Production of Probiotic Fermented Mixture of Carrot, Beet and Apple Juices

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ABSTRACT

In this study, production of fermented functional beverage based on the mixture of carrot, beet and apple juices using the *Lactobacillus casei* was carried out. Factors of consumption of reduction sugars, brix and bacterial growth were examined after fermentation and during the storage for 28 days and at 4˚C. To produce probiotic fermented mixture of Carrot, Beet and apple juices and *Lactobacillus casei* suspension with initial concentration of about $1.5x10^7$, $1.5x10^6$ cfu/ml was prepared and added to the mixture of juices to the amount of 20, 30 and 40% , respectively. The fermentation process was completed during 48 hours and at 37 ˚C. Data analysis was conducted using the multiplied Duncan test including 6 test treatments and 1 control treatment and was repeated 3 times. During the fermentation process within all of the treatments, the number of probiotic bacteria increased due to the usage of sugar and nutrients inside the juice while sugars and brix levels decreased. The results revealed that the sample of A3B1 with concentration of 40% and $1.5x10^6$ cfu/ml of *Lactobacillus casei* was considered as the best treatment which had the maximum rate of cell viability during 4 weeks of storage at 4 ˚C. In the sensory evaluation that was conducted by the examiners after the first and the forth weeks at 4˚C, control treatment had the highest score and A3B1 treatment had the lowest score with concentration of 40% and the 10^6 cfu/ml of probiotic bacteria.

Key words: Probiotic beverage; Apple, Carrot; Beet; *Lactobacillus casei*

INTRODUCTION

Today in most communities, the role of food is of great importance in health and human nutrition. The initial role of food as energy and growth source is changed into the biological role of its components on human health and the food consumption and production market is guided into "Functional food". Functional food is the product providing basic nutrition and improving health. Among functional foods, the foods with probiotic microorganisms are of great importance. As probiotics have positive impact on microbial balance of intestine and entire body health, the production and consumption market of this group of food is developing [1]. Probiotic products are divided into two types:Dairy probiotic products as different yogurt, cheese, cream, yogurt water, butter, ice-cream, infant formula, drinks with whey and dairy desserts [2]. Non-dairy probiotic products as grain, candy, different drinks as juice and nonalcoholic beer, baby food and meat products [3]. In addition to food products, probiotic pharmacological products are used as tablet or capsule for treating different diseases as intestine infection, diarrhea, vaginal infection, constipation and lactose intolerance. In pharmacological products, there are many probiotic microorganisms as dried frozen [4]. Probiotic survival during transfer in digestive system is affected by physical-chemical features with food carriers. Resistance to gastric acid and bile, attaching to the digestive system mucus and production of acid by probiotics are affected by constituents of food carrying probiotics [5]. The studies show that probiotics in fruit and vegetable juice can be a good alternative for a group of people with special needs of vegetarians and people with allergic reactions to milk proteins [6-7]. Fruit juices improve health widely and have high amount of antioxidants, vitamins, minerals, dietary fiber and other useful nutritional substances [8-9]. The study aimed at producing drink of probiotic fermented mixture of carrot,

beet and apple juices and determining optimal storage time, considering concentration of fruit juice, inoculation ratio and density of probiotic bacteria and total sugar, brix and survival of probiotic bacteria in periods after fermentation and during 28 days of keeping at 4 ˚C temperature.

MATERIALS AND METHODS

The required materials to produce probiotic product

Providing fresh fruit juice

To produce probiotic product, selection of basic environment and suitable growth for inoculation of useful probiotic microorganisms is of great importance. To do this, apple, beet and carrot juices are used and their extract is frozen in freezer.

The applied microorganisms to produce probiotic drink

L.casei 1608 is provided from Iranian Research Organization for Science and Technology as lyophilized vial. The fruit juice extract is reached to 40, 30, and 20% by sterile distilled water as 100cc and is poured into flask. For pasteurization of specimen, it is put into water bath. [10- 11].

