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Quality Characteristics of Apple Wine Fermented with *Rosa rugosa* Thunb.

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해당화로 가향한 사과 발효주의 품질특성

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Abstract

Changes in physiochemical properties and polyphenol activities of wine fermented with apples and *Rosa rugosa Thun* were investigated. To this end, four different mixing ratios of apple and *R. rugosa*, including *R. rugosa*:apple 2:1 (Apple 33), *R. rugosa*:apple 1:2 (Apple 67), *R. rugosa*:apple 1:5 (Apple 83), and apple alone (Apple 100), were prepared and fermented by *Saccharomyces bayanus* for 14 days at 24℃ with a further 14 days of post-fermentation at 20℃. The final ethanol content ranged from 8.2 - 10.2%, with no significant difference between the four groups after fermentation and post-fermentation. Final Brix, pH, and total acidity of the four samples ranged from 7.1 - 7.5 Brix, pH 3.85 - 4.07, and acidity 0.73 - 1.19%. As the proportion of *R. rugosa* increased, the anthocyanin content and color intensity of wine also increased, whereas the free amino acid concentration decreased.

Key words : apple, fermentation, malic acid, *Rosa rugosa* Thunb., wine

Introduction

With increasing interest in the well-being life style, fermentation beverage has been received attention. According to the annual beverage consumption report in 2007 by National Tax Service, the wine consumption in Korea was 3.8×10^7 L in 2007, which is 2.5 times higher than that in year 2000(1).

Among various fruits, apple (*Malus domestica*) is currently one of the most cultivated fruits in Korea. Most apple grown in Korea are of the Fuji varities, but other varities, including Ryohong, Seokwang, and Mihong, are also available. It is a rich source of vitamin C, minerals, sugars, organic acids, cellulose, and pectic substances(2). Apple usually consumed as raw material but also can be used for making juice, vinegar, and wine(3). It is very difficult to obtain an apple variety which would have all of the important constituents, such as carbohydates, color and flavor, organic acids, and phenolic compounds, in an ideal proportion. Therefore, a combination of varieties should be used to obtain the most desirable composition in the apple wine(4).

Korean medicinal herbs including Nidium offoconale, Paeonia lactiflora Pall, Chrysanthemi Flos, Zizyphi Fructus, Glycyrrhiza glabra, Angelica acutiloba Kitag, Lycii Fructus, and Astragallus Membranaceus, were used for the rice wine fermentation(5). Yoon *et al.*(6) demonstrated that the supplementation of ginseng into the mash improved the flavor of Korean rice wine. Using the similar strategy, mixed fermentation system containing *Rosa rugosa* Thun and apple was applied in the present study. *Rosa rugosa* Thun, which contains phenolic compounds, has been used as a traditonal

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medicine in Korea and Japan(7-8). *Rosa species* are rich in essential oils and rose essential oils are applicable for fragrance and production of perfumes and cosmetic. It is used as a flavor in tea and liquire(9). It has been reported that *Rosa rugosa* Thun has antioxidant, antiobesity, and antidiabetes activities(7-8).

This study was aimed to investigate the physiochemical properties and polyphenol activities of wine fermented with apple and *Rosa rugosa* Thun.

Materials and Methods

Materials

Apple (Fuji, *Malus domestica*) was purchased from a commercial market in Jecheon-si (Chungbuk province, Korea) in 2006. Fruits of *Rosa rugosa* Thunb. was harvested in Samcheok-si (Kangwon province, Korea) in 2007. *Saccharomyces bayanus* was from Red Star Premier Cuvee (LeSaffre, France). Sugar and $K_2S_2O_5$ were from Cheiljedang (Seoul, Korea) and Sigma (St. Louis, MO., U.S.A.), respectively.

Preparation of Enzymatic Lysate of *Rosa rugosa* Thunb.

