



Some Properties of Probiotic Yoghurt Produced for Babies by Adding Fruit Puree, Containing *B. infantis*, *B. bifidum*, *B. longum*, *L. paracasei*

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ABSTRACT

Probiotic yoghurt with fruit was produced to enrich the intestinal flora of infants and to prevent various ailments in infants when the flora is inadequate. Peach, apple and pear purees (10% and 20% each), cow milk, milk powder, starter culture (combination of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Bifidobacterium infantis*, *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Lactobacillus paracasei*) were used in the production of probiotic yogurt for babies. Some properties of yoghurt samples were investigated during fermentation and on the 1st, 7th, 14th and 21st days of storage. After ten hours of fermentation, the lowest pH was observed in samples with apple puree. It has been determined that syneresis increases with increasing concentrations of fruit purees. The water holding capacity was less in yoghurts containing fruit puree compared to control yoghurt and in 20% fruit puree compared to yoghurts containing 10% fruit puree. The number of *L. bulgaricus* generally increased in all samples during storage. It was determined that the number of *S. thermophilus* in control sample was higher than other samples during storage. The number of *L. paracasei* and *Bifidobacterium* spp. decreased during storage. While the control sample remained probiotic until the 14th day of storage, other samples lost its probiotic properties before the 7th day of storage. Considering that the number of probiotic microorganisms in a probiotic product should be at least 10⁶-10⁷ CFU/g according to FAO, it has been decided that the most suitable fruits for probiotic yogurt with fruit puree are peach and apple, respectively. Considering the structural features, it is more appropriate to use 10% fruit puree, and considering the probiotic feature, it is more appropriate to use 20% fruit puree. Choosing the appropriate packaging and fixing suitable storage conditions will help probiotic microorganisms to preserve their vitality for a long time.

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Introduction

Some lactic acid bacteria isolated from the gastrointestinal tract of humans and animals are known as probiotics (Saccaro et al., 2009). Yoghurt is one of the most popular foods in terms of transporting probiotic microorganisms. With the use of probiotic bacteria in yoghurt production, the health benefits of the product are increased, while the quality of the product is positively affected (Comak Gocer et al., 2016). Yoghurt is rich in protein, calcium, phosphorus and riboflavin. Probiotic culture also contributes favorably to the sensory properties of the product (Kristo et al., 2003). The buttery acetaldehyde flavor, which is felt predominantly in traditional yoghurt, is not felt in these products (Comak Gocer et al., 2016).

Probiotics develop slowly in milk because they do not have essential proteolytic activity (Saccaro et al., 2009). The addition of *Lactobacillus delbrueckii* ssp. *bulgaricus*

and *Streptococcus thermophilus* is required to shorten the fermentation time (Shah and Lankaputhra, 1997).

S. thermophilus one of the classic yoghurt starter bacteria, due to its oxygen-consuming properties, prepares the anaerobic conditions required especially for *Bifidobacterium* spp. and promotes their development (Ozer, 2006). Streptococci ferment fructose, mannose and lactose (Sagdic and Arici, 2010). Due to this feature, it can be quite active in fruit yoghurt.

In addition to its positive effects on health, *Lactobacillus paracasei* is recommended as a suitable microorganism in the production of fermented milk due to its organoleptic properties (Xanthopoulos et al., 2000). In a study, *L. paracasei* isolated from healthy people showed antibacterial and anticandidal activities against oral pathogens such as, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Staphylococcus aureus*,

Actinomyces viscosus, *Porphyromonas gingivalis*, *Candida albicans*, *Candida tropicalis* and *Candida grabata* (Sookkhee et al., 2001). In developing countries where environmental conditions are generally insufficient diarrhea attacks are encountered in infants and children. Fermented milk products have been reported to reduce the duration of diarrhea by half in children with diarrhea. In addition to organic acids produced by probiotic bacteria, bacteriocins and inhibitory proteins such as lactocidin, acidolin, acidophilin, lactacium-B have antimicrobial effects on pathogens (Ozer, 2010).

Although the proportion of *Bifidobacterium* species varies according to the type of nutrition, as people get older, their numbers in the digestive system of people decrease and their species also changes. *Bifidobacterium* spp. constitute 25% of the total population in adults and 95% in newborn babies (Larpen and Larpen Gourgaut, 1997). Predominant species in breastfeeding infants are *B. longum*, *B. infantis* and *B. breve*. Predominant species in infants and children fed with food are *B. adolescentis*, *B. infantis*, *B. breve*, *B. bifidum* and *B. longum* (Salminen et al., 2004).

Bifidobacteria exhibit phosphatase activity, which can increase the absorption of protein from breast milk. Some *Bifidobacterium* species produce vitamins B₁, B₉ and B₁₂ (Ceyhan and Alic, 2012). *B. infantis* mostly produces thiamine (vitamin B₁), folic acid (vitamin B₉), nicotinic acid and biotin (vitamin B₇ or H) (Salminen et al., 2004).

Since the importance of intestinal flora in babies has been understood, foods containing probiotics and prebiotics have been produced for feeding babies (Ceyhan and Alic, 2012). Although breast milk is continued from the 6th month, yoghurt, fruit juice, vegetable juice and puree should also be given to the baby (Koksal and Gokmen Ozel, 2008).

Fruits improve the nutritional value and taste of yoghurt (Cakmakci et al., 2012). Fresh vegetables and fruits are efficient sources of non-digestible carbohydrates such as vitamins, minerals, carbohydrates and cellulose, especially for preschool children. Fruits help intestinal activity due to their cellulose content (Unver and Unusan, 2005). Fibers that can be fermented in the colon act as prebiotics, promoting the development of health-friendly probiotics such as lactobacilli and bifidobacteria, increasing the beneficial bacterial mass (Celik, 2013).