Table 1. Study treatments

Bacteria density	**Concentration of fruit juice mixture (apple, beet, carrot)**	**Treatments**
10^6 cfu/ml	20%(7%apple, 6% beet, 7% carrot)	A_1B_1
10^6 cfu/ml	30%(10%apple, 10% beet, 10% carrot)	A_2B_1
10^6 cfu/ml	40%(14%apple, 12% beet, 14% carrot)	A_3B_1
10^7 cfu/ml	20%(7%apple, 6% beet, 7% carrot)	A_1B_2
10^7cfu/ml	30%(7%apple, 10% beet, 10% carrot)	A_2B_2
10^7cfu/ml	40%(14%apple, 12% beet, 14% carrot)	A_3B_2
-	30%(10%apple, 10% beet, 10% carrot)	Control

Preparation of strain for inoculation

MRS agar medium is prepared according to the instruction on the can, Then, from MRS, broth with microorganism as mixed well with shaker is taken by sterile needle from microbial suspension and it was cultured as surface on solid environment. Then, para film was used around the plates and it was placed in incubator at 37 ℃ for 48 hours. Then, the bacteria growing on solid media were used in next stages of study.[12]

Microorganism inoculation

For microbial inoculation, McFarland method was used as a method to determine bacteria at two levels of 1.5×10^7 cfu/ml and 1.5×10^8 cfu/ml [13]. From the turbidity as 1.5×10^8 cfu/ml of required bacteria strain (Suspension 1), to provide 1.5×10^7 cfu/ml, 1cc was taken by sterile pipette and was transferred to the falcon with 9cc sterile distilled water (Suspension 2). After this stage, the strains were prepared for inoculation[14]. To reach concentration of 10^6 cfu/ml and 10^7 cfu/ml, dilution operation was made.

Specimen fermentation

For fermentation, falcons were transferred to incubator at temperature 37°C for 48 hours. According to the report of Saxelin *et al*., 1999, temperature 37-40°C is a suitable temperature for growth of probiotic bacteria such as *Lactobacillus casei.* After fermentation**,** Erlenmeyer flask were transferred to evaluate required factors during 4 weeks to fridge at temperature 4°C [15-16].

Tests

Lactic acid bacteria counting

For live microbial cells counting, decimal dilution pour plate as SPC (Standard plate count) is used. The number of colonies are multiplied by inverse dilution and the final value is defined as the number of colonies per mL (cfu/ml) as Equation (1) [17].

$$N = \frac{\Sigma C}{V(n1+0/1n2)d} \qquad \text{Equation (1)}$$

ΣC: The sum of counted colonies in all representative plates of two consecutive dilutions
V: The volume of inoculated dilution in each plate in mL
n1: The number of counted plates in the first dilution (strong)
n2: The number of counted plates in the second dilution (diluted)
D: Dilution coefficient based on the first counted dilution (strong).[18]

The measurement of total sugar

According to the method in national standard of Iran, No. 2685, Equation is as follows:

$$n = \frac{F \times 100 \times 100}{V \times 25} \qquad \text{Equation (2)}$$

n =the amount of reducing sugars (before hydrolysis) in gram per 100 gram.
F = *Fehling's Factor*
V =Consumption volume of neutralized solution (a)

The test of solid dissolved in water (Brix at 20℃)

To measure Brix, digital refractometer (RX- 7000 α) is applied. [18]

Statistical design and statistical analysis

To investigate the results, fully randomized design by factorial method is applied. The data is analyzed by SPSS 22 Software and the comparison of means is done by Duncan test at level 95%.

RESULTS

The evaluation of changes of total sugar in drink after 48 hours of fermentation at temperature 37℃ and four weeks of keeping at temperature 4℃:

The results of tests showed that concentration of mixture of juices, bacteria density and keeping time had significant effect on total sugar of probiotic drink ($P<0.01$).As shown in Figure 1, kinetic of total sugar of probiotic drink after 48 hours of fermentation and during 28 days of keeping, the results show that total sugar changes for all treatments are not similar during different times but after 28 days of keeping, total sugar of drink in all treatments is reduced significantly ($P<0.01$) in all treatments.