To prepare enzymatic lysate of *Rosa rugosa* Thun paste, *Rosa rugosa* Thun was trimmed, cut into 4 pieces, mixed together with water at a ratio of 1:1 (*Rosa rugosa* Thun/water), and then homogenized together using a mixer (HMF 370, Hanil Electric Co., Seoul, Korea) for 1 min. After supplementation of 4 mL of α amylase (Termamyl 120L, Novo Nordisk, Denmark) to 2 L of the mixture, *Rosa rugosa* Thun paste were incubated at 80℃ for 2 hr. Subsequently, after cooling down temperature to 65℃, 4 mL of gluco-amylase (AMG300L, Novo Nordisk) was supplemented to the mixture, then further incubated at 65℃ for 2 hr. This was used as the source of enzymatic lysate of *Rosa rugosa* Thun paste.

Fermentation

As seen in Table 1, four different mixing ratios of apple and *Rosa rugosa* Thun, including *Rosa rugosa* Thun two vs. apple one (Apple 33), *Rosa rugosa* Thun one vs. apple two (Apple 67), *Rosa rugosa* Thun one vs. apple five (Apple 83), and apple (Apple 100), were prepared. Concentrations of sugar in four groups in 1.6-L fermentor were adjusted to 24 °brix using sucrose, then supplemented by the addition of 0.5 g of pectinase (Pectinex 100 L, Novozyme, Denmark) and 0.2 g of $K_2S_2O_5$. After incubation at room temperature for 16 hr, fermentation was started by the addition of 0.5 g of yeast to the fermentor. Fermentation was continued at 23-24℃ for 14 days, and post-fermentation process was followed at 20℃ for 14 days.

Table 1. Proportion of *Rosa rugosa* Thunb.-apple for wine brewing

	Apple 33[1)]	Apple 67	Apple 83	Apple 100
Rosa rugosa Thunb. (mL)[2)]	500	250	125	
Apple (mL)	250	500	625	750
Pectinase (g)	0.25	0.25	0.25	0.25
$K_2S_2O_5$ (g)	0.1	0.1	0.1	0.1

[1)] *Rosa rugosa* Thun two vs. apple one (Apple 33), *Rosa rugosa* Thun one vs. apple two (Apple 67), *Rosa rugosa* Thun one vs. apple five (Apple 83), and apple (Apple 100).
[2)] Enzymatic lysate of *Rosa rugosa* Thun paste were prepared as described in the Materials and methods section.

General Analysis

The pH vlaues was determined using a pH meter (lstek Co., Seoul, Korea). The titratable acidity (TA) was determined by titrating 10 mL of the filtrate with 0.1 N NaOH to a pH of 8.0, and was expressed as the quantity of tartaric acid produced in wine. Sugar concentration(°brix) was determined using a hand-held refractometer (model N-1α, Atago, Japan). Concentration of frucose, glucose and sucrose were determined using high performance liquid chromatography (HPLC). Briefly, wine was passed through a 0.44 ❍m filter, then 20 μL of the filtrate was injected directly into the HPLC (LC-10 Avp, Shimadzu, Tokyo, Japan). The HPLC was equipped with a column (Kromasil 100-10NH2 column, Eka Chemicals, Bohus, Sweden). The HPLC conditions were as follows: mobile phase, acetonitrile 75%/water 25%; flow rate, 1.0 mL/min; detector, refractive index; running time: 30 min. For determination of the ethanol content, wine were centrifuged at 10,000 × g for 10 min, then the supernatant was passed through the 0.45 μm filter. The filtrate of eluted was injected directly into a gas chromatograph (6890, Agilent Technologies Inc., Santa Clara, CA, U.S.A.) equipped with flame ionization detector. HP Innowax column(0.25 μm, 30 m × 0.25 mm, Agilent Technologies Inc.) was used for the analysis. The oven temperature was programmed from 35℃ (5 min) to 150℃ (0 min) at a rate of 5℃/min and then to 250℃ (2 min) 20℃/min. Injection volumn was 10 μL and split ratio was 10:1. Injection port temperature and detector port temperature were 225℃ and 260℃, respectively. Coloring degree and ultraviolet absorption were obtained by absorbance measurement at 280, 320, 420, and 520 nm, respectively using a UV-1650 PC UV-visible spectrophotometer

(Shimadzu, Japan) and 10 mm glass cuvette.