In this study, peach, apple and pear rich in prebiotics were used in the production of probiotic fruit yoghurt. Pear is one of the foods rich in dietary fiber. Rhamnogalacturono-oligosaccharides, which are prebiotics, are found in apples (Rastall and Maitin, 2002). In some studies, the applicability of apples as an ingredient to increase probiotic viability in foods has been noted. It is stated that apple and pear pieces are suitable material for *L. casei* immobilization due to their cellulosic structure (do Espírito Santo et al., 2011). Kourkoutas et al. (2006) stated that because cellulose is not digested, fruit pieces have a possible protective effect during passage through the intestinal tract, which may help *L. casei* reach the colon. In one study, apple was found to be a appropriate prebiotic for *L. rhamnosus* (Alegre et al., 2011).

In this study, the properties of probiotic yoghurts with fruit, rich in fiber and produced specifically for babies were

examined during storage and the most suitable yoghurts were determined.

Materials and Methods

Materials

Peach, apple and pear purees, cow milk, milk powder, starter culture obtained from commercially producing companies were used as materials. Organic peach puree was obtained from HIPP Dış Tic. Ltd. Şti., Turkey. It is composed of 89% fruit, water, corn flour and vitamin C (energy 52 kcal, sugar 8 g, fiber 1.9 g/100 g peach puree). Organic apple and pear puree were obtained from Numil Gıda Ürünleri San. ve Tic. A.Ş., Turkey (Milupa Brand). They contained 99% fruit and vitamin C (energy 43 kcal, sugar 9.2 g, fiber 1.7 g/100 g apple puree, energy 55 kcal, sugar 8.5 g, fiber 2 g/100 g pear puree). Pasteurized whole cow milk (fat 3.1% and protein 2.8%) was obtained from Ak Gıda San. ve Tic. A.Ş., Turkey (İçim brand). Medium heat skim milk powder obtained from Pınar Süt Mamülleri San. A.Ş., Turkey. The starter culture was supplied from Doğadan Bizim Gıda ve Süt Ürünleri San. ve Tic. Ltd. Şti, Turkey (Bizim Baby for Combiotic Yoghurt). It is a combination of, *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*, *B. infantis*, *B. bifidum*, *B. longum* and *L. paracasei*.

Production of Set-Type Yoghurts

According to the company's yoghurt production instructions, 2 g of lyophilized culture should be inoculated in 5 L milk. Lyophilized culture (2 g) was activated by incubating for 30 min at 42°C using 100 mL of pasteurized milk with 5% milk powder added. After adding 5% milk powder, the milk (4900 mL) was pasteurized at 90°C for 10 min and then cooled to 43°C. The activated culture was added to pasteurized milk for yoghurt production and the mix was divided into seven experimental lots, peach (10%, 20%), apple (10%, 20%) and pear (10%, 20%) purees and control yoghurt (before starting the main study, preliminary tests were carried out using various proportions of fruit purees and milk powder. Preliminary tests were repeated until the most structurally appropriate yoghurt was obtained). The fruit purees were added to yoghurt milk before the fermentation. In order to determine the pH values during fermentation, 30 mL of fruit puree added yoghurt milk and control yoghurt milk were put into each of 50 mL 50 disposable polipropilen (PP) plastic packages (total 1500 mL). For the analysis to be carried out on the 1st, 7th, 14th and 21st days of the storage, 125 mL of fruit puree added yoghurt milk and control yoghurt milk were put into each of 150 mL 28 disposable PP plastic packages (total 3500 mL). All samples were incubated at 43°C until the pH values of yoghurts were reached to 4.5. Fermentation lasted about 10 hours at 43°C. They were cooled at the room temperature and stored at 4°C for 12 hours and analysis were performed on the 1st, 7th, 14th and 21st days of store.

Physicochemical Analysis

pH values of samples were measured with pH meter (HI2002-02, Hanna Instruments, Inc., USA). Syneresis was determined according to Tamime et al. (1996). 25 g of sample was weighted to Whatman No.1 filter paper and

stored at 4°C for 2 hours. It was calculated as the ratio of whey weight to sample weight as percentage. Titratable acidity and the total solid amount of samples were carried out using AOAC method (Helrich, 1990). For the determination of water holding capacity (WHC), 10 g of yoghurt was placed into falcon tube and centrifuged at 5000 rpm for 20 min at 4°C (Celik and Bakirci, 2003). L*, a*, b* color measurements of samples were made using Konica Minolta Chroma Meter CR-5. Color measurements and total solid values of the samples were carried out on the first day of storage.

Microbiological Analysis

The counts of microorganisms were determined during storage at 4°C (1st, 7th, 14th and 21st days). One gram of sample was diluted with 9 mL of sterile 0.1% (w/v) peptone water (Oxoid, Basingstoke, UK), mixed with a vortex and subsequently serially diluted. The spread plate method was used to evaluate of microbial counts. M17 agar (Merck, Germany) was used for enumeration of *S. thermophilus* at 37°C for 48 hours under aerobic conditions (Rybka and Kailasapathy, 1996). MRS agar (Oxoid CM 361, Thermo-Fisher Scientific Inc., USA) was acidified with HCl to reach 5.2 pH value. It was used to determine the count of *L. delbrueckii* ssp. *bulgaricus* and incubated anaerobically at 37°C for 72 hours (Dave and Shah, 1996). The counts of *L. paracasei* ssp. *paracasei* were determined with using Vancomycin added MRS agar and incubated anaerobically at 37°C for 72 hours. MRS agar was supplemented with cysteine chloride and lithium mupirocin (69732, Sigma-Aldrich) to determine the count of *Bifidobacterium* strains. The incubation was carried out anaerobically (5% CO₂ atmosphere) at 37°C for 72 hours (Tharmaraj and Shah, 2003). After the incubation, plates were counted and results were expressed as log CFU/g.

