

Comparison of Takju Characteristics Manufactured using *Rosa rugosa* Thunb. and Two Different Pre-treatments of Rice

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쌀의 전처리 과정을 달리하고 해당화로 가향한 탁주의 발효특성 비교

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Abstract

Takju was manufactured using 150 g *Rosa rugosa* Thunb. (Haedanghwa) and two different preparations of 3,000 g each of cooked and uncooked rice. Nuruk (150 g) and yeast (60 g) were inoculated into both mixtures for fermentation, and physiochemical changes were investigated during 14 days of fermentation. The final brix value, pH, and ethanol concentration of Haedanghwa (uncooked rice Takju) were 13.0°Bx, 4.3, and 11.8%, respectively, whereas those of Haedanghwa (cooked rice Takju) were 14.0°Bx, 4.6, and 14.4%, respectively. Lactic and acetic acid levels in Haedanghwa (uncooked rice Takju) were significantly higher than those in Haedanghwa (cooked rice Takju) ($p < 0.05$). The level of γ -amino-*n*-butyric acid in Haedanghwa Takju (uncooked rice) was twice than that of Haedanghwa Takju (cooked rice). Our results demonstrate that the use of uncooked rice in the preparation of Takju may help improve the taste and function of the final product.

Key words : Ethanol fermentation, cooked rice, *Rosa rugosa* Thunb., Takju, uncooked rice.

INTRODUCTION

Recent focus on the improvement of lifestyle for health and well-being has raised the interest in Korean traditional fermented rice wines. The quality and product diversity of Korean traditional alcoholic beverages, such as Takju, Yakju, and Soju, need to be improved to be able to compete with imported brands (Cho *et al* 2009). Among the alcoholic beverages, Takju is the fastest-growing commodity in South Korea. The draft Takju (Makgeolli), which is prepared without sterilization, is enriched in viable *Lactobacillus* sp. and nutrients however, its short shelf life limits the marketing area to around the brewing plant. To solve this problem, a cold distribution system and the use of diverse components for alcohol production are

being implemented. Traditional Korean rice wines are prepared by cooking the rice, adding Nuruk as a starter, and then allowing the mixture to ferment (Bae HJ 2003, Bae SM 2009, Cho *et al* 2004, Jeong & No 2004, Shon *et al* 1990). Although the production and export size of Takju is growing and its quality is improving, relatively few studies have been performed on rice. Rice, as the source of fermentative carbohydrate and nutrients for the Nuruk starter, has a major contribution in the resulting characteristics of the final Takju product. These characteristics make Takju distinct, in terms of flavor, from alcoholic products derived from fruit and vegetable products. The starter culture for Takju fermentation consists of a mixture of microorganisms, such as bacteria, yeasts, and fungi. Some important microbes believed to be responsible for Takju fermentation include *Lactobacillus* species, *Leuconostoc* species, yeasts, and *Rhizopus* species. The latter is responsible for production of the saccharogenic enzyme, whereas yeast promotes ethanol

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fermentation. Therefore, yeast strains are selected based on the required brewing characteristics such as fermentation rate taste and flavor of the final product fusel oil, ester, and organo sulfur production consistently high cell viability and ethanol production and tolerance (Stewart & Russell 1985).

Haedanghwa (*Rosa rugosa* Thunb.) is a native rose species of Korea that grows on coasts, often on sand dunes. It contains essential oils such as citronellol, geraniol, nerol, phenyl ethyl alcohol, *cis*-3-hexenyl acetate, eugenol, quercitrin, tannin, and gallic acid (Hashidoko *et al* 2002, Lee *et al* 2004, Lee & Seo 2008). Haedanghwa is used for tea and beverage (Kong *et al* 2003) its volatile flavor component (Lee & Seo 2008) and antibacterial and antiviral activities (Kim *et al* 2007) were published as patents. However, the use of Haedanghwa is limited by its season-dependent production its short shelf life due to the soft texture of fruits and its fast spoilage rate during harvest, distribution, and storage. Therefore, food products using Haedanghwa fruits are processed and fermented for distribution and storage (Lee *et al* 2003a,b). In addition, because of its antibacterial activity and the lack of a carbon source, the use of Haedanghwa in fermented food products require the supplementation of an extra material that would serve as the carbon source. In this study, we investigated the fermentation characteristics of Takju prepared using Haedanghwa and rice, with or without external steam application.

