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Alcohol Fermentation of *Opuntia ficus* Fruit Juice

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Abstract

Prickly pear juice (PPJ) extracted from prickly pear fruit (*Opuntia ficus-indica*) was used as a raw material for the production of alcoholic beverages. Prickly pear juice (PPJ) had 0.88 °Brix of soluble solid, pH 3.96 and 0.14% of total acidity. Alcohol fermentation of 25% PPJ including 22 °Brix of sugar was not successful, but was promoted by addition of grape juice (GJ). In the presence of GJ, the 22 °Brix of sugar and 1×10^6 of inoculum was suitable for alcohol fermentation indicating the rapid decrease of sugar content. The 22 °Brix of sugar in PPJ (25%)/GJ (50%) mixture was changed to 6.5 °Brix after fermentation at 30°C for 7 days. The alcohol content was 9.2% (w/v). PPJ (70%)/GJ (30%) mixture produced alcoholic beverage with 6.9% alcohol content resulting in the gradual decrease of soluble solid for 7 days. On the other hand, PPJ (50%)/GJ (50%) mixture carried out completely the alcohol fermentation at 22°C for 6 days and enhanced the red color of alcoholic beverages.

Key words: prickly pear juice, alcohol fermentation, grape juice

INTRODUCTION

Prickly pear (*Opuntia* sp.) is in the Cactaceae family and is grown in arid and semiarid regions (1). Cacti produce fruits, green vegetables, gum for adhesives and thickening foods (2). Specifically, prickly pear fruit is a many-seeded berry with a thick fruit wall (1). Prickly pear fruits are also sources of nutrients and vitamins, and are eaten fresh or preserved in jams, syrups, or candies (3-5).

According to various types of *Opuntia* sp., the difference in composition and quality of prickly pear cactus was reported (6). The most abundant component of the pulp and skin of prickly pear fruit (*Opuntia ficus-indica*) was ethanol-soluble carbohydrates (7). Contents of minerals, free sugars, free amino acids and total phenolic compounds in the stem, fruit and seeds of prickly pear cactus was reported (8). The predominant soluble sugars in the prickly pear fruit are glucose and fructose with smaller amounts of sucrose (3).

Prickly pear cactus has been known as a therapeutic medical plant (9). It was reported that pectin isolated from prickly pear (*Opuntia* sp.) modified low density lipoprotein metabolism in cholesterol-fed guinea pigs (10,11). An extract purified from prickly pear cactus (*Opuntia fuliginosa*) was able to control diabetes induced in rats (11).

It has been known that commercial production of prickly pear fruit flourished in Argentina, Chile, Israel, Italy, Mexico, Spain, South Africa, and the United States (1). In Korea, Cheju island is a region which grows prickly pear cactus (*Opuntia ficus-indica*). Prickly pear cactus is now considered a valuable crop because of its medicinal effect as well as various functional properties as food ingredients. The red color of prickly pear juice (*Opuntia* sp.) was identified as betalains. The thermostability of the red pigment was dependent on temperature and heating time (12). Previously, the functional properties of mucilage and red pigment extracted from *Opuntia ficus-indica* was evaluated (13).

Because of various functional properties of prickly pear juice, its application in food systems has been carried out. Previously, prickly pear fruit was used for preparing food products such as jam and juice (4). To produce single cell protein, *Candida utilis* was grown in batch and continuous culture using prickly pear juice as the sole carbon and energy source (14). A cocoa butter product from prickly pear juice was produced by the fermentation of *Cryptococcus curvatus* (15). Recently, it was reported that production of red pigments was carried out by *Monascus purpureus* grown on prickly pear juice (16). However, in spite of various applications of prickly pear juice, little is reported about the alcohol fermentation with prickly pear juice. In particular, prickly pear juice with red pigment and mucilage was considered an ingredient for wine production. The objectives of our study were to produce alcoholic beverages with prickly pear juice obtained from *Opuntia ficus-indica* var. *sabolne* and determine the optimum conditions for alcohol fermentation.

MATERIALS AND METHODS

Materials

Opunita ficus-indica var. *sabolen* MAKINO grown in Cheju island was purchased and stored at -18°C. Grapes (*Campbell Early*) harvested from Youngchun in Kyungpook 1999 were obtained. Alcohol reagents for determining alcohol contents were obtained from Sigma Chemical Co. (St Louis, MO, USA). *Saccharomyces cereviseae* (No, SM102)

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was purchased from Gist-brocades (Delft, Netherland). All other chemicals used for this study were analytical grade.

