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Investigation of the Viability of Probiotic Microorganisms in non – dairy Probiotic Beverages

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ABSTRACT

In recent years, many variations have been occurred in human nutrition such that there has been increasing tendency towards consumption of functional foods, i.e. foods having medicinal as well as nutritional value in addition of basic nutritional properties. Probiotic products are among important functional foods, thus, over last decade many studies on these special products also have been carried out in Iran. Probiotic products are divided into two groups: dairy and non – dairy products of which dairy probiotic products are widely consumed, however problems such as increased cholesterol level and lactose intolerance make some people avoiding consumption of such products. Thus demand for non – dairy probiotic products is growing especially by the vegetarians worldwide. Probiotics prevent or reduce the incidence of a wide range of infectious diseases while establishing a balanced flora in the intestine and stomach thereby promoting the host's health. Along similar lines, the viability of probiotics in these products to confer their healthful effects on the host's body is of enormous importance. Therefore, in the present study, we investigate the viability of probiotics and the factors affecting their survival.

Keywords: Beverage, Non-dairy, Probiotic, Viability.

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INTRODUCTION

Antibiotics have been long used for prevention and treatment of diseases and also to enhance the growth of animals. This continuous and sometimes, irregular use of antibiotics in humans and animals has caused many problems. The scientists, thus, sought to find some alternatives with the probiotics being on the top. Probiotics, prebiotics, symbiotic as well as nutritionally fortified products all are among functional foods. Presently over 75 dairy products containing probiotics are produced worldwide and the attempts to use prebiotics along with probiotics have commenced. The tendency of humans towards using probiotic to benefit from their healthful effects dates back 1908 when Elie Metschnikoff a Russian researcher, announced that human beings should consume milk fermented with lactobacillus for longevity. Probiotics have been identified as living microorganisms which in sufficient amounts are able to balance microbial flora of the host. Many evidence is available that consumption of foods containing useful microorganisms known as probiotics, help the survival of indigenous intestinal flora and keep the bacteria in balance thereby conferring healthful effects on humans health [1]. Probiotics are commonly added to yogurt and other dairy fermented products, however, their high cholesterol content as well as lactose – intolerance in some susceptible people have limited their consumption [2]. In recent years demand for non-dairy probiotic products has been growing and today probiotic products in form of beverages is increasingly manufactured [3]. Fruits and vegetables are rich in functional components such as minerals, vitamins, fiber and antioxidants (phytochemicals). Also they are free from those allergens contained in dairy products limiting their consumption [4].

The objective of this study is to review the viability of probiotics in different non-dairy beverages produced in recent years.

Probiotic means something enlivening in latin and conceptually is opposite of antibiotic meaning contralife which was used by kollath, for the first time, for malnourished patients [5]. Among many

definitions presented for probiotics so far, the definition proposed by FAO and WHO, best represents the attributes of probiotics, "living microorganisms that in sufficient amounts have healthful effects on the host" [6]. Probiotics, for the first time, were used in fermented milk and then they were recognized by their application in animal feed [7]. Today the role of many probiotic species in human health is well recognized [8].

Probiotics are living microorganisms conferring healthful effects on the host and improving the balance of intestinal microflora if they are taken in sufficient amount [9,10]. Today, probiotics are defined as non-pathogenic living microbes that are taken singly or in combination by humans or animals promoting the host health via increasing the number of beneficial intestinal bacteria [11].

The healthful effects of living microorganisms especially lactic acid bacteria (LAB) on human health were identified many years ago [12]. Probiotics improve the balance of intestinal microflora and inhibit the harmful bacteria growth. They also improve digestion, promote the function of immune system, and boost resistance to infection [13, 14]. Various studies have shown the effectiveness of these bacteria in preventing and treating different diseases and health problems including hypercholesterolemia [15], constipation food allergy [16], osteoporosis [18], hypertension [18], ulcerative colitis [19], colon diseases [20], unbalanced immune system [21, 22], lactose intolerance [23], bowel inflammatory diseases [3], atopic eczema [21], teeth decay [24, 25], different types of cancer [26, 27], Helicobacter pylori – caused gastritis [28], and obesity [29]. Probiotics may reduce lactose and cholesterol level as well as blood pressure. Also they are able to prevent colon cancer and treat irrigative bowl syndrome (IBS) and acute diarrhea [7, 30, 31, 19]. The activity and viability of probiotics in the intestine are necessary if their healthful effects are to achieve. Thus minimum 10^7 living probiotic bacteria per g of dairy products on consumption are required [32, 33, 34].

