose hip is not used in fruit yoghurt production[6].

Rose hip is the ripe reddish-orange fruit. The fruits are gained from the species *Rosa canina* that is widely grown in East Black Sea Region of Turkey[7]. Flesh of the hip can be used to make jellies, purees, syrups, fruit soups, tea and health drinks. Fruits of rose hips are an important source of antioxidants, as they are rich in vitamins C and E, carotenoids (especially lycopene and β-carotene) and phenolic compounds such as flavonoid glycosides and proanthocyanidin aglycones and minerals including K, P, Mg, Ca, Fe. For these reasons rose hips are consumed as a food source and as medicine in ethnopharmacology. For example, *Rosa canina* fruits are used in Turkish folk medicine to treat a great variety of ailments *i.e.* cancer, eczema, psoriasis, bronchitis, colds, cough, asthma, diabetes, urinary inflammations or as hypotensive[8-13]. In addition, *Rosa canina* recently reported to have antiinflammatory properties, on the symptoms of osteoarthritis[14].

Bio-yoghurts are often manufactured for dietetic and/or therapeutic purposes. Most strains of *L. bulgaricus* and *S. thermophilus* do not survive in the intestinal tract. The survival of ingested probiotics at different levels of the gastrointestinal tract differs between strains. Some strains are rapidly killed in the stomach while others, such as strains of bifidobacteria or *L. acidophilus*, can pass through the entire gut at very high concentrations. Milk as a vehicle appears to protect probiotics against gastric conditions. The main probiotic microorganisms belong to genera of *Lactobacillus, Bifidobacterium, Enterococcus* and *Pediococcus*. Although the incorporation of *L. acidophilus* and *Bifidobacterium* species into the yoghurt starter culture may contravene some existing definitions of yoghurt, the resultant milk product is reported to be of excellent therapeutic value[15-17].

In this research, rose hip fruit and *L. acidophilus* strain are used in fruit yoghurt production and the organoleptic, nutritional and therapeutic properties of yoghurts are studied to improve. For this purpose, yoghurts samples are analyzed for some microbiological and physico-chemical characteristics.

EXPERIMENTAL

Milk sample using in yoghurt production was obtained from Atatürk University Pilot Dairy Plant. Starter cultures were used in yoghurt production (*L. bulgaricus, S. thermophilus, L. acidophilus* DSMZ 20079) were obtained from Atatürk University, Agricultural Faculty, Department of Food Engineering, Food Microbiology Laboratory, Erzurum.

Preparation of yoghurts: The milk was pasteurized by heating for 10 min at 95 °C. Next the milk is cooled to *ca.* 40 °C, which is an optimum growth temperature for the yoghurt starter culture (*L. bulgaricus, S. thermophilus* and, *L. acidophilus*) that is added to the milk in a fermentation tank. A temperature of 40 °C is maintained for 3-4 h. The acid development of yoghurt is carefully monitored until the pH reached 4.0 to 4.7. Then, the fermentation is stopped by rapid cooling. Yoghurts were cooled to 4 ± 1 °C and kept at same temperature for 1 d. The mix of rosehip marmalade (50 %) and sucrose (50 %) were prepared and then added 15 g mix/100 g yoghurt, homogenized. Four different yoghurts were produced as plain yoghurt (Y), bio-yoghurt (BY), rose hip yoghurt (RY) and rosehip bio-yoghurt (RBY). All the samples were filled in glass jars and stored at 4 ± 1 °C for 15 d and then subjected to some microbiological and physicochemical analyses in certain intervals (0, 2, 4, 6, 9 and 15 d).

Microbiological analyses: 25 g of yoghurt were homogenized with 225 mL of a 0.85 % NaCl and 0.1 % peptone solution in a Bagmixer 400 W (Interscience, St. Nom, France). Tenfold dilution of yoghurt homogenates were prepared with sterile Ringer's solution (Merck) and plated onto specific media (in duplicate). Aerobic mesophilic bacterial counts were determined on plate count agar (PCA; Merck) which was incubated at 30 ºC for 72 h; coliforms were determined on Violet Red Bile Agar (VRBA, Merck) after incubation at 35 ºC for 24 h; yeasts and moulds were counted on Potato Dextrose Agar (PDA, Merck) acidified with 10 % tartaric acid (pH 3.5 ± 0.1), incubated 25 ºC for 5 d. *Staphylococcus aureus* was determined on Baird-Parker Agar (Fluka) added with egg yolk telluride solution (Merck), incubated 35 ºC for 24 h[18]. *S. thermophilus* was counted on M17 Agar (Merck) after incubation at 37 ºC for 72 h. *Lactobacillus bulgaricus* was counted on MRS Agar after incubation at 37 ºC for 72 h considering to colony and bacterial morphology. Bacterial morphology was determined by using a microscope (BX51T, Olympus, Japan) combined with digital imaging apparatus (C7070, Olympus, Japan). *L. acidophilus* counts were determined on Bile-MRS Agar after incubation at 37 ºC for 72 h. *L. bulgaricus* and *L. acidophilus* were incubated under anaerobic conditions in anaerobic jar (Merck) with Anaerocult A (Merck)[19,20].