Statistical Analysis

During the evaluation of physicochemical and microbiological analysis results, the difference between the groups was determined using the univariate general linear model procedure of the SPSS statistical software programme (version 18; SPSS, Inc., Chicago, IL, USA). Duncan's multiple comparison test was used to determine significant differences among the means at P<0.05 (Duzgunes et al., 1978). All analyses and measurements were repeated in triplicates.

Results and Discussion

The fermentation of all samples was completed at the end of the 10th hour. pH decreased earlier in probiotic yoghurt samples containing apple and pear puree than other samples (Table 1). In yoghurt production, the incubation period is 2.5-3.0 hours when standard cultures are used, and 6-9 hours when cultures with moderately acidifying properties are used. When pH decreases below 5, acid gel formation begins to appear. Coagulation is complete when the pH value is less than the isoelectric point (pH 4.6) of the casein (Ucuncu, 2005). Since probiotic bacteria growth slowly in milk, it is necessary to add yoghurt starter cultures to shorten the fermentation time (Shah and Lankaputhra, 1997). Probiotic bacteria may adversely affect the development of other starter bacteria through the

metabolites they produce during fermentation and extend the fermentation process (Ozer, 2010).

pH and Titratable Acidity

The pH and titratable acidity of the samples containing peach, pear and apple puree (0%, 10% and 20%) were measured during cold storage of the samples. The results obtained from the pH and titratable acidity analysis of the samples during the storage periods are shown in Figure 1 and Figure 2. The pH values of yoghurt samples decreased until the 14th day of storage. While the pH values of the samples containing 10% apple and 10% pear puree continued to decrease after the 14th day of storage, the pH values of the other samples increased. The findings of the current study are consistent with those of do Espirito Santo et al. (2012) who found fluctuating results for pH values of control sample during cold storage. Demirci et al. (2017) demonstrated that pH values of the yoghurt samples decreased with the time of cold storage. The pH results of samples were found significant at the P<0.05 level. The pH values of samples containing 10% apple puree and 10% pear puree decreased considerably at the end of the storage. The current study found that the pH values of yoghurt samples containing 10% and 20 % apple puree are 4.14 and 4.26 on the 21st day of storage. The present findings seem to be consistent with other research which found the pH value of apple pomace added yoghurts were 4.3 (Wang et al., 2019). The data of pH can be compared with the data in Figure 2 which shows the titratable acidity values of samples. Generally, the pH of samples showed consistency with their titratable acidity. The titratable acidity values of yoghurt samples varied from 0.84% to 1.17% lactic acid during cold storage for all samples. This finding is in agreement with do Espirito Santo et al. (2012) findings which showed that the titratable acidity of passion fruit added yoghurt ranged from 0.87 to 0.74% lactic acid. As shown in Figure 2, there was an increase in the titratable acidity values. The difference between storage days in the pH of the yogurt sample containing 10% peach puree was insignificant (P>0.05)

The increase in the titratable acidity values induced during the storage period was statistically significant in all samples with the exception of the control sample and sample containing 10% peach puree (P<0.05). The storage time was not having a significant effect in the titratable acidity of sample containing 10% peach puree. However, the titratable acidity of control samples significantly increased to the 14th day, but then titratable acidity of the control yoghurt fell slightly. Karaca et al. (2019) produced yoghurt that fortified with the apricot fibre. They found that there has been a steadily decrease in the titratable acidity values with the increased ratio of apricot fibre (from 1.00 to 0.97% lactic acid). The findings of them seem to be consistent with the current study which showed that the increased peach ratio resulted in the lower titratable acidity (from 1.02 to 0.89% lactic acid) at the first day of the cold storage.

Physicochemical Properties

Table 2 shows syneresis and the water holding capacity results of the yoghurt samples. The reason of syneresis defined as the shrinkage of the yoghurt gel and resulted in the whey separation (Lucey, 2004).

Table 1. pH values of the yoghurt milk sample during the fermentation

Fermentation time (h)	Control	10% Peach	20% Peach	10% Apple	20% Apple	10% Pear	20% Pear
0	6.48±0.04 ^{Aa}	6.26±0.03 ^{Ac}	6.05±0.03 ^{Be}	6.27±0.03 ^{Ac}	6.11±0.04 ^{Ad}	6.36±0.03 ^{Ab}	6.23±0.04 ^{Ac}
2	6.50±0.03 ^{Aa}	6.30±0.03 ^{Ac}	6.11±0.02 ^{Ae}	6.29±0.03 ^{AcD}	6.10±0.02 ^{Ae}	6.35±0.04 ^{Ab}	6.25±0.02 ^{Ad}
4	6.51±0.04 ^{Aa}	6.30±0.02 ^{Ac}	6.10±0.03 ^{Ae}	6.31±0.02 ^{Ac}	6.14±0.04 ^{Ae}	6.38±0.02 ^{Ab}	6.24±0.02 ^{Ad}
6	6.20±0.05 ^{Ba}	5.92±0.03 ^{Bc}	5.84±0.02 ^{Cd}	5.78±0.02 ^{Be}	5.47±0.03 ^{Bf}	6.03±0.04 ^{Bb}	5.87±0.03 ^{Bcd}
8	5.10±0.03 ^{Ca}	4.91±0.04 ^{Cb}	4.91±0.01 ^{Db}	4.70±0.03 ^{Cd}	4.69±0.02 ^{Cd}	4.79±0.02 ^{Cc}	4.83±0.00 ^{Cc}
9	4.89±0.02 ^{Da}	4.78±0.02 ^{Db}	4.74±0.02 ^{Ec}	4.57±0.01 ^{De}	4.51±0.01 ^{Df}	4.55±0.04 ^{De}	4.70±0.01 ^{Dd}
10	4.84±0.00 ^{Da}	4.70±0.03 ^{Eb}	4.67±0.01 ^{Fb}	4.50±0.02 ^{Ed}	4.51±0.05 ^{Dd}	4.50±0.03 ^{Dd}	4.56±0.02 ^{Ec}

