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Effect of Pectinase Treatment on the Extraction Yield Improvement from *Rubus coreanus* Juice and Physicochemical Characteristics during Alcohol Fermentation

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Pectinase 처리가 복분자 과즙의 추출 수율 향상과 알코올 발효 중 이화학적 특성에 미치는 영향

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Abstract

The effects of pectinase treatment and other processing conditions on juice yield from *Rubus coreanus*, and physicochemical changes during alcohol fermentation, were investigated. The yield from *R. coreanus* increased by 8.60% with Pectinex 100L treatment (500 ppm, 30 min) compared to a control group. The soluble solid content in the group fermented at 24 °Brix by addition of sucrose (24B-group), and the group fermented at 8 °Brix by addition of 16% sucrose after 4 days of fermentation (8B-group) decreased to 8.2～8.3 and 7.7～8.0% after 10 days of fermentation, respectively, and no significant differences were observed with Pectinex 100L treatment. Initial titratable acidity in the enzyme treatment was slightly higher (1.18～1.22%) than for the control group (1.02%). The initial L* and b* values of *R. coreanus* juice decreased with enzyme treatment, and the a* value increased, but the color difference (ΔE) between the control and enzyme treatment gradually decreased with fermentation time. The ethanol contents in the 24B-group and the 8B-group were 16.01～16.22% and 13.29～13.52%, respectively, after 10 days of fermentation. The methanol contents in the enzyme treatment and the control were 0.359～0.404 and 0.520～0.604 mg/mL, respectively, and within standard regulations (1 mg/mL).

Key words : *Rubus coreanus*, pectinase, yield, sugar addition, alcohol fermentation

Introduction

Rubus coreanus (*Bokbunja*) is a deciduous prickly shrub belonging to the family Rosaceae which is grown on sunny slopes of mountains in southern Korea. The shrub reaches 2～3 m in height and has thorny branches covered characteristically by a white powder. The fruit of *R. coreanus* forms a black compound with a half moon shape by gather bunch. It flowers from May to June and the black fruits are fully ripened in July and August(1). The fruit of *R. coreanus* is edible, and is known to have beneficially effects on kidneys, reproduction, robustness, clearing of the blood, and strengthening of the liver(2). Unripe fruit is often boiled and used as a medical herb in Korea. In addition, fully ripened fruit of *R. coreanus* is used to make local wines(1).

The composition of *R. coreanus* is different in different varieties, but in general it contains 87.09% moisture, yields

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pH 3.52 with 1.03% total acidity, 1.37% protein, and 1.52% fat(1). The major free sugars are sucrose 1.52%, fructose 3.98%, and glucose 1.23%(3). The major organic acids are citric, succinic, and fumaric acid(1). Physiological properties of *R. coreanus* are typically high content of total polyphenolic compounds in the leaf and ripe fruit and high levels of superoxide dismutase (SOD). The fruits also show antimicrobial activities against *Bacillus cereus*(4) and its extracts have antioxidative effects(5). Compounds isolated from the fruits have been confirmed as gallic acid, 3-(*S*)-HHDP-D-glucopyranose, sanguiin H-4, and sanguiin H-6(6). In addition, metabolites of lactic acid fermentation of *R. coreanus* are known for their electron donating ability, SOD activities, nitrite scavenging effects, and antimicrobial activities(7). Cardiovascular activities of other Korean traditional wines and liquors have also been reported(8).

The *R. coreanus* wine industry is developing in Korea in keeping up with an increasing consumption as alcoholic liquor. The color of the fruits is similar to that of grapes used in grape-based wines, and the flavor compares favorably to the grape-based wines. The yield of *R. coreanus* juice, however, is low for the price of *R. coreanus* starter fruit. The application of heat to grape juice(9) and combination of heat and enzyme treatments for peach-based wine production(10) reportedly increase the yield of fruit juice. Further, the enzyme pectinase effectively clarifies apple juice(11,12), persimmon vinegar(13), and the processing of most juices(14,15).

In this study we have estimated the effects of pectinase treatment on improving the juice extraction yield of *R. coreanus* and to characterize the physicochemical characteristics of the juice during alcohol fermentation.

