



Study on production possibility of probiotic fermented beverage based on mixture of pineapple, apple and mango juices

Somayeh Mashayekh¹, Mahnaz Hashemiravan^{1*} and Fahim Dokht Mokhtari²

¹Department of Food Science and Technology, Varamin- Pishva Branch, Islamic Azad University, Varamin, Iran

²Research group Manager of Microbiology, Institute of Standards and Industrial Research of Iran

ABSTRACT

Probiotics are live microorganisms and if they are applied adequately can have useful effect on the health of host. One of the advantages of fruits as the basic medium to produce probiotic drinks is the lack of consumption limitation by specific people due to the lack of lactose and cholesterol, enriched with nutrients. The present study evaluates fermented functional drink production based on the mixture of pineapple, apple and mangifera by *Lactobacillus casei* PTCC 1608. Factors such as reducing sugars at times after fermentation and acidity, survival of probiotic bacteria after fermentation during 28 days at temperature 4°C are evaluated. To produce probiotic fermented drink based on the mixture of pineapple, apple and mangifera, microbe suspension with initial concentration 10^6 , 10^7 cfu/ml is provided and is inoculated to concentrate mixture of juice with concentration of 20, 30, 40% of juice and fermentation process is performed for 72 hours at temperature 37°C. The data analysis is done by multi-range Duncan test in fully randomized design consisting of 6 treatments with 3 control treatments with three replications. During fermentation in all treatments, the population of probiotic bacteria was increased due to using sugar and nutrients in juice, acidity is increased and reducing sugar is reduced. Based on the results, F_2T_2 treatment with concentration of 30% of juice (including 15% pineapple juice, 7.5% apple juice and 7.5% mangifera juice and density 10^7 cfu/ml is the best treatment and it has the highest bacteria after 28 days. The results of study show that mixture of pineapple, apple and mangifera juice is a good medium for the growth of lactic acid bacteria and functional drink production.

Keywords: Probiotic fermented drink, Mixture of pineapple, apple and mangifera juice, *Lactobacillus casei*

INTRODUCTION

The increasing consumption of useful food products without pathogens or toxics or different types of microorganisms changing chemical compound can increase variety of food products. On the other hand, attention of users to food diets have useful and preventive effects on chronic diseases and this increases the tendency of using natural foods and functional foods by people [5].

Probiotics is one of the most famous functional products with great importance in this regard [12].

Current industrial probiotic foods are basically dairy products, which may represent inconveniences due to their lactose and cholesterol content of these products and this has led into the consumption limitation by some people. In recent years, the demand of consumers for non-dairy probiotic foods is increased. Useful effects of fruit and vegetable are improved with a biological process as lactic fermentation. In addition, some fruits and vegetables consist of probiotics stimulating specific probiotic growth [3], [9].

In this study apple is used as it is full of nutrients as vitamin, pectin, fiber and antioxidant and due to its great therapy features [19] ,Krchegani&Mokhtari,2012).

In addition to apple, tropical fruits as pineapple and mangifera are applied. Mangifera has high concentration of sugar, antioxidants and enriched with vitamin A, acid with good organoleptic properties [7].

Pineapple is a good source of vitamin A, C, B, protein and ash and it is also an antioxidant [13].

This study aimed to produce probiotic ferment beverage based on mixture of pineapple, apple and mangifera and determining optimal keeping time of product by considering concentration of fruits juice, inoculation ratio and density of probiotic bacteria and evaluation of reducing sugar after fermentation and evaluation of acidity and survival of probiotic bacteria after fermentation and during 28 days being kept at temperature 4 °C.

EXPERIMENTAL SECTION

Materials

The applied material in probiotic beverage production process is concentrate of pineapple, apple mangifera juice from Alifardcompany (Sanich), “*Lactobacillus casei* 1608” from scientific and industrial research organization in Iran. mixture of pineapple, apple and mangifera is provided as ratios 20, 30, 40% and reaches volume 100cc by distilled water and then the specimen are kept in water bath for pasteurization and temperature 80 °C is used for 5min [20].

Table 1- Introduction of tested treatments

Bacterial density (Cfu/ml)	mangifera juice concentration (%)	Apple juice concentration (%)	Pineapple juice concentration (%)	Fruit juice concentration (%)	Treatments
10 ⁶	5	5	10	20	F ₁ T ₁
10 ⁶	7/5	7/5	15	30	F ₂ T ₁
10 ⁶	10	10	20	40	F ₃ T ₁
10 ⁷	5	5	10	20	F ₁ T ₂
10 ⁷	7/5	7/5	15	30	F ₂ T ₂
10 ⁷	10	10	20	40	F ₃ T ₂
-	5	5	10	20	C ₁
-	7/5	7/5	15	30	C ₂
-	10	10	20	40	C ₃

Preparation of strain for inoculation

Activation of *Lactobacillus casei* 1608 is performed at liquid culture medium MRS and temperature 37 ° C for 24 hours [8].

