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Fermentation of Beet Juice by Beneficial Lactobacillus Bacteria

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ABSTRACT

This research was conducted to determine the suitability of beet calyces as a raw material for the production of probiotic beet juice by lactic acid bacteria (Lactobacillus plantarum and Lactobacillus casei). The juice was fermented for 72 h at 30°C and 37°C separately and then analyzed for pH, acidity, sugar content and viable cell counts. Both lactic cultures were found capable of rapidly utilizing beet juice for cell synthesis and lactic acid production. They grew significantly better at 30°C for the first 24 h with an increase in cell numbers greater than 1.5 log cfu/ml. However, the viable cell count at 72 h of both temperatures was not much different (less than 0.6 log cfu/ml). Both strains produced a similar amount of titrable acidity expressed as lactic acid. However, the titrable acidity produced was about two-times higher at 30°C compared with that at 37°C (0.5% vs. 0.2-0.3% lactic acid). After 4 weeks of cold storage at 7°C, Lb. plantarum grown at 30°C survived significantly better than that grown at 37°C (1.8×10^6 cfu/ml vs. 2.9×10^4 cfu/ml). The addition of sucrose at the beginning of fermentation increased the amount of titrable acidity by at least two times (more than 1.1% lactic acid). However, both cultures did not survive well at the low pH and high acidity conditions in fermented sugar-added beet juice, the viable cell count was up to 4×10^4 cfu/ml after 4 weeks of cold storage at 7°C.

Keywords: Food, beverages, probiotics, Lactobacillus plantarum, Lactobacillus casei, Hibiscus sabdariffa.

INTRODUCTION

Research has shown that the addition of probiotics to food provides several health benefits including improved gastrointestinal function, enhanced immune system and lower risk of colon cancer [1-3]. The majority of probiotics recommended are the species of *Lactobacillus* including *Lb. acidophilus, Lb. plantarum, Lb. casei* and *Streptococcus lactis*, etc. [4]. For health benefits, probiotic bacteria must be viable and available at a high concentration, typically above 10^6 cfu/ml or per gram of product [5].

Currently, probiotic products are usually marketed in the form of fermented milk and yogurt. However, lactose intolerance and the cholesterol content are two drawbacks related to their consumption, particularly in Asia. It has been suggested that fruit juice could serve as a good medium for cultivating probiotics [6]. Fruit and vegetables are healthy food, because they are rich in antioxidants, vitamins, dietary fibre and minerals. Furthermore, fruit and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population [7].

Beet (*Hibiscus sabdariffa* L.) is widely cultivated in Thailand where it is known as *krachiap daeng*. Beet calyces contain 9% moisture, 1.14% protein, 2.61% fat, 12% fibre and 6.9% ash. It is high in calcium, niacin, riboflavin and iron [8] and it is commonly used to make beverage and food colouring. The brilliant red colour and unique flavour make it a valuable food product [9]. It is claimed as a Thai traditional medicine for kidney stones. It is also used as an antibacterial, antifungal, hypocholesterolemic, diuretic, mild laxative and antihypertensive substance [10]. The objective of this study was to determine the suitability of beet calyces as a raw material for production of probiotic beet juice by *Lb. plantarum* and *Lb. casei*.

METHODOLOGY

Preparation of beet juice

Dry beet calyces (*Hibiscus sabdariffa* L.) were purchased from a local store and kept at 4 °C prior to use. Beet juice was prepared by boiling the dried calyces with water for 15 minutes using low heat. The boiled beet juice was filtered

through a thin white cloth to separate the calyces and the pH of the juice was adjusted with $CaCO_3$ before being sterilized for 15 min at 110 °C.

Strains and cultures

Probiotic lactic acid bacteria (*Lactobacillus plantarum* TISTR 863 and *Lactobacillus casei* TISTR 390) were obtained from the Thailand Institute of Scientific and Technological Research, Pathumthani**.** The cultures were grown at 37 °C for 24 h in MRS broth and were used as an inoculum.

Fermentation of probiotic beet juice

Fermentation experiments were conducted in 250-ml erylenmeyer flasks, each containing 100 ml of sterile beet juice. All samples were inoculated with a 24-h culture (>10^5 cfu/ml) and incubated at 30 or 37 °C for 72 h. Samples were taken every 24 h for chemical and microbiological analyses. The effect of sucrose concentration on the growth of lactic acid bacteria in beet juice was also studied using 7% and 14% final sucrose concentration.

