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Study on Chemical and Sensory Changes of probiotic fermented beverage based on mixture of pineapple,apple and mango juices

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K E Y W O R D S: Lactobacillus casei , Probiotic fermented mixture of pineapple, apple ,mangifera juice

ABSTRACT: Lactic bacteria play important role in production and keeping ferment food and providing probiotic products. The present study evaluates fermented drink production based on the mixture of pineapple, apple and mangifera by Lactobacillus casei PTCC 1608. Factors such as Brix at times after fermentation and pH after fermentation and during 28 days at temperature 4℃ 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℃. 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, pH and Brix are reduced. Based on the results, F_2T_2 treatment with concentration of 30% of juice and density 10^7 cfu/ml is the best treatment and it has the highest bacteria after 28 days. Sensory evaluation is done after 7, 28 days at temperature 4℃ by 5 specialist analyzers. C_3 control treatment with concentration of 40% of juice is selected as the best treatment. Among fermented samples, F_3T_1 treatment with concentration of 40% of juice and bacteria density 10^6 cfu/ml is selected as the best treatment. The results of study show that mixture of pineapple, apple and mangifera juice is a good medium for the growth of lactic acid bacteria .

Introduction

The recent economic and social progresses have created many problems for human health. The tension and busy mind of people have led into heart attack, high blood pressure, intestine disorder and different cancers. One of the effects ways for prevention of these diseases is using probiotic products (Nematolahi et al., 2012).

The term probiotic is defined by the committee of experts as live microorganisms as when they are in suitable values in gastric system, they have useful effects on host (Lotfi et al., 2009).

The most important effect of probiotics is their replacement in small intestine stimulating intestine and cleaning it and it avoids sticking of pathogens and inhibits the toxic effect of toxins (Farnworth et al., 2007; Tsen et al., 2007).

The nutrition and biological potential of fruit juice and vegetables has caused that these foods are turned into products with multiple properties to maintain the balance of microorganisms. These useful effects cause that the water of these products is used to heal various diseases. They are rich in nutrients. Fruit juice doesn't consist of starters competing with food with probiotics and fruit juice has high sugar improving the growth of probiotics (Ding&Shah, 2008; Morarue et al., 2007).

Most of studies show that probiotics have bad flavor and they lead to dissatisfaction of users (Nematolahi et al., 2012).

By pineapple and mango and with their strong flavor, we can improve bad flavor of probiotics. 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 brix after fermentation and evaluation of pH after fermentation and during 28 days being kept at temperature 4 ˚C. Sensory evaluation is done after 7, 28 days at temperature 4℃ by 5 specialist analyzers.

Materials and Methods

Materials

The applied material in probiotic beverage production process is concentrate of pineapple, apple and mangifera juice from Alifard company (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 (Mousavi et al.,2011).

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_1T_1
10^6	5/7	5/7	15	30	F_2T_1
10^6	10	10	20	40	F_3T_1
10^7	5	5	10	20	F_1T_2
10^7	5/7	5/7	15	30	F_2T_2
10^7	10	10	20	40	F_3T_2
-	5	5	10	20	C_1
-	5/7	5/7	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 (Mokarram et al., 2009).

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 (Mousavi et al., 2011).

Microorganisms inoculation

The microbial inoculation was conducted by the McFarland method (Ashrafi, 2006) .which was used to determine the amount of bacteria at the two levels of 1.5×10^7 cfu/ml and 1.5×10^6 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 (Mokarram et al., 2009).

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 $1/5\times10^8$ 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$1/5\times10^7$ cfu/ml and microbial dilution$1/5\times10^6$ cfu/ml is achieved similarly (Yahyaei,2014).

Specimen 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 (Yoon et al., 2004).

Tests

The test of sold soluble in water (Brix at 20°C).

Brix shows the soluble solid in gram in 100gram of sample. Refractometer is also used (Anonymous, 2007).

The test of measuring pH

To measure pH, pH meter is used (Anonymous, 1997).

Sensory test

After fermentation of beverage for panel test, the test is kept at the first and fourth weeks and is taken of five fixed and specialized people in food industry (Luckow et al., 2006).

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

Brix changes during fermentation

As shown in Chart 1, synthetic of brix of fermentation beverage is shown before fermentation and after 72 hours of fermentation at temperature 37℃. The results show that brix of beverages was reduced significantly after 72 hours of fermentation in all treatments ($P \leq 0.05$).

