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Research note

Fermentation of beet juice by beneficial lactic acid bacteria

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Abstract

Red beets were evaluated as a potential substrate for the production of probiotic beet juice by four species of lactic acid bacteria (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus plantarum*). All the lactic cultures were found capable of rapidly utilizing beet juice for cell synthesis and lactic acid production. However, *L. acidophilus* and *L. plantarum* produced a greater amount of lactic acid than other cultures and reduced the pH of fermented beet juice from an initial value of 6.3 to below 4.5 after 48 h of fermentation at 30°C. Although the lactic cultures in fermented beet juice gradually lost their viability during cold storage, the viable cell counts of these lactic acid bacteria except for *L. acidophilus* in the fermented beet juice still remained at 10^6–10^8 CFU/ml after 4 weeks of cold storage at 4°C.

Keywords: Beet juice; Probiotic; Lactic acid bacteria; *Lactobacillus acidophilus*; *Lactobacillus casei*; *Lactobacillus delbrueckii*; *Lactobacillus plantarum*

1. Introduction

Research has shown that probiotic bacteria can colonize and proliferate in the intestinal tract of humans and animals to prevent the growth of intestinal pathogens (Fuller, 1989; Abe, Ishibashi, & Shimamura 1995). Lactic acid bacteria have been added to a variety of dairy-based products such as fermented milks and yogurts for their probiotic human health benefits (Siuta-Cruce & Goulet, 2001). In recent years, consumers' demand for nondairy-based probiotic products has increased. Red beets (*Beta vulgaris*) are mostly grown in New York, California, Colorado, Ohio, Texas and New Jersey. Beets contain 87.3% moisture, 1.6% protein, 9.1% carbohydrates, 0.8% fiber, 0.1% fat, and 1.1% ash (Altman & Dittmer, 1968). Beets contain red pigments that can be used for coloration of food (Von Elbe & Schwartz, 1981). Small beets are tender and tasty and can be eaten fresh or processed into pickled or canned products (Cruess, 1958; Pederson, 1979). Oversized or larger red beets, however, are currently underutilized because their texture is fibrous or tough. The objective of this study was to determine the suitability of larger beets as a raw material for production of probiotic beet juice by *L. acidophilus* and other beneficial lactic acid bacteria.

2. Materials and methods

Red beets were purchased from a local store and kept at 4°C prior to use. Beet juice was obtained with a Loomis press operated at 2000 psi. Four lactic cultures, *L. acidophilus* LA 39, *L. casei* A4, *L. delbrueckii* D7, and *L. plantarum* C3 (John Churey, New York State Agricultural Experiment Station Culture Collection, Geneva, New York) were used in the study. The inoculum was prepared by growing the culture at 30°C for 24 h in MRS broth (dextrose 20.0 g/l; meat peptone 10.0 g/l; beef extract 10.0 g/l; yeast extract 5.0 g/l; sodium acetate 5.0 g/l; disodium phosphate 2.0 g/l; ammonium citrate 2.0 g/l; Tween 80 1.0 g/l; magnesium sulfate 0.1 g/l, manganese sulfate 0.05 g/l). Viable cell counts (CFU/ml) of the inoculum were determined by the standard plate method with MRS medium after 48 h of incubation at 30°C.

Fermentation experiments were conducted in test tubes (25 × 200 mm), each containing 15 ml of sterile

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beet juice (15 min at 121°C in an autoclave). All samples were inoculated with a 24-h-old culture and incubated at 30°C. The viable cell counts (CFU/ml) of *L. acidophilus*, *L. plantarum*, *L. casei* and *L. delbrueckii* as an inoculum in beet juice at the beginning of the fermentation (0 h) were given in Tables 2, 3, 4 and 5, respectively.

pH was measured with a pH meter. Total acidity expressed as percent lactic acid was determined by titrating beet juice samples with 0.02 N NaOH to pH 8.2. Viable cell counts (CFU/ml) were determined by the standard plate method with Lactobacilli MRS medium after 48 h of incubation at 30°C. Sugar was analyzed by HPLC under the following conditions: column, Aminex HPX-87C (300×7.8 mm); temperature, 85°C; mobile phase, water; flow rate, 0.6 ml/min; and RI detector (Knauer K-2300, Sonntek Inc., Upper Saddle River, NJ, USA).

