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Original Research Article

A Survey About Plain and Apricot Probiotic Drinks in Shelf Life Process by Different Microbiological Parameters

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ABSTRACT

In recent years, people tend to consume probiotic products because of their health benefits. In accordance with this purpose, lots of different probiotic products, especially dairy products with or without various fruit contents are produced. Therefore, two commercial probiotic dairy drinks which were plain (PPD) and containing apricot (APD) and control group without any probiotic supplement were investigated in this study. For this aim, coliform (*Klebsiella pneumonia* (KPA12) as a test microorganism), total lactic acid bacteria, bifidobacteria, *Lactobacillus acidophilus*, mould and yeast were enumerated and pH alterations were observed during to 21-day shelf life of the drinks. *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strain mix were used as probiotics. Then, KPA12 (6.08 log CFU/mL) was totally prevented at 15th, 16th and 19th days on PPD, APD and Control, respectively. In the meanwhile, any mould and yeast growth were not observed. Additionally, number of total lactic acid bacteria, *L. acidophilus* and bifidobacteria almost protected to their 7 log CFU/mL level. Similarly, any dramatic changes were not seen on pH levels that ranged 4.15-4.25 of the drinks during the storage.

Keywords: Dairy drinks, *Klebsiella pneumonia*, Probiotics

INTRODUCTION

Definition of Probiotics comes from a Greek word which is meaning 'for life' and it is referred to living microorganisms that have beneficial effects on human gut or host when supplied in adequate amounts (Chan and Liu, 2022; Lee et al., 2013; Pandey et al., 2015; Quin et al., 2018). In addition to that, *Bifidobacterium*, *Lactobacillus* and non-pathogenic yeasts are most commonly used as probiotic microorganisms (Cosme et al., 2022; Özoğlu et al., 2020; Reid et al., 2003; Vijaya Kumar et al., 2015). Probiotic microorganisms are added various food products due to their many health benefits that are including positive effect on gastrointestinal infections, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, antimicrobial, antimutagenic, anti-carcinogenic and anti-diarrheal properties, countering seasonal allergies, decreasing the duration of acute respiratory infections, effecting on adults as laxative and increasing immune parameters in HIV patients (Agrawal et al., 2009; Hemsworth et al., 2012; King et al., 2014; Rahimi and Himmat, 2022; Rashidi et al., 2021; Syiemlieh and Morya, 2022; Tran and Li, 2022; De Vrese et al., 2011). Thus, there is a rising demand by consumers on probiotic products as well as their sales. Then, many food matrices, especially dairy products commonly yogurt are used for producing probiotic food products (Özoğlu et al., 2020; Reid, 2015; Shori, 2015). Dairy products are generally chosen for providing probiotic food products because of their lactic acid bacteria (LAB) content. LAB can produce several various antimicrobial compounds supporting probiotic characteristics (Leroy and De Vuyst, 2004; Özoğlu et al., 2022; Shori, 2015). Besides, milk (as fluid) and dairy products are quite suitable medium to carry or generate live and active probiotic cultures (Kandylis et al., 2016; Khan, 2014). Apart from that; many factors like pH, titratable acidity, presence of antibiotics, microbial competition, availability of micronutrients, packaging materials, salt concentration, incubation and storage temperature, antimicrobial ingredients effect on viability of probiotic cultures at food products (Shori, 2015; Terpou et al., 2018). In addition to dairy products, fruit content food products are become very popular for producing probiotic foods because of their bioactive components like phenolic compounds (Chaikham et al., 2013; Monteiro et al., 2022).

It is known that *Klebsiella pneumonia* is one of the important pathogen microorganisms according to food microbiology. It is the second most common Gram-negative pathogen that reasons of wide spectrum infections (Sabota et al., 1998; Vading et al., 2018). *K. pneumonia* is a coliform bacterium (some strains could be fecal