STUDIES ON NON – DAIRY PROBIOTIC BEVERAGES WORLDWIDE

Yoon et al., (2004) used species L[1].acidophilus, *L.plantarum*, *L.casei*, and *L.delbrueckii* in order to produce probiotic tomato juice. L.plantarum consumed the sugar at a higher rate, thus produced more acid and reduced pH to a lower value as compared to other species. Also the number of living cells was greater than 10^8 cfu/ml after 48h of fermentation. The results showed that the number of living cells was greater than 10^6 cfu/ml after 4-week storage at 4°C (especially for L. acidophilus, L. casei and *L. delbrueckii*) [35].

Yoon et al., (2005) examined beet root juice as a suitable substrate for producing a probiotic product by L. acidophilus, L. casei, *L. delbrueckii*, and L. plantarum. They also investigated the biovailability of the probiotic species as well as living cells count during refrigerated storage at 4 °C for 4 weeks (once a week). Sterile beet juice was inoculated with the probiotic bacteria and the samples were incubated at 30°C for 72h. at the time intervals of 48, 24 , 0 and 72 h, microbial count and chemical analysis (acidity , pH , sugar , Organic acids) were done. After 72 h of incubation, the samples were transferred to refrigerator and kept at 4°C for 4 weeks in order to study the bioavailability of probiotics in the final product. All used species held the potential for using beet juice for cell synthesis and lactic acid production. The number of live cells reached 10^9cfu/ml after fermentation at 30°C for 48h. Extending the time of fermentation from 48h to 72h had no significant effect on the living cells count. Although only L. acidophilus and L. plantarum decreased pH value from 6.3 to less than 4.5 after 48h of fermentation due to their ability of producing more acid as compared to L. casei as compared to L. casei and *L. delbrueckii*. The latter produced only 0.25% acidity resulting in a decrease in pH value to 5 after 72 h of fermentation. The number of living cells but L. acidophilus, in fermented beet juice reached 10^6-10^8 cfu/ml after 4-w storage at 4 °C. for example L.casei, L. plantarum and *L. delbrueckii* count measured 9×10^6 , 7.7×10^7 and 7.2×10^7 cfu/ml, respectively, after 4-w refrigerated storage at 4°C. the factors affecting the bioavailability of probiotics included acidity, pH, oxygen content, lack of nutrients and the presence of antimicrobial agents[11].

Yoon et al., (2006) used cabbage as a raw material for production of probiotic cabbage juice by lactic acid bacteria (L. casei A4, L. plantarum C3, *L. delbrueckii* D7). After fermentation, the samples were refrigerated at 4°C for 4w in order to investigate the effect of refrigerated storage on the bioavaiability of probiotics. Cabbage juice was inoculated with probiotic species and the samples were incubated at 30°C for 72h. Microbial and chemical analyses were done at the time intervals of 48, 24 , 0 and 72h. the results showed that all three species could easily grow in the sterile cabbage juice without any nutrients as the number of living cells reached 10×10^8 cfu/ml after 48h of fermentation. Extending the time of fermentation from 48h to 72h had no significant effect on living cell count. L. platarum and *L. delbrueckii* could produce greater amount of lactic acid (1%) than L. casei (0.74%). L. casei seems to reqire some nutrients which are not present in sufficient amount in cabbage juice. Previous experiments have shown

[1] lactobacillus

that cabbage contains some antimicrobial compounds including carbohydrates naturally occurring in cabbage which are inactivated when steamed for 10 min before extraction. The results revealed that *L. delbrueckii* and L. plantarum retained their bioavailability during several weeks at 4°C and could grow at low pH and acidity reaching 10^5-10^7 cfu/ml, while L. casei lost its viability completely after 2-w refrigerated storage [36].