Then, from liquid MRS media with microorganism as shaken well with shaker, microbial suspension is taken by sterile needle and is cultured on solid medium as surface and it is put inside jar and anaerobic device is used and then is kept in incubator 37 ° C for 48 hours. Then, the bacteria being grown in solid medium are used in next stages of study [20].

Microorganisms inoculation

The microbial inoculation was conducted by the McFarland method [1]. which was used to determine the amount of bacteria at the two levels of 1.5×10⁷ cfu/ml and 1.5×10⁶ cfu/ml.

Inoculation of microorganisms to specimen

10 cc of MRS broth was transferred to a sterile falcon by a sterile pipette in a laminar hood under sterile conditions and centrifuged at a speed of 4000 for 10 minutes. Then the supernatant fluid was separated and some amount of sterilized distilled water was poured into the sediment by sterile pipette and was mixed well with shaker and it was centrifuged again with the above conditions. This was repeated twice to wash the bacteria completely [8].

Then some amount of sterilized distilled water was poured into the falcon containing sediment to wash the remaining medium. The spectrophotometer was adjusted to 0.5 McFarland, in fact the turbidity was equal to 0.5 McFarland. Then, a little amount of created turbidity (about 3cc) was transferred separately to specific cell of

spectrophotometer and after putting cell in optic absorption at wavelength 623nm (as applied for bacteria) was measured and recorded for bacteria species. Of created turbidities equal to $1/5 \times 10^8$ cfu/ml of bacteria strain, 1cc is taken by sterile pipette and is added to the tube with 9cc sterile distilled water to achieve dilution $1/5 \times 10^7$ cfu/ml and microbial dilution $1/5 \times 10^6$ cfu/ml is achieved similarly [15].

fermentation

The specimen flasks were transferred into incubator at 37°C for 72 h for fermentation. After fermentation, the flasks were stored at 4°C for 4 weeks [16].

Tests

1- Lactic acid bacteria counting

For counting live microbial cells, serial dilution and pour plate methods are used in accordance to SPC (Standard plate counts). Ringer solution was prepared for dilution. 9cc of mentioned ringer was transferred into tube and then it was sterilized in autoclave 121°C for 15min. After cooling solutions, 1cc of specimen with microorganism was added to ringer solution and was mixed by shaker well. The dilution is 10^{-1} . This trend was continued to dilution 10^{-8} . The final dilutions were used to count the number of microorganisms. Of selected dilutions, 1mL of microbial suspension was transferred into sterile plate and sterile solid culture medium MRS was added adequately. After closing cultural media, plates were transferred into jar and anaerobic system was used. Then, plates were kept in incubator at temperature 37°C for 72h. The numbers of grown colonies were counted after 72 hours. The number of colonies as in equation (1) was multiplied by dilution inverse and final value was defined as number of colonies in each milliliter (cfu/ml) [14].

Equation (1): Number of colonies per mL (cfu/ml) = Number of colonies \times inverse of dilution factor

2- Measurement of reducing sugars

According to the mentioned method in national standard of Iran, NO. 2685, Equation (2) is calculated.

$M = \text{Equation (2)}$

M = the amount of reducing sugars (sugar before hydrolysis) gram percent mL

F = Fehling's Factor

V = Consumption volume of neutralized solution (a)

3- Measurement of acidity in terms of lactic acid

The method of titration with 0.1 N, NaOH to create a stable pale pink color was applied to measure the lactic acid content. It is noteworthy that 1 ml of 0.1 N NaOH is equal to 0.009008 g of lactic acid [4].

Statistical analysis

To investigate the results of present study including 6 treatments with 3 control treatments with 3 replications, fully random design by factory method is used. The data is analyzed by SPSS 22 software. The comparison of means is compared by multi-range Duncan test at level 95%. To plot the charts, Excel software is used.

RESULTS AND DISCUSSION

The changes of reducing sugars during fermentation

As shown in Chart (1), general changes of reducing sugar of beverage before and after 72 hours of fermentation at temperature 37°C showed that the trend of changes of reducing sugars during the storage time for all treatments were equal. As shown, beverage reducing sugar had significant reduction after 72 hours fermentation in all treatments ($P \leq 0.05$).