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coliform that are never permitted to present in foods) which is Gram-negative, facultative anaerobe, non-motile, encapsulated, rod-shaped, generated gas from lactose within 48 hours and member of *Enterobacteriaceae* family (Gundogan and Avci, 2013; Vieira et al., 2016). *Klebsiellae* are opportunistic pathogens and lead to many diseases like septicemia, UTI (urinary tract infections), soft tissue infection and also *K. pneumonia* is a reason of pneumonia that presents to intense lung injury and mortality that are correlated with infective exacerbations (Gundogan and Avci, 2013; Podschun and Ullmann, 1998; Vading et al., 2018; Vieira et al., 2016). Additionally, it is the main reason of Klebsiella infections which are nosocomial. *K. pneumoniae* is found as a saprophyte in the nasopharynx and in the intestinal tract in humans (Podschun and Ullmann, 1998; Yu and Chuang, 2018). Therefore, preventing of *K. pneumoniae* spread is required. Because of the increasing antibiotic-resistance of *K. pneumonia*, efficient therapies gain majority (Ahmadi et al., 2021; Vieira et al., 2016). Thus, probiotics are seen a strong alternative to conflict with this pathogen (Melia and Kurnia, 2022; Vieira et al., 2016).

In the light of these information, effects of probiotics based on *K. pneumonia*, growth of total LAB, *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus acidophilus*, mold and yeast, pH were evaluated in the present study at commercial plain and apricot probiotic dairy drinks according to their 21-day shelf life which was determined by the producer company (Bursa, TURKEY).The study has a significance due to effectiveness of probiotics on *K. pneumonia* has not commonly studied as well as other food-borne pathogens in the literature especially on food matrices.

MATERIALS AND METHODS

Probiotic drink products and bacterial strains

Plain probiotic drink (PPD), apricot probiotic drink (APD) and dairy drink without added any probiotics (Control) were provided from production lines of a company in Bursa, TURKEY. Control was taken before adding probiotic microorganisms of the production line.

Klebsiella pneumonia (KPA12) which was chosen as coliform bacteria was isolated from ayran (drink made of yogurt and water) sample at Bursa Uludağ University Agriculture Faculty Food Engineering Department Laboratory. Then, identification of the isolate was made at Mérieux NutriSciences Laboratory in terms of MALDI-TOF. *Bifidobacterium animalis* subsp. *lactis* (Nu-trish® ABY-Premium BB-12®, Chr. Hansen, Hønsholm, Denmark), *Lactobacillus acidophilus* (Nu-trish® ABY-Premium LA-5®, Chr. Hansen, Hønsholm, Denmark), *Lactobacillus delbrueckii* subsp. *bulgaricus* (Nu-trish® ABY-Premium, Chr. Hansen, Hønsholm, Denmark), *Streptococcus thermophilus* (Nu-trish® ABY-Premium, Chr. Hansen, Hønsholm, Denmark) culture composition was used as probiotic microorganisms.

Enumeration of coliforms for controlling growth of *Klebsiella pneumonia*

Enumeration of coliforms for controlling growth of KPA12 was done according to ISO 4832 Colony Count Technique in triplicate at sterile UV cabinet ("ISO 4832:2006 - Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coliforms -- Colony-count technique," n.d.). For this aim, KPA12 that adjusted 6.08 log CFU/mL was added probiotic drink samples. As regards to the protocol of the method; samples were diluted in ways that been five-fold serial dilutions and inoculation was done from 3rd,4th and 5th dilutions to Petri Dishes content VRBL (Violet Red Bile Lactose) Agar (Oxoid) by Pour Plate Technique. Then, incubated at 30℃ for 24 hours. After incubation process, minimum 0.5 mm in diameter, dark red colored colonies were counted and colony forming units were calculated for number of between 10-150 colonies.

Enumeration of total lactic acid bacteria

Number of *S.thermophilus* and *L. bulgaricus* were calculated and gathered. Inoculation was done to Double Layered Pour Plate Technique from 3rd,4th and 5th dilutions of five-fold serial dilutions at sterile UV cabinet. In line with this purpose, M17 Agar (Oxoid) for *S.thermophilus* and MRS (De Man, Rogosa, Sharpe) Agar (Oxoid) for *L. bulgaricus* were used as media. Meanwhile Petri Dishes content M17 Agar were incubated at 37℃ for 48

hours and 3-4 mm in diameter opaque colonies were calculated, Petri Dishes content MRS Agar were incubated at 37℃ for 72 hours and opaque colonies were calculated (Moreno et al., 2006).

