Survival of Lactobacillus Spp. in Fruit Based Fermented Dairy Beverages

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***Abstract*—In this study fruit based (apple and bluberry) fermented dairy beverages were made with *L. acidophilus* and *L. rhamnosus.* Viability of probiotic bacteria and sensory analysis were determined. The type of fruit and probiotic bacteria used were significantly effective on microbiological and sensory properties of fermented beverage (*p<0.01*). The growth proportion index (GPI) of *L. rhamnosus* was significantly higher than *L. acidophilus* in all samples during storage. In this study, both *Lactobacillus* strains showed good probiotic viability (>7 log cfu g^{-1}) and remain at this satisfactory viability levels even after 28 days of storage. All the products were evaluated with high sensory scores.**

***Index Terms*—dairy beverage, prozbiotic, *lactobacillus* spp.**

I. Introduction

Intestinal tract of humans constitutes a complex ecosystem of microorganisms. It is widely accepted that the change of intestinal microbiota depends on the nutrition style and health conditions of the person. The bacterial population in the large intestine can reach a maximum of 10^{12} cfu g^{-1}, however, it is considerably lower at only 10^4-10^8 cfu g^{-1} in the small intestine and in the stomach only 10^1-10^2 cfu g^{-1} due to the low pH. In addition, use of antibiotics can damage the equilibrium of intestinal microbiota, reducing counts of *Bifidobacteria* and *Lactobacilli* while increasing *Clostridia*. Thus in order to stimulate the growth of preferred microorganisms, improve the balance of intestinal microbiota, inactivate potentially harmful bacteria, and enhance the body's autoimmune system probiotic microorganisms can be added to the diet [1]-[5].

According to the most widely accepted definition, probiotics are live microorganisms which confer health benefits on the host via their effects in the gut when administered in adequate amounts [6], and have a role in prevention of many diseases [7], [8].

Probiotic administration mainly result in an increase the number of health-promoting microorganisms in gut microbiota such as *Bifidobacteria* and *Lactobacilli*, a decrease in fecal pH, a decline in those bacterial enzyme activities that are associated with the development of colon cancer, production of antibacterial substances, improvement of intestinal barrier function, stimulation of the immune system, modulation of cholesterol uptake and reduction in the incidence of gastrointestinal disorders, cardiovascular diseases, diarrhea and osteoporosis [7], [9]-[15].

The global probiotic market size has increased rapidly in the last years, and the yoghurt and fermented milk beverage sector accounts for the highest market share in this area. There is a growing demand for development of new yoghurt-like probiotic foods [16], [17]. Fermented milks or beverages offer an attractive food-based delivery vehicle for probiotic cultures, and fruity or cereal-based ingredients are new trends for development of probiotic dairy products, particularly the use of high-phenolic containing fruit juice as a medium for probiotics [18].

The effects of the fruity food matrices on the probiotic survival and/or activity and a positive effect of this interaction in the host are important approches for many studies. Recent studies point out that probiotic strains such as of *L. acidophilus* and *L. rhamnosus* are the most utilized bacteria in the formulation of new fruity probiotic products [19]. The technological properties that a probiotic culture should have are to remain viable for large-scale production, to remain stable and viable during storage and use, and to survive in the intestinal ecosystem [20]. A sufficient number of viable microorganisms must be present throughout the entire shelf life of the product in order to produce therapeutic benefits. In this regard, minimum levels for probiotic bacteria in fermented milks should be between 10^5-10^6 cfu mL^{-1} [21]. Bacterial populations of 10^6-10^7 g^{-1} in the final product have been shown to be more acceptable as efficient levels of probiotic cultures in processed foods. However, these organisms often show poor viability in market preparations [22]-[24].

The vitality of probiotics cultures in the food matrix depends on such factors as pH, acidity, process and storage temperatures, oxygen content, production of hydrogen peroxide due to bacterial metabolism, the strains used, the presence of other microorganisms, interaction between species present, culture conditions, the presence of competitive microorganisms and inhibitors [25]-[28].

