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산·학·연 논단

Production of Fermented Beverage with Prickly Pear Extract

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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 fruit as the nutritive and functional ingredients has been eaten freshly or are used for the processed foodstuffs such as jam, syrups or candies (2,3).

The red pigment of prickly pear fruit was known as betalains, was stable in acidic pH (4). The red pigment was well maintained for long time during cold storage, and showed the good stability by heating at 80°C (5).

According to the various types of *Opuntia* sp. the difference in composition and quality of prickly pear was reported (6). Contents of minerals, free sugars, free amino acids and total phenolic compounds in the stem, fruit and seeds of prickly pear cactus was reported (7,8). It has been known that content of viscous mucilage from prickly pear is variable according to the cultivar and harvesting season.

Prickly pear cactus has been known as a therapeutic medical plant (9). Fernandez et al. (10) reported that the pectin contained in the prickly pear pulp lowers levels of bad cholesterol while leaving good cholesterol levels unchanged. Another study found that the fibrous pectin in the fruit may lower diabetics need for insulin (11).

Considering the nutritionally important components of the prickly pear and its potential uses in functional foods, one of the major needs is the development of new processed prickly pear products to utilize prickly pear. Because of various functional and nutritional properties of prickly pear fruit, it has been used as substrate for fermentation. To produce biomass of *Candida utilis*, prickly pear juice was used for batch and continuous culture (12). It has been reported that the production of red pigments was carried out by *Monascus purpureus* grown on prickly pear juice (13). As the national drink (pulque) of Mexico, a white, viscous and acidic alcoholic beverage was made by lactic acid bacteria and ethanol-producing yeast with Agave plant (14).

Prickly pear extract with red pigment and mucilage is considered as a valuable ingredient for fermentation. This study was designed to optimize the lactic acid fermentation and alcohol fermentation of prickly pear extract.

MATERIALS AND METHODS

Preparation of prickly pear extract

Prickly pear which was harvested in Jeju on February 2002 was purchased and stored at -18°C. Two hundred grams of prickly pear was thawed slightly and sliced, and then mixed with 800 mL water for 2 h by shaking at 150 rpm in a shaker (KMC-1205S, Vision Scientific Co., Korea). Prickly pear extract (PPE) was obtained by filtration with cotton fabrics and kept at 4°C. The PPE with red color and viscous mucilage was used as culture medium for seed culture and beverage production by lactic acid bacteria (LAB)(15).

Alcohol fermentation

To inactivate enzymes and indigenous microorganism in PPE, PPE was pasteurized by heating at 80°C for 10 min. Various amounts of PPE (0~100%)

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was mixed with grape juice (GJ). A 150 ppm of SO_2 was added to PPE and/or PPE/GJ mixture were adjusted to 20~28°Brix by adding white sugar. To determine viable cell counts, the freeze dried *S. cerevisiae* (30 mg) was resuspended on sterilized water (5 mL), and then incubated on YM plate at 30°C for 24 h. A single colony was selected and inoculated in GJ (10 mL). After growing at 30°C for 24 h, a GJ culture (3 mL) as a mother starter was transferred to PPE or/and GJ (100 mL). During fermentation, the alcoholic beverages were analyzed to determine soluble solid and alcohol content.

Determination of alcohol content in PPE/GJ wines

The alcohol content of alcoholic beverages was determined by enzymatic assay. Wine samples (1 mL) diluted with distilled water were reacted with an alcohol reagent (2 mL) and then incubated at 30°C for 5 min. Absorbance at 340 nm was converted to the alcohol content.

Seed starter for lactic acid fermentation

Lactobacillus rhamnosus LS isolated from soymilk curd residue previously was used for preparation of PPE beverage (16). *Lactobacillus bulgaricus* (KCTC3188), and *Lactobacillus brevis* (KCTC3498) were used for lactic acid fermentation of PPE. LAB were grown on MRS agar plate 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% edible yeast extract, 0.1% edible calcium carbonate and 1% glucose. The seed culture was prepared by incubating at 30°C for 24 h. To prepare an active seed starter the culture (100 μL) grown in PPE for overnight was transferred to the same PPE medium (10 mL), and cultured at 30°C for 24 h.

