

# Eastern Prickly Pear (*Opuntia humifusa* Raf.) Power Enhanced the Quality of Korean Traditional Wine, *Takju*

Young-Joon Choi<sup>1</sup>, Sanjeev Kumar Dhungana<sup>2</sup>, Il-Doo Kim<sup>3</sup> 

<sup>1</sup>Food Beverage and Culinary Arts, Daegu Technical University, Daegu 42734, Korea

<sup>2</sup>School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea

<sup>3</sup>International Institute of Research & Development, Kyungpook National University, Daegu 41566, Korea

**Abstract:** *Takju*, also known as *makgeolli*, is a famous traditional alcoholic beverage in Korea. Various studies on the effect of raw materials on the quality of *takju* have been carried out. This study aimed to investigate the influence of *Opuntia humifusa* powder on the quality of *takju*. Chemical characteristics, color value, antioxidant potential as well as sensory characteristics were considered for the quality evaluation. Addition of the powder improved the functional as well as organoleptic properties of *takju*. The optimum proportion of *O. humifusa* powder to be added for having better impact was 0.5% (w/v). Results of this study suggested that *O. humifusa* could enhance the antioxidant potential as well as overall acceptance of Korean traditional rice wine, *takju*.

**Keywords:** antioxidant potential, Korean traditional wine, *Opuntia humifusa*, overall acceptance

## Introduction

*Takju*, also known as *Makgeolli*, is a traditional alcoholic beverage with an alcohol concentration of 6–8%, a characteristic fragrance with a slightly acidic character, and a sweet taste. It is a turbid wine prepared from cereals, mostly glutinous rice, and Nuruk, a fermentation starter, containing yeast and several types of fungi. It is a famous Korean traditional rice wine. Majority of native Koreans as well as foreign visitors to Korea enjoy drinking *Takju*. It is rich in protein and carbohydrates and also possesses a small amount of organic compounds. Beneficial health effect of this wine has received attention among consumers and researchers. However, due to a lack of unique characteristics and inferior acceptability, *Takju* has received an attention by food scientists to improve the quality so that its popularity could be increased. Various kinds of cocktail *Takju* have been developed and studies on the microbial activities, functional characteristics, utilization of raw materials, manufacture processes, extension of shelf life, and more have been carried out (Lee et al., 2002a; Han et al., 1997; Song and Park, 2003; Kim et al., 2004; Cho et al., 2010).

In order to improve the overall quality of *takju*, various raw materials and additives, including dandelion (Kim et al., 2000), chamomile (Lee et al., 2002b), acasia (Seo et al., 2002), and *Paecilomyces*

*japonica* (Lee et al., 2002c) blueberry (Kim et al., 2015), banana (Kim et al., 2013a), mulberry (Kim et al., 2013b), *Laminaria japonica* (Choi et al., 2014), have been added. The quality of traditional wine and liquor has gradually improved, resulting in increased sales in recent years; however, problems still exist.

Different active substances such as polyphenols, polysaccharides, and polysaccharide-peptide complexes have been isolated from *takju* (Lee et al., 1996; Park and Lee, 2002). These active substances found in *takju* show various chemical characteristics and biological effects, including antioxidant and immunomodulating activities. Bae et al. (2010) mentioned that some *takju* samples possessed significantly high total polyphenol contents compared to white wine.

*Opuntia humifusa* (Raf.), commonly known as eastern prickly pear (EPP) is a kind of cactus, contains good radical scavenging and anti-inflammatory activity (Han et al., 2007). EPP also lowered the blood glucose and cholesterol levels in streptozotocin-induced diabetic rats (Hahm et al., 2011). In addition, EPP showed anti-cancer effect by inhibiting the growth of MCF-7 Human Breast Cancer Cells (Yoon et al., 2009) and U87MG Human Glioblastoma Cells (Hahm et al., 2010). EPP has also been used in cookies (Han et al., 2007). Considering

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Il-Doo Kim (Correspondence)



ildookim@hanmail.net



+82-53-950-5707, Fax: +82-53-958-6880

the functional and medicinal values of EPP and effect of raw material on wine quality the objective of this study was to investigate the quality characteristics of traditional Korean rice wine, *takju*, prepared with EPP.