However, it is a challenge to maintain the viability of probiotics in fruit juices because of the detrimental effects of the low pH environment (<pH 4.0) [20], [29], [30]. Probiotic viability in fruit juice is also affected by strain, method of culture preparation, state of the cells inoculated, storage temperature, oxygen level, and the presence of fibres [29]-[31]. Consequently, the objective

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of this study was to investigate viability of probiotic bacteria, namely *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*, in fermented dairy beverages supplemented with apple and blubbery juices.

II. Materials and Methods

A. Preparation of Probiotic Cultures

Probiotic cultures were prepared according to Ozcan *et al.* [32] using 1g of lyophilized culture in 100 mL 12% (w/v) reconstituted sterile non-fat milk at 121℃ for 15min. The cultures of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* (Danisco, Madison WI, USA) were incubated at 37±1℃ for 72h. The necessary inoculums was calculated as to give approximately 8 or 9.0 $\log_{10}$ colony forming units mL^{-1} in yogurt after inoculation.

B. Milk Fermented with Lactobacillus Acidophilus and Lactobacillus Rhamnosus

Skim milk powder was reconstituted in distilled water at 10.70% (w/w) to yield reconstituted skim milk of the same overall composition as the raw skim milk for the fermented milks production. Reconstituted milks were heat-treated at 90℃ for 10min and were cooled to 37℃. Yogurt mixes inoculated with each probiotic bacteria, such as BA (*Lactobacillus acidophilus*) and BR (*Lactobacillus rhamnosus*). Incubation was carried out at 37℃ until the final pH value reached 4.7. The samples were kept at room temperature (22±1℃) for 30min., stored at 4±1℃ for 12 hours.

Apple juice and blueberry juice was obtained from a commercial fruit juice manufacturing company (Elite Ltd. Company, Ankara, Turkey). For the fermented beverages manufacture probiotic yogurt samples were mixed with fruit juice concentrate at 1:1 ratio according to Akin [33]. Fermented fruit-based probiotic beverages (BAA: fruit based fermented milk containing *L. acidophilus* and apple juice, BAB: fruit based fermented milk containing *L. acidophilus* and blueberry; BRA: fruit based fermented milk containing *L. rhamnosus* and apple juice; BRB: fruit based fermented milk containing *L. rhamnosus* and blueberry) were stored for 28 days at 4±1℃.

C. Evaluation of Probiotic Bacteria

Probiotic strains were enumerated on selective medium - Man, Rogosa and Sharpe Agar (MRS) (Merck, Darmstadt, Germany) during 28 days of refrigerated storage. *L. acidophilus* was counted in MRS-Bile (MRS agar with 0.15% (w/v) of bile) [34], whereas MRS-vancomycin agar (MRS V, with 20mg mL^{-1} of vancomycin, pH 6.2) was used for *Lactobacillus rhamnosus* [35]. The plates were incubated at 37℃ for 72h under anaerobiosis in jars with the AnaeroGen Gas Packs (Oxoid, Basingstoke, UK). The cell concentrations were expressed in logarithm of colony forming units per gram of product (log cfu g^{-1}). Growth proportion index (GPI) of probiotic microorganisms was calculated as following [36]:

GPI = Final cell population ($\log_{10}$ cfu g^{-1}) ⁄ initial cell population ($\log_{10}$ cfu g^{-1})

D. Analysis

In samples pH values analysed according to methodology recommended by the Association of Official Analytical Chemist Methods AOAC [37], and sensory parameters evaluated using the method of Gomes *et al.* [38]. The organoleptic attributes analysed were: appearance (uniformity), texture (viscosity), aroma intensity (fruity, acidity), flavor (milky and acid flavor), taste (acid, sweet, bitter), color and overall acceptability. The test was conducted with a 5-point hedonic scale of 1 to 5 (1 = unacceptable and 5 = excellent).