Enumeration of bifidobacteria

ISO 29981|IDF 220:2010 Milk products - Enumeration of presumptive bifidobacteria – Colony count technique at 37℃ protocol was succeeded in triplicate at sterile UV cabinet. MRS (Difco 288210) Agar with addition of 5 ml of CyHCl (Merck 2839) stock solution and 2.5 ml Mupirocin (LGC promochem, art no. EPM3806000) stock solution per liter of medium was prepared as a medium. Inoculation was performed by Pour Plate Technique from 3rd,4th and 5th dilutions of five-fold serial dilutions. Moreover, incubation was supplied at 37℃, 3 days under anaerobic conditions. In the end, all colonies are counted as bifidobacteria ("ISO 29981:2010 - Milk products -- Enumeration of presumptive bifidobacteria -- Colony count technique at 37 degrees C," n.d.).

Enumeration of *Lactobacillus acidophilus*

International standard ISO 20128, IDF 192: Milk products - Enumeration of presumptive *L. acidophilus* on selective medium – Colony- count technique at 37℃ protocol was followed in triplicate at sterile UV cabinet. MRS (Difco 288210) Agar with addition of 0.5 ml of clindamycine (Sigma C5269) stock solution per liter of medium was prepared as a medium. Inoculation was performed by Pour Plate Technique from 3rd,4th and 5th dilutions of five-fold serial dilutions. Furthermore, anaerobic incubation was supplied at 37℃, 3 days. Finally, all colonies are counted as *L. acidophilus* ("ISO 20128:2006 - Milk products -- Enumeration of presumptive *L. acidophilus* on a selective medium -- Colony-count technique at 37 degrees C," n.d.).

Enumeration of mould and yeast

ISO 6611 / IDF 094:2004 Milk and milk products - Enumeration of colony-forming units of yeasts and/or moulds - Colony-count technique at 25℃ method was taken as a reference and applied in triplicate at sterile UV cabinet ("ISO 6611:2004 - Milk and milk products -- Enumeration of colony-forming units of yeasts and/or moulds -- Colony-count technique at 25 degrees C," n.d.). According to the method, samples were diluted in ways that been five-fold serial dilutions and inoculation was done from 3rd,4th and 5th dilutions to Malt Extract Agar (Oxoid) by Pour Plate Technique and aerobically incubated at 25℃ for 5 days. Therefore, samples were checked for 19-day process and samples were renewed in each day.

Determination of pH value

pH values were measured directly using hand type pH meter with standard pH probe as in triplicate (Mettler Toledo- SG2-FK).

Statistical analysis

Data were statistically analyzed by IBM SPSS Software, Version 2.1. and Minitab ANOVA. Results were double checked with these two programmes. Besides, the difference between the values was determined by Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Effects of probiotics on *Klebsiella pneumonia* in drink samples

Inhibition of *K. pneumonia* in drink samples was shown in Figure 1. It is clearly seen that the results were given for 19 days even so the shelf life of the products is 21 days. The reason of that KPA12 was completely inhibited at the end of the 19th day in all the samples. Thus, the experiment was not continued for 20th and 21st days. Besides, the other results were given until the end of 19th day for considering uniformity of the study.