METHODOLOGY

1. Materials

Haedanghwa (*Rosa rugosa* Thunb.) fruits were harvested in Samcheok (Kangwon, Korea) in 2007. The fruits were washed and dried before use. The rice was harvested in Kimje (Chunbuk, Korea) in October 2007. For Haedanghwa-uncooked rice fermentation, the rice (3 kg) was soaked in water (3.8 L) at 20°C for 10~12 hr, and was ground using a mixer (HMF 370, Hanil Electric Co., Seoul, Korea) for 1 min. For Haedanghwa-cooked rice fermentation, the soaked rice was cooked using an electric cooker (Cuckoo Electronics Co., Yangsan, Korea). Bio-Nuruk-R (Korea Enzyme Co. Ltd., Gyeonggi-do, Korea), containing yeasts (*Saccharomyces cerevisiae* Engevita™) and Nuruks, was used for Takju fermentation. Sucrose was purchased from Cheiljedang (Icheon, Gyeonggi-do, Korea) and used as carbon source for microbial growth. Potassium metabisulfite (K₂S₂O₅) was obtained from Sigma Co. (St. Louis, MO, USA), and used for sterilization of broth.

2. Preparation of Haedanghwa Sugar Extracts

The sliced Haedanghwa fruits (1 kg in 1 L distilled water) were ground by an electric mixer (HMF 370, Hanil Electric Co.) for 1 min, and were incubated with 4 mL of Termamyl 120 L (Novo, Denmark) with agitation for 2 hr at 80°C. Then, 4 mL of AMG 300 L (glucoamylase, Novo) obtained from *Aspergillus niger*, was added to the mixture in water bath for 2 hr at 65°C.

3. Fermentation of Takju Using Rice and Haedanghwa Extracts

For the fermentation using uncooked rice, Haedanghwa extract (150 mL) and ground rice (3 kg) were mixed with water to 10 L. For the fermentation using cooked rice, the soaked rice (3 kg) was cooked in an electric cooker and mixed with Haedanghwa extracts (150 mL) and water to 10 L. Subsequently, 60 g of dry yeast (*S. cerevisiae*) and 150 g of Nuruk were inoculated into both mixtures, which were then fermented at 25°C for 14 days (Table 1). Samples (50 mL) were taken during the fermentation period and were stored at -20°C for analysis.

4. Determination of Total Carbohydrate Contents and pH

The sugar contents of the fermented mixture were determined by a hand-held refractometer (model N-1 α , ATAGO, Japan) using a 50 μ L sample. The change in pH was measured by a pH meter (model 725p, Istek Co., Seoul, Korea).

5. Determination of Ethanol Content

The ethanol content of the fermented mixture was measured by a gas chromatograph (6890, Agilent Technologies, Inc., Santa Clara, CA, USA) with an HP INNO-Wax column (0.25 μ m, 30 m \times 0.25 mm, Agilent Technologies Inc.). The column temperature was programmed to hold constant at 35°C for 5 min, to increase to 150°C with 5°C/min and to 250°C with 20°C/min, and to hold at 250°C for 2 min. The injection volume was 10 μ L, and the temperature of the injection port was 225°C. The temperature of the detector port with the FID detector (split ratio 10:1) was 260°C.

6. Color Measurement

The color of the fermented mixture was determined by a spectrophotometer (UV-visible spectrophotometer UV-1650 PC,

Shimadzu, Japan) in a 10-mm quartz cuvette, with distilled water used as a blank. Total phenol content, hydroxycinnamate content, anthocyanin content, color intensity, and brightness were expressed as A_{280} , A_{320} , A_{520} , $A_{420} + A_{520}$, and A_{420}/A_{520} , respectively.

7. Analysis of Organic Acids

For the measurement of organic acids in the samples, the fermented mixture was centrifuged at 10,000 g for 10 min, and the colorants and proteins were removed by SEP-PAK cartridge. Organic acid was detected by a high-performance liquid chromatograph with C-610 column (30 cm × 7.8 mm ID, Supelco Co., Bellefonte, PA, USA), using 0.1% phosphoric acid as a mobile phase (0.5 mL/min), and was quantified as the absorbance at 280 nm.

