Short communication

Applied Food Biotechnology, 2016, 3 (1): 59-62
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

Effect of Refrigerated Storage on Sensory Properties and Viability of Probiotic in Grape Drink

*Shirin Malganji[1], Sara Sohrabvandi[*2], Mahshid Jahadi[1], Ameneh Nematollahi[3], Bahareh Sarmadi[3]*

1. Department of Food Science and Technology, Faculty of Food Science and Technology, Islamic Azad University, Khorasgan (Isfahan) Branch, Isfahan, Iran.
2. Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences, Food Science and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Students' Research Committee, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

The popularity of non-dairy probiotic products continues to persist as the consumers prefer functional foods satisfying their health needs. Among these promising foods, probiotic grape drink would have beneficial effects on modifying gastrointestinal flora and human health. In this study, the pasteurized grape drink was inoculated by three species of lactic acid bacteria (*Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus*) separately, and the samples were subjected to non-fermented conditions. The samples were kept in the refrigerator at 4°C for 4 weeks to determine microbial viability and sensory evaluation during cold storage. Based on the results obtained, *Lactobacillus rhamnosus* and *Lactobacillus delbrueckii* displayed greater surviving than *Lactobacillus plantarum* during cold storage. Sensory evaluation outcome indicated that grape juice inoculated with *Lactobacillus rhamnosus* showed higher overall acceptability over 4 weeks of storage. The findings revealed that sustainability and sensory properties of probiotic products are important from the consumers' point of view; therefore, production of probiotic grape juice by *Lactobacillus rhamnosus*, due to its higher viability and desirable organoleptic properties, is suggested.

Article Information

Article history:
Received 14 Nov 2015
Revised 30 Nov 2015
Accepted 22 Dec 2015

Keywords:
Grape,
Lactic Acid Bacteria
Drink,
Refrigerated storage

Correspondence to:
Sara Sohrabvandi
Departmentof Food Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition and Food Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Tel: +98-21-22086348
Fax: +98-21-22360660
E-mail: sohrabv@sbmu.ac.ir

1. Introduction

According to the definition by the Food and Agriculture Organization and World Health Organization, consumption of desired amount of probiotics may lead to several health benefits e.g. in the intestinal micro-flora and improvements in intestinal functioning [1]. In order to receive the beneficial effects of probiotics, the viable cell count of these microorganisms should be 10^6 CFU ml^{-1} in the product. Fermentation time, storage temperature and oxygen content of the product have important effect on the viable cell count of probiotics [2]. Several researches are performed worldwide to explore the viability of probiotics and suitability of fruit juices as an alternative medium for non-dairy probiotic products. Yoong et al. (2004) determined the suitability of tomato juice, as a raw material, for the production of probiotic drink by *Lb. acidophilus* LA39, *Lb. plantarum* C3, *Lb. casei* A4 and *Lb. delbrueckii* D7. Their study indicated that the viable cell count of *Lb. acidophilus* and *Lb. delbrueckii* ranged from 10^6 to 10^8 CFU ml^{-1} after 4 weeks of storage at 4°C [3]. Yoon et al. (2006) also reported the viability of *Lb. casei A4*, *Lb. plantarum* C3 and *Lb. delbrueckii* D7 in cabbage juice during cold storage (4°C) for four weeks. *Lb. delbrueckii* and *Lb. plantarum* grew well in low pH and high acidity, and reached to 10^5-10^7 CFU ml^{-1}. However, *Lb. casei* lost its viability after two weeks of cold storage [4].

Due to beneficial effects of probiotics on gastrointestinal tract and the important role of sensory properties based on the consumers' point of view, in this study, the viability and overall acceptability of three strains (*Lb. plantarum*, *Lb. rhamnosus* and *Lb. delbrueckii*) were studied in non-fermented grape drink for 4 weeks at 4°C.

2. Materials and Methods

Commercial concentrated grape juice was supplied from Takdaneh Iran Co. (Tehran, Iran) and kept at 4˚C prior to use.