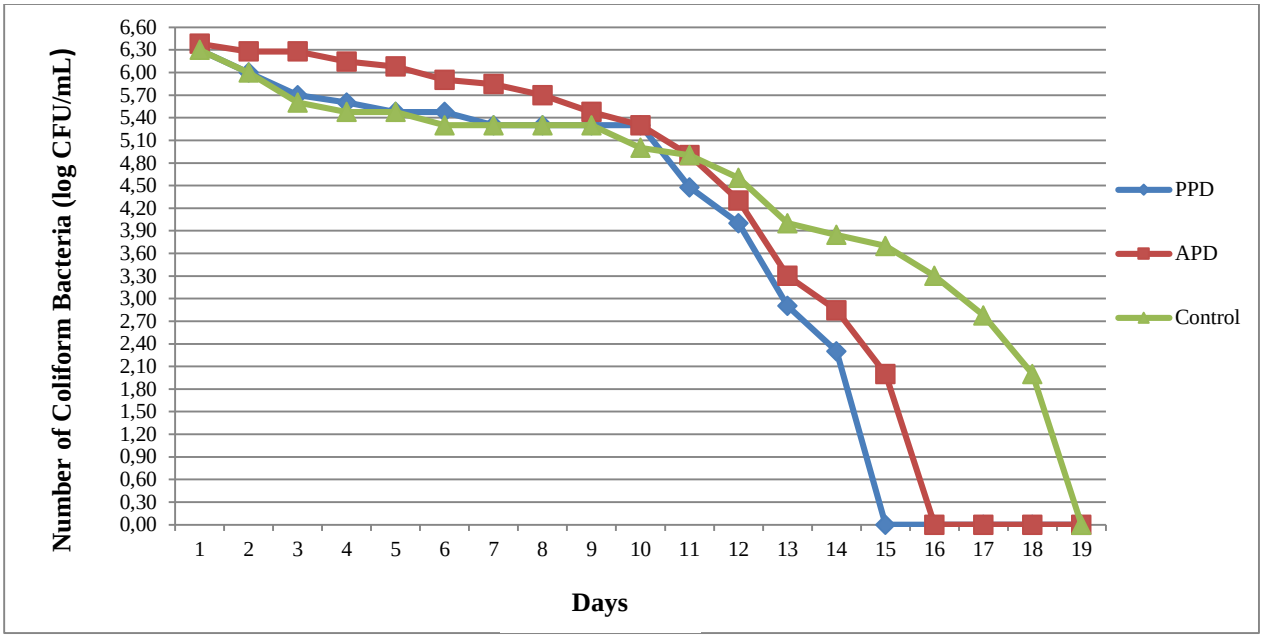

Figure 1. The inhibition of KPA12 in PPD, APD and Control in 19 days. (The results were given as averages of triplicates).

According to the Figure 1, it is clearly seen that the number of KPA12 was smoothly decreased day by day. Then, it was totally inhibited at 15th, 16th and 19th days for PPD, APD and Control, respectively. Thus, it was deduced that probiotics were more rapidly effects on KPA12 in the drinks. Besides, PPD was affected more quickly then APD when compared probiotic drinks. It was expected due to fact that, fruit contents adjusted could damage to the viability of some species and strains of probiotics in food products (Shori, 2015; Syiemlieh and Morya, 2022).

The results were supported the inhibition effects of probiotic microorganisms on *K. pneumonia*. Similarly, Tunçer and Karaçam (2020) were indicated the effect of probiotic *Streptococcus salivarius* M18 on *Pseudomonas aeruginosa* and *K. pneumonia* in their study. According to the study cell-free supernatant of *S. salivarius* M18 were significantly inhibited the growth of the pathogens and their antibiotic sensitivity (Tunçer and Karaçam, 2020). Another study about inhibition effects of probiotics on *K. pneumonia* was done by Radiati et al. (2022). Antimicrobial activity of *Saccharomyces cerevisiae* that is a probiotic yeast concentration levels on kefir against *Escherichia coli*, *Salmonella* Typhi and *K. pneumoniae* was investigated on the study. Then, *E. coli*, *S.* Typhi and *K. pneumoniae* were inhibited in all the samples at 56.00, 64.28 and 68.18%, respectively (Radiati et al., 2022). Thus, it is an advantage that totally inhibition was observed in the current study.

Additionally, KPA12 was inhibited in the control even not to contain any probiotic microorganisms. The reason of that could be LAB content and also lower pH values that could be related LAB content of the dairy products like the food matrices in the current study. LAB are naturally found in fermented products like cheese, yogurt and olive etc. Then, their antimicrobial effects on pathogen microorganisms including *K. pneumoniae* was known (Balayan et al., 2019; El-Mokhtar et al., 2020; Fidan et al., 2022; Özoğlu et al., 2022; Savinova et al., 2021).

Evaluation of total lactic acid bacteria, *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus acidophilus* in drink samples

Enumeration of total LAB, *B. lactis* and *L. acidophilus* in PPD, APD and Control at 1st and 19th days of the storage were shown in Table 1. In line with the results, number of total LAB, *B. lactis* and *L. acidophilus* in the samples were protect their 7 log CFU/mL level at between 1st and 19th days. Then, the changes at between days were not found statistically significant ($p > 0.05$). Furthermore, levels of total LAB, *B. lactis* and *L. acidophilus* in probiotic drinks are higher than the control group drinks, which were not content any additional probiotic microorganisms

expectedly due to these microorganisms are probiotic or potential probiotic microorganisms (Karami, 2018; Semjonovs et al., 2014; Shori, 2015). Also, total LAB was higher in all samples than others and it was statistically meaningful as a result of naturally LAB content of the drinks.