Preparation of prickly pear juice

Prickly pear fruit (75 g) was washed, severed and mixed with distilled water (440 ml) using a shaker (Flask Shaker KMC1205S, Vision Scientific Co., Korea) at 20°C for 5hr. Prickly pear juice (PPJ) was prepared after removing insoluble solids such as seeds and pulp by centrifugation at 7,000 rpm for 20 min. PPJ including red pigment and mucilage was used as an ingredient for alcohol fermentation.

Physicochemical analysis of PPJ

The solid content of PPJ was determined by drying at 105°C (17). The pH was determined with a pH meter (Digital pH meter 110, Wheaton, USA). The titrable acidity (%) was determined by titrating with 0.1 N NaOH to pH 8.3 and expressed as a percentage of tartaric acid (18). A hand refractormeter (Atagoni Brix 0~32%, Japan) was used for determining the soluble solid of PPJ and grape juice. The relative viscosity was determined by a capillary viscometer (Cannon Instrument, #75, USA). The total phenol content of PPJ was determined with the Folin-Ciocalteu Reagent (18). To prepare the Folin-Ciocalteu Reagent, 5 g of sodium tungstate and 1.25 g of sodium molybdate were dissolved in 35 ml of distilled water. 2.5 ml of 85% phosphoric acid and 5 ml of hydrochloric acid (conc.) were added and then boiled in a reflux condenser for 7 hr. The solution recovered was mixed with 7.5 g of lithium sulfate and a few drops of bromine and then boiled for 15 min in a hood. The solution with yellow color was cooled down, filtered and stored in an amber bottle. Caffeic acid solution (1 mg/ml) was used for the preparation of the calibration curve. 2 ml of diluted PPJ was mixed with 0.2 ml of Folin-Ciocalteu Reagent and 0.4 ml of the 20% sodium carbonate solution and then adjusted to 4 ml volume with water. The mixture was incubated for 2 hrs at 20°C and then determined the absorbance at 725 nm. The color (L, a, b) of both the PPJ and the alcoholic beverage fermented with PPJ/GJ mixture were measured with a Color Reader (Minolta CR-10, Japan). After adjusting the PPJ to pH 3.2 and 4.0 with 10% tartaric acid, the red color of the heat-treated PPJ was also determined by absorbance measured at 534 nm using a Spectrophotometer (UVICON, Kontron Instruments, France).

Alcohol fermentation using PPJ

In order to determine the optimum conditions for alcohol fermentation with PPJ, the effects of heat treatment, pH, fermentation temperature and PPJ content were evaluated. To inactivate enzymes in PPJ, PPJ was pasteurized by heating at 85°C for 5 min. GJ was obtained by the gauze filtration of crushed grape. Various amounts of PPJ (0~100%) was mixed with GJ. A 150 ppm of SO_2 was added to PPJ and/or PPJ/GJ mixture with 10% Na_2SO_3. Acidity was adjusted to 0.7% with 10% tartaric acid. The soluble solid of PPJ or PPJ/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 in sterilized water (5 ml), and then incubated on YM plate at 30°C for 2~3 days. A single colony was selected and inoculated in GJ (10 ml). After growing at 30°C for 1 day, a GJ culture (3 ml) as a mother starter was transferred to PPJ or/and GJ (100 ml). During fermentation, the alcoholic beverages were analyzed to determine their color, titrable acidity, soluble solid and alcohol content.

Determination of alcohol content in PPJ/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 mins. Absorbance at 340 nm was converted to the alcohol content.

RESULTS AND DISCUSSION

Physicochemical properties of PPJ

PPJ had an 8.6 of apparent viscosity (cp) and a bright red color (L: 19.1; a: 24.9). The solid content, pH and titrable acidity were 0.88% (w/v), 3.96 and 0.14% (w/v), respectively (Table 1). Because of the viscosity and red pigment of PPJ, PPJ may enhance the color and viscosity of various processed foods. Also, the low acidity of PPJ can reduce the high acidity in GJ used for alcohol fermentation. In particular, the total phenolic compound in PPJ was 0.9 mg/g (w/w). It was reported that the contents of the total polyphenols in prickly pear fruit were changed by type and temperature of the extraction solvent (8). Generally, phenolic substances are very important to wine characteristics and quality. They include the red pigments, the astringent flavors, and the bitterness (19). Previously, the antioxidant effect was also determined with the pigment syrup prepared from PPJ (20). The physicochemical properties of PPJ give us important information for its application in fermented foods.