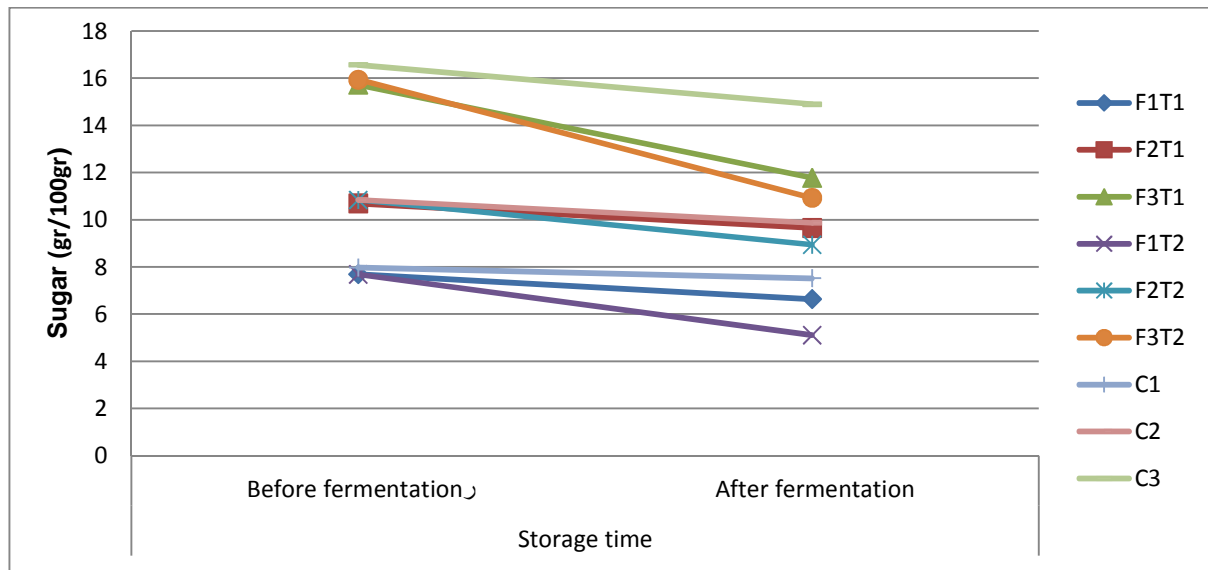


Chart 1: The general changes of reducing sugar of beverage before and after 72 hours fermentation at temperature 37°C

As shown in the results, it was shown that bacteria growth led into reduction of reducing sugar during fermentation and the main reason is regarding the consumption of sugar and production of organic acid.

The results of this study are consistent with Kumar *et al*[7] applying grape, mangifera, melon as a good substrate to produce probiotic beverage by *Lactobacillus casei*. The results showed that the amount of reducing sugars was reduced during fermentation and this sugar reduction was due to using *Lactobacillus casei* of reducing sugars to produce organic acids. The results of this study are consistent with the study of Yahyaei[15] and Mousaviet *al*[20].

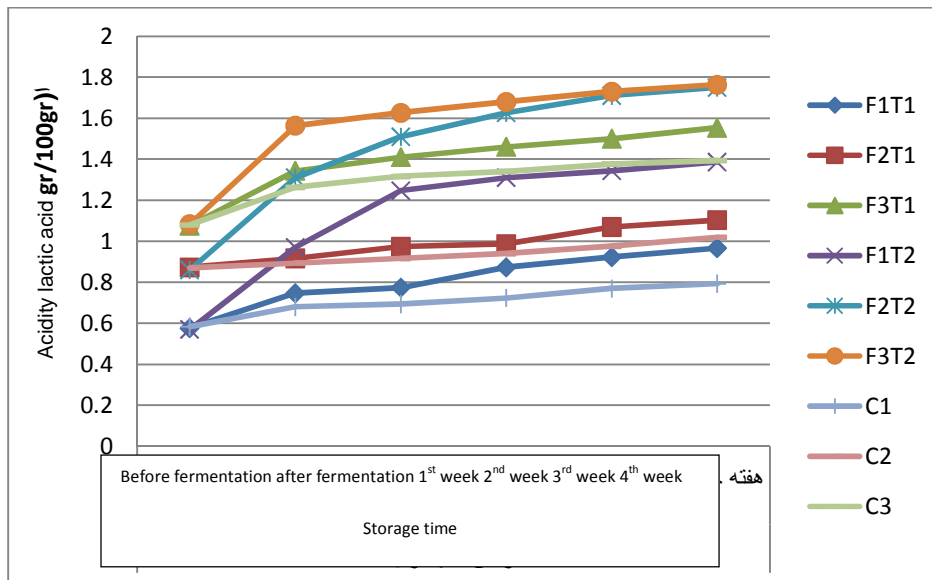


Chart 2-General changes of acidity of beverage before fermentation and after 72h of fermentation at temperature 37°C and during four weeks of storage at temperature 4°C