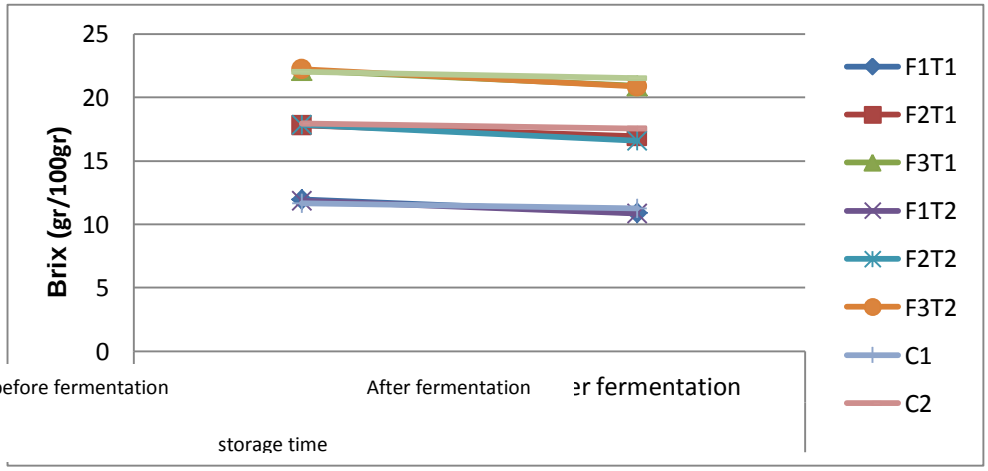

Chart 1. Syntetic of brix of fermentation beverage before and after 72 hours at temperature 37℃

As shown in the results, it was shown that bacteria growth led into reduction of brix 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 the results of study of Zomorodi&Ghasemi, (2014) investigating survival of Lactobacillus acidophilus as free and capsulated in probiotic apple juice. In three samples, brix was reduced during keeping. The highest brix reduction in apple juice specimen with free bacteria and lowest brix reduction was in capsulated treatment and the reason was capsule barrier for easy access of probiotics to sugar. In apple juice with free bacteria, due to easy access to sugar, brix reduction was significant.

The results of this study are consistent with the results of study of Yahyaei,(2014) and Kumar et al.,(2013).

pH changes during fermentation and keeping

As shown in chart 2, synthetic of pH of fermentation beverage during keeping shows that the pH changes during keeping time is equal for all treatments. As shown, pH of beverages during keeping is significantly reduced in all treatments ($P \leq 0.05$).

According to the results, the bacteria growth led into pH reduction during fermentation and storage and the main reason is regarding consumption of sugar and production of organic acids.

The results of study are consistent with the study of Sharma&Mishra,(2013) regarding Lactobacillus plantarum, Lactobacillus acidophilus and Pediococcus pantosaceus to produce probiotic carrot and squash mixture . The results showed that during storage, pH was reduced from 5.2 to 3.5 and pH reduction during storage was observed at temperature 4℃.

Shukla et al., (2013) investigated the production of probiotic pineapple juice by Lactobacillus acidophilus and whey. PH was reduced from 4.36 to 3.87 during fermentation. Compared to the studies in the present study, pH reduction in fermentation was similar to the results of study of Shukla et al. The results of study are consistent with the results of study of Yahyaei, (2014) and Yoon et al.,(2006); Kumar et al.,(2013) .

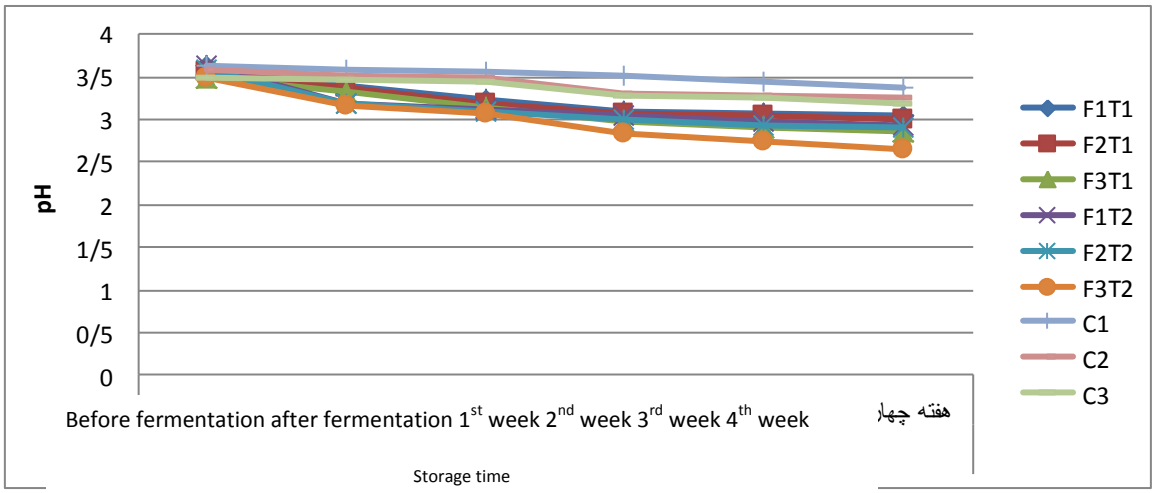

Chart 2. Synthetic of pH of fermented beverage during storage

Sensory evaluation

As shown in Chart 3, the general changes of sensory test of beverage after 1 week of storage at temperature 4℃ show that the highest score is regarding control treatment C_3 and lowest score is regarding F_1T_2 treatment and among the fermented specimen, F_3T_1 treatment has the highest score.