2.1. Strains and Cultures

Lb. plantarum PTCC, *Lb. delbrueckii* PTCC 1737 and *Lb. rhamnosus* PTCC 1657 were obtained from Iranian Research Organization for Science and Technology (Tehran, Iran). All bacterial cultures were grown at 30˚C for 24 h in MRS broth and were used as an inoculum. 10^7 CFU ml^{-1} was inoculated to grape juice and all the samples were kept in the refrigerator for 4 weeks at 4°C. The microbiological analysis and sensory properties were assessed on the 0, 7, 14, 21 and 28 days of cold storage.

2.2. Microbiological analysis

The viable count of *Lb. plantarum*, *Lb. rhamnosus* and *Lb. delbrueckii* was determined by the standard plate count method using Man-Rogosa-Sharpe agar (MRS agar) on the 0, 7, 14, 21 and 28 days of cold storage, and the results were expressed as CFU ml^{-1} juice.

2.3. Sensory evaluation

Ten trained panelists pointed out the qualitative aspects of non-fermented grape juice as color, flavor, aroma and overall acceptability. Furthermore, the hedonic rating of the non-fermented grape juice was used for evaluating the consumers' preference [5].

2.4 Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean±SD. The SAS software (ver. 9) was used to analyze the experimental data (SAS Institute, Cary, NC, USA). Differences at $p<0.05$ were considered to be significant.

3. Results and Discussion

3.1. Viability of probiotics during cold storage

Table 1 illustrates the effect of cold storage on the viability of three lactic acid bacteria in non-fermented grape drink during 4 weeks at 4°C. There was a significant difference ($p<0.05$) in the initial viable counts of *Lb. plantarum* compared to *Lb. rhamnosus* and *Lb. delbrueckii*. In addition, this significant decrease ($p<0.05$) in viability of *Lb. plantarum* was continued during the 7th and 14th days of cold storage. Notably, *Lb. rhamnosus* and *Lb. delbrueckii* showed no significant decrease in viability during the 7th and 14th days of cold storage. As shown in the Table, during the 21st and 28th days of cold storage, there was a significant difference ($p<0.05$) in the viable count of all three strains in the samples. Therefore, the type of incorporated species (such as *Lb. plantarum*, *Lb. rhamnosus* and *Lb. delbrueckii*) and storage period have a significant effect on the viable counts of probiotics.

According to the research conducted on orange, pineapple and cranberry juice by Sheehan et al. (2007), *Lb. rhamnosus* GG, *Lb. casei* DN-114 001 and *Lb. paracasei* NBFC43338 could grow well on these juices, and their viable cell counts reached to 10^7 CFU ml^{-1} in orange juice and 10^6 CFU ml^{-1} in pineapple juice during 12 weeks of cold storage [6]. Champagen et al. (2008) reported that *Lb. rhamnosus* could grow well on fruit juice (mixture of different fruits) compared to *Lb. acidophilus* [7].

Yoon et al. (2005) studied the viability of probiotics in red beet juice during the cold storage. According to the results, the viable cell count of *Lb. acidophilus* remained 10^6-10^8 CFU ml^{-1} after 4 weeks of cold storage. However, *Lb. plantarum* lost its viability during the cold storage [8]. The same result was reported in the current study, and *Lb. plantarum* had less viability than the other two strains.

Mousavi et al. (2011) studied the viability of probiotics in pomegranate juice during the cold storage. There was 2 CFU ml^{-1} decrease in the viable cell count of *Lb. plantarum* after one week of cold storage. This decrease was due to the low pH and existence of some metabolites such as organic acids [9]. A similar result was obtained in the present study on non-fermented grape juice. After two weeks of storage, the viable cell count of probiotics was reduced in a significant amount.

Daneshi et al. (2012) examined the effect of cold storage on viability of probiotic bacteria in carrot fortified milk. *Lb. plantarum*, *Lb. rhamnosus* and *B.lactis* showed a slight reduction in cell counts; however, in contrast to three other strains, *Lb. acidophilus* count remained stable at 6.6 log CFU ml^{-1} during the cold storage [10]. In our study, *Lb. rhamnosus* showed higher viability than the other two strains; this finding is incompatible with Daneshi et al. results. Alegre et al. (2011) reported that *Lb. rhamnosus* remained viable in orange juice and apple wedges over 12 and 4 weeks of cold storage at 4˚C, respectively [11]. The same process was performed on grape juice in the present study, the findings of which reflect similar viability results.