Sheehan et al., (2007) studied the resistance of lactic bacteria to acid as well as their technological resistance in juice environment. They examined the bioavailability of five lactobacillus species and one Bifidobacter species in orange juice (pH 3.65) pineapple juice (pH 3.40) and cranberry juice (pH 2.5). all species could grow well in orange and pineapple juice but not in cranberry juice. The number of living cells of L. casei DN-114001 , L. rhamnosus GG and L. paracasei SSP.Salivarius UCC500 in orange juice and pineapple juice reached 10^7cfu/ml and 10^6 cfu/ml, respectively for at least 12 weeks. These three species held great potential for being used in juice due to their resistance to acidic environment. The survival of *Bifidobacterium lactis* BB-12 in considerably over first 4 weeks of refrigerated storage and the number of living cells measured greater than 10^6 cfu/ml at least for 6 weeks, decreasing by 2 log within 6 and 10w. The number of living cells in pineapple juice measured greater than 10^6 cfu/ml at least for 4 weeks decreasing by 3.5 log from 4 w to 6w. *B. lactis* however, has been recognized as one of the most resistant species against human gastrointestinal conditions. Exposure to oxygen during refrigerated storage results in decreased bacterial population over time. *L. rhamnosus, L. casei* and *L. paracasei* showed satisfactory bioavailability as their living cells count measured >10^7 cfu/ml in orange juice and >10^6 cfu/ ml in pineapple juice over 12 weeks. It should be noted that in pineapple juice, due to its low pH the viability of probiotics decreased to a greater extent than in orange juice. Cranberry juice also did not provide a favorable environment for growth and viability of probiotics. In order to determine whether low pH and or benzoic acid, and lacton contained in cranberry juice reduce the bioavailability of probiotics the bioavailability of *L. paracasei* juice with different pH values, 2.5 , 3.5 , 4.5 and 5.5. At high pH value living cells count remained >10^7 cfu/ml for 9 days whereas the number was found only for 2 days at pH 2.5 thus it was concluded that the main reason for the observed decrease in bioavailability of probiotics in cranberry juice was its low pH value. Also the bioavailability of probiotic species in orange juice was studied before pasteurization process. The results suggested that the probiotic species lost their bioavailability and similar results were obtained when non-thermal pasteurization (high – pressure) process was exerted where exerting and removing high temperature, respectively, decrease and increase pH value thus these pH variations increase the susceptibility of probiotic species and reduce their resistance to acidic conditions in the product[37].

Mousavi et al., [38] investigated the viability of *L.rhamnosum, L.gasri*, and *L.fermentum* in orange juice and tomato juice at 4°C, 23°C, and 37°C for four weeks. Their results revealed that *L.rhamnosus* and L. gasri were viable in both media.

Champagne et al., [39] inoculated apple – pear – raspberry juice mixture with 4.5×10^9 cfu/250ml L. rhamnosus and examined its viability during storage (2-4 w) at 2-7°C under conditions similar to those of consumption. The results suggested that if the product is refrigerated the consumers could expect a satisfactory viability of L. rhamnosus (1.5×10^9 cfu/ 250ml) over several weeks and that L. rhamnosus shows desirable viability even when the package is opened and exposed to oxygen.

Kun et al., (2008) studied carrot juice as a suitable substrate for manufacturing probiotic foods by use of *Bifidobacterium* species (B. lactis BB-12, B. bifidum B7.1 and B. bifidium B3.2).All *Bifidobacterium* species were able to grow in carrot juice without any supplied nutrients. The initial number of 10^7cfu/ml increased to 10^8 cfu/ml after 6 h of incubation and remained viable up until the end of fermentation process (24h). Due to intense metabolism of bacteria, pH of carrot juice dropped below 4.5 during fermentation. Also the amount of glucose and saccharose considerably reduced while the amount of fructose did not change. Decomposition of carotenoids (α- and β- carotene) ranged 15-45% depending on the used species. The amounts of produced lactic and acetic acids were 14.8-16.7 mg/ml and 3.3-5.3 mg/ml repectively. The results showed that pasteurization process leads to decomposition of vitamins and enzymes and induction of maillard reaction reducing the availability of amino acids as well as carbohydrates in carrot juice. Pasteurization did not change the chemical composition including carbohydrates, carotenoids content and pH value. In order to examine the effect of initial inoculation concentration on the capability of microorganisms in fermentation, carrot juice was inoculated with two inocfulation volume of *Bifidobacterium* 10^6 and 10^7 cfu/ml. For 10^6 cfu/ml, the number of bifidobacteria reached 10^8 cfu/ml after 24h of fermentation and they remained viable up until 32h while pH value decreased from6 to below 5. For 10^7 cfu/ml, after only 24 h of fermentation, increased at 12 h and remained viable up until 24 h. Some researchers suggest that initial amount of 10^7 cfu/ml is sufficient for inoculation and fermentation of fruits and vegetables juice as well as acceleration of the process. The results of evaluating bifidobacteria growth and organic acids production in carrot juice showed that after

