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Optimization of Lactic Acid Fermentation of Prickly Pear Extract

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Abstract

Lactic acid fermentation of prickly pear extract (PPE) was performed by *Lactobacillus rhamnosus* LS, *Lactobacillus bulgaricus*, and *Lactobacillus brevis*. The PPE was pasteurized to eliminate indigenous microorganisms as well as to dissolve the partially insoluble pulp. The PPE fermented without yeast extract by *L. rhamnosus* LS exhibited 0.57% acidity and 3.5×10^8 CFU/mL bacteria count. With the addition of 0.2% edible yeast extract the PPE fermented by *L. rhamnosus* LS exhibited 1.15% acidity, 2.7×10^9 CFU/mL bacteria count and 95.0% retention of red color. When 5% fructose syrup was added, the PPE fermented by *L. rhamnosus* LS had 1.09% acidity, 6.5×10^8 CFU/mL, and 97.7% retention of red color. With 1~3% (w/v) concentrations of starter, the PPE fermented by *L. bulgaricus* and *L. brevis* showed 0.97% and 0.65% acidities, respectively. The viable cell counts from *L. rhamnosus* LS fermentation were higher compared with those of other LAB. During cold storage at 4°C, the viable cell count was well maintained for 3 weeks, but then rapidly decreased. The red pigment was highly stable during cold storage for 4 weeks. The pasteurized PPE fortified with 5% fructose syrup, 0.2% yeast extract, and 0.05% $CaCO_3$ was successfully fermented by inoculating with 3% LAB and incubating at 30°C for 2 days. Both viable cell counts and the red color of the fermented PPE were well maintained during cold storage for 3 weeks.

Key words: lactic acid bacteria, prickly pear extract, pigment

INTRODUCTION

The prickly pear cactus (*Opuntia ficus-indica* var. *saboten* MAKINO) belongs to the *Cactaceae* family, and is abundantly distributed in the arid and semi-arid regions of many countries (1). Prickly pear cactus has been used as an important food source, providing essential nutrients. In particular, prickly pear fruit is a berry with a thick peel enclosing a delicately flavored seedy pulp (2). Prickly pear fruit is a source of functional ingredients such as vitamins, calcium, red pigment and mucilage, and has been eaten raw or as an ingredient in processed foodstuffs such as jams, syrups or candies (3,4).

The red pigments in prickly pear fruit are alkaloids known as betalains that are similar to the betacyanins of red beets (5,6). In general, the red color of betanin is stable in acidic pH (7). The red pigment remains stable for long periods of cold storage, and has good stability when heated at 80°C (8).

The different *Opuntia* sp. have been compared for composition and quality of prickly pear fruit (9). Concentrations of minerals, free sugars, free amino acids and total phenolic compounds were reported for the stem, fruit and seeds of prickly pear cactus (10,11). The content of viscous mucilage from prickly pear varies according to the cultivar and harvesting season. Amin et al. (12) reported that the mucilage is a neutral polysaccharide with approximately 55 sugar residues without uronic acid, composed of arabinose, rhamnose, galactose, and xylose.

Prickly pear cactus has been recognized as a therapeutic medicinal plant (13). Fernandez et al. (14) reported that pectin from prickly pear pulp lowers serum LDL cholesterol while leaving HDL cholesterol unchanged. Another study found that the fibrous pectin in the fruit may lower diabetics need for insulin (15).

In order for the important nutrients and functional components in prickly pear fruit to be fully utilized, new products need to be developed and introduced into the market. The various functional and nutritional properties of prickly pear fruit have resulted in its use as a substrate for fermentation. To produce biomass of *Candida utilis*, prickly pear juice was used for batch and continuous culture (16). It has been reported that the production of red pigments was carried out by *Monascus purpureus* grown on prickly pear juice (17). Recently, an alcoholic beverage was successfully prepared by fermenting a blend of prickly pear juice blended with grape juice (18).

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The national drink (pulque) of Mexico is a white, viscous, and acidic alcoholic beverage made by fermenting fresh Agave juice with lactic acid bacteria and ethanol-producing yeast (19).