Materials and Methods

Materials and microorganism

R. coreanus fruits were obtained from Asan National Agricultural Cooperative Federation (Gochang, Korea) and stored at -20℃ until use. Pectinex 100L (pectintranseliminase + polygalacturonase + pectinesterase, standard activity of 5,000 FDU/mL) and Viscozyme L (arabanase + cellulase + β-glucanase + hemicellulase + xylanase, unit 100 FBG/g) were obtained from Novozyme Co. (Bagsvaerd, Denmark), and the Rapidase C80 MAX (pectinase + arabanase, unit 132,000 AVJP/g) and Rapidase press (pectinase + hemicellulase, unit 180,000 AVJP/g) were obtained from Gist-Brocades Co. (S.A, France).

The *R. coreanus* fruit used for enzyme treatment and alcohol fermentation was thawed by slow warming in an incubator at 25℃ and chopped by a Waring blender (Blender 31BL91, Waring Co., New Hartford, NY, U.S.A.) for 10 sec.

Wg-15 yeast, which has a high alcohol productivity and acceptable flavor as noted in a preliminary study, was isolated from a locally produced wild-grape wine and used for alcohol fermentation of the *R. coreanus* juice. Yeasts were grown at 30℃ for 18 hr in malt extract broth and agar medium (Oxoid, Basingstoke, Hampshire, England).

Enzyme treatment and yield measurement

Pectinex 100L, Viscozyme L, Rapidase C80 max, and Rapidase press were used to study yield improvement of the *R. coreanus* juice. The conditions of enzyme treatment were 0, 50, 100, 500, and 1,000 ppm of concentration (w/w), and 30, 60, and 120 min at 30 or 50℃ (optimum temperature of each enzyme). Twenty grams of ground *R. coreanus* fruit were transferred to a centrifuge tube, treated with the enzymes, and repeat centrifuged (Model J2-21, Beckman Instruments, Inc., Palo Alto, CA, U.S.A.) for 10 min at 3,024×g, and the supernatant was then weighed. The calculation of extraction yield was calculated using the formula: Extraction yield (%) = [weight of supernatant / weight of sample (20 g)] × 100.

Alcohol fermentation

The Pectinex 100L that had demonstrated excellent results previously in our enzyme treatment test was applied to *R. coreanus* juice (8% soluble solid) at a level of 500 ppm at 50℃ for 30 min. The enzyme was inactivated by heating at 85℃ for 3 min and then combined with $K_2S_2O_5$ (200 ppm), and each group (treated and control) was divided into two subgroups; the group fermented at 24°Brix by sucrose addition at the initial stage (24B-Group), and the group fermented at 8°Brix without sucrose addition at the initial stage and added with 16% sucrose after 4-days of fermentation (8B-Group). The control group also received 200 ppm of $K_2S_2O_5$. These groups were then inoculated with 3% (v/v) of culture fluid of the Wg-15 yeast grown in a malt extract broth (Oxoid Ltd., Basingstoke, Hampshire, England) for 18 hr. Changes in physicochemical characteristics were analyzed at 2 days-intervals during alcohol fermentation for 10 days at 25℃.

Soluble solid content, pH, and titratable acidity

Soluble solid content was presented as % using a refractometer (Atago Co. Ltd., Tokyo, Japan) and the pH values were measured using a pH meter (Orion Research Inc. 520A, Beverly, MA, U.S.A.). Titratable acidity was determined as the amount of citric acid by titration with 0.1 N NaOH solution to a pH of 8.3.

Color measurement

The CIE L*a*b* values were measured using a color and color difference meter (SP-80, Tokyo Denshoku, Tokyo, Japan). L* value is a measure of lightness and varies from 0 (black) to 100 (white); a* value varies from -100 (green) to +100 (red); and b* value varies from -100 (blue) to +100 (yellow). △E is the color difference of control group and enzyme treatment group. △E value is 0 to 0.5: few color difference, 0.5 to 1.0: a shade of color difference, 1.5 to 3.0: the sensible color difference, 3.0 to 6.0: distinguished color difference, 6.0 to 12.0: more distinguished color difference, and over 12.0: a different color(16).