Effect of cold storage on cell viability in probiotic beet juice

After 72-h of fermentation, the samples were stored at 7 °C for 4 weeks. Samples were taken at weekly intervals and the viability of probiotic cultures in the juice was determined and expressed as colony forming units (cfu/ml).

Chemical and microbiological analyses

The pH of probiotic beet juice was measured with a pH meter. Total acidity, expressed as percent lactic acid, was determined by titrating beet juice samples with 0.1 N NaOH using phenolphthalein as indicator. Sugar content was analyzed as glucose by the phenol sulphuric acid method of Dubois *et al.* [11]. Viable cell counts (cfu/ml) were determined by the standard plate method with MRS agar after 48 h of incubation at 37 °C.

STATISTICAL ANALYSIS

All experiments were carried out in triplicate and the results expressed as mean □ SD (standard deviation). Data generated from the experiments were analyzed for significance by the Student's t-test. The values within rows that have no common superscript are significantly different ($p < 0.05$) according to this testing method. Any two means not marked by the same superscript (for example, a and b or b and c within rows) are significantly different ($p < 0.05$). Any two means marked by the same superscript (for example, a and a or b and b within rows) are not significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Viable cell counts, pH and titrable acidity of sucrose-free beet juice during 72-h fermentation period

Both *Lb. plantarum* and *Lb. casei* were found capable of growing well in sterilized beet juice without nutrient supplementation. The time courses of lactic acidfermentation of beet juices by these species are presented in Tables 1-4, respectively,and show that *Lb. plantarum* and *Lb. casei* grew significantly better at 30 °C than at 37 °C for the first 24 h. The increase in cell numbers was more than 1.5 log cfu/ml at 30 °C,compared with less than 0.7 log cfu/ml at 37 °C.

This observation was different from thatreported by Ma and Marquis [12] who found that the optimum temperature for the growthof *Lb. casei* was 37°C, not 32 or 45°C. On the other hand, Shamala *et al.* [13] reported that the optimum temperature for the growth of *Lb. plantarum* was 30°C. Extending the fermentation beyond 24 h resulted in a significant decrease in the viable cell counts oflactic acid bacteria at 30 °C, but not at 37 °C. However, the viable cell number of both strains at 72-h fermentation at both temperatures was not much different (less than 0.6log cfu/ml).

According to Tables 1-4, the beet juice fermented at 37 °C showed a significantly faster reduction in pH for the first 24 h, compared with the juices fermented at 30 °C (1.2 units*vs.* 0.8 units). However, after 48-h fermentation the pH of the juices incubated at 30 and 37 °C showed nearly the same final pH, especially that of the beet juice fermented by *Lb. plantarum*. This observation agrees with Narvhus *et al.* [14], who studied the production of fermented milk at 22, 30 and 37°C by *Lactococci* and observed that products incubated at 37°C showed a faster reduction in pH early in the fermentation period, but that after 18 h the products incubated at 30 and 37°C showed the same final pH. Both *Lb. plantarum* and *Lb. casei* caused a stable pH in beet juice during prolonged incubation between 24 and 72 h at both 30 and 37 °C.

This observation was different from Ostlie *et al* [15], who studied the effect of temperature on metabolism of probiotic bacteria in milk and observed that after 24-h incubation *Lactobacillus reuteri* SD2112 and *Lb. rhamnosus* GG caused a further decrease in pH on prolonged incubation between 24 and 48 h at 30 °C, but showed a stable pH during prolonged incubation at 37 °C from 24 to 48 h.

Both *Lb. plantarum* and *Lb. casei* produced significantly more titrable acidity expressed as lactic acid at 30 °C of fermentation than that at 37 °C, even though at 37 °C the cultures can utilize sugar in the juice more so than at 30 °C (Tables 1-4). For example, *Lb. plantarum* produced only 0.2% acidity and reduced the pH to 4.0 at 72 h of fermentationat 37 °C, but it produced 0.44% acidity and reduced the pH to 3.9 at 72 h of fermentationat 30 °C. This was different from that found by Ostlie *et al* [15]. They reported that the amount of lactic acid produced in fermented milk by lactobacilli was highest at 37 °C.