Lactic acid fermentation

The PPE was fortified with 0.05% edible calcium carbonate, 0.2% edible yeast extract and 5% fructose and fermented by *L. rhamnosus* LS, *L. bulgaricus* or *L. brevis* at 30°C for 2 days.

L. rhamnosus LS was used as a starter for lactic acid fermentation of PPE by polysaccharides. For the lactic acid fermentation of PPE, the PPE was fortified with 0.2% polysaccharide and then pasteurized at 80°C for 10 min. As food polysaccharides carrageenan and glucomannan were purchased from MSC Co. (Korea). Xanthan gum and gellan gum were obtained from Guigdao F.T.Z. Co., LTD (China). Alginic acid, pectin and dextran were purchased from Sigma Chemical Co. (USA). The lactic acid fermentation of PPE fortified by polysaccharides was carried out by inoculating 3% seed starter.

Measurement of viable cell counts and titratable acidity

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

Color and viscosity of PPE fermented

The red color of PPE fermented was evaluated by measuring the absorbance of fermented PPE diluted at 528 nm, and also determined by using Hunter color difference meter (CR-10, Minolta, Japan). During the cold storage, the red color change of PPE fermented was evaluated.

Flow rheological experiments of natural or fer-

Table 1. Physicochemical properties of prickly pear extract

Prickly pear	Moisture (%)	85.2
	Total solid (%)	14.8
Prickly pear extract (PPE)	pH	3.99
	Titratable 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.

mented PPE was performed using RheoStrss 1 controlled stress rheometer (HAAKE, Thermo Electron Corporation, Germany) with a double gap concentric cylinder geometry adapted with a measuring cup DG43. The sample (13 mL) was loaded to a measuring cup and determined the shear stress (Pa) at the shear rate (1/s) in the range of 0.1 and 100. For determining the flow behavior of fluid, consistency index and flow behavior index as the parameter of flow behavior were determined based on the power law equation.

RESULTS AND DISCUSSION

Physicochemical properties of PPE

PPE had a 10.3 of apparent viscosity (cp) and a bright red color (L: 18.6; a: 14.8). The total solid, pH and titratable acidity were 14.8% (w/v), 3.99 and 0.35% (w/v), respectively (Table 1). Because of the viscosity and red pigment of PPE, PPE may enhance the color and viscosity of various processed foods. Also, the phenolic compound in PPE was 5.7 mg% (w/v). Generally, phenolic substances are very important to beverage characteristics and quality. They include the red pigment, the astringent flavors, and the bitterness. Therefore, The physicochemical properties of PPE give us important information for its application in fermented foods.

Alcohol fermentation

To determine the optimum concentration of PPE for alcohol fermentation, PPE was mixed with GJ (12.8°Brix, 0.67% acidity) and then fermented at 30°C. Table 2 shows the sugar (°Brix) and alcohol content in alcoholic beverages fermented with various PPE/GJ mixtures. The higher concentration of alcohol in alcoholic beverages. It was concluded that in the presence of GJ, both 25% and 50% of PPE content was a suitable concentration for alcohol fermentation.

Table 2. Analysis of alcoholic beverages fermented with different composition of PPE and GJ

PPE (%)	GJ (%)	Sugar content (°Brix)	Alcohol content (%)
0	100	7.2	9.6
25	0	23.8	-
25	75	7.0	9.1
50	50	7.4	8.9
70	30	10.6	6.9
90	10	14.6	0.9

Alcoholic beverages were fermented at 30°C for 7 days.