However, no data exists concerning lactic acid fermentation of prickly pear extract prepared from prickly pear fruit. Prickly pear extract with red pigments and mucilage has potential as an ingredient in lactic acid fermented beverages. This study was designed to develop methods to optimize the lactic acid fermentation of prickly pear extract for producing a lactic acid fermented beverage.

MATERIALS AND METHODS

Preparation of prickly pear extract

Prickly pear fruit that was harvested in Jeju, Korea in February, 2002 was purchased and stored at -18°C. Two hundred grams of prickly pear fruit were thawed slightly, sliced, and then mixed with 800 mL water for 2 hr by shaking at 150 rpm in a shaker (KMC-1205S, Vision Scientific Co., Korea). Prickly pear extract (PPE) was obtained by filtering the solution through a cotton cloth, and stored at 4°C. The PPE containing red pigments and viscous mucilage was used as a culture medium for seed culture and beverage production by lactic acid bacteria (LAB).

Physicochemical analysis of PPE

The solid content of PPE was determined by drying at 105°C (20). A hand refractometer (Atagoni Brix 0~32%, Japan) was used for determining the soluble solid of PPE. The total phenol content of PPE was determined using the Folin-Ciocalteu method (21). To isolate viscous mucilage in the PPE, the PPE extract was centrifuged at 10,600×g for 15 min, and then polysaccharide in supernatant was recovered by isopropanol precipitation. Polysaccharide content was determined by drying at 105°C and weighing.

Effect of heat treatment on the pigment stability and viscosity of PPE

The PPE was heat treated at 80°C for 10~30 min. The relative viscosities of PPE and heat treated PPE were measured in a capillary viscometer (size 75, Cannon Instrument, USA) at 20°C and compared with the viscosity of water. Also, the apparent viscosity of PPE was determined using a Brookfield viscometer (Brookfield Engineering, MA, USA). To measure the intensity of red pigment, PPE was diluted seven-fold in distilled water, and the absorbance measured at 550 nm using a Spectrometer (UVICON, Kontron Instruments, France). The red color of fermented PPE was also determined by measuring Hunter color values using a Hunter colorimeter (CR-10, Minolta, Japan).

Preparation of seed starter

Lactobacillus rhamnosus LS previously isolated from soymilk curd residue, was used for preparation of PPE beverage. *Lactobacillus bulgaricus* (KCTC3188), and *Lactobacillus brevis* (KCTC3498) were used for lactic acid fermentation of PPE. LAB were grown on MRS agar at 30°C for 1 day. One loop of colony was inoculated in 10 mL of PPE sterilized at 121°C for 15 min, containing 0.1% yeast extract, 0.1% $CaCO_3$ and 1% glucose. The seed culture was prepared by incubating at 30°C for 24 h. To prepare an active seed starter, the culture (1 mL) was grown in PPE overnight and then transferred to the same PPE medium (10 mL), and cultured at 30°C for 24 h.

Effect of yeast extract on lactic acid fermentation

A stock solution of edible yeast extract (20% w/v) was prepared and autoclaved at 121°C for 15 min. To elucidate the effect of yeast extract on the fermentation, the PPE medium was fortified with 0.1~0.6% yeast extract.

Measurement of viable cell counts and titratable acidity

Viable cell counts were determined by plating on MRS agar plates with 20 μL of serial diluted PPE after fermenting for 48 hr. The fermented PPE was stored in a cold room for 4 weeks, and then viable cell counts were determined. The titratable acidity (lactic acid, %) was determined by measuring the amount of 0.1 N NaOH necessary to adjust to pH 8.3. The pH of fermented PPE was measured with a pH meter (Digital pH meter 110, Wheaton, USA).

Lactic acid fermentation of PPE

The PPE was fermented by *L. rhamnosus* LS, *L. bulgaricus* or *L. brevis*, and then the lactic acid produced was neutralized by adding 0.05% $CaCO_3$. Lactic acid fermentation conditions were evaluated according to the concentration of fructose syrup, yeast extract, amount of seed starter and dilution of PPE in the culture medium. Fructose syrup was added to the PPE at concentrations up to 20% (v/v). Yeast extract was added at final concentrations of up to 0.6%. The LAB starter culture was inoculated at levels of 1~5% (v/v), and fermented at 30°C for 2 days.