Table 1. Time courses of lactic acid fermentation of beet by *Lb. plantarum* at 30 ºC.

Time (h)	pH	Acidity (% lactic acid)	Sugar (mg/ml)	Cell number (cfu/ml)
0	4.79 ± 0.01	0.25 ± 0.00	9.66 ± 0.9 [a]	$3.3 \pm 0.4 \times 10^7$
24	3.90 ± 0.00	0.50 ± 0.00	2.75 ± 0.28 [b]	$2.7 \pm 0.3 \times 10^9$
48	3.89 ± 0.00	0.46 ± 0.01	1.82 ± 0.35 [c]	$5.1 \pm 1.0 \times 10^8$
72	3.91 ± 0.00	0.44 ± 0.01	1.82 ± 0.21 [c]	$4.6 \pm 0.5 \times 10^8$

Mean and standard deviation for n = 3. Means with different letters within thesame row are statistically significant ($p<0.05$) according to t-test range.

Table 2. Time courses of lactic acid fermentation of beet by *Lb. casei* at 30 ºC.

Time (h)	pH	Acidity (% lactic acid)	Sugar (mg/ml)	Cell number (cfu/ml)
0	4.74 ± 0.04	0.25 ± 0.00	9.36 ± 0.76	$8.2 \pm 3.7 \times 10^7$
24	3.92 ± 0.02	0.51 ± 0.01	4.71 ± 0.42	$4.2 \pm 0.7 \times 10^9$
48	3.88 ± 0.00	0.50 ± 0.04	4.76 ± 1.45	$3.0 \pm 0.2 \times 10^8$
72	3.90 ± 0.00	0.52 ± 0.00	4.07 ± 0.48	$2.7 \pm 0.2 \times 10^8$

Mean and standard deviation for n = 3. Means with different letters within thesame row are statistically significant ($p<0.05$) according to t-test range.

Table 3. Time courses of lactic acid fermentation of beet by *Lb. plantarum* at 37 ºC.

Time (h)	pH	Acidity (% lactic acid)	Sugar (mg/ml)	Cell number (cfu/ml)
0	5.32 ± 0.03	0.17 ± 0.01	13.09 ± 4.5	$4.5 \pm 1.5 \times 10^7$
24	4.07 ± 0.05	0.24 ± 0.02	3.53 ± 0.83	$2.0 \pm 0.5 \times 10^8$
48	4.15 ± 0.12	0.20 ± 0.00	3.49 ± 0.90	$1.6 \pm 0.2 \times 10^8$
72	4.06 ± 0.13	0.21 ± 0.01	3.22 ± 0.52	$2.2 \pm 0.5 \times 10^8$

Mean and standard deviation for n = 3. Means with different letters within the samerow are statistically significant ($p<0.05$) according to t-test range.

Table 4. Time courses of lactic fermentation of beet juice by *Lb. casei* at 37 ºC.

Time (h)	pH	Acidity (% lactic acid)	Sugar (mg/ml)	Cell number (cfu/ml)
0	4.97 ± 0.04	0.17 ± 0.01	20.69 ± 4.99	$2.6 \pm 0.1 \times 10^7$
24	3.74 ± 0.01	0.31 ± 0.02	1.67 ± 0.13	$4.2 \pm 0.8 \times 10^7$
48	3.76 ± 0.03	0.28 ± 0.01	0.98 ± 0.14	$2.4 \pm 0.1 \times 10^8$
72	3.79 ± 0.01	0.32 ± 0.00	0.44 ± 0.07	$9.3 \pm 2.2 \times 10^8$

Mean and standard deviation for n = 3. Means with different letters within the samerow are statistically significant ($p<0.05$) according to t-test range.

Table 6. Time courses of lactic fermentation of beet juice by *Lb. plantarum* at 30 ºC with the addition of 7% sucrose.

Time (h)	pH	Acidity (% lactic acid)	Cell number (cfu/ml)
0	4.80 ± 0.00	0.24 ± 0.02	$1.1 \pm 0.90 \times 10^8$
24	3.52 ± 0.02	0.75 ± 0.04	$5.2 \pm 0.8 \times 10^9$
48	3.28 ± 0.00	1.17 ± 0.00	$2.7 \pm 0.2 \times 10^8$
72	3.26 ± 0.01	1.19 ± 0.05	$3.6 \pm 0.6 \times 10^8$

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$) according to t-test range.