## Materials and Methods

### *Chemicals and materials*

Folin-Ciocalteu phenol reagent and DPPH were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade. EPP [*Opuntia humifusa* (Raf.)] powder was obtained from a local market in Daegu, Korea.

### *Preparation of takju samples*

Typically, Korean traditional rice wine, *takju* is made of steamed rice with yeast and water fermented for about 6 d. *Takju* is prepared by 2-step fermentation of steamed rice without distillation. A mixture of rice *koji*, a commercially available steamed rice (2 kg), yeast (2.5 g) and water (3 L) fermented at 25°C for 1 d. In the second step, mixture of steamed rice (8 kg), *nuruk* (200 g), water (12 L), and different proportions of EPP powder was fermented at 25°C for 5 d. Addition of various proportions of additives (blueberry powder and black rice powder) does influence the physicochemical properties of *takju* differently. Different varieties of *takju* prepared with various proportion of EPP were named as Control: Ordinary *takju* containing no added EPP, OH-A: *takju* containing 0.75 g of EPP in 750 mL of raw *takju*, OH-B: *takju* containing 3.75 g of EPP in 750 mL of raw *takju*, and OH-C: *takju* containing 7.5 g of EPP in 750 mL of raw *takju*. A range of additive concentrations was used for formulation of optimum proportions of Eastern prickly pear powders so that a superior quality *takju* could be prepared. After preparation, *takju* varieties were pasteurized at 85°C for 5 min using a sterilizer (CK-25; Hanil Co.) and were stored at 4°C for subsequent analyses.

### *Physicochemical parameters*

The pH value of *takju* was measured using a pH Meter (Model 250; Beckman Coulter, Inc., Fullerton, CA, USA). Titratable acidity (lactic acid in g/L) was measured using addition of 5 mL of *takju* to 125 mL of deionized water, followed by titration using 0.1N sodium hydroxide to an endpoint pH of 8.2. Alcohol concentration was determined following the method of Ough and Amerine (1988). The brix (°Bx) value was measured following the Official Methods of the National Tax Service (2006). All the chemical measurements were replicated 3 times and mean values were reported.

### *Color measurement*

L\* (lightness), a\* (redness, + or greenness, -), and b\* (yellowness, + or blueness, -) values of *takju* samples were measured using a chroma meter (CR-300; Minolta Corp., Osaka, Japan). A Minolta calibration plate (YCIE=94.5, XCIE=0.3160, YCIE=0.330) and a Hunter Lab standard plate (L\*=97.51, a\*= -0.18, b\*= +1.67) were used to standardize the instrument using a D65 illuminant (Kim et al., 2013). Color values were measured directly from 3 zones of *takju* and mean values were calculated.

### *Determination of the total phenol content*

The total phenol contents of *takju* samples were estimated according to the Folin-Ciocalteu method (Singleton et al., 1999). The undiluted Folin-Ciocalteu reagent (250- $\mu$ L) was added to *takju* sample (50  $\mu$ L). After 1 min, 750  $\mu$ L of 20% (w/v) aqueous Na<sub>2</sub>CO<sub>3</sub> was added to the mixture, and the volume was made up to 5.0 mL using distilled water. A control sample contained all the reaction reagents except the *takju* sample. After incubation at room temperature under dark condition for 2 h, the absorbance value was measured at 760 nm using microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland). Gallic acid was used to prepare a calibration curve and the total phenol contents were determined as gallic acid equivalents ( $\mu$ g/mL of *takju*).

### *DPPH radical scavenging activity*

The DPPH free radical scavenging potential was determined following the methods described by Cheung et al. (2003) with some modifications. A 0.8-mL of 0.2 mM DPPH ethanol solution was mixed with 0.2 mL of a *takju* sample. The mixture was thoroughly mixed and left to stand for 30 min at room temperature under dark condition. The absorbance value was measured at 520 nm using microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific).

### *Evaluation of sensory properties*

The sensory properties of *takju* samples was evaluated with the freshly prepared samples. The samples were rated for color, flavor, and overall acceptance using a scale: 1= very poor, 2= poor, 3= fair, 4= good, 5= very good. Twenty volunteer panelists (10 women and 10 men) selected from the list of graduate students of College of Agriculture and Life Sciences of Kyungpook National University, Daegu, Korea were employed for the sensory evaluation.