To examine effect of cold storage on cell viability in probiotic beet juice, the samples were fermented for 72 h at 30°C and then stored at 4°C for 4 weeks. Samples were taken at weekly intervals, and the viability of probiotic cultures in probiotic beet juice was determined and expressed as colony forming units (CFU/ml).

All fermentation experiments were conducted in triplicate and the results are expressed as mean ±S.D (standard deviation). The SAS statistical computer package (SAS Institute, Inc., Gary, NC, USA) was used to analyze the experimental data. The values that have no common superscript are significantly different ($P<0.05$) according to Duncan's multiple range test.

Table 2
Time course of lactic fermentation of beet juice by *L. plantarum*

Time (h)	pH	Acidity (%)	CFU/ml
0	6.3	0.13	$22.0 \times 10^6 \pm 4.38 \times 10^6$
24	4.2±0.01	0.44±0.01	$14.5 \times 10^7 \pm 0.50 \times 10^7$
48	4.1±0.0	0.52±0.01	$11.7 \times 10^8 \pm 0.80 \times 10^8$
72	4.1±0.1	0.56±0.0	$9.2 \times 10^8 \pm 5.0 \times 10^8$

Experimental conditions were the same as described above except that sterile beet juice was inoculated with *L. plantarum* ($N = 3$).

Table 3
Time course of lactic fermentation of beet juice by *L. casei*

Time (h)	pH	Acidity (%)	CFU/ml
0	6.3	0.13	$59.9 \times 10^5 \pm 6.5 \times 10^5$
24	5.0±0.0	0.23±0.01	$17.1 \times 10^7 \pm 0.8 \times 10^7$
48	5.0±0.0	0.25±0.01	$16.6 \times 10^8 \pm 0.8 \times 10^8$
72	5.0±0.1	0.25±0.0	$16.7 \times 10^8 \pm 7.7 \times 10^8$

Experimental conditions were the same as described above except that *L. casei* was used in the fermentation ($N = 3$).

Table 4
Time course of lactic fermentation of beet juice by *L. delbrueckii*

Time (h)	pH	Acidity (%)	CFU/ml
0	6.3	0.13	$23.3 \times 10^5 \pm 4.6 \times 10^5$
24	5.0±0.0	0.23±0.02	$11.5 \times 10^7 \pm 0.9 \times 10^7$
48	5.0±0.0	0.23±0.01	$15.5 \times 10^8 \pm 0.6 \times 10^8$
72	5.0±0.0	0.23±0.02	$15.3 \times 10^8 \pm 2.3 \times 10^8$

Experimental conditions were the same as described above except that *L. delbrueckii* was used as an inoculum ($N = 3$).

3. Results and discussion

Autoclaved beet juice as analyzed by HPLC was found to contain 57.8 g/l of sucrose. Glucose and fructose were not detected under similar analytical conditions. As shown in Tables 1–4, *L. acidophilus, L. plantarum, L. casei,* and *L. delbrueckii,* were capable of utilized beet juice for growth and lactic acid production, respectively. The viable cell counts of four lactic cultures reached 10^9 CFU/ml after 48 h of fermentation at 30°C. Extending the fermentation time from 48 to 72 h did not result in a significant increase in viable counts (CFU/ml). However, only *L. acidophilus* and *L. plantarum* reduced the pH of beet juice from an initial value of 6.3 to lower than 4.5 after 48 h of fermentation due to their ability to produce a greater amount of lactic acid than *L. casei* and *L. delbrueckii*. As shown in Tables 3 and 4, *L. casei* and *L. delbrueckii* produced only 0.25% acidity and reduced the pH to 5.0 even after 72 h of fermentation. Tuorila and Cardello (2002) suggested that fruit and vegetable juices could serve as a good medium for growing probiotics. Mårtensson, Öste, and Holst (2002) reported that probiotic cultures, *L. reuteri, L. acidophilus* and *Bifidovacterium bifidum*, grew well in nondairy oat-based products. Wheat and barley extracts were found to exhibit a significant protective effect on the viability of *L. plantarum, L. acidophilus* and *L. reuteri* under acidic condition (Charalampopoulos, Pandiella, & Webb, 2003). In a recent study (Luckow & Delahunty, 2004), consumers did not display a preference for either probiotic or conventional fruit juice drinks.