Table 7. Time courses of lactic fermentation of beet juice by *Lb. plantarum* at 30 °C with the addition of 14% sucrose.

Time (h)	pH	Acidity (% lactic acid)	Cell number (cfu/ml)
0	4.79 ± 0.02	0.24 ± 0.02	$2.6 \pm 0.75 \times 10^8$
24	3.49 ± 0.02	0.72 ± 0.03	$6.0 \pm 0.6 \times 10^9$
48	3.24 ± 0.01	1.17 ± 0.00	$7.4 \pm 0.5 \times 10^8$
72	3.19 ± 0.00	1.40 ± 0.00	$6.1 \pm 0.8 \times 10^8$

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$) according to t-test range.

Table 8. Time courses of lactic fermentation of beet juice by *Lb. casei* at 30 °C with the addition of 7% sucrose.

Time (h)	pH	Acidity (% lactic acid)	Cell number (cfu/ml)
0	4.80 ± 0.00	0.25 ± 0.00	$8.9 \pm 0.90 \times 10^7$
24	3.80 ± 0.03	0.54 ± 0.03	$7.9 \pm 0.5 \times 10^9$
48	3.39 ± 0.01	0.90 ± 0.00	$3.9 \pm 0.6 \times 10^9$
72	3.30 ± 0.01	1.13 ± 0.04	$4.7 \pm 0.4 \times 10^9$

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$) according to t-test range.

Table 9. Time courses of lactic fermentation of beet juice by *Lb. casei* at 30 °C with the addition of 14% sucrose.

Time (h)	pH	Acidity (% lactic acid)	Cell number (cfu/ml)
0	4.75 ± 0.02	0.24 ± 0.02	$8.2 \pm 1.6 \times 10^7$
24	3.77 ± 0.03	0.54 ± 0.03	$6.4 \pm 0.8 \times 10^9$
48	3.37 ± 0.01	0.91 ± 0.01	$5.0 \pm 0.4 \times 10^9$
72	3.26 ± 0.01	1.24 ± 0.06	$6.4 \pm 0.5 \times 10^9$

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$) according to t-test range.

Table 10. Effect of cold storage on the viability of lactic cultures in fermented beet juice at different sugar concentrations

Time	cfu/ml (7% sucrose)		cfu/ml (14% sucrose)	
(Weeks)	*Lb. plantarum*	*Lb. casei*	*Lb. plantarum*	*Lb. casei*
0	$3.6 \pm 0.6 \times 10^8$	$4.7 \pm 0.4 \times 10^9$	$6.1 \pm 0.8 \times 10^8$	$6.4 \pm 0.5 \times 10^9$
1	$3.9 \pm 0.9 \times 10^6$	$3.0 \pm 0.2 \times 10^6$	$3.6 \pm 0.6 \times 10^6$	$2.6 \pm 0.1 \times 10^6$
2	$1.1 \pm 0.5 \times 10^5$	$8.8 \pm 3.4 \times 10^4$	$3.5 \pm 0.7 \times 10^4$	$1.2 \pm 0.6 \times 10^4$
3	$5.2 \pm 0.3 \times 10^4$	$8.1 \pm 3.4 \times 10^4$	$1.9 \pm 0.3 \times 10^4$	$2.5 \pm 1.3 \times 10^4$
4	$4.4 \pm 0.4 \times 10^4$	$3.7 \pm 0.3 \times 10^4$	$1.3 \pm 0.1 \times 10^4$	$5.0 \pm 0.9 \times 10^3$

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$) according to t-test range.

The survival of lactic acid bacteria in fermented sucrose-free beet juice during cold storage

The data in Table 5 illustrates the effect of cold storage on the viability of both species of lactic acid bacteria in fermented beet juice. *Lb. plantarum* grown in 30 ºC can survivefor several weeks in the fermented beet juice at 7 ºC, significantly better than thatgrown in 37 ºC. For example, the viable cell counts of *Lb. plantarum* grown at 30 ºC was still 1.8×10^6 cfu/ml after 4 weeks of storage at 7 ºC, while that grown at 37 ºC was reduced to 2.9×10^4 cfu/ml. In contrast, the growth temperature did not have much effect on the survival of *Lb. casei* during cold storage. The viable cell counts of *Lb. casei* was approximately 1.6×10^6 cfu/ml after 4 weeks of storage at 7 ºC previously incubated at either 30 ºC or 37 ºC. For the maximum health benefits, not less than a million viable cells/ml of probiotic product have to be present for transfer of the 'probiotic' effect to consumers.