Data are means ± SD (n=3). Lower-case letters present the differences between the different samples in the same fermentation time and upper-case letters show differences between the different fermentation times of each sample (P<0.05).

Table 2. Syneresis and water holding capacity (%) values of yoghurt samples during storage

Analysis	Samples	Day 1	Day 7	Day 14	Day 21
Syneresis (%)	Control	28.66±4.04	28.92±2.54 ^a	20.26±1.10 ^{cd}	29.64±0.65 ^a
	10% Peach	30.00±1.69	27.64±1.07 ^a	26.46±0.93 ^b	29.12±0.56 ^a
	20% Peach	28.84±0.39 ^A	29.24±0.56 ^{A,a}	30.30±0.02 ^{A,a}	26.86±0.29 ^{B,a}
	10% Apple	24.48±3.28 ^A	16.14±1.27 ^{B,bc}	14.50±0.19 ^{B,f}	6.20±0.59 ^{C,c}
	20% Apple	24.66±0.08 ^A	23.78±0.82 ^{A,ab}	21.44±1.07 ^{A,c}	12.02±0.41 ^{B,bc}
	10% Pear	22.06±0.93 ^A	15.18±0.25 ^{A,c}	14.90±0.02 ^{A,ef}	6.44±1.72 ^{B,c}
	20% Pear	24.82±1.32	19.10±4.10 ^{bc}	17.90±1.27 ^{de}	17.54±1.20 ^b
Water Holding Capacity (%)	Control	55.90±1.27 ^{AB,a}	51.05±0.91 ^B	58.50±1.13 ^{A,a}	53.55±2.05 ^{AB}
	10% Peach	55.30±0.00 ^{A,ab}	51.60±1.97 ^{AB}	50.45±0.49 ^{B,bc}	55.25±1.20 ^{AB}
	20% Peach	55.90±4.94 ^a	49.20±3.11	50.40±0.28 ^{bc}	53.05±0.63
	10% Apple	47.75±0.63 ^{C,bc}	52.15±1.06 ^B	49.55±0.07 ^{BC,bc}	55.35±0.77 ^A
	20% Apple	46.50±0.28 ^c	46.25±0.49	47.00±0.00 ^c	51.40±3.25
	10% Pear	48.15±0.07 ^{abc}	49.40±5.51	51.30±1.55 ^b	54.15±0.63
	20% Pear	47.75±1.34 ^{B,bc}	52.90±0.56 ^A	47.65±1.34 ^{B,c}	49.40±0.56 ^{AB}

Lower-case letters present the differences between the different samples in the same storage time and upper-case letters show differences between the storage times of each sample (P<0.05). The difference between storage days in the pH of the yogurt sample containing 10% apple puree was insignificant (P>0.05).

It is found that syneresis increased with the increase of the concentration of all fruit purees. The findings of the current study are consistent with those of Wang et al. (2019) who found the increased amount of the apple pomace in yoghurt resulted in high ratio of syneresis. It seems possible that these results are due to the insoluble fiber content of fruit purees. The insoluble fibers can cause an increase in syneresis values as a result of their disruption potential of gel structure (Wang et al., 2019). There were no significant differences during cold storage period of control sample, samples containing 10% peach puree and 20% pear puree (P>0.05). On the other hand, the syneresis values of the other samples were found statistically important with the progress of cold storage period (P<0.05). The addition of peach puree increased syneresis of yoghurt samples significantly compared to yoghurts containing pear and apple puree. Syneresis was higher in samples containing 20% fruit puree than containing 10% fruit puree. The water holding capacity provide an information about the gel stability of samples (Brückner-Gühmann et al., 2019). The water holding capacity was higher in the samples containing 10% fruit puree than in the ones containing 20% fruit puree.

Color values of fruit purees is shown in Table 3. The total solid and color values of probiotic yogurt samples are shown in Table 4. Total solid values of probiotic yogurt samples containing fruit puree were lower than that of control sample. The increase in the fruit puree added caused a decrease in the total solid value. Except for the control yoghurt, the yoghurt sample with peach puree had the highest total solid value. In the study of Debashis Kumar et al. (Debashis Kumar et al., 2015) as the fruit pulp ratio

increased in yoghurts containing papaya and watermelon pulps, total solid values decreased. In that study, as the rate of banana pulp increased, the total solid value of yoghurt increased slightly. In the same study, the total solid values of yoghurts containing fruit pulp were lower than that of the control yoghurt. These results are similar to those in our study.

Although the L* values of the samples containing fruit puree were lower than that of the control sample, the a* and b* values of the samples containing fruit puree were higher than that of the control sample. The increase in the fruit puree rates caused the L* values to decrease and the a* and b* values to increase.