Enumeration of Yeast

Following sampling, 10-fold serial dilutions of cells were made and appropriate dilutions spread onto YPD agar(Difco, St. Louis, MO, U.S.A.) to enumerate viable cells of the yeast. Numbers were counted in triplicate after incubation at 30℃ for 36 hr. Results of viable cell counts were presented as the average values of colony forming units (CFU) per mL of wine.

Sensory Evaluation

After the post-fermentation period was terminated, *Rosa rugosa* Thun-apple wines were stored in a refrigerator for 20 hr. The sensory evaluation was conducted with 21-student panel of university. Nine-point hedonic scales (1 = dislike extremely, 9 = like extremely) for color, taste and flavor were used.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) at 95% level of significance(10). All experiments were replicated twice and all analyses were carried out in triplicates. The results presented are a mean of 6 observations ± standard deviations (SD), unless otherwise stated.

Results and Discussion

Characterisitics of Apple and *Rosa rugosa* Thunb.

At the most beginning of the fermentation, the viable yeast cells was $6.4 \times 10^5 \pm 1.2 \times 10^2$ CFU/mL. In group Apple 100, the most abundant sugar was fructose (6.6%), but glucose (4.4%) was also found. pH in apple and enzymatic lysate of *Rosa rugosa* Thunb. paste were 3.9 and 4.0, respectively. Enzymatic lysate of *Rosa rugosa* Thunb. paste contained 1.7% fructose and 1.6% glucose.

pH and Titratable Acidity

As seen in Table 2 and 3, the pH increased during the fermentation, then remained relatively constant during the following 14 days in post fermentaton. As increased the proportion of *Rosa rugosa* Thunb. in apple wine, final pHs were significantly decreased (Table 3). In contrast, titratable acidity was higher in group Apple 33 than in group Apple 100 (Table 3). Chung *et al.*(11) reported that final pH of apple wine made from Fuji apple with a concentration of 20 °brix was 3.81 after 14 days in fermentation at 25℃. According to the previous results by others, the titratable acidity varied dependent upon several factors. These include: apple varieties, time of harvest, growing conditions, storage and maturation (12-13).

Table 2. Changes in pH and sugar concentration during alcohol fermentation for 14 days at 24℃

Time (day)	Apple 33[1)]	Apple 67	Apple 83	Apple 100
pH				
5	3.81 ± 0.02^{aA}	3.86 ± 0.01^{abA}	3.86 ± 0.01^{abA}	3.96 ± 0.06^{bA}
14	3.87 ± 0.01^{aA}	3.96 ± 0.04^{abB}	3.98 ± 0.03^{abB}	4.80 ± 0.04^{bA}
Sugar (°Brix)				
5	11.0 ± 0.0^{aA}	11.5 ± 0.7^{aA}	12.4 ± 0.6^{aA}	14.0 ± 0.0^{bA}
14	7.5 ± 0.7^{aB}	8.0 ± 0.0^{aB}	7.5 ± 0.1^{aB}	7.0 ± 0.0^{aB}

[1)] See Table 1.
Values are the means of 3 observations at 95% level of confidence.
[abc] Means in the same column with different alphabets are significantly different within a particular treatment.
[ABC] Means in the same row with different alphabets are significantly different for a particular day of fermentation.

Ethanol Concentration and Brix

Final ethanol concentration ranged between 8.20~10.20, but there was no significant difference among the group. Chung *et al.*(14) prepared an apple wine by propagation at 25℃ for 2 weeks using *S. cerevisiae* KCCM 12224, following aging process at 15℃. These authors found that the ethanol concentration was changed from 7% at the most beginning of aging to 12% at the 8 weeks in aging. By the same authors, it was also found that there were little change in titratable acidity and pH during aging, whilst sugar concentration was gradually reduced as increased the aging. As far as sugar content is concern, a decrease during fermentation and post-fermentation appears to be the general trend of changes(13-14). Thus, the present results seen in Table 2 and Table 3 are also supportive to the results reported previously.