Temperature, which enhances the mortality effect of organic acids, is one of the most important factors on the viability of probiotics. The cell wall of lactic acid bacteria consists of saturated, unsaturated and cyclic carbon chains, which will vary depending on parameters like temperature, pH, NaCl concentration and medium content. Linoleic and oleic synthesis will occur at acidic situations. These acids will absorb hydrogen in an acidic environment increasing the permeability of proton in membranes, and therefore, leading to viability increase when confronted with hostile conditions throughout acidic situation during storage at refrigeration temperature [6].

Table 1. Effect of cold storage on the viability of probiotic microorganism ((log CFU ml^{-1}) in grape drink

Time (days) Treatment	0	7	14	21	28
Lb. delbrueckii	8.6[a]	8.03[bcd]	8.14[bcd]	7.95[cde]	7.77[de]
Lb. plantarum	7.59[ef]	7.25[gf]	7.18[g]	7.15[g]	6.94[g]
Lb. rhamnosus	8.44[ab]	8.42[ab]	8.41[ab]	8.38[ab]	8.3[abc]

The means in the column shown with different letters are significantly different ($p<0.05$).

3.2. Effect of cold storage on sensory evaluation in non-fermented probiotic grape juice

Ten trained panelists assessed the sensory evaluation of non-fermented samples on 0, 7, 14, 21 and 28 days of cold storage. Sensory properties included flavor, odor, color and overall acceptability.

Table 2 shows flavor changes during the 0, 7, 14, 21 and 28 days of cold storage. *Lb. plantarum* and control grape drink showed sharp flavors during 7 days of storage. However, in the study conducted by Daneshi et al. (2012), the milk/carrot juice inoculated with *Lb. plantarum* had higher sensory acceptability over 20 days of storage [10]. After 14 days of storage, *Lb. rhamnosus* had the highest flavor acceptance among the other samples. Significant difference was observed for *Lb. rhamnosus* and *Lb. delbrueckii* compared to *Lb. plantarum* after 21 days of storage ($p<0.05$). After 28 days of storage, *Lb. rhamnosus* showed higher overall acceptability in flavor comparing to the other strains. These findings indicate that several factors should be taken into account, including appropriate selection of probiotic cultures and storage time. The flavor changes are time-dependent and will alter during the storage time.

Table 2 shows the effect of cold storage on the odor of non-fermented grape juice. *Lb. rhamnosus* and control showed better odor than *Lb. plantarum* and *Lb. delbrueckii* during 28 days of cold storage. The effect of cold storage on the color of non-fermented grape drink is presented in Tables 2. There was no significant difference observed in the color of samples during 28 days of cold storage. A same scenario was observed for the color of the samples.

Table 2 illustrates the effect of cold storage on the overall acceptability of non-fermented grape drink. After 14 days of storage, *Lb. rhamnosus* showed the highest acceptability comparing to the other samples.

Table 2. Effect of cold storage on the sensory properties of treatments with *Lb. delbrueckii, Lb. plantarum, Lb. rhamnosus and control*

Time (days)	Taste	Odour	Color	Overall acceptance
Lb. delbrueckii				
0	6[a]	6.6[a]	5.6[a]	20.6[a]
7	5.3[ab]	4.9[b]	5.2[ab]	18.05[b]
14	5.1[b]	5[b]	5[b]	17.65[bc]
21	4[c]	4[c]	5.1[b]	15[d]
28	3[cd]	3.9[c]	5.1[b]	13.5[e]
Lb. plantarum				
0	6[a]	6.6[a]	5.6[a]	20.6[a]
7	4.8[b]	5.2[b]	4.9[b]	17.3[b]
14	5.3[ab]	4.7[bc]	5[ab]	17.65[b]
21	3[c]	3.9[d]	5[ab]	13.4[c]
28	2.9[c]	4[d]	5[ab]	13.35[c]
Lb. rhamnosus				
0	6[a]	6.7[a]	5.5[ab]	20.61±1.39
7	4.9[b]	5.6[b]	5.2[b]	18.15±0.97
14	5.5[ab]	5.9[ab]	5.9[a]	20.05±1.57
21	4[d]	5[c]	4.8[bc]	15.8±1.47
28	4[d]	5[c]	5[b]	16±1.98
Control				
0	6[a]	6.7[a]	5.2[a]	20[a]
7	5.3[ab]	5.1[d]	5[a]	17.95[c]
14	5.4[ab]	4.8[d]	5.1[a]	18[c]
21	5.4[ab]	6[b]	5.1[a]	18.6[b]
28	5[b]	5.7[bc]	5[a]	18.2[bc]