Effect of temperature on the alcohol fermentation

GJ and GJ/PPE mixtures were fermented at 22°C and 30°C. As shown in Fig. 1, both GJ and PPJ/GJ mixtures were fermented for 6 days. The sugar contents of GJ was quickly decreased compared with that of PPE/GJ mixture. Alcohol fermentation of GJ was completed after 4 days. The fermentation in the PPE/GJ mixtures was slightly different between 22°C and 30°C. Alcohol production of the PPE/GJ mixtures was more effective at 22°C than at 30°C. Although the PPE/GJ mixtures produced alcohol slowly, they produced a similar concentration of alcohol to that of GJ after fermentation for 6 days. Both 22°C and 30°C PPE/GJ mixtures produced alcoholic beverages with alcohol contents of 10.4% and 10.6%, respectively. GJ only produced alcoholic beverages with 9.8% and 9.6%. Compared with previous results, the fermentation of the PPE/GJ mixtures was completed within a shorter fermentation time. This may be due to an inoculum of higher concentration.

Lactic acid fermentation according to LAB

In the optimum condition the PPE was fermented by *L. rhamnosus* LS, *L. brevis* or *L. bulgaricus* (Table 3). The PPE fermented by *L. bulgaricus* showed the

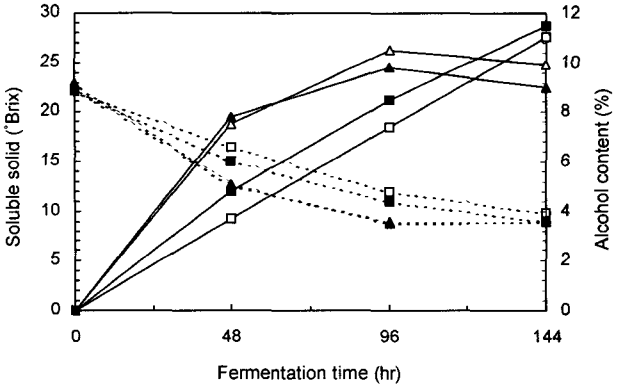

Fig. 1. Comparison of alcohol production and soluble solid content during fermentation of PPE/GJ mixtures at different temperatures.

△: GJ100, 30°C; □: PPE50/GJ50, 30°C; ▲: GJ100, 22°C; ■: PPE50/GJ50, 22°C; —: alcohol content; ····: soluble solid.

Table 3. Effects of the type of starter on lactic acid fermentation and pigment stability of PPE

Strains	pH	Titratable acidity (%)	Viable cell counts ($\times 10^9$ CFU/mL)	Relative absorbance (%)
Control	4.27	0.20	0	89.3
L. rhamnosus LS	3.12	1.03	2.10	97.3
L. brevis	3.58	0.65	1.00	94.7
L. bulgaricus	3.11	0.97	1.20	97.8

similar results in acidity, pH and stability of red pigment, but indicated 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* indicated the lowest acidity with 0.64%. Lower acidity is due to the different pattern in the production of lactic acid as a heterofermentative strain. But the stability of red pigment was well maintained with about 94.7% of relative absorbance. Therefore, *L. rhamnosus* LS was superior to produce 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, indicating 1.3×10^9, 7.33×10^8 and 6×10^8, respectively. It concludes that culture broth containing PPE as a main ingredient is necessary to be fortified with more nutritive ingredient for *L. brevis* and *L. bulgaricus* than *L. rhamnosus* LS even if PPE was fortified with yeast extract. In particular, the PPE fermented by *L. rhamnosus* LS showed higher viable cell counts with 2.10×10^9 CFU/mL.

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 4, *L. rhamnosus* LS was superior to the cell growth compared with those of *L. bulgaricus* and *L. brevis*. The PPE fermented by *L. rhamnosus* LS, *L. bulgaricus* or *L. brevis* indicated 2.1×10^9, 1.2×10^9 and 1.0×10^9 of viable cell counts, respectively. The viable cell counts of PPE fermented by *L. rhamnosus* LS was not decreased during the cold storage for 3 weeks, maintaining initial viable cell counts of 2.1×10^9, but was decreased

Table 4. Changes in viable cell counts during cold storage of PPE fermented by LAB

	Viable cell counts ($\times 10^8$ CFU/mL)				
Strains	0	1[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 period of cold storage with PPE fermented (weeks).