Besides, it is known that fruit or fruit juice or pulps adjusted could damage to the viability of some species and strains of probiotics in food products, due to fact that acidity and the presence of antimicrobial compounds (Shori, 2015; Syiemlieh and Morya, 2022). Thus, the PPD had more number of total LAB, *B. lactis* and *L. acidophilus* than APD as expected.

Table 1. Number of total LAB, Bifidobacteria and *L. acidophilus* in PPD, APD and Control Group at 1st and 19th days.

Samples	Days	Total Lactic Acid Bacteria (Log CFU/mL)	Bifidobacteria (Log CFU/mL)	*Lactobacillus acidophilus* (Log CFU/mL)
PPD	**1**	$7.83\pm0.06^{A,a}$	$7.64\pm0.05^{A,b}$	$7.65\pm0.15^{A,b}$
	19	$7.78\pm0.30^{A,a}$	$7.63\pm0.14^{A,b}$	$7.64\pm0.21^{A,b}$
APD	**1**	$7.73\pm0.15^{A,a}$	$7.52\pm0.21^{A,b}$	$7.39\pm0.21^{A,b}$
	19	$7.72\pm0.21^{A,a}$	$7.49\pm0.10^{A,b}$	$7.34\pm0.10^{A,b}$
Control	**1**	$7.78\pm0.09^{A,a}$	$7.64\pm0.15^{A,b}$	$7.65\pm0.05^{A,b}$
	19	$7.77\pm0.50^{A,a}$	$7.64\pm0.14^{A,b}$	$7.64\pm0.06^{A,b}$

Values are Mean ± SD. Means in the same column followed by different capital superscript letters and same row followed by different lower-case superscript letters are significantly different at $p < 0.05$ level of probability

These results are shown similarity with the results of Terpou et al.'s (2018) study. In their study, a novel potential probiotic strain *Lactobacillus paracasei* K5 was accreted by free or immobilised on industrial white brined cheese production and the culture was conserved its viability during 70 days ripening and storage time of the cheese. In the meantime, the culture was protected its high levels (7 to 8 log CFU/g) (Terpou et al., 2018). Besides, the level of the microorganism(6-7 log CFU/g-mL) is the minimum required live cell level for acceptance a food as a probiotic (Özoğlu et al., 2020; Shori, 2015; Terpou et al., 2018).

Similarly, another study with probiotic fermented milk beverages with black mulberry (MFM), red grape (GFM) and cornelian cherry (CFM) were produced as probiotic drinks and *S. thermophilus*, *L. bulgaricus*, *L. acidophilus* and *B. lactis* were used as counting viable cell. As regards of the results, all strains were almost saved their logarithmic levels like the current study in 28-day storage except for *L. acidophilus* at CFM (Barat and Ozcan, 2018).

Growth of mould and yeast in drink samples

Growth of mould and yeast in the all samples were checked during 21-day shelf life of the drinks. Then, number of mould and yeast were found below 1 log CFU/g (<10 CFU/g) during the shelf life in all samples. The results were expected based on inhibition effects of probiotic microorganisms and LAB on pathogen bacteria, mould and yeast (Fidan et al., 2022; Özoğlu et al., 2020; Papadopoulou et al., 2018; Tatsadjieu et al., 2016). Additionally, working in sterile conditions (from producing to end of the shelf life) might have effected on not to be observed growth of mould or/and yeast.

Papadopoulou et al. (2018) studied about the performance of *Lactobacillus plantarum* T571 with probiotic potential as a co-starter culture in Feta cheese production and its long-term storage. Then, the number of yeast/mould was decreased during ripening times and 362-day storage even it was found 2 log CFU/g during the 2nd ripening period. When compared the current study, using cheese as food matrices is a significant difference of the study. Therefore, cheese contains yeast because of their ripening process(Papadopoulou et al., 2018).

pH values change in drink samples

pH values of all drink samples were measured ate 1st and 19th day. Alterations on the values were indicated with mean values and standard deviations at Figure 2. As a result of pH measurement at 1st and 19th days in the samples, pH values grades were protected except for APD as it was seen in Figure 2. The cause of its could be that fruits

have high acidity and contents are dropped pH values and viability of probiotic microorganism in food matrices (Shori, 2015; Syiemlieh and Morya, 2022).