The means in the column shown with different letters are significantly different ($p<0.05$)

Moreover, there was a similar trend after 21 and 28 days of storage and *Lb. rhamnosus* samples did not fail to meet consumer expectations. This result is similar to the findings of Krasakapot and Kitsaw research on the sensory properties of probiotic grape and orange juice in 2010, which showed the importance of flavor compared to odor from the panelists points of view [5]. Periara et al. (2011) studied the sensory properties of apple juice during the cold storage. The results indicated that transparency was reduced due to high biomass of lactobacillus inoculated into apple juice; therefore, the turbidity was increased. Besides, yellowness in the color of apple juice during the cold storage was reported to be caused by low pH [12].

4. Conclusion

The present study was undertaken to identify the viability and sensory properties of probiotic grape drink during cold storage at 4ºC for 4 weeks. The results showed that the viable cell count of *Lb. rhamnosus* was greater than that of the other two strains during the cold storage. Moreover, *Lb. rhamnosus* was a suitable strain for incorporation into grape drink in comparison with *Lb. plantarum* and *Lb. delbrueckii*. According to the sensory evaluation results, *Lb. rhamnosus* had higher overall acceptability compared to *Lb. delbrueckii* and *Lb. plantarum*. Finally, *Lb. rhamnosus* has promising potential to be used as functional supplement in grape drink. It is noteworthy to say that sensory properties of probiotic products should be taken into account if they are consumed in the form of food products rather than medicine.

5. Acknowledgement

The authors would like to thank Takdaneh Fruit Juice Co., for the support provided for this work.

6. Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

1. Rivera-Espinoza Y, Gallardo-Navarro Y. Non-dairy probiotic products. Food Microbiol. 2010; 27(1): 1-11.
2. Yoon KY, Woodams EE, Hang YD. Fermentation of beet juice by beneficial lactic acid bacteria. LWT-Food Sci Technol. 2005; 38(1): 73-75.
3. Yoon KY, Woodams EE, Hang YD. Probiotication of tomato juice by lactic acid bacteria. J Microbiol. 2004; 42(4): 315-318.
4. Yoon KY, Woodams EE, Hang YD. Production of probiotic cabbage juice by lactic acid bacteria. Bioresource Technol. 2006; 97(12): 1427-1430.
5. Krasaekoopt W, Pianjareonlap R, Kittisuriyanont K, 2007, editors. Probiotic fruit juices. The 2nd International Conference on Fermentation Technology for Value added Agricultural Products, Kosa Hotel, Khon Kaen, Thailand.
6. Sheehan VM, Ross P, Fitzgerald GF. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. Innov Food Sci Emerg Technol. 2007; 8(2): 279-284.
7. Champagne CP, Gardner NJ. Effect of storage in a fruit drink on subsequent survival of probiotic lactobacilli to gastro-intestinal stresses. Food Res Int. 2008; 41(5): 539-543.
8. Yoon KY, Woodams EE, Hang YD. Fermentation of beet juice by beneficial lactic acid bacteria. LWT-Food Sci and Tech. 2005; 38 (1):73-75.
9. Mousavi Z, Mousavi S, Razavi S, Emam-Djomeh Z, Kiani H. Fermentation of pomegranate juice by probiotic lactic acid bacteria. World J Microbiol Biotechnol. 2011; 27(1): 123-128.
10. Daneshi M, Ehsani M, Razavi H, Labbafi M, Rezaee M. Effect of cold storage on viability of probiotic bacteria in carrot fortified milk. Nutr Food Sci. 2012; 2(9): 1-4.
11. Alegre I, Vinas I, Usall J, Anguera M. and Abadias M. Microbiological and physicochemical quality of fresh-cut apple enriched with the probiotic strain *Lactobacillus rhamnosus* GG. Food Microbiol. 2011; 28(1): 59-66.
12. Pereira ALF, Maciel TC, Rodrigues S. Probiotic beverage from cashew apple juice fermented with *Lactobacillus casei*. Food Res Int. 2011; 44(5): 1276-1283.