Ethanol and methanol analysis

Ethanol and methanol content was analyzed by gas-chromatography using a sample pretreated by the distillation method(17). Twenty-five mL of distilled water was added to 100 mL of fermented wine and distilled on a heating mantle (Hana Co., HMI-F200, Seoul, Korea) until distilled to 90 mL volume. The volume was adjusted to 100 mL by adding distilled water. A gas chromatograph (Agilent 6890N, Agilent, Palo Alto, CA, U.S.A.) equipped with a HP-innowax (30 m × 0.32 mm × 0.25 μm, capillary column) was used for the ethanol and methanol analysis. Other conditions of operation included: N_2 carrier gas at a flow rate of 30 mL/min; oven temperature of 40℃ (hold 2 min) - 2℃/min - 80℃ (hold 1 min); injector temperature of 250℃; and a detector temperature of 260℃. The injection volumes were 1 μL with a spilt ratio of 30:1.

Statistical analysis

Data was analyzed using the Statistic Analysis System package software(18) for the analysis of variance to determine differences between samples. When applicable, Duncan's multiple comparison was performed to separate the means of test samples. Evaluations were performed in triplicate. A probability level of less than 0.05 was taken to indicate statistical significance.

Results and Discussion

Changes of yield by enzyme treatment

The changes of yield due to enzyme treatment are shown in Fig. 1. The yield of *R. coreanus* juice extract not treated with enzyme ranged from 52.36 to 52.42% depending on the treatment duration and increased from 1.97% to 8.60% according to enzyme treatments at levels of 500 or 1,000 ppm. The extraction yield was the highest at 60.98% (8.60% increases) by the treatment of Pectinex 100L at 500 ppm for 120 min among treatment groups, but this was not a statistical difference from the 1,000 ppm treatment (60.52%). Yields were increased by 6.76~7.20% by Viscozyme L, 1.97~4.82% by Rapidase C80, and 6.24~7.52% by Rapidase

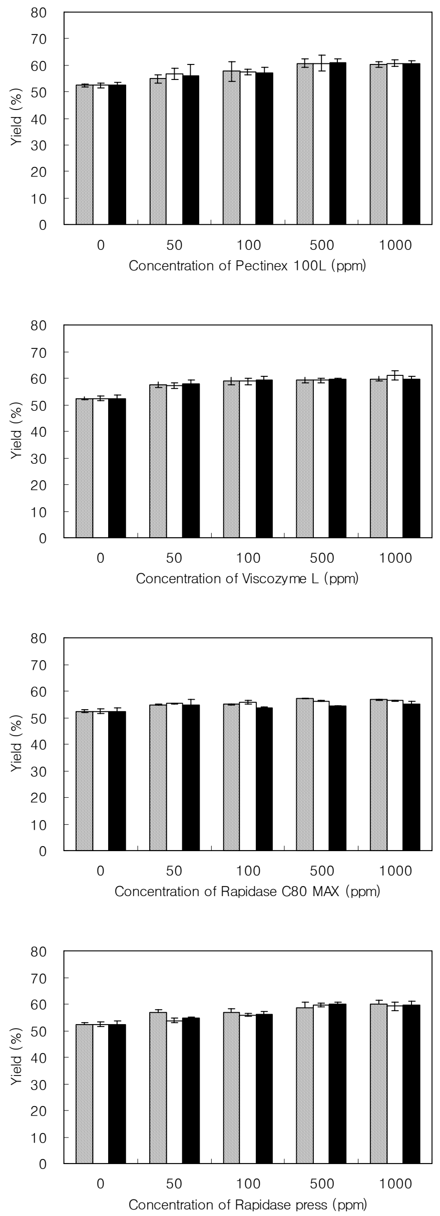

Fig. 1. Extraction yield of *Rubus coreanus* juice treated with various enzymes at different concentrations and time.

▨: 30 min, □: 60 min, ■: 120 min.
Vertical bars represent standard deviation (n=3).

press treatment depending on treatment time. Statistical differences did not occur in any treatment intervals (30, 60, 120 min) in all treatments. Peach juice was reportedly increased by 12.1% with a pectinase treatment for 8 hr using compressed method(10), which was 6 hr longer than our longest treatment time for the *R. coreanus* juice. The Pectinex 100L in our study was selected for ethanol fermentation at the level of 500 ppm for 30 min.