Physicochemical analyses: The pH value of yoghurt samples was measured at 17-20 °C using a pH meter (pH 211, Hanna Ins., Portugal) as described by Dave and Shah[21]. The concentration of ascorbic acid was determined according to the AOAC official methods of analysis[22]. The ascorbic acid was extracted in the precipitant (metaphosphoric, acetic acid and water mixture) solution and the clear filtrate titrated against the sodium salt of 2,6-dichlorophenol.

Statistical analysis: Data obtained from analysis of the samples were evaluated statistically using a variance analysis and the differences among means were detected by the Duncan's multiple range tests[23].

RESULTS AND DISCUSSION

The change in the viable counts of aerobic mesophilic bacteria in yoghurt samples during refrigerated storage are shown in Fig. 1. There were minimal differences in the aerobic mesophilic bacteria counts among yoghurt types and the viable counts were not changed remarkably during the storage. Aerobic mesophilic bacteria counts were changed between 7.81 and 9.44 log cfu g^{-1} and average of the samples was found as 8.72 log cfu g^{-1}. Aerobic mesophilic bacteria counts were not effected by fruit, storage period and *L. acidophilus* addition, but there was a positive correlation ($p < 0.05$) with *S. thermophilus* count.

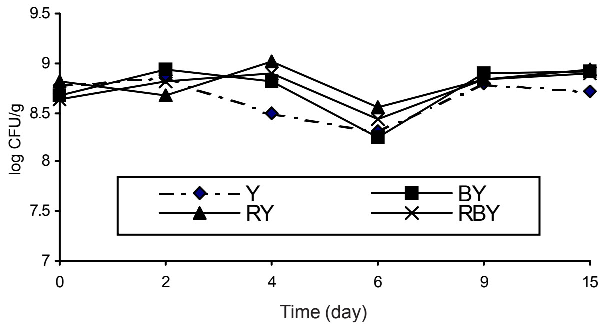

Fig. 1. Aerobic mesophilic bacteria count of yoghurt samples during storage period

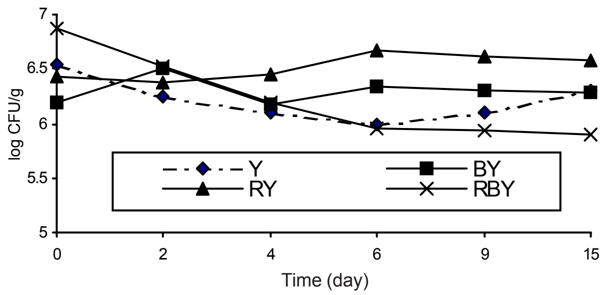

Fig. 2. *S. thermophilus* count of yoghurt samples during storage period

As seen from Fig. 2, *S. thermophilus* counts of the samples in general decreased slightly during the storage. *S. thermophilus* counts were ranged from 5.41 to 6.97 log cfu g^{-1} and average of the samples was found as 6.32 log cfu g^{-1} during the storage period. The viable counts of *S. thermophilus* were not influenced by fruit, storage period and *L. acidodhilus* addition. *S. thermophilus* counts of yoghurt samples similarly decreased with concentration of ascorbic acid ($p < 0.05$) and *L. bulgaricus* count ($p < 0.01$). A similar relation was also seen between *S. thermophilus* counts and ascorbic acid level in the study of Dave and Shah[21].

The alteration in *L. bulgaricus* counts of yoghurt samples was similar to *S. thermophilus*. As a matter of fact, it was observed a important decrease ($p < 0.01$) in *L. bulgaricus* counts of yoghurt samples during the storage (Fig. 3). *L. bulgaricus* counts were found in 7.17-9.83 log cfu g^{-1} and average 8.49 log cfu g^{-1} in all the samples. Change in yoghurt bacteria (*L. bulgaricus* and *S. thermophilus*) during the storage period was similar to previous studies[24-28].

L. acidophilus counts changed from 7.21 to 2.01 log cfu g^{-1}. Average of the samples was found as 5.10 log cfu g^{-1}. Fruit, storage period and ascorbic acid remarkably affected on the viable counts of *L. acidophilus* ($p < 0.01$). The minimal concen-tration for probiotic bacteria suggested by many researchers is 106 cfu/g of a product to achieve optimal therapeutic effects[17,29-31]. Survival of these bacteria during shelf life and until consumption is therefore an important consideration.