Physicochemical and biological characteristics of fermented PPE during cold storage

PPE fortified with 0.2% yeast extract and 5% fructose syrup and fermented by *L. rhamnosus* LS, *L. bulgaricus*

or *L. brevis* at 30°C for 2 days was stored in a cold room for 4 weeks. The PPE was sampled every week, and the acidity, viable cell counts and pigment stability were determined by the previously described methods.

RESULTS AND DISCUSSION

Effect of heat treatment on the red pigment and viscosity of PPE

The physicochemical properties of prickly pear and PPE are shown in Table 1. The pulp of prickly pear without seeds contained 14.8% total solid and 85.2% moisture. The PPE had a pH of 3.99 and 0.35% (w/v) acidity as lactic acid. Phenolic compound and polysaccharide contents were 5.7 mg% and 0.5% (w/v), respectively. The apparent viscosity of PPE was 10.3 cP, and decreased to 4.7 cP after heating at 80°C for 10 min, indicating that PPE is heat labile. The relative viscosity and intensity of the red pigments in PPE gradually decreased over time when heated at 80°C. After heating for 10 min, the relative viscosity of PPE was decreased, and the intensity of red pigment showed 84% retention. Heat treatment for more than 10 min resulted in a slight reduction in the relative viscosity of PPE. The indigenous microorganisms in PPE were completely destroyed by heating at 80°C for 10 min (Table 2). Initial viable cell counts in PPE were 1.3×10^4 on an MRS agar plate, and exhibited characteristics consistent with lactic acid bacteria. We concluded, therefore, that heat-treatment of PPE at 80°C for 10 min effectively eliminates the indigenous microorganisms, as well as dissolves the pulp. Previously, PPE was heat-treated at 85°C for 30 min, resulting in drastic reductions in the red color (8). The optimization of the heat-treatment of PPE is a very critical step in the production of a lactic acid fermented beverage with red color. Therefore, the heat pre-treatment of PPE at 80°C for 10 min was successful while preserving the organoleptic qualities. Mucilage (cholla gum) from PPE is considered a potential source of industrial hydrocolloid and dietary fiber, and is composed of galactose units as the main chain, with D-galacturonic acid, D-galactose, D-xylose, and L-rhamnose side chains (12). Therefore, PPE containing mucilage and red pigments can be utilized as an ingredient for the production of a functional lactic acid-fermented beverage.

Table 1. Physicochemical properties of prickly pear extract

Prickly pear	Moisture (%)	85.2
	Total solid (%)	14.8
Prickly pear extract (PPE)	pH	3.99
	Total acidity (%)	0.35
	°Brix	0.80
	Apparent viscosity[1] (cP)	10.3
	Apparent viscosity[2] (cP)	4.70
	Color value (L, a, b)	18.6, 14.8, 1.9
	Polysaccharide (%, w/v)	0.50
	Phenolic compound (mg%, w/v)	5.7

[1]Viscosity was determined at 60 rpm (spindle #1).
[2]The PPE was pasteurized at 80°C for 10 min.

Table 2. Changes in pigment stability, relative viscosity, and viable cell counts in heat treated PPE

Heating time (min)	Absorbance (528 nm)	Relative viscosity	Viable cell counts (CFU/mL)
0	1.99	ND[1]	1.3×10^4
5	1.74	4.00	30
10	1.67	2.56	0
15	1.60	2.37	0
20	1.55	2.26	0

[1]ND: Non-determination because of high viscoisty.