8. Analysis of Amino Acids

The fermented mixture was centrifuged at 10,000 g for 10 min, and the supernatant was filtered with a 0.45- μ m membrane. Amino acids in the sample (20 μ L) were then analyzed (L-8800, Hitachi, Tokyo, Japan).

9. Statistical Analysis

All results were expressed as mean±standard deviation (SD) and were analyzed using 1-way analysis of variance (ANOVA) (Albright *et al* 1999). Results were considered statistically significant when *P* values were <0.05.

RESULTS AND DISCUSSIONS

1. Fermentation Characteristics of Haedanghwa Takju

Rice was prepared in two different ways, with or without

Table 1. Proportion of rice and *Rosa rugosa* Thunb. used for Takju preparation

	Haedanghwa-uncooked rice Takju	Haedanghwa-cooked rice Takju
Paste(mL) ¹⁾	150	150
Rice(g)	3,000	3,000
Water(mL)	6,700	6,700
Nuruk(g)	150	150
Yeast(g)	60	60

¹⁾ Paste: *Rosa rugosa* Thunb. paste

steaming process, then used for Haedanghwa Takju fermentation for 14 days (Table 1). The sugar content of Haedanghwa extract was 0.5°Bx, and the Haedanghwa extract and rice mixture was fermented without sugar supplementation. For Haedanghwa-uncooked rice Takju, the total sugar content rapidly increased from 5°Bx to 12°Bx for the first 2 days of fermentation, which was then maintained at 13~14°Bx during 14 days of fermentation (Table 2, Fig. 1). The pH of Haedanghwa-uncooked rice Takju decreased from pH 4.9 (0 day) to pH 4.3 (14 days), and the ethanol content increased to 11.8% at 14 days of fermentation (Table 2, Fig. 1). The initial total sugar content for Haedanghwa-uncooked rice Takju was 17.4°Bx, the highest value obtained, which then decreased to 14°Bx at 7 days until the end of fermentation (Fig. 1). This result shows that the starch in Haedanghwa-uncooked rice Takju was simultaneously liquefied and saccharified by Nuruk and yeasts. This increased sugar content, then subsequently utilized for ethanol fermentation. On the other hand, in the Haedanghwa-cooked rice Takju, the rice starch was converted to small-molecule carbohydrates by the cooking process before fermentation, and was easily used by Nuruk and yeasts for Takju fermentation thus, ethanol fermentation rapidly progressed in this process as compared with Haedanghwa-uncooked rice Takju.

Uncooked rice Tapioca fermentation, using amylolytic enzymes and yeasts, provided a 12~13% ethanol yield for 120 hr of fermentation (Jeong & No 2004). Moreover, Bae SM (2009) reported that uncooked rice Takju fermentation (25~

Table 2. Components of Haedanghwa-uncooked rice Takju and Haedanghwa-cooked rice Takju after fermentation at 25°C for 14 days

Absorbances	Haedanghwa-uncooked rice Takju	Haedanghwa-cooked rice Takju
Ethanol(%)	11.8	14.4
pH	4.3	4.6
°Bx	13.0	14.0
A_{280}	29.4	29.6
A_{420}	0.57	0.33
A_{520}	0.38	0.27
A_{320}	4.32	4.50
Color intensity	1.34	0.77
Shade	1.14	1.87

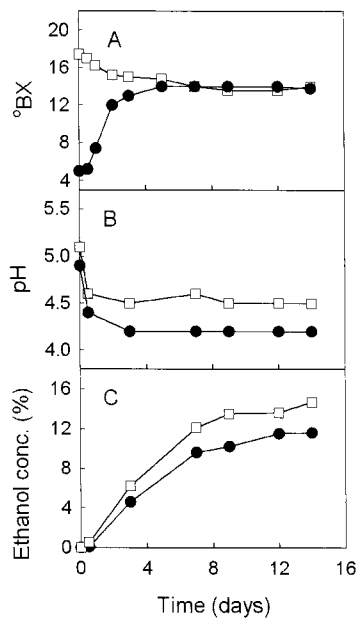


Fig. 1. Changes in sugar concentration (°Bx) (A), pH (B), and ethanol concentration (C) of Haedanghwa-uncooked rice Takju (closed symbol) and Haedanghwa-cooked rice Takju (open symbol) from fermentation at 25°C for 14 days.