Estimation of the effect of probiotic bacteria and time of storage was conducted using ANOVA, and the significance of differences between the means was determined on the basis of Duncan's test at the significance level of $p<0.01$.

III. Results and Discussion

A. Viability of Probiotic Bacteria and Post-Acidification

The viable counts of probiotic bacteria in the fruit-based fermented beverage during 28 days of storage are shown in Fig. 1a and Fig. 1b. Storage time significantly affected viable cell counts (at 7 days intervals) of *L. rhamnosus* in fermented beverage with apple and blueberry depending on the growth of probiotic bacteria in fruit matrix ($p<0.01$) (Table I).

The physicochemical properties of food influence probiotic bacteria survival. Table I shows the viability and growth proportion index (GPI) of probiotic microorganisms in fermented beverage during 28 days of refrigerated storage per 7 day intervals. The viability and GPI of *L. rhamnosus* were significantly higher than *L. acidophilus* in all fermented beverages. The decrease in viability of *L. acidophilus* than of *L. rhamnosus* (Fig. 1) during storage in acid foods were reported by Garro *et al.* [39] and [40]. It has been suggested that the probiotics should be present in a food at a minimum level of 10^6–10^7 cfu mL^{-1} or cfu g^{-1} in order to be recommended as a functional food [19], since 6 $\log_{10}$ viable bacteria cfu g^{-1} of product is required to confer health benefits [41]-[43].

The GPI for all strains at the end of storage ranged between 0.95-0.96 in apple juice beverage, whereas it was higher the in fermented beverage with blueberry with 0.99-1.02. GPI for all strains were highest in 14^{th} day of storage. Viability and survival of *L. acidophilus* and *L. rhamnosus* in products were still higher than satisfactory therapeutic levels at the end of the recommended shelf-life (Table I). Generally when growth conditions arenot controlled, most probiotic bacteria may promptly lose viability leading a sharp reduction in its functionality [42]. The survival of *Lactobacillus* spp. varied due to the probiotic strain used as a result of different sensitivity to environmental stresses of these bacteria such as low pH and high titratable acidity [44]-[49]. Vinderola *et al.* [50] reported that pH 4.5 or lower negatively affects the cell viability of probiotic bacteria. Therefore, variations in strain stability observed in this study may be due to pH, fruit juice composition or oxygen present.

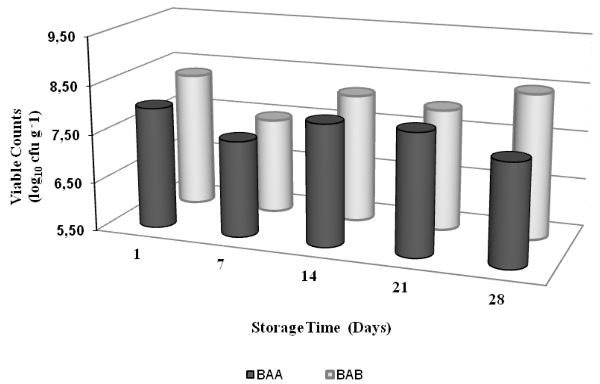
TABLE I. VIABILITY AND GROWTH PROPORTION INDEX (GPI) OF PROBIOTIC MICROORGANISMS IN DIFFERENT TREATMENTS AT DURING STORAGE*

Probiotic Yogurt	Viable Counts During Storage ($\log_{10}$ cfu g^{-1})					GPI 0	GPI 7	GPI 14	GPI 21	GPI 28
	0	7	14	21	28					
BAA	8.00^{aA}	7.48^{aA}	8.00^{aA}	8.00^{aA}	7.60^{aA}	-	0.94^{a}	1.00^{a}	1.00^{a}	0.95^{b}
BAB	8.30^{aA}	7.48^{aA}	8.15^{aA}	8.00^{aA}	8.48^{aA}	-	0.90^{b}	0.98^{b}	0.96^{b}	1.02^{a}
BRA	9.00^{aB}	9.60^{aA}	9.15^{aAB}	8.30^{aC}	8.71^{aBC}	-	1.07^{a}	1.01^{a}	0.92^{a}	0.96^{b}
BRB	9.00^{aA}	8.23^{bB}	8.85^{aA}	8.30^{aB}	8.93^{aA}	-	0.91^{b}	0.98b	0.92^{a}	0.99^{a}