Effect of fructose syrup on the lactic acid fermentation of PPE

PPE was fermented with different amounts of fructose syrup, from 0~20%, as the carbon source at 30°C with a static culture by inoculating 3% seed starter of *L. rhamnosus* LS. Previously, it was ascertained that the red color of pigment was drastically decreased during lactic acid fermentation at 37°C. Thus, to minimize the discoloration of red pigment, the fermentation temperature of PPE was carried out at 30°C rather than 37°C. As shown in Table 3, the PPE fermented without fructose syrup had a pH of 3.48, 0.53% titratable acidity and 5.16×10^8 viable cell counts (CFU/mL). With the addition of 5% (v/v) fructose syrup, the acidity increased, with 1.09% acidity and pH 3.1. Also, the intensity of red pigment of fermented PPE was well maintained after fermentation for 2 days, exhibiting about 95% relative absorbance. The addition of fructose syrup up to 15% did not further affect the acidity or pH. At the concentrations of 10~20%, the PPE had lower viable cell counts than PPE fermented with 0 or 5% fructose. Nancib et al. (22) reported maximal lactic acid production by *Lactobacillus casei* subsp. *rhamnosus* with date juice containing 6% (w/v) glucose. Therefore, we conclude that 5% fructose syrup as a carbon source is suitable for LAB fermentation of PPE.

Effect of yeast extract

To determine the effect of edible yeast extract on lactic acid fermentation, PPE was fortified with different amounts of 20% yeast extract, and then fermented by inoculating with 3% *L. rhamnosus* LS. As shown in Table 3, the PPE medium fermented without yeast extract had an acidity of 0.57% and viable cell count of 3.5×10^8 (CFU/mL). With the addition of 0.2% (v/v) yeast extract, the acidity and viable cell counts were

Table 3. Effects of yeast extract and fructose syrup on lactic acid fermentation and pigment stability

Ingredient	Concentration (%)	pH	Titratable acidity (%)	Viable cell counts ($\times 10^8$ CFU/mL)	Relative abs. (%)
Fructose syrup	0	3.48	0.53	5.2	95.0
	5	3.11	1.09	6.5	97.7
	10	3.10	1.00	3.5	96.6
	15	3.10	1.05	4.9	98.7
	20	3.08	0.98	4.8	89.6
Yeast extract	0	3.23	0.57	3.5	102.0
	0.2	2.92	1.15	27.0	95.0
	0.4	2.88	1.34	27.2	93.5
	0.6	2.90	1.46	28.0	91.9

Starter culture of *L. rhamnosus* LS was inoculated at a 3% level.

drastically increased to 1.15% and 2.7×10^9, respectively. The acidity and viable cell counts increased slightly more when higher concentration of yeast extract were added, but the added yeast extract adversely affected the flavor (unpublished data). Schepers et al. (23) reported that yeast extract significantly affected the growth of *Lactobacillus helveticus* in whey permeate medium, resulting in a gradual increase in biomass up to 2.0% (w/v) of the yeast extract. It is necessary to minimize the addition of yeast extract because it is expensive and contributes excessively to the cost of lactic acid production (24). Thus, for PPE beverage production, fortification with 0.2% yeast extract was judged optimal before lactic acid fermentation. As shown in Table 3, the red color of the pigment was also quite stable, indicating more than 91% relative absorbance.

Effects of type and concentration of starter on PPE fermentation

PPE was fermented by *L. rhamnosus* LS, *L. brevis* or *L. bulgaricus* under conditions determined to be optimal. The acidity, pH and viable cell counts of PPE fermented were evaluated according to the inoculum volume of each LAB starter (Table 4). With inoculation of 1% seed starter, the PPE fermented by *L. rhamnosus* LS showed higher acidity at 0.98% and higher viable cell counts at 2.0×10^9. The color of red pigment of PPE was well retained with 97.6% of relative absorbance, but PPE fermented without starter had a lower acidity (0.2%) and 89.3% relative absorbance. These results suggest that the stability of the red pigment is partially affected by LAB fermentation. Titratable acidity and viable cell counts were slightly increased by increase of starter concentration. The PPE fermented with 5% inoculum indicated the highest viable cell counts with 3.66×10^9 (CFU/mL). The PPE fermented by *L. bulgaricus* showed similar results for acidity, pH and stability of red pigment, but had slightly lower viable cell counts compared with that of *L. rhamnosus* LS. However, in spite of the similar value of viable cell counts, the PPE fermented by *L. brevis* had the lowest acidity at 0.65%. The lower acidity was due to lower production in lactic acid by hetero-fermentative strains of LAB. However, the stability of red pigment was also well maintained with about 95% of relative absorbance. Therefore, *L. rhamnosus* LS was superior to the other bacteria for producing lactic acid with higher viable cell counts during fermentation of PPE for 2 days. The seed culture of *L. rhamnosus* LS, *L. bulgaricus* or *L. brevis* showed different viable cell counts, with counts of 1.3×10^9, 7.33×10^8 and 6×10^8, respectively. Therefore, we concluded that culture broth containing PPE as a main ingredient