27°C) using a 2-stage soaking method produced values of pH 4.0, 6.4°Bx sugar content, and 3.2% ethanol content at 1 day fermentation, and pH 4.5, 11.5°Bx sugar content, and 15.6% ethanol content at 6 days fermentation. Additionally, uncooked rice Takju fermentation by *Rhizopus delemar* 1476 produced 17% ethanol content (Bae HJ 2003), whereas uncooked and cooked rice Takju fermentation produced 16~17% and 14~15% ethanol content, respectively (Shon *et al* 1990).

Novel techniques for the improvement of ethanol production in uncooked-rice fermentation were recently reported. For example, low-temperature fermentation of Takju can produce more than 20% ethanol content (So & Yu 1993). The selective suppression of *Lactobacillus* and anaerobic microorganisms by a sterile air supply and an intermittent agitation produced a high ethanol yield and low-acidic Takju (Kim *et al* 2007). Fermentation of Takju usually completes within 7~10 days however, in this report, Haedanghwa Takju fermentation was allowed to progress over 12 days. This long-term fermentation of Haedanghwa Takju might be due to the antibacterial activity of Haedanghwa (Kim *et al* 2007). For the improvement of ethanol production in Haedanghwa Takju, low-temperature fermentation and an intermittent oxygen supply will need to be tested, and identification of antibacterial components in Haedanghwa is warranted.

The color of Takju is dependent on its ingredients. Brownness (A_{520}), color intensity ($A_{420} + A_{520}$), and brightness (A_{420}/A_{520}) represent the anthocyanin content, the presence of color, and the "orangeness", respectively (Kim SK, 1996). In addition, the absorbances at 280 and 320 nm indicate the total phenolic content and hydroxycinnamate level, respectively. The phenolic compounds in wine provide its characteristic color, taste, preference, and antibacterial activity (Hwang & Ahn 1975, Sarneckis *et al* 2006). Haedanghwa-uncooked rice Takju showed stronger color intensity than Haedanghwa-cooked rice Takju (Table 2).

2. Organic Acid and Amino Acid Contents of Haedanghwa Takju

Microorganisms in Nuruk are mainly molds, bacteria, and yeasts, but may vary from batch to batch. Among the various microbes in Nuruk, yeast is one of the main organisms responsible for ethanol production during Takju fermentation. Therefore, supplementation of yeast (*S. cerevisiae*) as a starter to Nuruk before ethanol fermentation may reasonably aid in the successful fermentation of Takju. Nuruk used for the fermentation of Takju contains many *Lactobacillus* sp. and aerobic microorganisms such as *Rhizopus* sp. These microorganisms produce organic acids, such as lactic, acetic, fumaric, and malic acids, during fermentation, which often cause spoilage, bad taste, and bad flavor. *Lactobacillus plantarum*, a homofermentative strain, and *Leuconostoc mesenteroides*, a heterofermentative strain, are also found in Nuruk (Seo *et al* 2007). Therefore, lactic acid from a homofermentative pathway and acetic and lactic acids from a heterofermentative pathway were also found in Takju. Lactic acid, a major organic acid, and acetic acid were found in Haedanghwa-cooked rice Takju and Haedanghwa-uncooked rice Takju. The acetic acid content was higher in Haedanghwa-uncooked rice Takju, with a significant difference (Table 3). On the other hand, the total organic acid content of Haedanghwa-uncooked rice Takju fermentation also had higher value but was not significantly different.

Amino acids play an important role in the resulting flavor and taste of wines. The major amino acids before fermentation were in the order of glutamic acid > arginine (Table 4). However, during Takju fermentation, amino acid compositions change to arginine > leucine > lysine > tyrosine > glutamic acid in Haedanghwa-uncooked rice Takju, and to glutamic acid >

Table 3. Changes in the concentration of organic acids (mg%, w/v) in Haedanghwa-uncooked rice Takju and Haedanghwa-cooked rice Takju after fermentation at 25°C for 14 days

Acids	Haedanghwa-uncooked rice Takju	Haedanghwa-cooked rice Takju
Citric+tartaric acid	9.5±16.5 ^{bb}	39.6±5.5 ^{ba}
Malic acid	41.2±10.3 ^b	49.9±7.3 ^b
Lactic acid	2,192.1±565.6 ^{aa}	1,581.3±207.6 ^{ab}
Formic acid	65.6±28.6 ^b	81.7±1.3 ^b
Acetic acid	80.7±4.4 ^{ba}	51.0±0.9 ^{bb}
Total	2,390.8±102.3 ^A	1,804.5±219.2 ^A

Values are the mean S.D. ($n = 3$) at the 95% confidence level.