Change of soluble solid content, pH, and titratable acidity

Soluble solid content of the 24B-Group rapidly decreased from 24.0% at initial stage to 8.8% after a 4-day fermentation period, and remained steady thereafter (8.3~8.2%) (Fig. 2). The sugar content of the 8B-Group decreased from 8.0% at the initial stage to 4.0% after 4-days of fermentation. After 6-days of fermentation, however, this group increased to 15. 0~16.7% by the addition of 16% sucrose after 4-days of fermentation and decreased 7.7~8.0% after 10-days of fermentation, which was similar to measures for the 24B-Group. Again, no significant differences were observed by treatment of the fruit with pectinase.

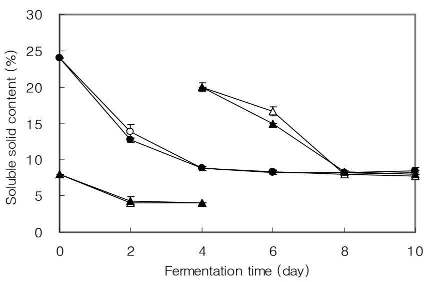

Fig. 2. Changes of soluble solid content during fermentation of *Rubus coreanus* wine treated with Pectinex 100L at 500 ppm for 30 min.

-○-: Not treated with enzyme, fermenting at 24°Brix soluble solid, -●-: Treated with enzyme, fermenting at 24°Brix soluble solid, -△-: Not treated with enzyme, fermenting at 8°Brix soluble solid and added with sucrose (16%) after 4-day of fermentation, -▲-: Treated with enzyme, fermenting at 8°Brix soluble solid and added with sucrose (16%) after 4-day of fermentation.
Vertical bars represent standard deviation (n=3).

The initial pH of the control group (not treated with pectinase) was slightly higher (pH 3.48~3.49) than those of enzyme treatment (pH 3.40~3.41) (Fig. 3). After 10-days of fermentation, the pH of the 24B-Group was slightly higher (3.63~3.65) than the 8B-Group (pH 3.54~3.58). However, the values of pH of 24B-Group and 8B-Group were not nearly as different from the control and enzyme treatment groups. Our results, therefore, were consistent with those reported by Kim and Kim(19), in that the pH of their new wild-grape wine increased during alcohol fermentation.

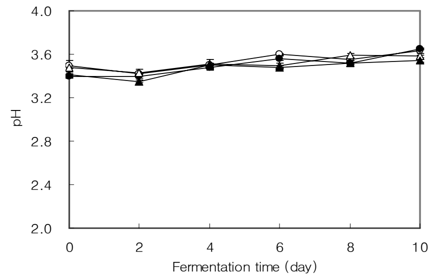

Fig. 3. Changes of pH in *Rubus coreanus* wine treated with Pectinex 100L during fermentation.

See Fig. 2 for symbols. Vertical bars represent standard deviation (n=3).

The initial titratable acidity (Fig. 4) of enzyme treatment group was slightly higher (1.18~1.22%) than that of the control group (1.02%). The titratable acidities of all treatments, however, except the control group of 24B-Group (1.09%) were similar (1.18~1.22%) after 10-days of fermentation.

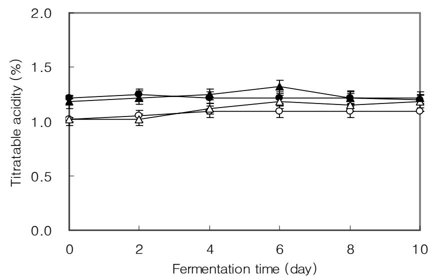

Fig. 4. Changes of titratable acidity in *Rubus coreanus* wine treated with Pectinex 100L during fermentation.

See Fig. 2 for symbols. Vertical bars represent standard deviation (n=3).

Change of color

The change of CIE L*a*b* values during fermentation of *R. coreanus* wine are presented in Table 1.

At the initial stages, L* value was 17.40 in the control group and 14.4 in enzyme treatment, a* values were 31.06 in the control group and 33.54 for enzyme treatment, and the b* value was 11.38 in the control group and 9.99 for the enzyme treatment group. The initial L* and b* values of *R. coreanus* juice were decreased by enzyme treatment and the a* values was increased. This result differed with that reported by Kim *et al.*(20), in which was L* and a* values of a mulberry preparation decreased, and the b* values increased by exposure to pectinase. CIE L*a*b* values of the control and enzyme treatment group decreased as fermentation continued. The color difference (△E*) between control group and enzyme treatment was measured as 4.13