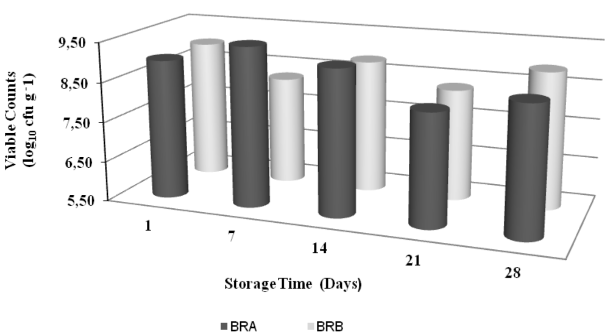
*Values presented are the means of three replicates trials

^{a,b}Different superscript lowercase letters denote significant differences (P<0.01) between probiotic bacteria with different fruits, ^{A,B}Different superscripts capital letters denote significant differences (P < 0.01) between different times

BAA: fruit based fermented beverage containing *L. acidophilus* and apple juice, BAB: fruit based fermented beverage containing *L. acidophilus* and blueberries, BRA: fruit based fermented beverage containing *L. rhamnosus* and apple juice, BRB: fruit based fermented beverage containing *L. rhamnosus* and blueberries.



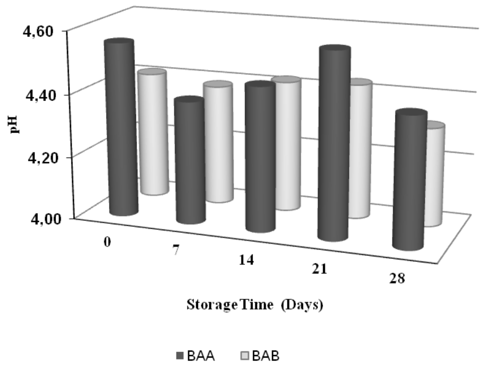
(a)



(b)

Figure 1. a) Viability of *L. acidophilus* in fruit based fermented beverage during storage b) Viability of *L. rhamnosus* in fruit based fermented beverage during storage.
BAA: fruit based fermented beverage containing *L. acidophilus* and apple juice, BAB: fruit based fermented beverage containing *L. acidophilus* and blueberry. BRA: fruit based fermented beverage containing *L. rhamnosus* and apple juice. BRB: fruit based fermented beverage containing *L. acidophilus* and blueberry.

Fruits, pulps and even the peels, have been successfully incorporated with probiotic dairy products as sources of prebiotic fibers and nutrients that stimulate the growth and activity of intestinal microbiota [51]-[54]. Fruits, especially berries, are a good source of polyphenols, like anthocyanins, micronutrients, and fibers [55]. It has been reported that phenolic compounds and some organic acids such as citric acid which are present in the fruits, are rapidly consumed by all the probiotic microorganisms and result in increased survival [56]-[59]. However, there is a lack of studies on the survival and activity of probiotic microorganisms demonstrating the effects of fruits as carrier matrices.



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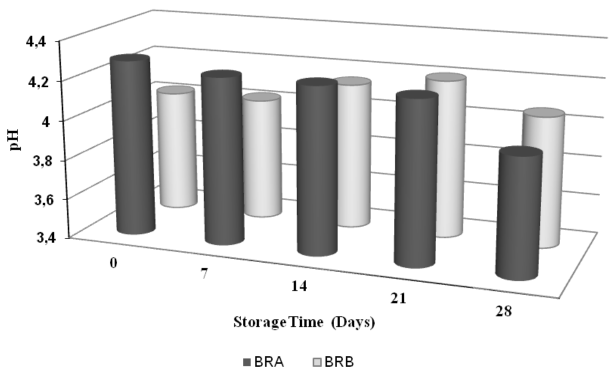