24 h of fermentation the number of B. lactis BB-12, B B7.1 and B. bifidium B3.2 reached 77.8 , 8.71 and 8.82 cfu/ml, respectively , suggesting the satisfactory bacterial growth and favorable environment of carrot juice for growth and viability of bifidobacteria. Initial pH value of carrot juice dropped from 6.4 to 4.2 because of acid production. Acid production by *Bifidobacterium* species used in this study varied within first 6 h of fermentation. Bifidobacteria also may inhibit undesirable microorganisms. The studies on the variations of sugar, ethanol and varotenoid content suggest that glucose and saccharose are good sugar sources for bifidobacteria. During fermentation 20% glucose and 80% saccharose were utilized due to inability of bifidobacteria to use other carbohydrate sources in carrot juice. Carotenoids content depends on different factors including carrot variety soil and weather conditions. α- and β- carotene content decreased after 24 h of fermentation. The greatest reduction was observed when carrot juice was fermented by *Bifidobacterium* B 3-2. The reason may be the bacterial metabolism or fermentation conditions (pH and temperature). During fermentation of carrot juice lactic acid was produced in greater amount than acetic acid lactic acid promotes the nutritional value of organic products and improves texture and flavor. The concentration of this acid also may increase as the amounts of nitrogen compounds and mineral salts increase [40].

Natural juice (green apple, kiwi, pineapple, pear and strawberry) added to the broth culture may exert an inhibitory effect on *Staphyloccus thermophilus*. Strawberry juice inhibit all probiotic species, but *L. casei* whereas pineapple juice and kiwi juice inhibit L. acidophilus species. Green apple juice inhibits *L. lactis* while pear juice has no effect on probiotic species [41].

In a study conducted in 2011, the conditions for L. casei growth in cashew apple juice were instigated and the proper level of inoculation as well as time of fermentation were determined. Also the bioavailability of L. casei during refrigerated storage at 4°C for 42 d was studied. The optimum conditions for manufacturing probiotic apple juice by use of L. casei included pH 4.6 fermentation temperature of 30°C inoculation level of 48.7 log cfu/ml and 16 h of fermentation. During fermentation and refrigerated storage transparency yellowness and total color variations increased and redness reduced. pH value biomass, living cell count and color were measured every two hours during 24h of fermentation in order to determine the best level of inoculation and time of fermentation. The above parameters were also measured during 42 d of refrigerated storage at 7-d intervals. The effects of initial pH and temperature of fermentation on the growth of microorganism depended on the used species and substrate. The effect of temperature on the growth of *L.casei* was greater than that of pH value. Initial pH had no significant effect on the biomass. The amount of *L. casei* biomass increased as the temperature did rise, thus the optimum temperature for L. casei growth in apple juice is ~ 35°C. A great number of living cells was observed at a mild temperature (~30°C) as higher temperatures diminished the viability of L. casei. The greatest viability was observed at pH 6.4 and fermentation temperature of 30°C. the optimal conditions (initial pH of 6.4 and fermentation temperature of 30°C) were selected for evaluation of time for fermentation and level of inoculation. The growth of L. casei was retarded within the initial hours of fermentation, however the accelerated growth was observed after 2 h of fermentation in apple juice inoculated with 7.3 and 7.48 log cfu/ml. More biomass was also observed in apple juice inoculated with 7.48 log cfu/ml. this level of inoculation thus was selected for manufacturing probiotic apple juice. Although the greatest bioavailability was found for the sample inoculated with 7.48 log cfu/ml at 14 h, the 16 th hour was selected due to ph < 4.6 and inhibition of growth of pathogenic for maintaining the bioavailability and survival of probiotics as well as optimum conditions of probiotic apple juice. During fermentation, the turbidity increased because of increase in biomass resulting in reduced transparency of apple juice. As the fermentation proceeded, the yellowness of apple juice inoculated with L.casei increased as a result of pH drop. Carotenoids are main pigments developing the color of apple juice. During 24 h of fermentation, pH drop in different samples with different levels of inoculation results in isomerization of carotenoids and converts them into transe isomer thereby weakening the color of juice. Apple juice was produced by initial pH of 6.4 fermentation temperature of 30 °C inoculation level of 7.48 log cfu/ml and 16 h of fermentation, and then refrigerated for 42 d in order to examine the bioavailability of L. casei. At 21 d the number of viable cells increased from 8.41 log cfu/ml to 8.72 log cfu/ml and then decreased to 8.62 at 35 d. the number of viable cells decreased at the end of storage period while they were still higher than 8 cfu/ml being acceptable for probiotic products. During refrigerated storage, pH value decreased from 4.28 to 3.79 at 42 d resulting in an insignificant decrease in an insignificant decrease in viable cells [42].