Table 1. Color changes of *Rubus coreanus* wine treated with Pectinex 100L during fermentation

Treatments			Fermentation time (day)					
			0	2	4	6	8	10
Fermenting at 24°Brix by sugar addition	Control	L*	17.40[aA]	14.83[bB]	11.79[bC]	11.24[aE]	11.55[aD]	9.79[aF]
		a*	31.06[bA]	23.14[bB]	19.18[cD]	18.41[bE]	19.33[aC]	17.29[bF]
		b*	11.38[aA]	9.14[bB]	7.47[bC]	7.00[dE]	7.16[aD]	6.25[aF]
	Enzyme treated	L*	14.40[bA]	11.59[cB]	9.05[dC]	8.42[dD]	8.24[dE]	8.27[cE]
		a*	33.54[aA]	20.88[cB]	17.18[dD]	16.40[dF]	17.07[cE]	17.34[aC]
		b*	9.99[bA]	7.76[cB]	6.08[dC]	5.62[cD]	5.59[dE]	5.65[cD]
	△E*[1)]		4.13	4.18	3.66	3.72	4.30	1.63
Fermenting at 8°Brix and added with sucrose (16%) after 4-day of fermentation	Control	L*	17.40[aB]	17.87[aA]	13.70[aC]	11.06[bD]	10.95[bE]	9.48[bF]
		a*	31.06[bA]	25.79[aB]	24.06[aC]	18.61[aD]	18.47[bE]	16.51[cF]
		b*	11.38[aA]	10.20[aB]	8.83[aC]	7.02[aD]	6.69[bE]	6.14[bF]
	Enzyme treated	L*	14.40[bA]	10.22[dB]	9.95[cC]	9.16[cD]	8.48[cE]	8.15[dF]
		a*	33.54[aA]	20.88[cB]	20.62[bC]	18.15[cD]	16.92[dE]	15.94[dF]
		b*	9.99[bA]	7.04[dB]	6.78[cC]	6.12[bD]	5.66[cE]	5.52[dF]
	△E*		4.13	9.62	5.48	4.23	3.09	1.57

[1)] $\triangle E^* = \{(\triangle L^*)^2 + (\triangle a^*)^2 + (\triangle b^*)^2\}^{1/2}$. △E means the color difference between the control group and the enzyme treatment.
[a-d]Means with the same alphabet in each column (L*, a*, or b*) are not significantly different.
[A-F]Means with the same alphabet in each row are not significantly different by fermentation time.
Evaluations were performed in triplicate.

at the initial stage of fermentation, exhibiting a distinguished color difference. However, △E* gradually decreased with fermentation time and decreased by 1.57~1.63 after 10-days of fermentation. In the present study, no difference was observed in the color of *R. coreanus* wine due to the sugar addition method. Therefore, we conclude that the color of *R. coreanus* wine was not affected by enzyme treatment.

Ethanol content

Ethanol content of the 24B-Group treated with pectinase increased sharply to 11.37% at 2-days of fermentation, 14.99% at 4-days of fermentation, and a maximum ethanol content of 16.01% after 10-days of fermentation (Fig. 5). The ethanol content of the 24B-Group not treated with pectinase was similar to enzyme treatment during fermentation except at 2-days, and 16.22% after 10-days of fermentation. The 8B-Group measured 2.98~3.21% ethanol after 4-days of fermentation, and after a 16% sugar addition at 4-days of fermentation the ethanol content sharply increased reaching 13.29~13.52% at 10-days of fermentation, although 2.49~2.93% lower than that of the 24B-Group. Ethanol contents of *R. coreanus* wine differed not by enzyme treatment but by addition of sugar.

The ethanol content of wine made with wild-grapes(19) and watermelon(21) was approximated as 11%, while citrus wine(22) and peach wines(10) reached 14%. Sugar contents at the initial stage for wild-grapes, watermelon, and citrus wine making were 24°Brix, and for citrus wine was 23°Brix. Enzyme treatment of *R. coreanus* juice did not have an affect on increasing phase and content of ethanol, which differed from the result of Jeong *et al.*(23), who reported increase in alcohol content. From these results, sugar addition at the initial stage of fermentation was beneficial to enhancement of ethanol production.