### *Statistical analysis*

Data were subjected to analysis of variance (ANOVA) using SAS9.4 (SAS Institute Inc., Cary, NC, USA). Differences between means were separated using Tukey test at  $p < 0.05$ .

**Results and Discussion**

*General chemical characteristics of takju*

The chemical composition of *takju* samples in terms of pH, alcohol, titratable acidity (TA), and soluble solid (SS) are shown in Table 1. The pH of ordinary *takju* (3.90) was significantly low compared to OH-A (4.00) and OH-C (4.02) but not different from OH-B (3.89). Although the pH of *takju* samples was significantly different, the values differed by only a small fraction.

However, the alcohol and SS contents of *takju* samples were not significantly altered by the addition of EPP powder. The unaltered alcohol concentration of EPP-added *takju* (6%) showed restoration of one of the fundamental attributes of Korean traditional rice wine. The TA of all the EPP-added *takju* samples were significantly higher (0.21–0.24) than the ordinary *takju* (0.18 g/100 mL of lactic acid). The variation in pH and TA of different *takju* might be due to the addition of EPP powder.

Table 1. pH, alcohol, titratable acidity (TA), and soluble solid (SS) content of *takju* samples

Properties	Sample <sup>1)</sup>			
	Control	OH-A	OH-B	OH-C
pH	3.90±0.05 <sup>b</sup>	4.00±0.04 <sup>a</sup>	3.89±0.17 <sup>b</sup>	4.02±0.01 <sup>a</sup>
Alcohol (%)	6.01±0.08 <sup>a</sup>	6.00±0.07 <sup>a</sup>	6.00±0.05 <sup>a</sup>	6.00±0.03 <sup>a</sup>
TA <sup>2)</sup> (g/100mL)	0.18±0.02 <sup>b</sup>	0.24±0.03 <sup>a</sup>	0.22±0.02 <sup>a</sup>	0.21±0.03 <sup>a</sup>
SS (°Bx)	3.40±0.20 <sup>a</sup>	3.70±0.20 <sup>a</sup>	3.50±0.10 <sup>a</sup>	3.50±0.10 <sup>a</sup>

<sup>1)</sup>Samples are defined in preparation of *takju* samples (Materials and Methods).

<sup>2)</sup>As lactic acid.

<sup>3)</sup>Quoted values are means±SD of triplicate measurements. Values followed by different superscripts in the same column are significantly different (p<0.05).

*Color value of takju*

The Hunter’s color value of *takju* samples was significantly altered with the addition of EPP (Table 2). The lightness value of EPP-added *takju* (9.51–16.51) was significantly lower than the ordinary *takju* (45.91). The redness value of *takju* was significantly increased (27.20–35.12) by the

addition of EPP compared to the ordinary *takju* (2.61). The yellowness value of ordinary *takju* (19.81) and EPP-added *takju* (13.91–28.12) was significantly different except for OH-B (19.88). The variations in lightness, redness, and yellowness values among blueberry *takjus* might be due to addition of different proportions of EPP powder.

Table 2. Hunter’s color value of *takju* samples

Sample <sup>1)</sup>	Color value <sup>2)</sup>		
	L*	a*	b*
Control	45.91±0.05 <sup>a</sup>	2.61±0.02 <sup>d</sup>	19.81±0.02 <sup>b</sup>
OH-A	16.51±0.04 <sup>b</sup>	35.12±0.07 <sup>a</sup>	28.12±0.03 <sup>a</sup>
OH-B	11.21±0.08 <sup>c</sup>	31.21±0.03 <sup>b</sup>	19.88±0.03 <sup>b</sup>
OH-C	9.51±0.05 <sup>d</sup>	27.20±0.03 <sup>c</sup>	13.91±0.02 <sup>c</sup>

<sup>1)</sup>Samples are defined in preparation of *takju* samples (Materials and Methods).

<sup>2)</sup>L\*, lightness (100, white; 0, black); a\*, redness (–, green; +, red); b\*, yellowness (–, blue; +, yellow).

<sup>3)</sup>Quoted values are means±SD of triplicate measurements. The values followed by different superscripts in the same column are significantly different (p<0.05).