The probiotic culture should be able to multiply to reach high cell counts in the fermented product and possess a high acid tolerance to ensure high viable cell numbers during storage [5]. The viability of probiotic organisms is dependent on many factors, such as the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature [16]. Furthermore, it is also affected by lactic acid produced during production and cold storage [17, 18].

Growth and acid production of lactic acid bacteria in sucrose-added beet juice during 72-h fermentation period

When sucrose was added to the beet juice and fermentation undertaken at 30 ºC, both *Lb. plantarum* and *Lb. casei* produced significantly more titrable acidity expressed as lactic acid than the sucrose-free juice (Tables 1, 2, and 6-10). The increase of acidity at 72-h fermentation was approximately 3 times higher in sucrose-added beet juice fermented with *Lb. plantarum* (Table 1, 6 and 7) and nearly 2.5 times higher in sucrose- added juices fermented with *Lb. casei* (Table 2, 8 and 9), compared with the sucrose-free juice. Greater reduction of pH for the first 24 h of fermentation in the sucrose-added beet juice was also observed. After 48 h, the pH of all fermented sucrose-added beet juice was reduced below 3.5. Haddadin [19] also reported the significant effect of sugar on the acidification of milk. He found that the addition of glucose and galactose caused the faster acidification of milk fermented by *Lb. casei* and *Lb. plantarum*, compared with the control. On the other hand, the addition of sucrose did not support good growth of *Lb. plantarum*, same as the results reported by Shamala *et al* [13]. However, sucrose supported the growth of *Lb. casei* in this study. Its cell number at 72-h of fermentation was $4.7 – 6.4 \times 10^9$ cfu/ml in sucrose-added beet juice, compared with 2.7×10^8 cfu/ml in sucrose-free juice.

The survival of lactic acid bacteria in fermented sucrose-added beet juice during cold storage

Both species were not able to survive well at the low pH and high acidity conditions in fermented sucrose-added beet juice at 7 ºC. As can be seen from Table 10, the viablecell counts of both species was reduced to approximately 10^4 cfu/ml in fermented sucrose-added juice after 3 weeks of cold storage at 7 ºC , compared with 10^6 cfu/ml in sucrose-free juices (Table 5). Nevertheless, the fermented juices with sugar had more acceptable taste and flavour than the fermented sucrose-free juice (personal observation). According to Luckow and Delahunty [7], the sensory characteristics of probiotic blackcurrant juice was 'perfumey' and 'dairy' in odour and 'sour' and 'savoury' in flavour. These off-flavours also occurred in beet juice. However, when sucrose was added at the beginning of fermentation, these flavours seemed to be reduced and the taste was more acceptable (personal observation).

CONCLUSIONS

Two lactic acid bacteria, *Lb. casei* and *Lb. plantarum* were examined for their ability to utilize beet juice for cell synthesis and lactic acid production without nutrient supplement. These lactic cultures grew better in beet juice at 30°C than at 37°C and the viable cell counts reached 10^9 cfu/ml at 24 h of fermentation at 30°C. Both *Lb. plantarum* and *Lb. casei* were capable of surviving the low pH and high acidic conditions in fermented beet juice during cold storage at 7°C. On the other hand, when sucrose was added to the beet juice at the beginning of fermentation, both cultures could not survive well at the low pH and high acidity in fermented sucrose-added beet juice. The cell number was reduced to approximately 10^4 cfu/ml after 3 weeks of cold storage at 7°C. From the results of this study, it is concluded that both *Lb. plantarum* and *Lb. casei* are suitable for use as probiotic cultures for production of a healthy beverage from that both *Lb. plantarum* and *Lb. casei* are suitable for use as probiotic cultures for production of a healthy beverage from beet calyces for vegetarians or consumers who are allergic to lactose present in probiotic dairy products.

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