Effect of pH on the thermostability of PPJ

In order to determine the thermostability of PPJ at different acidic pHs, PPJ adjusted with 10% tartaric acid was pasteurized at 85°C for 15 min. As shown in Fig. 1, the absorbance of red pigment was gradually decreased by increasing the heating time at both pH 3.2 and pH 4.0. It may indicate the destruction of red pigment due to the heat labile property.

Table 1. Physicochemical properties of prickly pear juice

Prickly pear juice	Value
pH	3.96
Titrable acidity (%)	0.14
Solid content (%)	0.88
Soluble solid (°Brix)	0.9
Apparent viscosity (cp)	8.6[1)]
Total phenolic compounds (mg/g)	0.9
Color (L, a, b)	(19.1, 24.9, 0.8)

[1)]Viscosity was determined at 60 rpm (spindle #1).

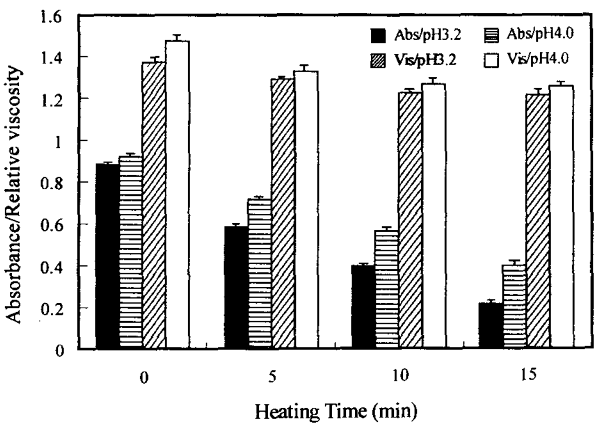

Fig. 1. Changes in color and viscosity of PPJ according to pH and heating time.

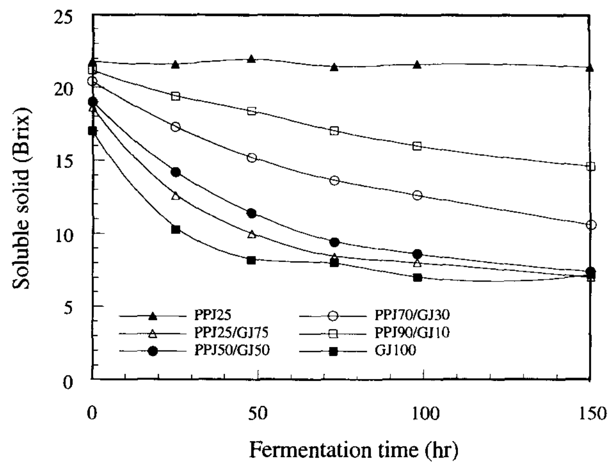

Fig. 2. Changes in soluble solid content of PPJ/GJ mixture during fermentation. PPJ25/GJ75 means a mixture of 25% PPJ and 75% GJ.

PPJ at pH 4.0 showed higher absorbance than PPJ at pH 3.2. It was similar to previous results that the relative absorbance of PPJ was more stable at pH 4.2 than at pH 3.0 during the storage period (13). Generally, the red colour of the betanin solutions remains unchanged from pH 3.0 to 7.0, exhibiting maximum absorption at 537 nm (21). However, the relative absorbance of PPJ was drastically decreased by heating at 80°C for 20 min. The thermostability of PPJ was deeply dependent on heating time and temperature (13). The relative viscosity of PPJ was also decreased by increasing heating time. The relative viscosity of PPJ of pH 4.0 was slightly higher than that of PPJ at pH 3.2. A 10% drop in the relative viscosity of PPJ was shown by heat treatment for 5 min. After 5 min, the relative viscosity was little changed. In practice, the heat treatment of PPJ reduced the indigenous off-flavor and viscosity. Thus, the heat-treatment of PPJ enhanced its functional properties for alcohol fermentation.