Table 4. Effects of the type and concentration of starter on lactic acid fermentation and pigment stability of PPE

Strains	Starter conc. (%)	pH	Titratable acidity (%)	Viable cell counts ($\times 10^9$ CFU/mL)	Relative abs. (%)
Control	0	4.27	0.2	0	89.3
L. rhamnosus LS	1	3.12	0.98	2.0	97.6
	3	3.12	1.03	2.1	97.3
	5	3.08	1.04	3.7	97.5
L. brevis	1	3.58	0.64	2.1	95.6
	3	3.58	0.65	1.0	94.7
	5	3.58	0.65	1.3	100.3
L. bulgaricus	1	3.10	0.97	1.6	97.8
	3	3.11	0.97	1.2	100.1
	5	3.10	0.98	1.3	98.5

should be fortified with additional nutritive ingredients for fermentation with *L. brevis* and *L. bulgaricus* than *L. rhamnosus* LS, even if the PPE is fortified with yeast extract. In particular, the full-strength PPE fermented by *L. rhamnosus* LS showed higher viable cell counts compared to diluted PPE. As shown in Table 3, the PPE diluted with distilled water (80 : 20) had only 6.5×10^8 viable cell counts after fermentation with 3% of *L. rhamnosus* LS starter, suggesting that dilution of PPE resulted in a nutrient deficiency in the PPE medium. In this case the stability of red pigment in PPE fermented was also drastically decreased during cold storage (unpublished results).

It was concluded that the initial concentration of PPE is a very important factor for sustaining cell growth during LAB fermentation as well as for maintaining the red color during cold storage.

Physicochemical and biological changes of fermented PPE during cold storage

The PPE fermented by *L. rhamnosus* LS, *L. bulgaricus* or *L. brevis* was evaluated to determine the stability of red pigment and viability of LAB during cold storage. As shown in Table 5, the growth of *L. rhamnosus* LS was superior to *L. bulgaricus* and *L. brevis*. The PPE fermented by *L. rhamnosus* LS, *L. bulgaricus* or *L. brevis* had viable cell counts of 2.1×10^9, 1.2×10^9 and 1.0×10^9, respectively. The viable cell counts of PPE fermented by *L. rhamnosus* LS did not decrease during the cold storage for 3 weeks, maintaining the initial viable cell counts of 2.1×10^9, but decreased to 3×10^8 after 4 weeks. In the case of PPE fermented by *L. bulgaricus*, the viability of a lactic acid bacterium was maintained during cold storage for 3 weeks, with viable cell counts of 7.1×10^8 (CFU/mL). However, the viable cell counts were decreased to 1.5×10^8 after cold storage for 4 weeks. The PPE fermented by *L. brevis* showed lower viable cell counts at 8.5×10^8 after cold storage for 2 weeks, and then was decreased to 2.5×10^8 after 4 weeks. It was concluded that *L. rhamnosus* LS used for fermenting PPE results in the highest viable cell counts and viability until cold storage for 3 weeks. After 4 weeks the viability of LAB was decreased to $1.5\sim3.0\times10^8$ viable cell counts. It has been reported that a decline in lactic acid bacterial numbers in plain yoghurt was observed under cold storage, reaching 7 log CFU/mL after 30 days (25). In the case of PPE fermented by *L. rhamnosus* LS, *L. brevis*, or *L. bulgaricus*, the viability for lactic acid bacteria was well maintained, indicating counts of more than 8 log CFU/mL.

