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Fermentation of Grape Juice by Probiotic Lactic Acid Bacteria: Short Communications

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Abstract: In this study, the pasteurized grape juice was inoculated by three species of lactic acid bacteria (*L. delbrueckii*, *L. plantarum* and *L. rhamnosus*) in order to determine the suitability of grape juice as a raw material for the production of probiotic grape juice. Viability of probiotics, pH, titrable acidity, sugar and sensory evaluation were measured during the fermentation at 30°C within 72 h. Results revealed that *L. rhamnosus* and *L. delbrueckii* grew well on grape juice and could survive in low pH and high acidity. The lactic acid cultures reduced the pH to 3.7 or below and increased the acidity to 0.27% or higher. Furthermore, high consumption of sugar was mentioned for *L. rhamnosus* and *L. plantarum*. Based on the results of the current study, *L. delbrueckii*, *L. plantarum* and *L. rhamnosus* having promising potential for exploitation as functional supplements in grape juice.

Key words: Fermentation, grape juice, Lactobacillus, probiotic, bacteria

INTRODUCTION

Probiotics are defined as live microorganism with favorable effects for host by supporting intestinal balance (Fuller, 1989). Although, Lactobacillus and Bifidobacterium are commercially used as starter cultures in probiotic dairy products, consumers demand for non-dairy-based probiotic products due to lactose intolerance and cholesterol content of dairy products has increased recently (Shah, 2001). Numerous reports have described that these "healthy" bacteria have beneficial effects such as reduction in cholesterol serum, immune system stimulation, anti-carcinogenic properties, acid folic production as well as the maintenance of a healthy gut microflora (Singh *et al*., 2011). Shah pointed out that the least amount of viable probiotics to insert human health benefits is typically 10^8 CFU day^{-1}, therefore, probiotic products should have at least 10^6 CFU g^{-1} (Shah, 2001).

Therefore, 10^6 CFU g^{-1} could serve as a minimum amount for every probiotic products. It has been suggested that fruit juices could apply as a suitable carriers for cultivating probiotic bacteria. Different researches have been performed to explore the suitability of fruit juices as an alternative media for non-dairy probiotic products.

Yoon *et al.* (2004) determined the suitability of tomato juice as a raw material for production of a probiotic drink by *Lb. acidophilus* LA39, *Lb. plantarum* C3, *L. casei* A4 and *Lb. delbrueckii* D7. As a result, the microbial population increased significantly after 48 h of fermentation and the viable cell counts of bacteria were ranged from 10^6-10^8 CFU mL^{-1} after 4 weeks of storage in 4°C (Yoon *et al*., 2004). Yoon *et al.* (2005) also evaluated the beet juice as a raw substrate for production of probiotic beet juice by the above four species of lactic acid bacteria. *Lb. acidophilus* and *Lb. plantarum* produced higher amounts of lactic acid and could reduce the pH of beet juice to below 4.5 after 48 h of fermentation. Furthermore, the viable cell counts after 4 weeks of cold storage, except *Lb. acidophilus* were

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remained 10^6-10^8 CFU mL^{-1} (Yoon *et al*., 2005). Moreover, these researchers developed a probiotic cabbage juice by *Lb. plantarum* C3, *Lb. casei* A4 and *Lb. delbrueckii* D7. The results indicate that the cultures could grow well and reached nearly 10^9 CFU mL^{-1} after 48 h of fermentation, however, *Lb. casei* lost its viability after 2 weeks of cold storage and could not survive in low pH (Yoon *et al*., 2006).

Grape is rich in functional food components such as antioxidants, phenolic compounds, dietary fibers and minerals (Garrido and Borges, 2013). Furthermore, grape juice is well recognized as one of the healthiest beverages and besides to contribute to its nutritive function, this beverage can be an ideal medium for cultivating probiotics. In the present study, we have assessed the suitability of grape juice and effect of fermentation on the production of probiotic juice by lactic acid bacteria.

MATERIALS AND METHODS

Starter culture: *Lactobacillus plantarum*, *Lactobacillus delbrueckii* and *Lactobacillus rhamnosus* 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.

Sample preparation: The grape juice concentrate was diluted to 20° Brix with distilled water and was pasteurized for 5 min at 80°C. All samples were inoculated with a 24 h culture and samples were incubated at 30°C for 72 h. Sampling was carried out at 0, 24, 48 and 72 h during fermentation at 30°C for chemical and microbiological analyses.

Biochemical, chemical and microbiological analysis: To measure the pH of probiotic grape juice, a digital pH meter (Mettler, MA 235, Switzerland) was used. Titrable acidity, expressed as percent tartaric acid was determined by titrating with 0.1 N NaOH (Merck, Germany) to pH 8.2. HPLC (Nomura chemical Co.) method was used to measure the sugar content of samples. The count of *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus delbrueckii* were determined by the standard plate count method using Man-Rogosa-Sharpe agar (MRS agar) after 72 h of incubation at 30°C. The results were expressed as CFU/mL juice.