The fermentation of tropical fruits by lactic acid bacteria (LAB) was also studied. The used strains included lactobacillus acidophilus L. casei *L. delbrueckii* and L. bulgaricus. The fruits included melon watermelon and Chinese pear. The results revealed that glucose was utilized well and lactic acid produced. The number of bacteria reached 10^9cfu/ml over 45 – h fermentation. Fructose was not utilized during fermentation. These fruits provided favorable medium for manufacturing non-dairy probiotic beverages [43].

Mousavi et al., [44] inoculated pomegranate juice with four species lactobacillus casei, *L. delbrueckii* L. plantarum, L. paracasei in the amount of 10^7 cfu/ml at 30°C for 72 h and then studied the microbial count, pH, acidity metabolism of organic acids and sugars. The viability of bacteria was measured during a 4-w period. The results showed that L.plantarum and L.delbrueckii increased the pH value rapidly within the first hours of fermentation and also utilized more sugars as compared to other two species. The greatest viability was observed for *L. delbrueckii* and L. plantarum within first two weeks as 1.5×10^5 cfu/ml and 2.8×10^5 cfu/ml respectively. It was demonstrated that pomegranate juice served as a favorable environment for production of probiotic fermented beverages [44].

Jahandide et al., (2012) studied borage – based drink fermented with four strains L.paracasei, L. acidophilus, L. delbrukii and *L. plantarum* at 30°C for 45h. Variations of pH and acidity kinetics of bacterial growth, sugar utilization organic acids production variations of phenolics and antioxidants were investigated. L. casei caused the greatest changes in pH acidity and sugar utilization. Lactic acid was produced in higher amount than other organic acides. The amount of phenolic compounds and antioxidants showed a significant increase during fermentation. After 2 weeks of refrigerated storage the number of bacteria was within the probiotic range. Thus it was concluded that borage extrac was a desirable medium for growth of lactic bacteria and production of functional drinks[45].

Marhamatzadeh et al., (2012) studied the feasibility of manufacturing probiotic apple and orange juice by L. acidophilus and *Bifidobacterium* bifidium. They supplemented the juice with milk maltose, lactose and glucose. Acidity pH and microbial count were explored during refrigerated storage (6w). the results revealed that glucose and lactose had positive effects on the extended shelf – life of the probiotic juice[46].

Pakbin et al., (2011a) produced a probiotic peach drink by using LAB (*L. delbrueckii* C3, L. casei A4, and L. plantarum D7). All samples were inoculated with 24-h cultured bacteria ($<10^5$ cfu/ml) and incubated at 30°C for 72 h. they concluded that the growth of *L. delbrueckii* and L. casei was more pronounced than that of L. plantarum. Also the rate of lactic acid production, pH drop and utilization of peach drink sugar by these two. Species was higher after 48 h of incubation demonstrating their accelerated growth during lactic fermentation and production of probiotic peach drink[47].

Pakbin et al., (2011b) produced a probiotic strawberry drink by used of LAB (L.delbrueckii C3, L. casei A4, and L.plantarum D7). All samples were inoculated with 24-h cultured bacteria ($<10^5$cfu/ml) and incubated at 30°C for 72 h. they concluded that the growth of L.delbrueckii and L. plantarum was more pronounced than that of L. plantarum. Also the rate of lactic acid production pH drop and utilization of strawberry drink sugar by these two species was higher after 48 h of incubation demonstrating their accelerated growth during lactic fermentation and production of probiotic strawberry drink [48].

Amini (2011) evaluated the microbial and sensory properties of probiotic apple juice and celery juice as well as apple – celery juice mixture produced by strains L. acidophilus and *L. delbrueckii*. Living cells count, pH , total acidity sugars utilization, organic acids production, total phenolics and sensory attributes during fermentation at 37°C for 24 h were investigated. The greatest pH drop was observed for the mixed drink as well as apple juice produced by *L. delbrueckii*, however the case was different for celery juice by L. acidophilus. All drinks were favorable for the activity of both strains however the curve of growth and organic acids produced by *L. delbrueckii* for the mixture of apple – celery juice and apple juice was higher than L. acidophilus and L. acidophilus did not show any activity in celery juice. This study showed the potential of probiotics for manufacturing valuable products by non-dairy apple – celery juice drink as well as apple juice and celery juice individually [49].