Table 5. Changes in viable cell counts during cold storage of PPE fermented by LAB

(Unit: viable cell counts $\times10^8$ CFU/mL)

Strains	Storage weeks[1)]				
	0	1	2	3	4
L. rhamnosus LS	21.0	11.9	23.5	21.0	3.0
L. brevis	10.0	10.0	8.5	3.1	2.5
L. bulgaricus	12.0	21.5	18.7	7.1	1.5

[1)]Numbers indicate the duration in weeks of cold storage after 2 days of fermentation.

Acidity and relative absorbance of fermented PPE were determined during 4 weeks of cold storage at the same time that cells were counted. As shown in Fig. 1, the PPE fermented by *L. rhamnosus* LS or *L. bulgaricus* were most acidic at 1.0%, but PPE fermented by *L. brevis* was less acidic at 0.65%. During cold storage at 4°C, the acidity of fermented PPE increased slightly. In particular, the acidities of *L. rhamnosus* LS and *L. bulgaricus* gradually increased in contrast to that of *L. brevis*. This implies that homo-fermentative LAB continued to produce lactic acid during cold storage. In spite of the cold storage for 4 weeks, the intensity of red pigment in PPE was retained up to 70% of relative absorbance (Fig. 1). The relative absorbance was determined in 7-fold diluted PPE samples; as a result, the relative absorbance is not suitable for evaluating the real red color of fermented PPE. The non-diluted fermented PPE retained an attractive pink/red color after the 4 weeks of cold storage. As shown in Table 6, the Hunter color values of fermented PPE remained stable for the red pigments during the 4 weeks of cold storage. Hunter color values (L, a, b) from PPE fermented by *L. rhamnosus LS*, *L. brevis* or *L. bulgaricus* were nearly identical to the initial color value after lactic acid fermentation for 2 days, indicating little change in red color during cold storage. Therefore, it was concluded that PPE can be used to produce a lactic acid fermented beverage with

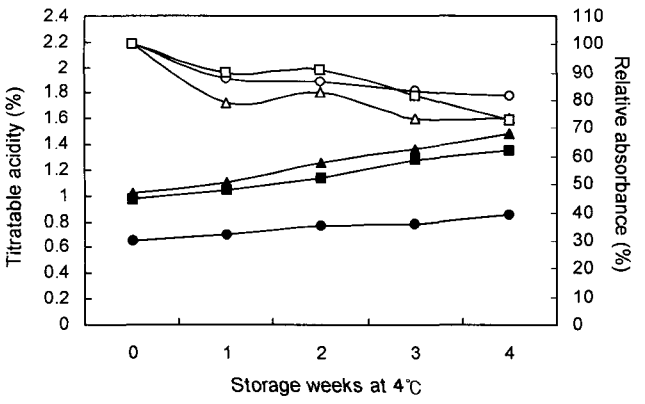

Fig. 1. Changes in the titratable acidity and relative absorbance of fermented PPE during cold storage.
Open symbols: relative absorbance, closed symbols: titratable acidity. ▲△ *Lactobacillus rhamnosus* LS, ●○ *Lactobacillus brevis*, ■□ *Lactobacillus bulgaricus*.

Table 6. Changes in Hunter color value of fermented PPE during cold storage

Strains		Storage weeks[1)]				
		0	1	2	3	4
L. rhamnosus LS	L	19.60	18.90	18.00	19.70	19.60
	a	12.33	10.20	11.60	11.56	13.50
	b	1.40	1.43	0.46	1.10	1.30
L. brevis	L	19.46	18.90	18.56	19.56	19.60
	a	12.17	11.37	11.03	11.87	13.97
	b	1.96	2.06	1.60	1.80	1.93
L. bulgaricus	L	19.80	18.76	18.20	19.50	19.50
	a	12.46	10.53	11.23	12.53	13.46
	b	1.40	1.33	0.53	1.33	1.40

[1)]Numbers indicate the duration in weeks of cold storage after 2 days of fermentation.

high viable cell counts and an attractive red/pink color.

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