Microbiological Enumeration

Microbiological analysis results are shown in Table 5. The numbers of *L. delbrueckii* ssp. *bulgaricus* were the highest on the 14th day of storage, except for samples containing apple puree. *L. delbrueckii* ssp. *bulgaricus* count was higher in samples containing 20% pear puree than samples containing 20% pear puree on all days of storage. *L. delbrueckii* ssp. *bulgaricus* counts were higher in samples containing 10% fruit puree than samples containing 20% fruit puree. According to the statistical analysis, the differences between the days of storage and samples in terms of *L. delbrueckii* ssp. *bulgaricus* were significant (P<0.05).

The number of *S. thermophilus* was high in control yoghurt from yoghurts with fruit during storage. In addition, its numbers in samples containing 10% fruit puree were higher than those containing 20% fruit puree. It can be said that the addition of fruit puree suppressed the growth of this bacteria.

Table 3. Colour values of fruit purees

Fruit Puree	L*	a*	b*
Peach puree	43.66±0.01	7.82±0.18	33.93±0.18
Apple puree	39.85±0.03	0.25±0.01	18.38±0.01
Pear puree	40.97±0.03	2.09±0.01	18.74±0.00

L*: lightness axis, a*: green-red axis, b*: blue-yellow axis

Table 4. Total solid (%) and color values of yoghurt samples

Yoghurts	Total solid (%)	Color values		
		L*	a*	b*
Control	16.50±0.33 ^a	83.92±0.03 ^a	-1.80±0.01 ^g	7.21±0.03 ^g
10% Peach	16.14±0.21 ^a	80.56±0.01 ^d	0.19±0.01 ^b	11.50±0.00 ^b
20% Peach	15.56±0.11 ^b	76.52±0.01 ^g	1.32±0.01 ^a	16.21±0.01 ^a
10% Apple	15.19±0.30 ^{bc}	82.30±0.01 ^b	-1.17±0.02 ^f	8.78±0.02 ^f
20% Apple	14.85±0.17 ^c	79.38±0.00 ^f	-0.93±0.03 ^e	10.21±0.05 ^d
10% Pear	14.91±0.08 ^c	81.76±0.01 ^c	-0.87±0.02 ^d	9.11±0.03 ^e
20% Pear	14.83±0.08 ^c	79.91±0.01 ^e	-0.38±0.01 ^c	10.51±0.02 ^c

Different letters in the same column are significantly different (P<0.05). Results are expressed as the mean ± standard deviation (n=3). *The results were given for day 1.

Table 5. The bacteria numbers of yoghurt samples during storage (log CFU/g)

Bacteria	Yoghurts	Day1	Day7	Day14	Day21
<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	Control	5.30±0.06 ^{D,f}	5.60±0.25 ^{C,c}	5.90±0.15 ^{A,e}	5.69±0.30 ^{B,f}
	10% Peach	5.45±0.04 ^{D,d}	6.18±0.60 ^{C,b}	6.30±0.90 ^{A,a}	6.26±0.21 ^{B,a}
	20% Peach	5.83±0.15 ^{C,a}	5.40±0.07 ^{D,e}	6.18±0.70 ^{B,c}	6.20±0.36 ^{A,b}
	10% Apple	5.58±0.21 ^{D,b}	6.40±0.45 ^{A,a}	6.23±0.45 ^{B,b}	6.11±0.30 ^{C,c}
	20% Apple	5.51±0.15 ^{B,c}	5.30±0.07 ^{C,e}	5.00±0.03 ^{D,f}	6.00±0.40 ^{A,d}
	10% Pear	5.08±0.04 ^{C,g}	5.30±0.09 ^{B,e}	6.08±0.40 ^{A,d}	5.30±0.03 ^{B,g}
	20% Pear	5.40±0.07 ^{D,e}	5.50±0.15 ^{C,d}	6.30±0.60 ^{A,a}	5.78±0.10 ^{B,e}
<i>Streptococcus thermophilus</i>	Control	8.48±0.70 ^{A,a}	8.08±0.36 ^{D,a}	8.20±0.35 ^{C,a}	8.26±0.40 ^{B,a}
	10% Peach	7.54±0.05 ^{BC,bc}	7.51±0.15 ^{C,b}	7.58±0.31 ^{AB,b}	7.61±0.21 ^{A,c}
	20% Peach	7.48±0.31 ^{A,c}	7.30±0.76 ^{B,d}	7.11±0.76 ^{C,e}	7.08±0.25 ^{C,f}
	10% Apple	7.32±0.72 ^{A,d}	7.40±0.57 ^{C,c}	7.48±0.91 ^{B,d}	7.60±1.52 ^{A,c}
	20% Apple	7.08±0.45 ^{C,e}	6.85±0.55 ^{D,e}	7.50±1.00 ^{A,cd}	7.48±0.58 ^{B,d}
	10% Pear	7.58±0.40 ^{B,b}	7.48±0.13 ^{C,b}	7.54±0.10 ^{B,c}	7.70±0.15 ^{Ab}
	20% Pear	7.53±0.21 ^{A,bc}	6.51±0.01 ^{C,f}	6.36±0.04 ^{C,f}	7.30±0.45 ^{B,e}
<i>Lactobasillus paracasei</i>	Control	5.00±0.30 ^{A,e}	4.00±0.06 ^{D,f}	4.79±0.15 ^{B,bc}	4.48±0.02 ^{C,g}
	10% Peach	5.00±0.45 ^{C,e}	4.00±0.04 ^{D,f}	5.60±0.15 ^{A,a}	5.54±0.11 ^{B,a}
	20% Peach	5.48±0.11 ^{A,a}	4.20±0.07 ^{D,e}	4.84±0.20 ^{C,b}	5.00±0.30 ^{B,c}
	10% Apple	5.00±0.35 ^{A,e}	4.59±0.31 ^{C,d}	4.46±0.03 ^{D,e}	4.70±0.21 ^{B,f}
	20% Apple	5.30±0.50 ^{A,b}	4.74±0.25 ^{BC,c}	4.70±0.15 ^{C,cd}	4.78±0.15 ^{B,e}
	10% Pear	5.11±0.49 ^{B,d}	4.95±0.36 ^{C,a}	4.59±0.21 ^{D,de}	5.28±0.17 ^{A,b}
	20% Pear	5.18±0.45 ^{A,c}	4.77±0.30 ^{C,b}	4.00±0.03 ^{A,f}	4.95±0.20 ^{B,d}
<i>Bifidobacterium</i> spp.	Control	7.81±1.15 ^{A,a}	6.56±0.11 ^{B,a}	6.38±0.06 ^{C,a}	5.18±0.05 ^{D,a}
	10% Peach	7.32±0.07 ^{A,b}	4.77±0.30 ^{B,d}	4.69±0.15 ^{B,c}	4.48±0.05 ^{B,d}
	20% Peach	6.97±0.30 ^{A,c}	4.70±0.10 ^{B,d}	4.48±0.05 ^{B,c}	4.00±0.01 ^{B,g}
	10% Apple	6.61±0.40 ^{A,e}	5.52±0.20 ^{B,c}	5.49±0.05 ^{B,b}	4.95±0.02 ^{B,b}
	20% Apple	6.81±0.20 ^{A,d}	5.62±0.25 ^{B,b}	4.60±0.05 ^{C,c}	4.38±0.03 ^{C,e}
	10% Pear	6.41±0.50 ^{A,f}	5.70±0.15 ^{B,b}	4.49±0.01 ^{C,c}	4.53±0.01 ^{C,c}
	20% Pear	5.49±0.10 ^{A,g}	4.90±0.25 ^{B,d}	4.30±0.08 ^{C,c}	4.23±0.02 ^{C,f}