in 3×10^8 after 4 weeks. In the case of PPE fermented by *L. bulgaricus* the viability of a lactic acid bacterium maintained during cold storage for 3 weeks, indicating 7.1×10^8 viable cell counts (CFU/mL). However, the viable cell counts was decreased in 1.5×10^8 after cold storage for 4 weeks. The PPE fermented by *L. brevis* showed lower viable cell counts with 8.5×10^8 after cold storage for 2 weeks. After then the viable cell counts was decreased in 2.5×10^8. It concludes that PPE fermented by *L. rhamnosus* LS indicates higher viable cell counts and viability until cold storage for 3 weeks. After 4 weeks the viability of LAB was decreased with $1.5\sim 3.0\times 10^8$ viable cell counts. It reported that a decline in lactic acid bacterial numbers in plain yoghurt was observed under cold storage condition, reaching 7 log CFU/mL after 30 days (17). 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 more than 8 log CFU/mL.

At the same time the acidity and relative absorbance of PPE fermented were determined during the cold storage for 4 weeks. As shown in Table 5, the Hunter color value of PPE fermented indicated the stability of red pigment during cold storage for 4 weeks. Hunter color value (L, a, b) from PPE fermented by *L. rhamnosus* LS, *L. brevis* or *L. bulgaricus* was closed to the initial color value after lactic

Table 5. Changes in Hunter color value of fermented PPE during cold storage

Strains		Color value 0	2[1)]	4
L. rhamnosus LS	L	19.60	18.00	19.60
	a	12.33	11.60	13.50
	b	1.40	0.46	1.30
L. brevis	L	19.46	18.56	19.60
	a	12.17	11.03	13.97
	b	1.96	1.60	1.93
L. bulgaricus	L	19.80	18.20	19.50
	a	12.46	11.23	13.46
	b	1.40	0.53	1.40

[1)]Numbers indicate the period of cold storage with PPE fermented (weeks).

acid fermentation for 2 days, indicating a little change in red color during cold storage. It concludes that PPE was able to convert to beverage with higher viable cell counts and attractive red/pink color by lactic acid fermentation.

Effect of food polysaccharides on the acidity and relative absorbance

Plant polysaccharides such as pectin, carrageenan, glucomannan, alginic acid and microbial polysaccharides such as xanthan gum, dextran, gellan gum were added to PPE with 0.2% level. Previously, higher concentration of xanthan gum was not completely dissolved because of its higher viscosity. Therefore, the concentration of polysaccharides added was fixed with 0.2%. As shown in Table 6, the acidity of PPE after fermentation for 2 days showed the similar value compared with PPE fermented without polysaccharides. After fermentation for 2 days, the titratable acidity of PPE fermented was 1.3~1.4%. But the addition of carrageenan and alginic acid as acidic polysaccharides resulted in the inhibition of acid production, indicating about 0.8% acidity. In addition, the acidity of PPE fermented by *L. rhamnosus* LS was gradually increased during the cold storage for 4 weeks, showing 18~38% increase in acidity. It turns out the *L. rhamnous* LS can produce the acid even at 4°C. But, PPE with carrageenan did not indicate the any change in acidity during the cold storage.

The thermal stability of betalain pigment of prickly pear fruits grown in southeastern Spain was evaluated. It was found that this pigment was very sensitive to temperature, causing a loss of almost 75% of the initial value by heating at 70°C, and treatment at 90°C resulting in 90% loss of pigment (18). In our studies the stability of red pigment in PPE could be successfully maintained, indicating 84% retention (16).

In the case of relative absorbance, the relative red color of PPE fermented was decreased during the cold storage for 4 weeks. The PPE fermented with xanthan gum and alginic acid showed the higher stability in red color, showing more than 70% retention. The PPE with carrageenan, glucomannan and dextran showed the moderate color stability of PPE, indicating more than 60% retention value. But, the PPE with pectin and gellan gum showed the similar value with the PPE fermented without polysaccharide. It concludes that the addition of certain polysaccharide may enhance the stability of red color during the cold storage

Table 6. Changes in titratable acidity and relative absorbance of PPE fermented by *L. rhamnosus* LS by the addition of food polysaccharide