Alcohol fermentation

To determine the optimum concentration of PPJ for alcohol fermentation, PPJ was mixed with GJ (12.8 °Brix, 0.67% acidity) and then fermented at 30°C. As shown in Fig. 2, 25% PPJ without GJ was not fermented at all for 7 days, showing the constant soluble solid. However, the alcohol fermentation of PPJ was promoted by the addition of GJ, and was successfully performed by increasing the amount of GJ. The sugar content of the PPJ (70%)/GJ (30%) mixture was slowly decreased by alcohol fermentation. In the case of 90% PPJ, 14.6 °Brix of sugar remained after fermentation for 7 days. In the presence of GJ, both 25% PPJ and 50% PPJ showed a similar fermentation pattern to that of 100% GJ. A PPJ (50%)/GJ (50%) mixture indicated 7.4 °Brix of sugar after fermentation. Table 2 shows the sugar (°Brix) and alcohol content in alcoholic beverages fermented with various PPJ/GJ mixtures. The higher concentration of PPJ produced the lower concentration of alcohol in alcoholic beverages. It was concluded that in the presence of GJ, Both 25% and 50% of PPJ content was a suitable concentration for alcohol fermentation.

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

Prickly pear juice (%)	Grape juice (%)	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	5.9

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

To evaluate the alcohol fermentation of PPJ according to the initial concentration of starter culture, PPJ (25%)/GJ (50%) mixture was inoculated with different amounts of starter. As shown in Fig. 3, an initial inoculum affected the decrease of the sugar content in PPJ (25%)/GJ (50%) mixture. With an inoculum of 1×10^6 cells, alcohol fermentation was more effective and the sugar content was changed from 22 °Brix to 7 °Brix after 7 days. It was reported that GJ inoculated with an inoculum of 5×10^6 cells was completely fermented at 25°C for 8 days, resulting in a decrease of sugar content from 24 °Brix to 8 °Brix (22).

Effect of sugar contents on the alcohol fermentation of the PPJ (25%)/GJ (50%) mixture was evaluated. White sugar was added to the PPJ/GJ mixture for adjusting the soluble solid (20~28 °Brix). As shown in Fig. 4, with sugar contents of 20~28 °Brix, the PPJ/GJ mixture was successfully fermented for 7 days showing a similar decrease of the soluble solid. A PPJ/GJ mixture with 28 °Brix produced an alcoholic beverage with 15 °Brix. of sugar. On the other hand, a mixture of PPJ/GJ with 20 °Brix produced a dry alcoholic beverage including 6 °Brix. Alcohol fermentation of GJ was completed at 30°C for 3 days. The alcohol contents of the PPJ/GJ mixture fermented with various sugar contents (20, 22, 24, 26, 28 °Brix) were 8.2%, 8.0%, 7.5%, 7.6% and 8.3%, respec-

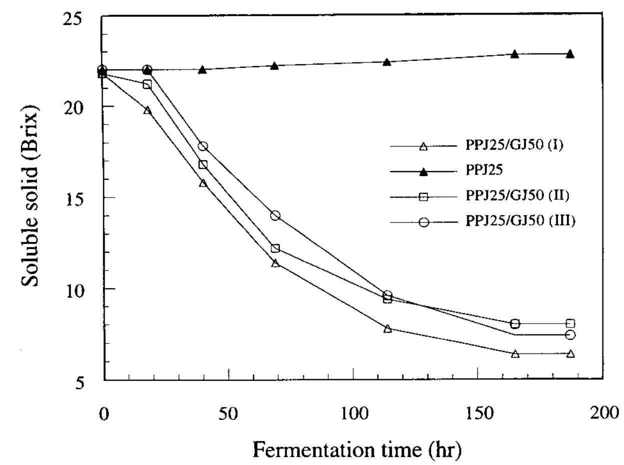

Fig. 3. Changes in soluble solid content of PPJ/GJ mixture fermented with different amounts of inoculum. I: 1×10^6 cfu/ml, II: 0.2×10^6 cfu/ml, III: 0.2×10^5 cfu/ml.

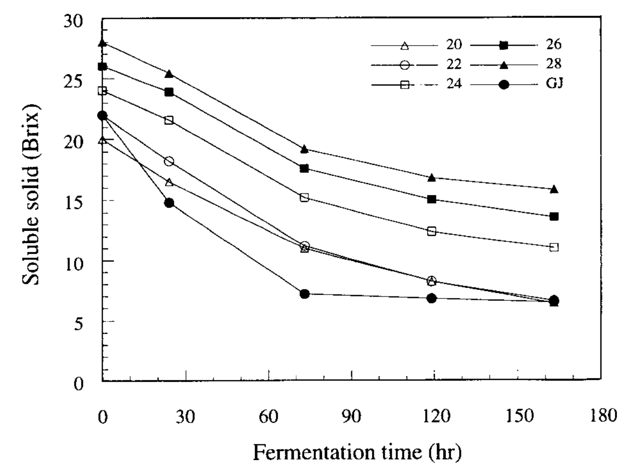

Fig. 4. Effect of soluble solid content on fermentation of PPJ/GJ mixtures. Alcoholic fermentation was performed with PPJ (25%)/GJ (50%) mixture. Number indicates initial sugar content (°Brix).

tively. GJ with 22 °Brix produced an alcoholic beverage with 8.8% alcohol content.