Acidity changes during fermentation and storage

As shown in Chart (2), general changes of acidity of beverage before fermentation and after 72h of fermentation are at temperature 37°C and during storage at temperature 4°C. The results show that acidity changes during storage time

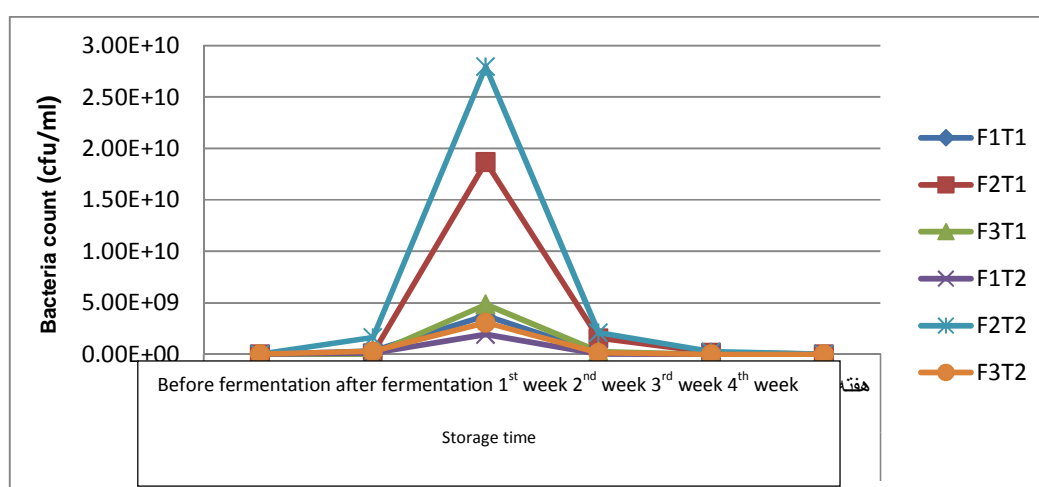
are equal for all treatments. As shown, acidity of beverages during storage is increased significantly in all treatments ($P \leq 0.05$).

As shown in the results, bacteria growth led into increase acidity during fermentation and storage. The main reason is regarding the consumption of sugars and organic acid.

The results of this study are consistent with the results of Costa *et al*[2] for the use of sonicated pineapple juice as substrate for producing a probiotic beverage by *Lactobacillus casei*. The results of study showed that during storage, acidity was increased and this acidity increase was due to using *Lactobacillus casei* from reducing sugars to produce organic acids. The results of study are consistent with the results of [18], [16], [7], [15].

The changes of probiotic bacteria growth during fermentation and storage

As shown in chart 3, the results show that bacteria growth trend of *Lactobacillus casei* after 72h of fermentation is increased at temperature 37°C and during four weeks of storage at temperature 4°C, *Lactobacillus casei* bacteria growth was reduced significantly over time ($P \leq 0.05$).



Linear chart 3- General changes of *Lactobacillus casei* bacteria growth in beverage after 72h fermentation at temperature 37°C and during four weeks of storage at temperature 4°C

As shown in the results, it was shown that probiotic bacteria were increased during fermentation and during four weeks of storage at temperature 4°C, probiotic bacteria were reduced. The maximum survival of bacteria in the recommended range from food and drug organization, for most of treatments during 4 weeks of storage were three weeks and only F₂T₁ treatments with 30% of fruit juice concentration (including 15% pineapple juice, 7.5% apple juice, 7.5% mangifera juice) and bacteria density 10⁶ cfu/ml and F₂T₂ with 30% of fruit juice concentration (Including 15% pineapple juice, 7.5% apple juice, 7.5% mangifera juice) and bacteria density 10⁷ cfu/ml to four weeks of storage and supported the results of tests of Yahyaei[15] During 28 days of storage, probiotic bacteria were reduced over time.

The results of study are consistent with the findings of Dogaheet *al*[3] and the number of live cells of probiotic bacteria in pineapple, apple and mangifera juice mixture during storage at temperature 4°C was reduced after two weeks and it is due to acidity conditions of beverage.

The results of study are consistent with the findings of Nagasivdu *et al* [10] and *Lactobacillus casei* in the mixture of watermelon and tomato juice incubated at temperature 37°C, had better survival during storage at temperature 4°C.