Lower-case letters present the differences between the different samples in the same storage time and upper-case letters show differences between the storage times of each sample (P<0.05).

The number of *S. thermophilus* decreased steadily from the beginning of storage in samples containing 20% peach puree. The numbers of this microorganism generally decreased until the 7th day of storage and then increased and reached the highest level on the 21st day of storage in samples containing 10% peach, 10% apple, 10% pear puree, but still could not exceed the number in the control sample. According to the statistical analysis, the differences between the days of storage and samples in terms of *S. thermophilus* were significant (P<0.05). In the

study conducted by Canganella et al. (2000), inoculated yoghurt starters, *L. acidophilus* and *B. infantis* in cow milk, and they obtained a yoghurt with a pH of 4.5 at the end of 42°C incubation. On the first day of storage, the number of each microorganism was approximately 10⁸ CFU/mL. Although the number of *L. bulgaricus* of plain cowmilk yoghurt sample stored at 4°C decreased up to the 5th day, then increased and decreased again after the 10th day of storage. The number of *S. thermophilus* was generally higher than *L. bulgaricus*. On the 10th day of storage, the

numbers of these microorganisms were close to each other. The situation was similar in the example of yogurt with raspberry.

The *L. paracasei* counts in all the samples containing puree were close to that in the control sample on the first day of storage. The *L. paracasei* in counts all yoghurt samples decreased until the 7th day of storage. While the number of that microorganism was higher in the samples with apple and pear puree on the 7th day of storage, it reached the highest number in the sample containing 10% peach puree on the 14th and 21st days of storage. The number of *L. paracasei* was higher in the sample containing 20% apple puree than the sample containing 10% apple puree on all days of storage. In other samples, the number of *L. paracasei* was higher in those that usually contain 10% fruit puree.

Kristo et al. (2003), in their study with fermented milk at 42°C, they found that the number of *L. paracasei* increased slightly during storage at 4°C for 21 days, unlike in our study. In study conducted by Pimentel et al. (2012), although the number of *L. paracasei* ssp. *paracasei* in set type yoghurts decreased slightly on the 14th day of storage, it remained above 8 log CFU/g for 28 days during storage. The number of *S. thermophilus* was higher than 9 log CFU/g in storage time. These numbers are higher than those in our study. This may be due to the high number of bacteria inoculated.

In general, *Bifidobacterium* spp. numbers were higher in all samples containing 10% fruit puree than all samples containing 20% fruit puree. At the beginning of storage, the number of *Bifidobacterium* spp. was observed mostly in the control sample and samples containing peach puree, while the number of *Bifidobacterium* spp. was the highest in yoghurt sample containing 10% apple puree and control sample at the end of storage. The number of *Bifidobacterium* spp. decreased during storage. The increase in the number of *L. delbrueckii* ssp. *bulgaricus* may have an effect on this.

In the study conducted by Pimentel et al. (2012), the number of *L. delbrueckii* ssp. *bulgaricus* was between 5.0-5.5 log CFU/g and showed a slight decrease during storage. It is advantageous to decrease the number of *L. delbrueckii* ssp. *bulgaricus*. Because it is responsible for the lowering of the pH. The increase in its number causes the probiotic number to decrease (Kailasapathy, 2006). According to the statistical analysis, the differences between the days of storage and samples were significant ($P < 0.05$). In study conducted by Canganella et al. (2000), the *B. infantis* number increased to 5th day of storage and then decreased as in this study. The number, which was 10^7 on the 20th day of storage, decreased rapidly after the 20th day and decreased to 10^1 levels on the 45th day of storage.