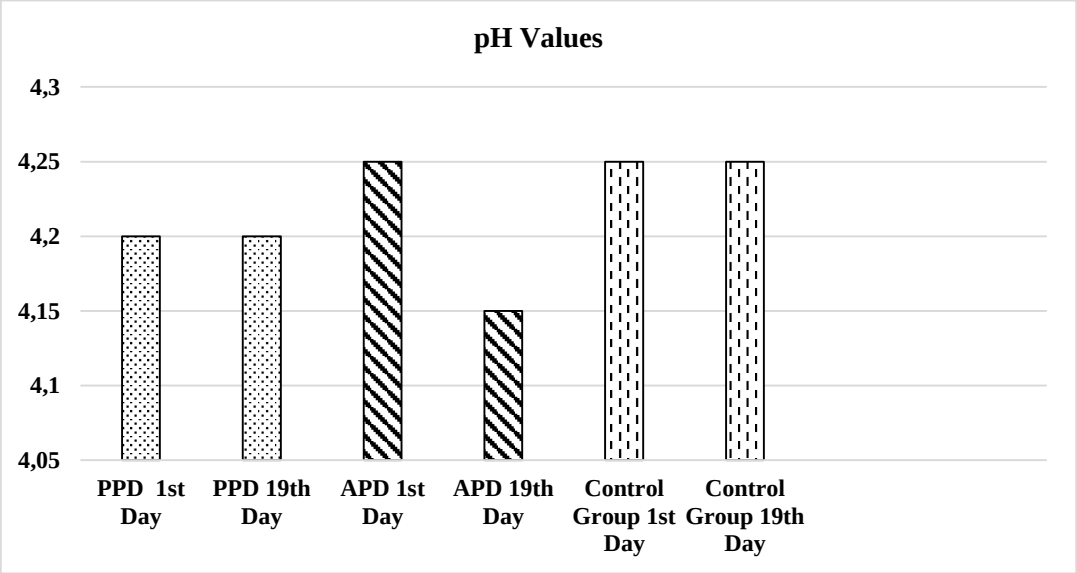

Figure 2. pH values alteration in PPD, APD and Control at 1st and 19th days

Similarly in reference to study of Chavan et al. (2018), there was not any significant pH changes found in all the germinated and ungerminated probiotic drink samples obtained from seeds of barley, finger millet and moth bean (Chavan et al., 2018). However, the pH of the green and black ice tea samples in another study reduced significantly with 28-day storage period from 4.85 to 3.92. The tea samples were produced with prebiotics mix (galacto- oligosaccharide (GOS), fructo-oligosaccharide (FOS), and inulin) or synbiotic ingredients (GOS, FOS, inulin, and *L. acidophilus*). Thus, the prebiotic mix could be effect on decreasing of the pH due to act as substrate for the probiotics. Then, acidic metabolites of the microorganism should be increased (Tewari et al., 2018).

CONCLUSIONS

In recent years, the consumers have become aware of probiotic foods as well as their health benefits. Thus, marketing of probiotic foods have shown rising in meantime. Thus, there are many probiotic food products with or without fruit content and dairy (mostly) or non-dairy produced. Besides, the inhibition effects of probiotics on pathogen microorganisms were known which was also promoted by the current study. Then, prevention growth of *Klebsiella pneumonia*, which is cause of nosocomial infections on commercial plain and apricot probiotic drinks as food matrices firstly were indicated in the present study. Thus, consuming of probiotic foods to hospitalized patients and children should be an alternative solution for reinforcing their immune system.

Apart from that, the shelf life of commercially dairy probiotic foods is generally 21 days recommended by their packaging system as well as the samples used in this study. In line with the results of the study, this period is quite enough for probiotic foods content fruit or not. During this period, pathogen microorganism is destroyed, not observed any yeast or mould growth, number of probiotic microorganisms and LAB are saved and pH levels are not altered, which is one of the most important parameter of probiotic drinks for staying stable and consumable at the end of the shelf life. However, supplement fruit ingredient is not showed any significant alteration. Therefore, people can consume probiotic foods with or without fruit content by their appetite.

To sum up, probiotics are a healthy solution for fighting pathogen microorganisms without required any chemicals or antibiotics supplementation. Thus, consuming of probiotic food products are so important for human wellness and it should be promoted.

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