Polysaccharides \ Days	Titratable acidity (%) After fermentation	Preservation (days) 14	28	Relative absorbance (%) After fermentation	Preservation (days) 14	28
	2			2		
Blank	1.06	1.31	1.40	94.8	76.9	55.0
Pectin	1.18	1.34	1.40	94.3	75.4	54.5
Carrageenan	0.76	0.78	0.77	96.9	86.5	69.6
Glucomannan	1.09	1.25	1.40	93.9	91.0	60.4
Xanthan gum	1.18	1.23	1.40	105.5	89.9	78.2
Alginic acid	0.70	0.73	0.88	88.2	78.7	76.9
Gellan gum	0.94	1.11	1.30	93.7	71.5	47.4
Dextran	0.94	1.12	1.37	92.6	83.4	67.8

of PPE fermented. It has been reported that the stability of red pigment of betalains could be affected by pH, temperature, light, oxygen, water activity and enzyme (19). It reported that anthocyanin-DNA copigmentation complex resulted in the mutual protection against oxidative damage. Generally, copigments are colourless substances which can form a coloured cluster with colourless forms of anthocyanins. Copigments include a large variety of structurally unrelated compounds, such as flavonoid and non-flavonoid phenols, amino acids and organic acid (20). In addition, thermal stability of anthocyanins was affected positively by copigmentation such as pullulan-anthocyanin mixture (21).

The mucilage, pectin-like polysaccharide, isolated from PPE by alcohol precipitation formed a complex with red pigment and its red pigment could be removed by repeating solubilization in water and precipitation in alcohol (unpublished results). It implies that the particular polysaccharide may interact with red pigment somehow, and contribute the increase of stabilization of red pigment.

To elucidate the hypothesis on the role of polysaccharides for enhancing color stabilization, various polysaccharides were added in PPE before lactic acid fermentation. Among them xanthan gum and alginic acid were the best stabilizer in keeping on the stability of red pigment during the cold storage. In terms of appearing color, all the PPE fermented with and without polysaccharides kept the acceptable red color, retaining the original red pigment after fermentation and during cold storage for 4 weeks.

Effect on viscosity

Among food polysaccharides applied, gellan gum and xanthan gum increased the viscosity of PPE fermented greatly. As shown in Fig. 2, the addition of xanthan gum in PPE indicated the highest viscosity, showing the distinguished flow pattern compared to those of other polysaccharides. Xanthan gum has the highest consistency index value, carrageenan and Mango beverage have a similar consistency index value. The apparent viscosity of PPE with xanthan gum or gellan gum was also higher than that of commercial Mango beverage. However, addition of other polysaccharides except for dextran, alginic acid, or pectin showed the slight increase in apparent viscosity. Therefore, xanthan gum could be utilized as best thickener for enhancing the viscosity in the acidic beverage such as PPE fermented by LAB. Considering overall effects of polysaccharides on the LAB fermentation in PPE, it concludes that xanthan gum is the best polysaccharide to be utilized in the PPE fermented by *L. rhamnosus* LS.

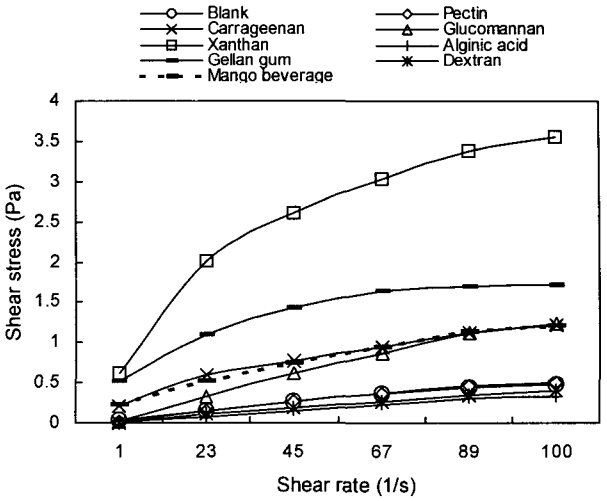

Fig. 2. Flow patterns of PPE fermented with food polysaccharides (Mango beverage is a commercial beverage of Haetae Co.).

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