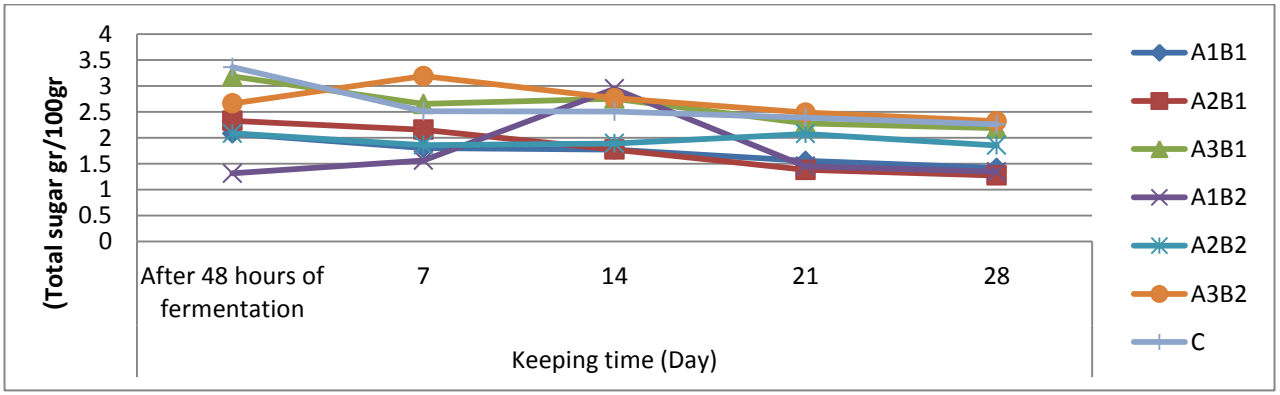

Figure 1. kinetic of total sugar of probiotic drink after 48 hours of fermentation and during 28 days of keeping at temperature of 4℃

A1B1: 20% Concentration of fruit juice, bacteria density 10^6 cfu/ml
A2B1: 30% Concentration of fruit juice, bacteria density 10^6 cfu/ml
A3B1: 40% Concentration of fruit juice, bacteria density 10^6 cfu/ml
A1B2: 20% Concentration of fruit juice, bacteria density 10^7 cfu/ml
A2B2: 30% Concentration of fruit juice, bacteria density 10^7 cfu/ml
A3B2: 40% Concentration of fruit juice, bacteria density 10^7 cfu/ml

C: 30% fruit juice concentration (control)

The evaluation of changes of brix in drink after 48 hours of fermentation at temperature 37℃ and four weeks of keeping at temperature of 4℃:

The results of tests showed that concentration of mixture of juices, bacteria density and keeping time had significant effect on brix of probiotic drink (P<0.01).

As shown in Figure 2, kinetic of brix of probiotic drink after 48 hours of fermentation and during 28 days of keeping, the results manifest that brix changes for all treatments are similar during different times and over time, brix is reduced significantly (P<0.01).

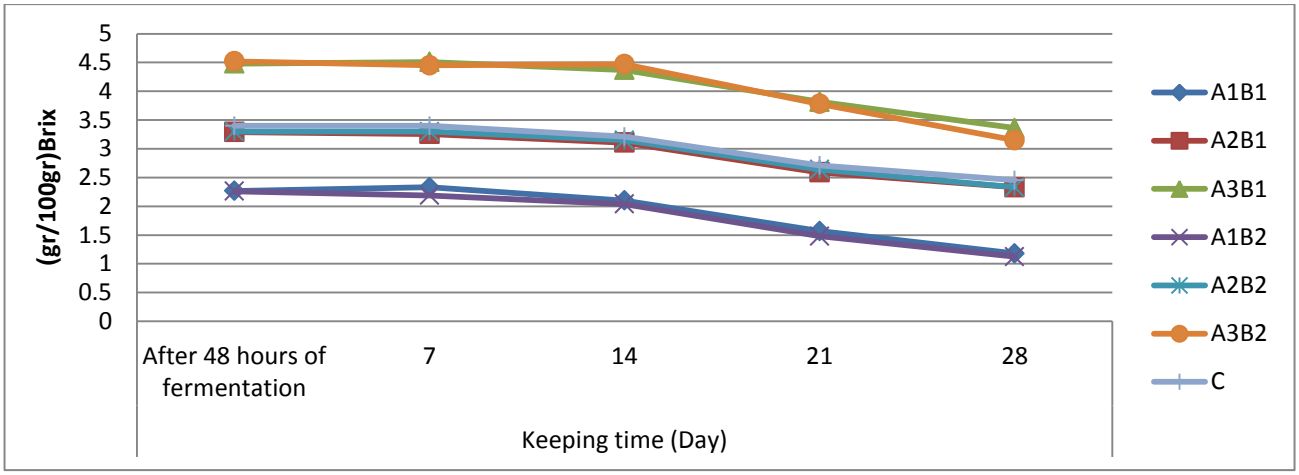

Figure 2. kinetic of brix of probiotic drink after 48 hours of fermentation and during 28 days of keeping at temperature of 4℃