However, studies showed low viability of probiotics in market preparations[31-33]. In this study, a significant decrease ($p < 0.01$) was also observed in yoghurt samples after 6th d of the storage and yoghurt samples lost its probiotic properties (Fig. 4).

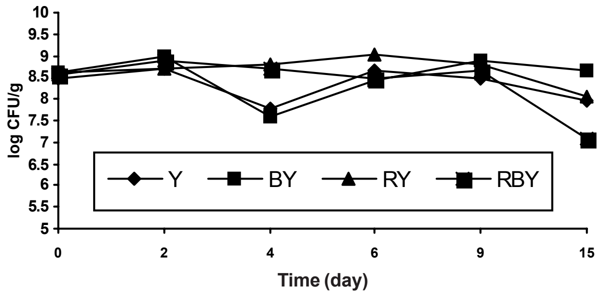

Fig. 3. *L. bulgaricus* count of yoghurt samples during storage period

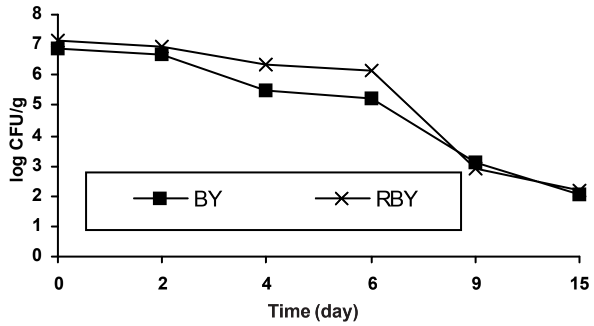

Fig. 4. *L. acidophilus* count of yoghurt samples during storage period

Coliform, *S. aureus*, yeast and mould were not determined any yoghurt samples during the storage period of 15 d. Coliform, *S. aureus*, yeast and mould counts were found in < 1, < 2 and < 1 log cfu g^{-1}, respectively. Similar results were also determined by Canganella *et al.*[24] and Lourens-Hattingh and Viljoen[34].

pH of milk and rose hip marmalade used in the fruit yoghurt production were 6.66 and 3.77. pH values decreased in the yoghurt samples during the storage period changed from 4.50 to 3.59 and average was found as 4.09 (Fig. 5). At the same time, many researchers stated decrease of pH in different fruit yoghurt samples[35-38].

Ascorbic acid levels of the milk, rose hip fruit and marmalade were determined as 1.86, 273.30 and 56.28 mg 100 mL^{-1}, respectively. While average of ascorbic acid in rose hip fruit yoghurts was 2.65 mg 100 mL^{-1}, it was 0.55 in yoghurts without rose hip marmalade. As seen from Fig. 6, amounts of ascorbic acid were decreased in all samples during the storage period of 15 d. Similarly, Zeytun[38] found that ascorbic acid level of the rose hip bio-yoghurt decreased during the storage period of 15 d. The high ascorbic acid level in fruit yoghurts helped to improve the viability of *L. acidophilus* and thus the probiotic quality of yoghurt increased.

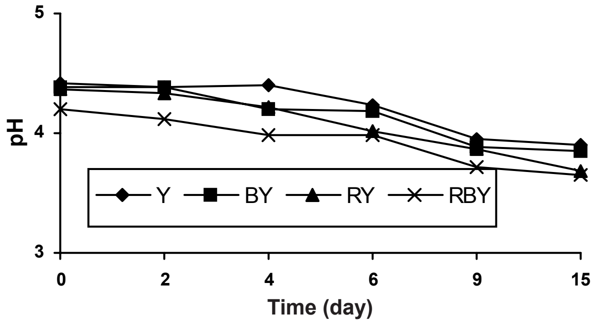

Fig. 5. Titratable acidity value of yoghurt samples during storage period

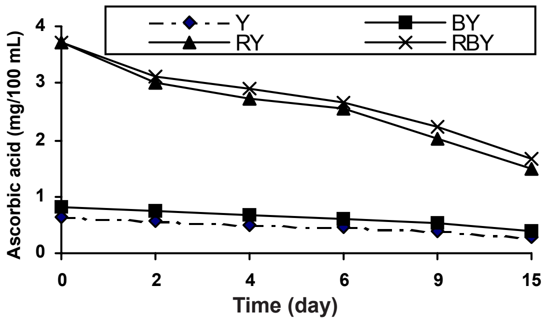

Fig. 6. Ascorbic acid level of yoghurt samples during storage period

Conclusion

In this research, rose hip yoghurt samples produced by adding *L. acidophilus* were stored for 15 d at 4 ± 1 °C. While aerobic mesophilic bacteria counts did not changed. The other microorganism counts decreased during the storage period. *L. acidophilus* count of rose hip yoghurts was found in < 10^6 cfu/g after 6 d. Therefore, it is concluded that this yoghurt should be consumed in 7 d for achive to optimal therapeutic effect.

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