Phenolic Compounds

In the apple wines, amounts of total phenolic compounds(A_{280}) were significantly increased in the dose dependent manner, as increased the ratio of *Rosa rugosa* Thunb. to apple(Table 3). In group Apple 33, total phenolic compounds was more than 2 times higher than that of group Apple 100. Anthocyanins (A_{520}) and color intensity ($A_{420}+A_{520}$) were tendency to increase as increased the ratio of *Rosa rugosa* Thun to apple, but there was no significant difference

among the groups. In cases of hydroxycinnamate (A_{320}) and browning (A_{420}), there were no significant difference among the groups. It was reported by Campo *et al.*(13), who were working with apple, the values of browning (A_{420}) in the five apple juices were ranged 0.29~3.56. Phenolic compounds are secondary metabolites of plant(15-16). Apples are rich in phenolics and contain substantial amounts of hydroxycinnamate derivatives, anthocyanins, flavonols, cyanidins, and dihydrochalcones(15,17). It was reviewed that apple-derived products in which anthocyanins are the main compounds responsible for the colour are cyanidin-3-glucoside, cyanidin-3-galactoside, and cyanidin-3-arabinoside (17). It was also suggested that there may be an association between the intake of phenolic compounds and protection against cancer and coronary heart disease(15,17). These compounds are also relevant to the appearance, taste and flavour of food products(15,17).

Table 3. Component of *Rosa rugosa* Thunb.-apple wine from fermentation at 24℃ for 14 days and post-fermentation at 20℃ for 14 days

Component	Apple 33[1)]	Apple 67	Apple 83	Apple 100
Ethanol(%)	8.40±2.97[a]	8.35±5.30[a]	10.20±4.67[a]	8.20±1.41[a]
pH	3.85±0.01[a]	3.93±0.01[b]	3.99±0.01c	4.07±0.01[d]
Total acidity(%)	1.19±0.08[a]	0.96±0.03[b]	0.82±0.04[c]	0.73±0.01[c]
Brix(%)	7.5±0.6[a]	7.1±0.1[a]	7.2±0.3[a]	7.5±0.1[a]
A_{280}	26.33±0.67[a]	19.33±2.39[b]	15.46±2.11[bc]	10.50±1.41[c]
A_{320}	8.52±0.13[a]	5.64±1.18[a]	6.62±0.56[a]	6.61±0.01[a]
A_{420}	0.76±0.25[a]	0.49±0.23[a]	0.35±0.15[a]	0.26±0.02[a]
A_{520}	0.32±0.05[a]	0.25±0.04[ab]	0.20±0.03[ab]	0.08±0.03[b]
Color intensity ($A_{420}+A_{520}$)	1.08	0.74	0.55	0.34

[1)]See Table 1.
Values are the means of 3 observations at 95% level of confidence.

Changes in Free Amino Acids

Free amino acids have been implicated as being responsible for the characteristic flavors of wine. Amino acid are produced by breaking down protein sources in fruits, but some of amino acids can be produced during fermentation(18-20). Some of amino acids (alanine, glycine, serine and threonine) confer a sweet taste. Leucine, phenylalanine, and valine confer a bitter taste. In apple juice, asparagine is most abundant, and aspartic and glutamic acids are other prominent amino acids(2). Ackerman *et al.*(2) reported that the total amino acid content of apple (cv. Glockenapfel) was 41 mg in 100 g of fresh weight of apple. According to the published report, Korean apple contains 2,400 mg of aspartic acid and 610 mg of glutamic acid per gram of nitrogen, respectively(21). In the present study, 33-100% of the wine is occupied respectively by apple. Amino acid analysis revealed that the concentration of free amino acids and their related compounds in group Apple 100 was 80.2 mg/100 g of wine (Table 4). After 4 weeks in fermentation and following post-fermentation, tyrosine was the dominant amino acid in group Apple 100, and cysteine and lysine followed (Table 4). Similar trends were seen in other three groups. Overall, total free amino acid in wine was high in group Apple 100 than in group Apple 33, indicating that supplementation of *Rosa rugosa* Thun to apple wine reduced the sensory characteristics of apple wine generated by free amino acids.