Statistical analysis: All experiments were performed in triplicate and each sample was analyzed in duplicate. The SAS:9 statistical package was used to analyze the experimental data (SAS Institute, Cary, NC, USA).

RESULTS

pH and acidity in different treatments during fermentation: Results revealed that all the three species of Lactobacillus (*L. plantarum*, *L. rhamnosus* and *L. delbrueckii*) were capable of growing in grape juice. The time courses of lactic acid fermentation of grape juice by *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus delbrueckii* are presented in Table 1-3, respectively. As presented in Table 1 and 2, there was a significant decrease in pH of grape juice treatments with *Lactobacillus delbrueckii* and *Lactobacillus plantarum* during the first 48 h of the fermentation. Also, the results indicated that both *L. plantarum* and *L. delbrueckii* produced significantly more titrable acidity expressed as lactic acid than *L. casei*. As it could be detected in Table 1-3, the major changes of the acidity was observed in the period between 48-72 h of fermentation; furthermore, extending the fermentation time (from 48-72) did not result in a significant change.

Residual sugar in different treatments during fermentation: Results obtained from sugar analysis reveals that *L. plantarum* and *L. rhamnosus* showed more affinity to sugar consumption during 72 h of fermentation and cause significant decrease during this period. However, in contrast to two other lactic acid bacteria, *L. delbrueckii* exhibited least ability in sugar consumption as illustrated in Table 1, *L. delbrueckii* reduced the sugar level from an initial value of 21 mg mL^{-1} to as low as 18.3 mg mL^{-1} after 72 h fermentation.

Viability of probiotic bacteria during the fermentation: As it is shown in Table 1-3, after 24 h of fermentation the

Table 1: Time course of lactic fermentation of grape juice by *Lactobacillus delbrueckii*

Time (h)	pH	Acidity (%)	Sugar (mg mL^{-1})	CFU mL^{-1}
0	4.06 ± 0.05^{a}	0.24 ± 0.005^{gh}	21 ± 0^{ab}	$7.49\pm0.1\times10^{7h}$
24	4.1 ± 0^{a}	0.26 ± 0.005^{efg}	20.6 ± 0.6^{abc}	$9.17\pm0.1\times10^{7d}$
48	3.7 ± 0^{b}	0.33 ± 0.02^{c}	21 ± 0^{ab}	$9.41\pm0.01\times10^{7cd}$
72	$3.6\pm0b^{c}$	0.35 ± 0.005^{b}	18.3 ± 0.1^{g}	$9.59\pm0.02\times10^{7cd}$

Table 2: Time course of lactic fermentation of grape juice by *Lactobacillus plantarum*

Time (h)	pH	Acidity (%)	Sugar (mg mL^{-1})	CFU mL^{-1}
0	4.1 ± 0^{a}	0.25 ± 0^{gh}	21 ± 0^{a}	$7.15\pm0.27\times10^{7bc}$
24	4.1 ± 0^{a}	0.27 ± 0.005^{e}	20.6 ± 0.06^{d}	$9.36\pm0.07\times10^{7dc}$
48	3.7 ± 0.1^{b}	0.32 ± 0.05^{c}	20.5 ± 0.05^{e}	$9.91\pm0.01\times10^{7a}$
72	3.6 ± 0.1^{c}	0.39 ± 0.005^{a}	18.06 ± 0.05^{g}	$9.73\pm0.03\times10^{7ab}$

Table 3: Time course of lactic fermentation of grape juice by *Lactobacillus rhamnosus*

Time (h)	pH	Acidity (%)	Sugar (mg mL^{-1})	CFU mL^{-1}
0	4.06 ± 0.05^{a}	0.24 ± 0.005^{h}	20.6 ± 0.6^{a}	$7.41\pm0.39\times10^{7h}$
24	4.1 ± 0.1^{a}	0.25 ± 0.005^{fgh}	21 ± 0^{bcd}	$8.17\pm0.2\times10^{7g}$
48	4.03 ± 0.05^{a}	0.27 ± 0.01^{ef}	21.6 ± 0.6^{d}	$8.50\pm0.13\times10^{7f}$
72	4 ± 0.1^{a}	0.29 ± 0.005^{d}	18.1 ± 0.1^{f}	$8.91\pm0.05\times10^{7e}$

[a-h]The means in a coloumn shown with different letters are significantly different ($p<0.05$)

viable cell counts showed significant changes. For instance, the viable cell counts of *L. delbrueckii* reached from 7.49-9.17 Log CFU mL^{-1} after 24 h of fermentation. Furthermore, *L. plantarum* reached from 9.36-9.91 Log CFU mL^{-1} in 48th h of fermentation.