To evaluate the effect of temperature on the alcohol fermentation of the PPJ (50%)/GJ (50%) mixture, GJ and GJ/PPJ mixtures were fermented at 22°C and 30°C. As shown in Fig. 5, both GJ and PPJ/GJ mixtures were fermented for 6 days. The sugar contents of GJ was quickly decreased compared with that of PPJ/GJ. Alcohol fermentation of GJ was completed after 4 days. The rate of fermentation in the PPJ/GJ mixtures was slightly different between 22°C and 30°C. Alcohol production of the PPJ/GJ mixtures was more effective at 22°C than at 30°C. On the other hand, GJ showed a similar pattern for sugar consumption as well as alcohol production. Although the PPJ/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 PPJ/GJ mixtures produced alcoholic beverages with alcohol contents of 10.4%

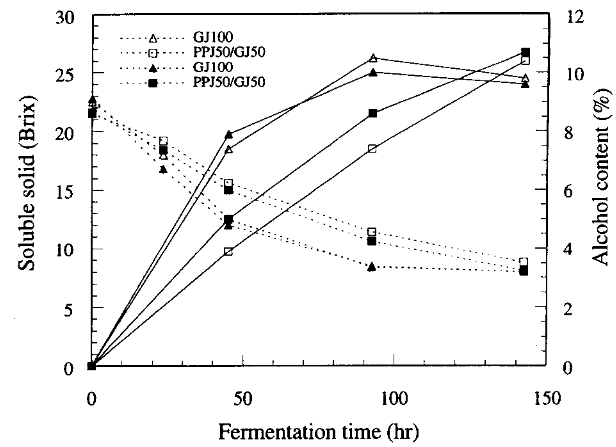

Fig. 5. Comparison of alcohol production and soluble solid content during fermentation of PPJ/GJ mixtures at different temperatures. Number indicates extract content (%). △, □: 30°C; ▲, ■: 22°C ; —: alcohol content; ···: soluble solid.

and 10.6%, respectively. GJ only produced alcoholic beverages with 9.8% and 9.6%. Compared with previous results, the fermentation of the PPJ/GJ mixtures was completed within a shorter fermentation time. This may be due to an inoculum of higher concentration (2×10^6 cfu/ml).

The presence of PPJ in the alcohol fermentation of the PPJ/GJ mixtures produced an enhanced red color in the alcoholic beverages. Table 3 indicates the color intensity of alcoholic beverages fermented for 6 days. The red color was slightly decreased during the fermentation period. In the presence of 50% PPJ, the red color of alcoholic beverages was greatly enhanced. The lightness of the alcoholic beverage produced from GJ was higher than that of the PPJ (50%)/GJ (50%) mixture. The red color of the alcoholic beverage fermented with PPJ/GJ at 22°C was slightly higher than that fermented at 30°C.

From these results, it can be concluded that PPJ (25% or 50%) is successfully fermented by the addition of GJ. The PPJ/GJ mixture fermented at 22°C allows an alcoholic beverage of 10.4% alcohol and enhanced red color to be produced. In addition, the PPJ/GJ mixtures may contribute to the nutritional fortification of alcoholic beverages. The PPJ with lower acidity, red pigment and viscous mucilage is con-

Table 3. Color value of alcoholic beverages fermented with PPJ and GJ mixtures at different temperatures.

Temp (°C)	PPJ (%)	GJ (%)	L	a	b
30	0	100	29.2 (30.4)	6.7 (9.4)	11.3 (12.1)
	50	50	19.7 (21.3)	17.8 (14.0)	-0.63 (3.0)
22	0	100	29.2 (30.5)	6.4 (9.0)	11.2 (12.9)
	50	50	20.4 (22.5)	18.6 (15.9)	-0.4 (4.6)

( ): Color value of alcoholic beverages fermented for 6 days.

sidered as a valuable ingredient for various food applications.

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