^{ab}Means in the same column with different letters are significantly different for the 2 groups for each organic acid.

^{A,B}Means in the same row with different letters are significantly different for particular organic acids for each group.

arginine > leucine > lysine in Haedanghwa-cooked rice Takju. The total amino acid content of cooked rice Takju was reported to be in the range of 0.21~0.44%, depending on the cooking temperature (So & Yu 1993). Shon *et al* (1990) reported that the major amino acids in cooked rice Takju were arginine, proline, and leucine, whereas those in uncooked rice Takju were arginine, tyrosine, and histidine. In addition, the amino acids in cooked rice Takju were in the order of alanine > leucine > arginine (Song & Park 2003). These amino acids were released from protein materials and were also produced by microorganisms during fermentation (Cho & Rhee 1979). Alanine, glycine, serine, and threonine are related to the sweet taste, whereas leucine, phenylalanine, and valine produce the bitter taste (Cho & Rhee 1979). Therefore, this result shows that the pre-treatment method used on the rice affects the taste of Takju. Additionally, the level of GABA of uncooked rice-Haedanghwa Takju was twice than that of cooked rice-Haedanghwa Takju. GABA is known to reduce blood pressure and plays an important role as a neurotransmitter this organic acid can be found in fermented foods such as kimchi and germinated-brown-rice wine (Ji *et al* 2009).

국문초록

해당화 150 g을 각각 증자법과 무증자법으로 처리한 쌀 3,000 g에 첨가하여 막걸리를 제조하였다. 이때 스타터균으로 누룩(150 g)과 효모(60 g)를 사용하여 25°C에서 14일 동안

Table 4. Changes in free amino acids (mg/100 g of Takju) in Haedanghwa-uncooked rice Takju and Haedanghwa-cooked rice Takju after fermentation at 25°C for 14 days

Amino acid	Haedanghwa-uncooked rice Takju		Haedanghwa-cooked rice Takju	
	Day 0	Day 28	Day 0	Day 28
Isoleucine	6.5	37.2	8.5	37.3
Leucine	13.6	82.5	15.0	90.4
Lysine	10.5	71.1	9.8	74.3
Methionine	4.9	28.4	3.5	29.6
Cystine	0.2	3.9	0.4	2.0
Phenylalanine	9.1	60.2	10.1	68.0
Tyrosine	7.4	65.3	8.3	59.3
Threonine	3.8	28.0	6.8	19.7
Valine	10.1	44.5	14.2	44.1
Arginine	15.5	112.8	17.6	123.0
Histidine	2.9	28.1	4.1	23.2
Alanine	10.2	57.2	17.4	69.1
Aspartic acid	6.4	25.1	13.3	28.9
Glutamic acid	21.6	62.8	29.4	148.8
Glycine	2.5	31.0	4.4	36.7
Proline	2.7	40.7	3.3	40.6
Serine	5.5	41.6	10.5	33.6
GABA ¹⁾	4.5	24.4	10.9	10.1
Total	137.9	844.8	187.2	938.7

¹⁾ GABA, γ -Amino-*n*-butyric acid.

발효하였다. 무증자법으로 제조한 막걸리는 당도 13.0°Bx, pH 4.3, 에탄올 농도 11.8%였으며, 증자법으로 제조한 막걸리는 당도 14.0°Bx, pH 4.6, 에탄올 농도 14.4%였다. 최종 막걸리의 유산과 초산 함량은 무증자법으로 제조한 막걸리에서 유의적으로 높게($p < 0.05$) 나타났다. 또한, 아미노산 분석에서는 무증자법으로 제조한 막걸리에서 γ -amino-*n*-butyric acid 함량이 증가하여 생리 기능성이 향상된 결과를 보였다. 결론적으로, 본 연구 결과는 쌀의 전처리 공정이 막걸리의 맛과 기능성에 큰 영향을 준다는 것을 보여준다.

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