A1B1: 20% Concentration of fruit juice, bacteria density 10^6 cfu/ml

A2B1: 30% Concentration of fruit juice, bacteria density 10^6 cfu/ml

A3B1: 40% Concentration of fruit juice, bacteria density 10^6 cfu/ml

A1B2: 20% Concentration of fruit juice, bacteria density 10^7 cfu/ml

A2B2: 30% Concentration of fruit juice, bacteria density 10^7 cfu/ml

A3B2: 40% Concentration of fruit juice, bacteria density 10^7 cfu/ml

C: 30% fruit juice concentration (control)

Correlation between brix and total sugar of drink after 48 hours of fermentation at temperature 37℃ and four weeks of keeping at temperature of 4℃

As shown in Figure (3), at confidence interval 99%, there is a positive significant correlation between brix and sugar of drink and with the increase in brix, sugar is also increased.

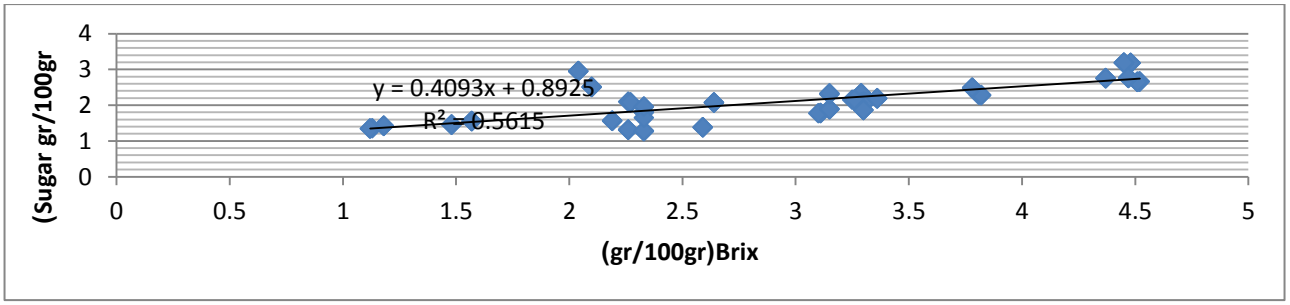

Figure 3. Correlation between Brix and Sugar

The evaluation of the changes of L.casei bacteria(cfu/ml) in drink after 48 hours of fermentation at temperature 37℃ and four weeks of keeping at 4℃ temperature

The results of tests show that concentration of mixture of fruit juices, bacteria density and keeping time has significant effect on counting probiotic drink bacteria (P<0.01).

As shown in Figure (4), kinetic of probiotic drink bacteria count after 48 hours of fermentation and during 28 days of keeping shows that the changes of bacteria count for all treatments except A1B2 during different times of keeping are similar. Regarding probiotic drink with bacteria density 10^7cfu/ml and 40% concentration (A3B2) at fourteen day of keeping, it is increased in terms of bacteria counting which significantly (P<0.01) reduces over time. In treatment A3B1, at fourteen day of keeping, the increase of probiotic drink bacteria count is observed and this is significantly increased over time. After 28 days of keeping, bacteria count of drink in all treatments except A3B1 of drink bacteria count is significantly (P<0.01) reduced.