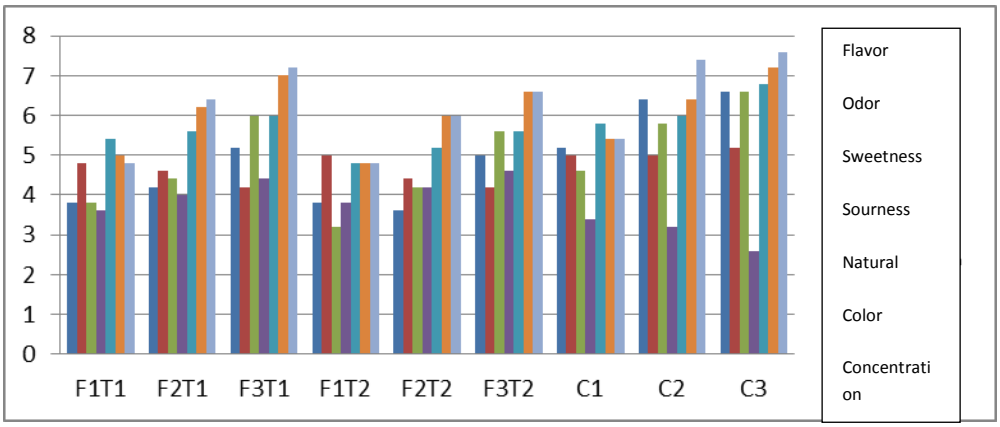

Chart 3. The general changes of sensory test of beverage after one week of storage at temperature 4℃

As shown in Chart4, the general changes of sensory test of beverage after 4 week of storage at temperature 4℃ show that the highest score is regarding control treatment C_3 and lowest score is regarding F_1T_2 treatment and among the fermented specimen, F_3T_1 treatment has the highest score.

As shown in the results of sensory test in this study, during beverage storage at temperature 4℃ in the first and fourth weeks, scores of flavor, odor, naturalness, sweetness, color and appearance and concentration of drink were reduced and sourness scores of beverage was increased.

Nematolahi et al., (2014) investigated the effect of storage in fridge on survival of probiotic strains and some physico-chemical features and sensory characteristics in Cornus mas. The evaluation of sensory test showed that there was a significant difference between control specimen and specimen inoculated with local strain during storage in terms of odor, flavor and total acceptance statistically. The results were consistent with the studies in present study.

The results of study are consistent with the results of study of Yahyaei,(2014) investigating physico-chemical and sensory properties of probiotic fermentation based on Malt extract and fermenting red fruit. The results showed that after 28 days of storage at temperature 4℃, scores of flavor, sweetness, naturalness, odor ,color and appearance and concentration of beverage are reduced and sourness scores of beverage are increased.

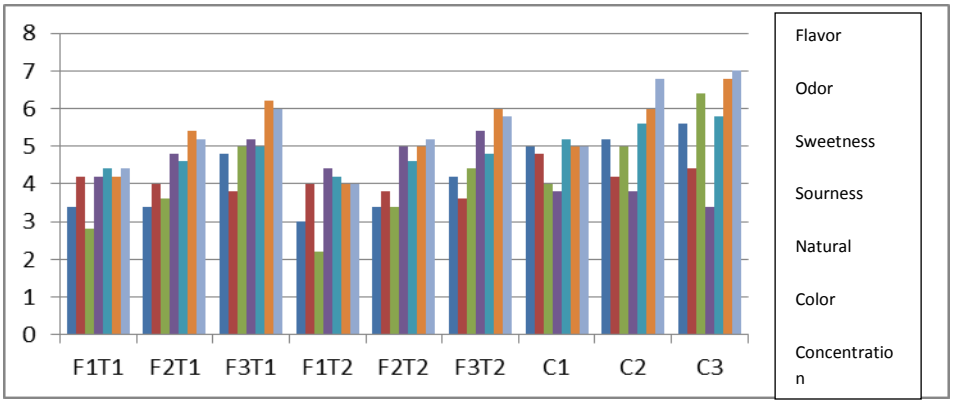

Chart 4. The general changes of sensory test of beverage after four week of storage at temperature 4°C

Conclusion

In this study, factors such as pH , brix and sensory evaluation of 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 were evaluated. 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 .Brix and pH were reduced . According to beverage sensory test during the first and fourth weeks at temperature 4°C, control treatment C_3 with 40% concentration of juice was the best treatment. Among the fermented specimen, F_3T_1 treatment with 40% concentration of fruit (20% pineapple, 10% apple juice, 10% mango juice) and bacteria density 10^6cfu/ml had the highest score. 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 beverage production.

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