Using L. acidophilus and B. lactis at different concentrations (25%, 75% and 50%) at initial inoculation level of 6×10^8 cfu/ml, Seyyedi et al., [50] produced a probiotic orange juice consisting of different concentrations of orange juice (20T 25 and 30%) and of cheese powder (0.1 , 0.2 and 0.3) and concluded that 25% orange juice and 0.2 cheese powder resulted in the greatest bacterial activity, growth and survival. Also the greatest viability was observed for the sample containing 75% L. acidophilus and 25% *B. lactis* .

Pakbin and Karami [51] produced a probiotic fig drink by use of LAB (L.delbrueckii C3, L. casei A4, and L. plantarum D7). All samples were inoculated with 24-h cultured bacteria ($<10^5$ cfu/ml) and incubated at 30°C for 72h. they concluded that the growth of *L. delbrueckii* and L. casei was more pronounced than that of L. plantarum. Also the rate of lactic acid production, pH drop and utilization of fig drink sugar by these two species was higher after 48 h of incubation demonstrating their accelerated growth during lactic fermentation and production of probiotic fig drink [51].

Tutunchi et al., (2012) produced probiotic organic red grapes juice by use of L. casei 431 at intial inoculation level of 10^8 cfu/ml and concluded that although the bacterial survival in fermented red grapes juice decreased gradually during refrigerated storage at 4°C living cells count was higher than 10^6 cfu/ml

even after 4-w storage at 4°C. The results suggested that organic red grapes juice could be used as raw material for manufacturing probiotic organic red grapes juice via lactic fermentation by L. casei [52].
Karbasi and Izadi (2013) produced a probiotic beverage by using cabbage – tomato juice fermented by LAB (*L. rhamnosum, L. paracasei*, and L. acidophilus). All samples were inoculated with 48-h cultured bacteria ($<10^9$ cfu/ml) and incubated at 37°C for 72 h. Then they were kept at 4°C for 4 weeks and the viability of the strains was determined. After four weeks the number of viable cells of L. rhamnosus and L. paracasei still measured 3.6×10^8 and 4.7×10^5 cfu/ml, respectively. These two strains thus could be used as probiotic microbial cultures for manufacturing a healthy drink [53].
Carrot juice and beet juice were inoculated with species *Bifidobacterium* and L. acidophilus and incubated at 37 °C for 24h. After 7 h of fermentation the number of viable cells carrot juice reached 10^8 cfu/ml and did not change up until the 24 th hour of fermentation. The pH value decreased from 6.24 to 4.06. After a lag phase, the number of viable cells increased and pH dropped slowly. The gap is important for bacteria to adapt to the new environment. After 5h of fermentation, pH value dropped below 5 due to intense bacterial metabolism. L. acidophilus showed enhanced growth in carrot juice as compared to beet juice because beet juice contains some growth inhibitors. At the end of incubation (24h), the bacterial count reached 2.3×10^8 cfu/ml and pH value was below 3.9. The carrot juice samples inoculated with L. acidophilus were kept at 4 °C for 4 weeks in order to investigate the bacterial viability. The number of cells showed a considerable decrease within the 1 st week and no viable cells were observed during next three weeks. The number of L. acidophilus in beet juice reached 5×10^8 cfu/ml after 48 h of fermentation and pH value decreased from 6.29 to 4.62 however the amount of lactic acid increased to 2.65 g/L.After 24 h of fermentation, lactic acid cotent increased to 3.69 associated with pH 4.28 (pH increased to 4.31 at 37°C during next 24h). the bioavailability of *Bifidobacterium* BB-12 also was studied during fermentation of carrot and beet juice. After 48 h of fermentation the number of viable cells in both media measured > 4.5×10^8 cfu/ml. pH value of carrot juice and beet juice decreased from 6.17 to 4.28 and from 6.03 to 4.41 respectively [54].

CONCLUSION

Although dairy products are the most common substrates for probiotic bacteria other substrates may be used because of high cholesterol content of these products and lactose intolerance in some lactose – sensitive people. Thus the optimum conditions for probiotics growth in non – dairy products are investigated so that all people could benefit from healthful effects of probiotic bacteria. The research showed that the viability of probiotics depended on the used species medium conditions O_2 content acidity of final product, pH accumulation of the acids produced through fermentation and the presence of growth inhibitors. The results suggested that the viability of probiotics in the first place depended on the pH value and acidity of the product. Finally given the variety of substrates and microbial species further studies are needed to determine the optimal conditions for probiotics growth in different non – dairy probiotic products.

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