Table 1
Time course of lactic fermentation of beet juice by *L. acidophilus*

Time (h)	pH	Acidity (%)	CFU/ml
0	6.3	0.13	$15.4 \times 10^6 \pm 4.65 \times 10^6$
24	4.0±0.0	0.53±0.01	$26.6 \times 10^7 \pm 0.30 \times 10^7$
48	3.8±0.0	0.81±0.02	$26.3 \times 10^8 \pm 0.30 \times 10^8$
72	3.7±0.0	0.98±0.02	$27.8 \times 10^8 \pm 0.02 \times 10^8$

Experimental conditions were the same as described above except that *L. acidophilus* was used as an inoculum ($N=3$).

Table 5 illustrates the effect of cold storage on the viability of four lactic acid bacteria in the beet juice that had been fermented for 72 h at 30°C. Although the lactic acid cultures in the fermented beet juice gradually

Table 5
Effect of cold storage on the viability of lactic cultures in fermented beet juice

Time (week)	Viability (CFU/ml)			
	L. acidophilus	*L. casei*	*L. plantarum*	*L. debrueckii*
0	$27.8 \times 10^8 \pm 0.02 \times 10^8$	$16.7 \times 10^8 \pm 7.7 \times 10^8$	$9.2 \times 10^8 \pm 5.0 \times 10^8$	$15.3 \times 10^8 \pm 2.3 \times 10^8$
1	$15.8 \times 10^8 \pm 2.72 \times 10^8$	$77.0 \times 10^7 \pm 10.04 \times 10^7$	$18.2 \times 10^7 \pm 2.70 \times 10^7$	$12.3 \times 10^8 \pm 4.88 \times 10^8$
2	$16.0 \times 10^6 \pm 6.95 \times 10^6$	$71.5 \times 10^7 \pm 7.05 \times 10^7$	$15.4 \times 10^7 \pm 4.38 \times 10^7$	$81.5 \times 10^7 \pm 10.04 \times 10^7$
3	$11.7 \times 10^5 \pm 2.03 \times 10^5$	$49.8 \times 10^7 \pm 3.01 \times 10^7$	$8.5 \times 10^7 \pm 1.73 \times 10^7$	$28.6 \times 10^7 \pm 24.49 \times 10^7$
4	$16.1 \times 10^4 \pm 1.82 \times 10^4$	$7.2 \times 10^7 \pm 3.53 \times 10^7$	$7.7 \times 10^7 \pm 1.19 \times 10^7$	$9.0 \times 10^6 \pm 3.61 \times 10^6$

Samples of beet juice were fermented at 30°C for 72 h and then stored at 4°C ($N = 3$).

reduced their cell viability during cold storage at 4°C, the viable cell counts of the lactic acid bacteria except for *L. acidophilus* in the fermented beet juice still remained at 10^6–10^8 CFU/ml after 4 weeks of cold storage at 4°C. For example, the viable cell counts of *L. casei*, *L. plantarum,* and *L. delbrueckii* were 7.2×10^7, 7.7×10^7, and 9.0×10^6 CFU/ml, respectively, after 4 weeks of cold storage at 4°C. It is important to have a significant number of viable lactic acid bacteria present in the finished product for maximum health benefits (Shah, 2001). Factors affecting the viability of probiotic cultures include acidity (pH), oxygen level, lack of nutrients, and presence of antimicrobial substances in the product (Shah, 2001). Microencapsulation technology is being utilized to coat probiotic bacteria to extend shelf life, increase heat resistance and enhance acid resistance (Siutra-Cruce & Goulet, 2001).

4. Conclusion

From the results of this study, it is concluded that larger red beets could serve as a raw material for the production of probiotic beet juice by lactic acid fermentation with *L. acidophilus* or *L. plantarum*. The fermented beet juice has a pH value of less than 4.5 (high acid) and contains a significant number of beneficial lactic acid bacteria (10^9 CFU/ml). *L. acidophilus* was considerably less stable in the fermented beet juice than other lactic acid cultures (*L. plantarum*, *L. casei*, and *L. delbrueckii*) during cold storage at 4°C.

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