DISCUSSION

pH and acidity in different treatments during fermentation: As presented in Table 1 and 2, there was a significant decrease in pH of grape juice treatments with *Lactobacillus delbrueckii* and *Lactobacillus plantarum* during the first 48 h of the fermentation. As expressed by Yoon *et al.* (2004), *L. plantarum* showed a more rapid drop in pH of tomato juice than the other three lactic acid cultures examined. Yoon *et al.* (2005) also reported that *L. plantarum* and *L. acidophilus* reduced the pH of beet juice. In addition, Yoon *et al.* (2006) developed probiotic cabbage juice by lactic acid bacteria. The results indicated that both *L. plantarum* and *L. delbrueckii* produced significantly more titrable acidity expressed as lactic acid than *L. casei.* According to Saw, the greatest decrease in pH of the tropical fruit juices was mentioned for *L. delbrueckii* ssp. bulgaricus during the first 48 h of fermentation. Accordingly, both *Lactobacillus plantarum* and *Lactobacillus delbrueckii* produced significantly more titrabel acidity expressed as tartaric acid than *Lactobacillus rhamnosus* after 72 h of fermentation at 30°C. As it could be detected in Table 1-3, the major changes of the acidity was observed in the period between 48-72 h of fermentation; furthermore, extending the fermentation time (from 48-72) did not result in a significant change. Yoon *et al.* (2005) reported the same results. For instance, *L. plantarum* and *L. delbrueckii* produced nearly 1% titrable acidity expressed as tartaric acid after 72 h of fermentation.

Residual sugar in different treatments during fermentation: Earlier studies have reported that adding sugar (Monosaccharides and disaccharides) to fermented probiotic product will affect the rise in the number of lactic acid bacteria during the fermentation. In addition, simulatory effect of reducing sugars on probiotics is due to existence of ATP-ase proton pump in lactobacillus's membrane during acidic conditions (low pH), hydrogen ions will transfer out of the cell by ATP-ase proton pump and ATP required for this process is supplied by reducing sugars and if pH decreases, due to the denaturation of enzymes thereby disrupting Saccharolytic activity and ATP for pump performance does not satisfy, the cells will lose their viability gradually.

Residual sugar in different treatments during fermentation: Similar to the results of studies on beet juice and cabbage juice by Yoon *et al.* (2005) extending the fermentation time from 24-48 h did not result in a significant change in the viable cell count especially for *L. delbrueckii* and *L. plantarum*. In addition, *L. rhamnosus* showed a significant change in the viable cell counts during 72 h of fermentation. Nazzaro *et al.* (2008) investigated the influence of the addition of the two probiotic strains, *Lactobacillus rhamnosus* and *Lactobacillus bulgaricus* and the prebiotic components, inulin and fructooligosaccharides on carrot juice as a raw material for production of probiotic juice. They reported that the presence of inulin and fructooligosaccharides did not change the viable cell counts and both mentioned probiotic strains showed a good viability in the carrot juice (Nazzaro *et al.*, 2008). Yoon *et al.* (2004) reported that some factors can have influence on probiotic viability such as acidity, pH, amount of oxygen, nutrition deficiencies and antimicrobial substances in the product. As expressed by Mousavi *et al.* (2011), *L. planratum* and *L. delbrueckii* had a better growth rate in pomegranate juice; furthermore, juice enrichment by yeasts before fermentation, leads to high amount of amino acids, vitamins, minerals and antioxidant activity in the final product (Mousavi *et al.*, 2011). Similar in addition, Mortazavian reported that increased compatibility to organic acids and low pH in fermented products is important, since the existence of probiotics in fermentation process results in increasing compatibility with product conditions. Moreover, some factors protect probiotics against high acidity and low pH such as buffering capacity, network microstructure and ingredients of probiotic products. First, increasing the buffering capacity stimulates probiotic growth rate and viability in *in vivo* and *in vitro* conditions. Also, high buffering capacity will protect and increase the viability of probiotics throughout the gastrointestinal tract. Second, it has been proved that solid matrixes are more sufficient than liquid matrixes for probiotic protection. Third, the fat content in product will increase the protection of probiotics against gastrointestinal condition (Shafiee *et al.*, 2010).

CONCLUSION

Results revealed that the viable cell counts of three lactic acid bacteria were increased significantly during 48 h of fermentation at 30°C and extending the fermentation time from 48-72 h did not result in a significant increase in viable counts. Furthermore, all the three species of lactic acid bacteria were capable of using grape juice as a raw material and the significant decrease in pH in 48 h of fermentation was mentioned for fermented treatments with *L. delbrueckii* and *L. plantarum*. Results from the sugar content showed

that the high consumption of sugars in fermented samples was mentioned for *L. rhamnosus* and *L. plantarum*. Based on the results of the current study, *L. delbrueckii*, *L. plantarum* and *L. rhamnosus* having promising potential for exploitation as functional supplements in grape juice.

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