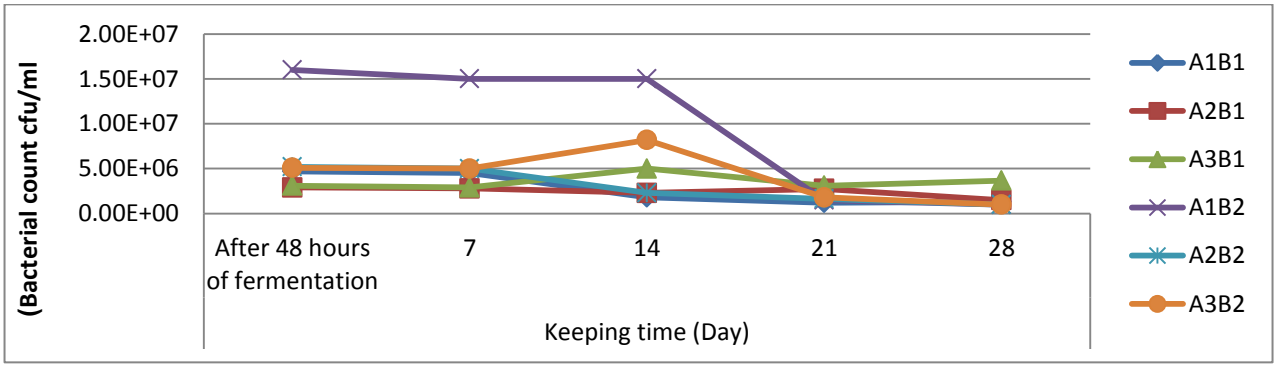

Figure 4. kinetic of bacteria count of probiotic drink after 48 hours of fermentation and during 28 days of keeping at 4℃ temperature

A1B1: 20% Concentration of fruit juice, bacteria density 10^6 cfu/ml
A2B1: 30% Concentration of fruit juice, bacteria density 10^6 cfu/ml
A3B1: 40% Concentration of fruit juice, bacteria density 10^6 cfu/ml
A1B2: 20% Concentration of fruit juice, bacteria density 10^7 cfu/ml
A2B2: 30% Concentration of fruit juice, bacteria density 10^7 cfu/ml
A3B2: 40% Concentration of fruit juice, bacteria density 10^7 cfu/ml
C: 30% fruit juice concentration (control)

DISCUSSION

The changes of total sugar and Brix during fermentation and keeping

According to the results of study, it is shown that bacteria growth leads to reduction of total sugar and reduction of Brix during 28 days of keeping, which proves to be consistent with the results of study of Moraru, (2007), Nosrati, (2013), and Yahyaei Soofyani, (2015). The main reason is related to the consumption of sugar and organic acid[19,20,21]. Ghasemi and Zomorodi (2014) investigated survival of Lactobacillus acidophilus as free and capsulated in probiotic apple juice. The highest Brix reduction is observed in apple juice with free bacteria and lowest brix reduction in capsulated treatment and the reason is avoiding capsule for easy access of probiotics to sugar. In apple juice with free bacteria, due to easy access to sugar, Brix reduction was significant and the results of this study are consistent with the results of present the study [22].

The changes of growth of probiotic bacteria during fermentation and keeping

According to the results of this study, during 4 weeks of keeping at 4℃ temperature, probiotic

bacteria are reduced as maximum survival of bacteria in the recommended range (10^6-10^7cfu-ml) from food and drug organization, which is the main reason for the reduction of probiotic cells' survival and lack of ability of bacteria to tolerate unsuitable conditions with low pH and high acidity as the result of bacteria growth and fermentation [12]. Yahyai (2014) investigated the production of functional drink based on a mixture of Malt extract and probiotic fermented red fruit juice by Lactobacillus casei. During 4 weeks of keeping at temperature of 4℃, the number of probiotic bacteria due to sugar consumption and nutrients in Malt extract and Red fruit juice are reduced. In comparison with the aforementioned studies, the similar results with Yahyai (2014) are achieved [23].

CONCLUSION

Based on the results of this study, it can concluded that: During fermentation, number of probiotic bacteria due to sugar and nutrient consumption in fruit juice can be increased and total sugar and brix are reduced (water soluble solid materials).In terms of sensory evaluation of drink during the first and fourth week of keeping at 4℃ temperature, highest score of control treatment and lowest score is regarding treatment A3B1 with concentration 40% (14% apple juice, 14% carrot juice and 12% beet) and density 10^6cfu/ml. Treatment A3B1 with concentration 40% (14% apple juice, 14% carrot juice and 12% beet juice) and density 1.5×10^6cfu/ml is the best treatment and it has the highest bacteria population after 4 weeks of keeping and has proved to be the best treatment. As A3B1 treatment is the best treatment, adding Malt extract or glucose syrup to drink may cause the probiotic drink to have a pleasurable flavor.

"The authors declare no conflict of interest"

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