Table 4. Changes in free amino acids (mg/100 g of wine) in *Rosa rugosa* Thunb.-apple wine from fermentation at 24℃ for 14 days and post-fermentation at 20℃ for 14 days

Amino acid	Apple 33[1)]	Apple 67	Apple 83	Apple 100
Tyrosine	7.64	11.75	11.62	48.35
Aspartic acid	0.08	0.13	0.21	0.03
Treonine	0.04	0.22	0.10	0.12
Serine	0.06	0.06	0.06	0.08
Asparagine	0.46	0.56	0.42	0.48
Glutamic acid	0.09	0.47	0.24	0.36
Glycine	0.26	0.41	0.44	0.69
Alanine	0.39	0.98	0.92	2.17
Valine	0.05	0.22	0.19	0.09
Cysteine	4.84	13.68	16.56	13.63
Methionine	0.06	0.08	0.09	0.06
Isoleucine	0.04	0.08	0.11	0.08
Leucine	0.10	0.37	0.31	0.18
Phenylalanine	0.12	0.12	0.14	0.10
Lysine	4.95	6.46	22.20	13.11
Histidine	0.03	0.03	0.03	0.03
Arginine	0.19	0.13	0.11	0.29
Proline	0.34	0.16	0.27	0.37
Total	19.68	35.87	53.96	80.22

[1)]See Table 1.

Sensory Evaluation

The mean sensory scores of four *Rosa rugosa* Thunb.-apple wine are given in Table 5. Since the result showed no significant difference between wines, we concluded that there were not any quality differences among these four groups. The typical sensory attributes of apples wine depends not

only acidity but also the taste sensations. In the present work, supplementation of *Rosa rugosa* Thunb. to apple wine prior to fermentation increased acidity but decreased free amino acids. In order to get more accurate information, different concentrations of apple to *Rosa rugosa* Thunb. wine prior to fermentation ranging from 83% to 100% are warranted. It is important to note that *Rosa rugosa* Thunb. is rich in terpenoid compounds, tocophenols and carotenes, in addition to sugars and ascorbic acid(22). The fruit color is mainly due to beta-carotene and lycopene(22). Beta-carotene act as a radical scavenger and a physical scavenger of singlet oxygen and is believed to have a protective role against cancer(23). The amount of beta-carotene in apple juice and *Rosa rugosa* Thun ranged from 1.4~2.3 μg/100 mL(23) and 13.8 mg/100 g in *Rosa rugosa* Thunb.(24), respectively.

In conclusion, the mixed combinations of *Rosa rugosa* Thunb.-apple wine on physiochemical properties and polyphenol activities of wine were investigated. As, increased the ratio of *Rosa rugosa* Thunb. to apple, the wine became sour with increase in polyphenol activities. Although the sensory score was not different between four *Rosa rugosa* Thunb.-apple wines, supplementation of *Rosa rugosa* Thun to apple juice prior to fermentation may help to improve the functionality of the apple wine.

Table 5. Sensory evaluation of *Rosa rugosa* Thunb.-apple wine from fermentation at 24℃ for 14 days and post-fermentation at 20℃ for 14 days

Sensory score[1)]	Apple 33[1)]	Apple 67	Apple 83	Apple 100
Taste	3.24±1.55[2)]	3.57±1.33	2.95±1.43	3.43±1.57
Color	5.95±1.07	5.48±1.08	5.71±1.15	5.67±1.91
Aroma	4.19±1.21	4.33±1.20	4.33±1.49	4.33±1.39

[1)]1=dislike extremely, 9 = like extremely.
[2)]Means in the same column with different alphabets are significantly different within a particular treatment.

요 약

사과와 해당화의 혼합비율을 4가지로 달리하여 제조후에 14일 동안의 발효와 다시 14일 동안의 숙성기간을 거쳐서 사과 해당화주를 만들었다. 4가지 그룹에서 발효와 숙성중에 에탄올 함량에서 유의적인 차이는 없었으며, 최종적인 에탄올 함량은 8.20~10.20%으로 나타났다. 4가지 그룹에서 최종제품은 각각 7.1~7.5 °brix, pH 3.85~4.07, 산도 0.73~1.19%를 보였다. 해당화즙의 첨가는 사과주의 anthocyanin 함량, 색도를 강화시키는 효과가 있는 것으로 나타났으나, 관능검사에서는 유의적인 차이는 보이지 않았으므로 해당화즙의 함량을 낮추어서 사과 해당화주의 기호성을 증가시키는 농도를 찾는 후속연구가 요구된다.

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