CONCLUSION

In this study, total acidity factors in terms of lactic acid, reducing sugars and survival of probiotic bacteria in probiotic fermentation beverage of concentrate mixture of pineapple, apple and mangifera juice in ratios 20, 30, 40% and *Lactobacillus casei* in two levels 10^6 , 10^7 cfu/ml. Based on the results, optimal temperature of fermentation and bacteria growth with high viability is 37°C for 72 hours. During fermentation of probiotic bacteria due to sugar and nutrient consumption in fruit juice was increased. Reducing sugars was reduced and acidity was increased due to reducing sugars by microorganisms. The maximum survival of bacteria in the recommended range of food and drug organization for most of treatments during 4 weeks of storage was three weeks and only F₂T₁ treatments with 30% of fruit juice concentration (including 15% pineapple juice, 7.5% apple juice, 7.5% mangifera juice) and bacteria density 10^6 cfu/ml and F₂T₂ with 30% of fruit juice concentration (Including 15% pineapple juice, 7.5% apple juice, 7.5% mangifera juice) and bacteria density 10^7 cfu/ml to four weeks of storage. Totally, the results of study showed that pineapple, apple and mangifera juice mixture was a good medium for the growth of lactic acid bacteria and functional beverage production.

REFERENCES

- [1] Ashrafi F, Practical microbiology, First published compilation, Ahsan Press, **2006**
- [2] Costa M.G.T; Vidal Fonteles A.L; Tiberis De Jesus S. Rodrigues; *Food Chemistry*, **2013**, 139, 261-266.
- [3] Dogahe K.H.M; K.K.Darani; A.Tofighi; M.Dadgar; A.M.Mortazavian, *British Biotechnology Journal*, **2015**, 5, 1, 37-50.
- [4] Emanuel V,V; Adrian P; Ovidiu C; Gheorghe, *African Journal of Biotechnology*, **2005**, 4,5, 103-408.
- [5] Hilliam M, *Word Food Ingred*, **2000**, 12, 50-52.
- [6] Karchegani M.M; K.Mokhtari, Natural treatment with fruits and vegetables, Agriculture science publications of Iran, **2012**, 116-150.
- [7] Kumar B.V; M.Sreedharamurthy; O.V.sarathireddy. *International journal of Applied Sciences and Biotechnology*, **2013**, 1,3, 131-137.
- [8] Mokarram P.R; S.Mortazavi; M.B.H.Najafi; F.Shahidi. *Food Res Int*, **2009**, 42 (8) ,1040-1045.
- [9] Moraru D; I.Bleoancal; R.Segal, *Food Technol*, **2007**, 4, 87-91.
- [10] Nagasivudu S; K.U.Mamahesh; O.V.S.Reddy. *International journal of Current Microbiology and Applied Sciences*, **2014**, 11, 977-984.
- [11] Nameless, Fruit juices - Methods of test. Iran National Standard No. 2685. Institute of Standards and Industrial Research of Iran, **2007**.
- [12] Sarrela M; G.Mogensen; R.Fonden; T.M. Sandholm, *journal of Biotechnology*, **2000**, 84, 197-215.
- [13] Tortoe C; P.T.Johnson; T.Slaghek; M.Miedema; T.Timmermans. *food And Nutrition Science*, **2013**, 4, 163-168.
- [14] Vinderola C.G; J.A.Reinheimer. *International Dairy Journal*, **2000**, 10,4, 271-275.
- [15] Yahyaei Sufiani. Z, The evaluation of production of beverage based on Malt extract and red fermented probiotic juice, MA thesis of food industry, Agriculture school, Azad University of Varamin Pishva branch, **2014**
- [16] Yoon K.Y; E.E.Woodams; Y.D.Hang, *The journal Of Microbiology*, **2004**, 42, 315-318.
- [17] Yoon K.y; E.E.Woodams; Y.D.Hang, Fermentation of Beet Juice By Beneficial Lactic Acid Bacteria, *Lebensm-Wiss U-Technol*, **2005**, 38, 73-75.
- [18] Yoon K.Y; E.E.Woodams; Y.D.Hang. Production of Probiotic Cabbage juice By Lactic Acid Bacteria, *Biore Source Technology*, **2006**, 97, 1427-1430.
- [19] Zargari A, Herbal plants, Tehran University publications, **1988**, 470-583.
- [20] Mousavi Z.E; S.M.Mousavi; S.H.Razavi; Z.E.mamdjomeh; H.Kiani, *World journal Biotechnol*, **2011**, 27, 123-128.