(b)

Figure 2. a) pH values of *L. acidophilus* in fruit based-fermented beverage during storage b) pH values of *L. rhamnosus* in fruit based fermented beverage during storage.
BAA: fruit based fermented beverage containing *L. acidophilus* and apple juice. BAB: fruit based fermented beverage containing *L. acidophilus* and blueberry. BRA: fruit based fermented beverage containing *L. rhamnosus* and apple juice. BRB: fruit based fermented beverage containing *L. acidophilus* and blueberry.

There were significant differences in the pH values of the samples either depending on *Lactobacillus* spp. used or t fruit variety (Fig. 2a and Fig. 2b) (*p<0.01*). The pH value of the samples decreased throughout the storage period, however, showed slight increases in some periods. The reason for the slight increase in pH value was the assimilation of lactic acid or other fruit organic acids and phenolic compounds by probiotic bacteria as energy source, the deamination of amino acids and the amphoteric properties of proteolysis products during the storage time.

B. Sensory Profile of Probiotic Fermented Beverages

Sensory properties including color, consistency or viscosity, taste, mouth feel, flavor is considered to be the most important properties for customer preferences. Sensory properties of fermented milk products are affected by several factors including raw materials such as milk or added fruits, production processes, fermentation conditions, food additives and starter cultures [60]. The sensory properties of yogurt samples were presented in Fig. 3. There were a significant differences in the sensory properties of the fermented dairy beverage samples ($p<0.01$). Fruit-based fermented milk containing *L. acidophilus* and apple juice (BAA), received the best scores for overall taste and acceptability (Fig. 3). In a sensory profile, sweetness and sourness correspond respectively to sugar and organic acid contents. Fructose, glucose and sucrose are the main sources for sweetness in fruit-based fermented dairy products. However, in fermented milk products, fructose and glucose are mostly consumed by lactic acic bacteria, resulting in organic acid and characteristic aroma compounds formation. The aroma is formed by non-volatile acids, volatile acids, carbonyl compounds and miscellaneous compounds [61], [62]. Fermented beverages containing *L. acidophilus* (BAA) and *L. rhamnosus* (BRA) with apple juice received higher scores for the flavor and aroma intensity, appearance and texture. All fermented beverages were acceptable for the color at the same level. Consequently, all beverages had high sensory acceptances from the first day to the end of shelf life. Fruit fibers and flavor compounds might contribute to the desired flavor of the final product.

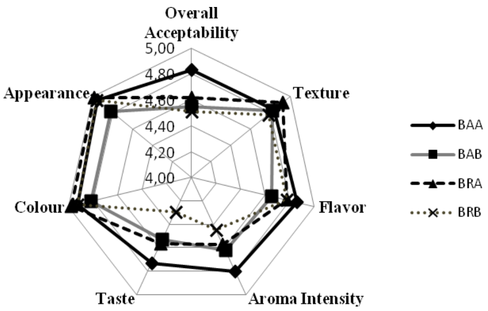

Figure 3. Average sensory ratings of fruit based fermented beverage. BAA: fruit based fermented beverage containing *L. acidophilus* and apple juice. BAB: fruit based-fermented beverage containing *acidophilus* and blueberries. BRA: fruit based-fermented beverage containing *L. rhamnosus* and apple juice. BRB: fruit based-fermented beverage containing *L. acidophilus* and blueberries.

IV. CONCLUSION

The microbial populations of probiotic fermented fruit-based beverages were high during storage within the designated shelf-life. The viable cell counts of the *L. acidophilus* and *L. rhamnosus* are satisfactory. Products produced with different bacteria and fruits showed high sensory characteristics. Future research needs to be conducted to develop fermented dairy beverages of improved storage stability of probiotic *Lactobacillus* strains in fruit juice matrices with functional properties.

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