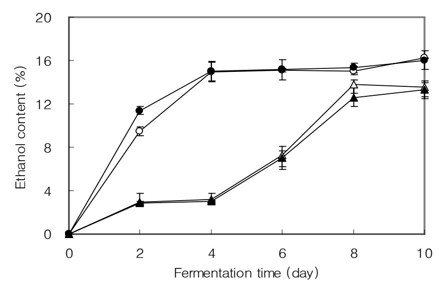

Fig. 5. Changes of ethanol content in *Rubus coreanus* wine treated with Pectinex 100L during fermentation.
See Fig. 2 for symbols. Vertical bars represent standard deviation (n=3).

Methanol content

Methanol is produced from pectin that hydrolyzed by methyl esterase in the fruit(24,25), but methanol causes vomiting, nausea, headache, and vision problems, including blindness(26). Therefore, the methanol content in all wine is regulated in accordance with food hygiene regulations(27). The methanol content of the 24B-Group treated with pectinase was higher (0.604 mg/mL) than that of the control group (0.404 mg/mL) after 10-days of fermentation (Fig. 6). The 8B-Group produced 0.520 mg/mL in enzyme treatment and 0.359 mg/mL in control group after 10-days of fermentation, a lesser level (0.044～0.084 mg/mL) than those of the 24B-Group. Methanol contents of 24B-Group and 8B-Group treated with pectinase were 0.201 and 0.161 mg/mL higher, respectively, than those of the control group. These results indicated that much more methanol is produced from pectin by pectinase treatment, but gradually decreased with fermentation time.

Kim and Kim(19) have reported that the methanol content of new wild-grape wine was less than 0.1 mg/mL, which was much lower than that of our *R. coreanus* wine. However, this difference was presumed to reflect pectin content of the raw material and insensitive analytic methods since the methanol produced was such a very small amount. The methanol content as a result of alcohol fermentation of *R. coreanus* juice was less than that allowed by law (1 mg/mL) (27).

From our results, we estimated that the *R. coreanus* juice yield by pectinase treatment was increased by 8.60% without the change of ethanol contents and physicochemical characteristics, and therefore the productivity of *R. coreanus* wine was increased. In addition, we have concluded that *R. coreanus* wine fermented by the pectinase treatment method was safe for human consumption. However, we need to continue with our efforts to further reduce the methanol content of the *R. coreanus* wine.

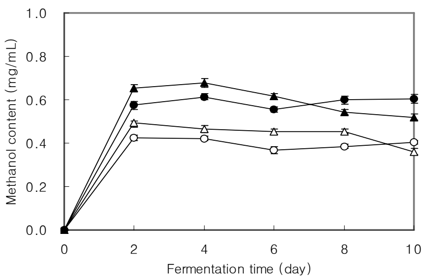

Fig. 6. Changes of methanol content in *Rubus coreanus* wine treated with Pectinex 100L during fermentation.

See Fig. 2 for symbols. Vertical bars represent standard deviation (n=3).

요 약

Pectinase 처리가 복분자 과즙의 추출 수율에 미치는 영향과 알코올 발효 중 이화학적 변화에 대하여 조사하였다. Pectinex 100L 처리(500 ppm, 30분)에 의해 복분자 과즙의 추출 수율은 대조구보다 8.60%가 증가되었다. 설탕 첨가에 의해 24°Brix에서 발효를 시작한 처리구(24B-group)와 8°Brix에서 발효를 시작하고 발효 4일 후에 설탕 16%를 첨가한 처리구(8B-group)의 고형분 함량은 발효 10일 후에 각각 8.2～8.3%와 7.7～8.0%로 감소하였으나 Pectinex 100L 처리에 의한 유의적 차이는 관찰되지 않았다. Pectinex 100L 처리구의 초기 적정산도는 1.18～1.22%로서 대조구(1.02%)보다 약간 높았다. 발효 초기에 복분자 과즙의 명도(L*)와 황색도(b*)는 효소처리에 의해 감소하였으나 적색도(a*)는 증가하였다. 대조구와 효소처리구 사이의 색차(△E*)는 발효기간에 따라 점차 감소하였다. 발효 10일 후 24B-group과 8B-group의 알코올 함량은 각각 16.01～16.22%와 13.29～ 13.52% 이었으며, 메탄올 함량은 각각 0.359～0.404 ppm과 0.520～0.604 ppm을 나타내어 법적 기준에 적합하였다.

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