*DPPH radical scavenging activities and total phenol contents of takju*

The addition of EPP powder significantly increased the DPPH radical scavenging activities and total phenolic contents of *takju* compared to the ordinary *takju* (Table 3). The DPPH radical scavenging activities of EPP-added *takju* (50.31–61.30%) were significantly higher than that of ordinary *takju* (16.31%). More than three times higher DPPH radical scavenging activities in EPP-added *takju* might be due to higher antioxidant potential of EPP (Han et al., 2007). Similarly, total phenol content of

EPP-added *takju* (292.30–445.98 µg/mL) was significantly high compared to that of ordinary *takju* (77.71 µg/mL). Almost six times higher total phenol content was detected in OH-C than that in ordinary *takju*. Such higher values of total phenols might be due to addition of EPP powder which is rich in phenolic compounds (Yoon et al., 2009). Park and Lee (2002) report that the polyphenols, flavonoids, and flavonols in *takju* account for its antioxidant activities. Report shows that there are multiple mechanisms involved in wine preparation, therefore, no single assay could reveal all the antioxidants

precisely within a mixed or complex system (Muntana and Prasong, 2010).

Table 3. DPPH radical scavenging activities and total phenol content (TPC) of *takju* samples

Sample <sup>1)</sup>	DPPH (% Inhibition)	TPC (GAE <sup>2)</sup> µg/mL of sample)
Control	16.31±2.22 <sup>c</sup>	77.71±2.20 <sup>d</sup>
OH-A	50.31±1.81 <sup>b</sup>	292.30±3.00 <sup>c</sup>
OH-B	60.20±1.90 <sup>a</sup>	415.31±3.75 <sup>b</sup>
OH-C	61.30±2.02 <sup>a</sup>	445.98±5.21 <sup>a</sup>

<sup>1)</sup>Samples are defined in preparation of *takju* samples (Materials and Methods).

<sup>2)</sup>Gallic acid equivalents.

<sup>3)</sup>Quoted values are means±SD of triplicate measurements. The values followed by different superscripts in the same column are significantly different (p<0.05).

#### Sensory characteristics of *takju*

Sensory characteristics of *takju* samples, except bitterness value, were significantly affected by the addition of EPP powder (Table 4). Based on the overall acceptance, OH-B (4.32) was the most

acceptable *takju*. The sweetness value of EPP-added *takju* was increased with the increased amount of EPP powder. However, the addition of EPP powder reduced the sourness value.

Table 4. Sensory characteristics of *takju* samples

Sample <sup>1)</sup>	Sensory characteristics			
	Sweetness	Sourness	Bitterness	Overall acceptance
Control	2.11±0.11 <sup>c</sup>	1.81±0.02 <sup>a</sup>	1.12±0.20 <sup>a</sup>	2.31±0.21 <sup>c</sup>
OH-A	3.00±0.25 <sup>b</sup>	1.21±0.03 <sup>b</sup>	1.05±0.31 <sup>a</sup>	3.40±0.37 <sup>b</sup>
OH-B	3.51±0.15 <sup>a</sup>	1.14±0.30 <sup>b</sup>	1.10±0.25 <sup>a</sup>	4.32±0.05 <sup>a</sup>
OH-C	3.61±0.21 <sup>a</sup>	1.11±0.21 <sup>b</sup>	1.00±0.21 <sup>a</sup>	3.41±0.21 <sup>b</sup>

<sup>1)</sup>Samples are defined in preparation of *takju* samples (Materials and Methods).

<sup>2)</sup>Quoted values are means±SD of triplicate experiments (n=20) based on 5 point scores (1, very poor; 2, poor; 3, fair; 4, good; 5, very good). Values followed by different superscripts in the same column are significantly different (p<0.05).

In conclusion, addition of *Opuntia humifusa* (Raf.) powder enhanced the functional value as well as overall acceptance of Korean traditional rice wine, *takju*. Addition of the powder did not cause any adverse effect on the basic chemical and organoleptic properties, instead contributed to have positive impacts in *takju*. Based on the overall acceptance and antioxidant potentials, the optimum proportions of *O. humifusa* (Raf.) powder to be added during *takju* fermentation was 0.5% (w/v). Results of this study showed that addition of optimum amount of *O. humifusa* (Raf.) could enhance the antioxidant potentiality as well as overall acceptance of Korean traditional rice wine, *takju*.

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