In the study of Shah and Lankaputhra (1997), the number of *B. longum* decreased during storage. Samples of five brands of commercial claimed to contain *L. acidophilus* and *B. bifidum* within 2-3 days after production was supplied by Shah et al. (1995) and then they stored at 4°C for 5 weeks. On the first day of storage, the number of *B. bifidum* was between 10^6 - 10^7 in two of these samples, while it was between 10^3 - 10^4 in the other three, and it has declined sharply since the 12th day of storage and has completely lost its vitality. *B. bifidum* number decreased faster than others in low pH samples. In a study, it was shown that acid adapted *Bifidobacterium breve* exhibits superior survival characteristics, in acidic conditions, in the presence of other environmental stresses such as bile, hydrogen peroxide and cold storage (Park et al., 1995). Acid resistant *Bifidobacterium* strains may prove useful for probiotic applications and may exhibit enhanced survival both in host environmental conditions and in food systems. Lactobacilli are generally more durable than bifidobacterial when compared to their rates of influencing from different factors (Ross et al., 2005). Lactobacilli are more resistant to low pH values and show more adaptation to milk and other food substrates (Lee and Salminen, 2009).

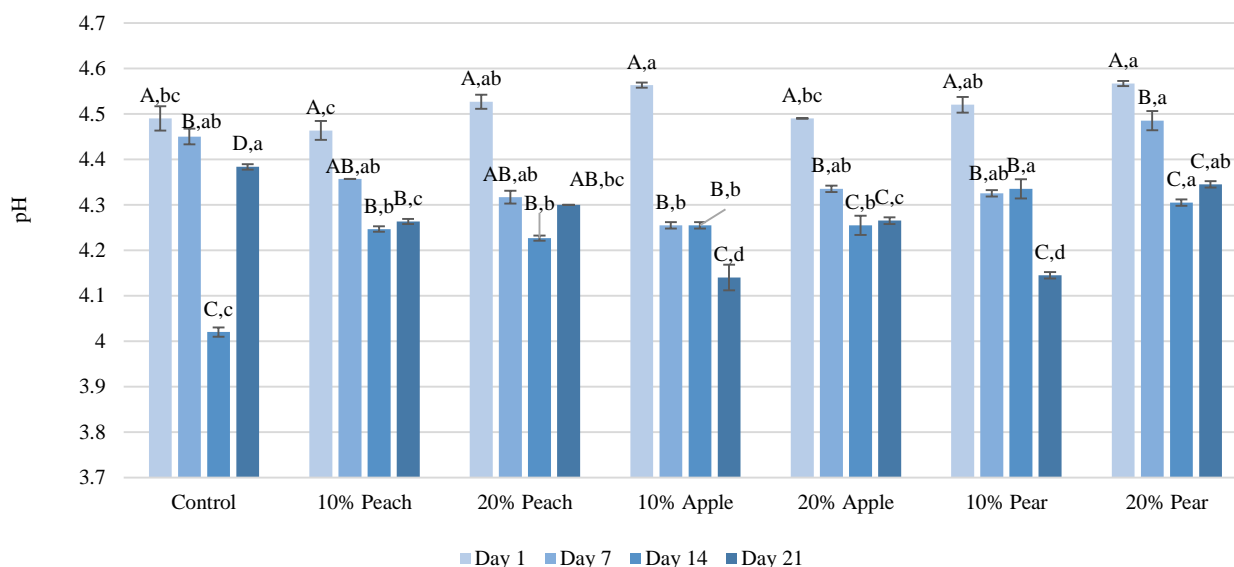


Figure 1. pH values of yoghurt samples during storage

Lower-case letters present the differences between the different samples in the same storage time and upper-case letters show differences between the storage times of each sample ($P < 0.05$).

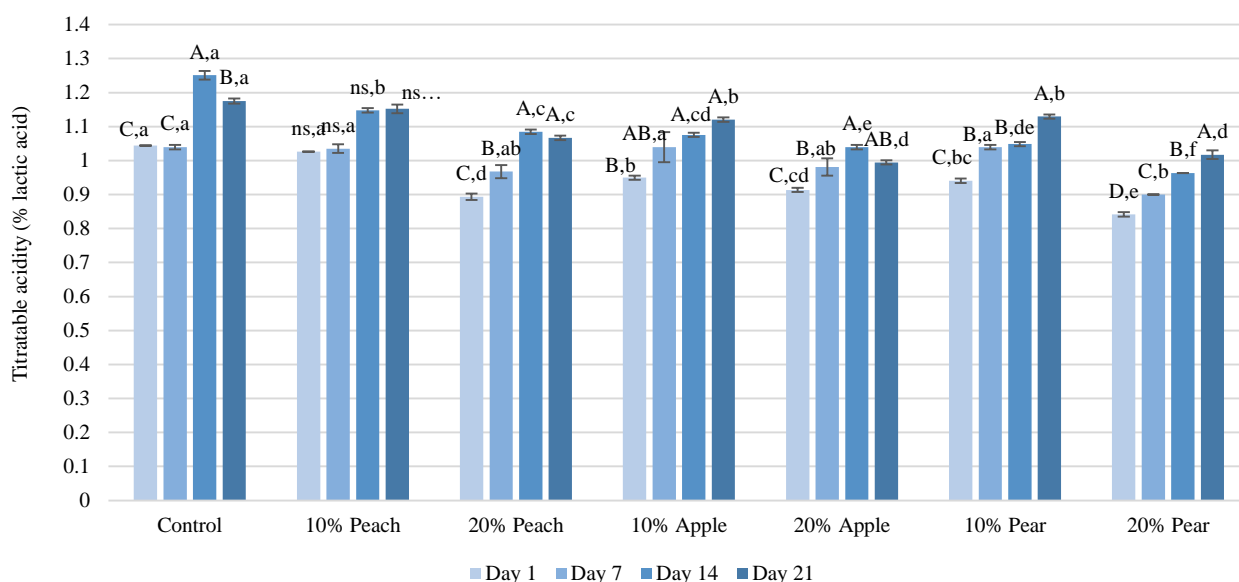


Figure 2. Titratable acidity (% lactic acid) of yoghurt samples during storage

Lower-case letters present the differences between the different samples in the same storage time and upper-case letters show differences between the storage times of each sample ($P < 0.05$). The difference between storage days in the pH of the yogurt sample containing 10% peach puree was insignificant (ns) ($P > 0.05$)

Çakmakçı et al. (2012) produced probiotic yoghurt by adding *B. bifidum*, *L. acidophilus* and yoghurt starters, added 15% banana marmalade after fermentation and kept it at 4°C for 14 days. The number of *B. bifidum* was above 10^6 in the yoghurt only *B. bifidum* added decreased rapidly after the 7th day of storage and dropped below 10^4 on the 14th day of storage. The number of *B. bifidum* was above 10^6 in the yoghurt only *B. bifidum* added, decreased rapidly after the 7th day of storage and dropped below 10^4 on the 14th day of storage. Similar results were obtained in yoghurt, where two probiotic cultures were used together. Banana yoghurts with probiotic cultures lost their probiotic properties after the 7th day. More research should be carried out for possible interactions between selected strains to produce a dairy product, the selection of the best combination (s) and optimization of the processes and their survival times during cold storage. Because these bacteria must reach the intestinal tract to perform their probiotic roles (Saccaro et al., 2009).

Research indicates that probiotic bacteria and especially bifidobacteria are not sufficiently viable in yoghurt preparations. It is claimed that there are various factors that affect the viability of probiotic bacteria in yoghurt, including the acid and hydrogen peroxide produced by the yoghurt bacteria, the amount of oxygen in the product and the oxygen permeability of the package (Shah and Lankaputhra, 1997).

Cruz et al. (2013) produced yoghurt by yoghurt starters, *B. longum* and *L. acidophilus* inoculating. Then, they stored the yoghurts in 4 plastic packages with different oxygen permeability for 28 days. While there was no significant change in the number of yoghurt starters during storage, there was a decrease in the number of *B. longum* and *L. acidophilus*. The decrease in the number of probiotic bacteria was higher in yoghurt stored in high permeability packaging. In probiotic products, the amount of oxygen in the package should be kept to a minimum to prevent toxicity and the development of microorganisms and to

protect the functionality of the product. In this case, the viability of *L. acidophilus* and bifidobacteria is negatively affected in fermented milk products (Dave and Shah, 1997). It has been determined that products formed as a result of lipid peroxidation damage DNA (Zayed and Roos, 2004). Therefore, a combination of vacuum storage and antioxidant is effective to minimize oxidation and maximize probiotic viability during storage (Weinbreck et al., 2010). The use of packaging materials with low oxygen permeability can be considered as the addition of oxygen scavengers and preventing oxygen transfer to the product during production (Talwalkar et al., 2004). While dissolved oxygen levels in HDPE packages increased significantly, oxygen levels in glass bottles remained at low levels for 35 days storage. In that study, it has been determined that the most suitable temperature for storing yoghurts containing *B. lactis* BB-12 is + 8°C (Mortazavian et al., 2007). So, *Bifidobacterium* cells cannot resist to low storage temperatures (Akan and Kımık, 2015). In current study, probiotic yoghurts were stored at +4°C. The use of plastic ambalage in the fermentation and storage of samples and also low storage temperature for bifidobacteria may be one of the reasons for the decrease in probiotics.

The FDA recommends that the amount of probiotics in probiotic foods should be at least 10^6 CFU/mL at the time of consumption. Considering the effect of storage on the digested amount and probiotic viability, it has been stated that the amount required for the probiotic effect in the human organism should be at least 10^8 - 10^9 (Tripathi and Giri, 2014). It is recommended to consume 100 grams of probiotic products daily for approximately 10^9 live probiotics to enter the digestive system (Akan and Kımık, 2015).

In current study, considering the sum of *L. paracasei* and *Bifidobacterium* spp. numbers, the control yoghurt preserved its probiotic characteristic until the 14th day of the storage. It is understood that fruit yoghurts can be consumed as probiotic yoghurt before the 7th day of storage. In current study, it was determined that it is more

appropriate to choose peach and then apple as the fruit that can be used in terms of having probiotic yogurt feature. It is more appropriate that the rate of fruit to be used is 20%. Since the *L. delbrueckii* ssp. *bulgaricus* count was higher in samples with 10% peach and apple puree, the probiotic bacteria count may have been lower. Especially, peach puree can be recommended for freshly consumed fruit probiotic yogurts. Considering the structural features, it is more appropriate to use 10% fruit puree.

Conclusions

Probiotic dairy products, especially probiotic yoghurt, are increasingly consumed in developed countries. Its consumption, especially in childhood, will contribute to the healthier growth of new generations. Recently, adding fruits is a very common method to make dairy products more nutritious and more attractive. If the fruits are added to probiotic products, they will also serve as prebiotics in the product. In order for yoghurt with fruit to be consumed as probiotic yoghurt for a longer time, the number of added probiotic bacteria should be increased and yoghurts should be stored under appropriate conditions.

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