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Monitoring on Alcohol Fermentations Properties for Aronia Juice for Aronia(*Aronia melanocarpa*) Vinegar

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Abstract : This study is to establish optimizing condition of alcohol fermentation in vinegar production with aronia, to confirm whether this can be industrially used, and to compare and analyze a change in anti-oxidative activity and quality characteristic according to alcohol fermentation of aronia. The optimized conditions for alcohol fermentation were as follows: *Saccharomyces cerevisiae* 5645 of yeast strain, a 5% inoculum size, aronia juice with a brix value of 14, and a glucose content of 7%. As a result to conduct scale up with optimizing conditions of alcohol fermentation of aronia, 8 days (192 hrs) of total alcohol fermentation time and 7.4% of the final alcohol content. The harvest volume accounted for approximately 90.2% with a loss of about 2.8%. As a result of antioxidant test, anti-oxidative activity of alcohol fermented liquor is lower than anti-oxidative activity of aronia extract, because of the decrease of antioxidant by oxidation of the fermentation process. However, the decrease of tannin by the fermentation process reduces acerbity of aronia, so increases overall preference

Keywords : *Alcohol fermentation, vinegar, aronia, saccharomyces cerevisiae, anti-oxidative activity*

1. Introduction

Vinegar, the oldest fermented food in human history, has been used not only as a seasoning in cooking but also as a food preservative and medicine[1,2]. According to the Korean Food Code, vinegar is classified into diluted vinegar, which is made by diluting acetic acid or glacial acetic acid with water, and vinegar made by through fermentation, which is made by adding acetobactor to various raw materials including grains, fruits, and alcoholic[3]. The traditional production method, parallel double fermentation using a traditional vinegar mother, involves fermenting fruits or grains to create a fermented liquid, which is then mixed with additives and weighed down to create vinegar. Meanwhile, the method used to produce commercial brewing vinegar involves diluting yeast with water and adding fruit juice or mineral salts to produce vinegar through two-stage fermentation[4,5]. There is a growing demand for fermented vinegar made from natural food materials such as fruits and herbs that are known for their functionality, driven by factors such as increasing income

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levels, a consumer culture that pursues LOHAS and well-being, health promotion of vinegar's functional properties, and strategies for upgrading vinegar. This trend is observed both domestically and internationally[6,7]. Two-stage fermentation method and simultaneous saccharification and fermentation (SSF) method are used in the production of natural brewed vinegar for mass production. The two-stage fermentation method involves alcohol fermentation and acetic acid fermentation using squeezed or crushed fruits, which has high efficiency in acetic acid production and fast fermentation rate. However, the loss of flavor components due to agitation and browning reactions caused by air injection can deteriorate the quality of vinegar. On the other hand, the SSF method involves natural fermentation of crushed or squeezed fruits in a raw state, which retains the nutritional value and characteristics of the raw material. However, the low yield and high residual alcohol content due to simultaneous alcohol fermentation and acetic acid fermentation are its drawbacks[8,9].

Aronia melanocarpa, also known as black chokeberry, is a perennial deciduous shrub belonging to the Rosaceae family, native to North America. Its fruit is used for medicinal and culinary purposes and is also used as a source of natural food coloring [10]. Aronia is divided into black chokeberry(*Aronia melanocarpa*), puple chokeberry (*Aronia prunifolia*), and red chokeberry(*Aronia arbutifolia*) according to scientific names, but it is commonly referred to as *Aronia melanocarpa*[11]. In 1978, Poland introduced Aronia from Russia and started commercial cultivation for the first time. Currently, about 90% of Aronia production is grown in Poland. Since the launch of juice using aronia in Poland in 1991, research results have been reported that aronia has anti-inflammatory, anti-obesity, cancer prevention, and immune enhancement functions due to its rich content of anthocyanins, polyphenols, and flavonoids. There is[12] Domestic ashes of Aronia began to be cultivated on a small scale in some farmhouses in 2007, and since 2010, cultivation has expanded nationwide, centering on Danyang, Chungcheongbuk-do[13]. In the early stage of aronia cultivation, it can be harvested from 3 years after planting, and despite the problem of raw fruit consumption due to the unique sour and astringent taste of aronia, it is recognized as a super-high-income crop. As the production rate is declining, it is necessary to develop products using various processing methods using aronia.

Therefore, the aim of this study is to determine the optimal conditions for both alcohol fermentation and acetic acid fermentation using aronia, while also proposing an industrial solution. To achieve this goal, the quality characteristics of aronia and the changes in antioxidants during alcohol fermentation will be examined to provide essential data for the future development of aronia vinegar products.

2. Experiment

2.1. Material

The aronia concentrate (65 brix, produced in Poland) used in this study was provided by Wellfine(WellFine orporation, Gyeonggi Province, Korea).

2.2. Culture preparation

The *Saccharomyces cerevisiae* KCTC 17798 and *S. cerevisiae* KCTC 7942A used in this study, were purchased from the Biological Resource Center of Korea Research Institute of Bioscience and Biotechnology. The *Saccharomyces cerevisiae* 5645 stored in this laboratory were used. The solid culture of the yeast strain for alcohol fermentation was plated on YM broth medium and cultured at 30℃. The fermentation liquid culture was prepared by preparing 200 mL of YM broth liquid medium in a 500 mL baffled flask. The

inoculum was prepared by transferring colonies of the yeast strain. The fermentation was performed in a shaking incubator (JSSI-100C, JS Research Inc., Korea) for 48 hrs at 30℃ and 180 rpm.

2.3. pH and acidity measurement

pH was measured at room temperature using a pH meter (B39267, Thermo scientific, USA). Acidity was expressed by neutralizing 10 mL of a 10-fold diluted sample with 0.1 N NaOH standard solution until the pH reached 8.2 to 8.5, and converting the titration value to acetic acid.

$$\text{total acidity (\%)} = \frac{V \times F \times D \times A}{S} \times 100$$

V: Consumption of 0.1 N NaOH (mL)
F: factor of 0.1 N NaOH
D: dilution factor
A: Amount of organic acid per 1 mL of 0.1 N NaOH (acetic acid: 0.006)
S: sample amount (mL)

2.4. Reducing sugar content measurement

The reducing sugar content was measured using the 3,5-dinitrosalicylic acid (DNS) method. Mix 0.5 mL of DNS reagent with 0.5 m of diluted sample at a constant rate, react in a water bath (BS-21, Jeio tech, Korea) at 100 ℃ for 5 min, cool, add 4 mL of distilled water, and measure the UV-spectrophotometer (Cary 100 Conc, VARIAN, USA) was used to measure the O.D value at 540 nm. As a standard material, glucose purchased from Sigma (USA) was diluted and used.

2.5. Determination of total phenolic compounds

Total phenolic compounds were colorimetrically quantified using the Folin-Denis method[14]. After diluting the sample at a certain ratio, 1 mL of 50% phenol reagent (Folin-Ciocalteu's reagent, Sigma-Aldrich, Co., ST. Louis, USA) was mixed with 1 mL of the diluted sample. After reacting at room temperature for 3 minutes, 1 mL of 10% Na_2CO_3 (SAMCHUN, Korea) was inoculated, mixed, and reacted at room temperature for 1 hr, and the O.D. value was measured at 700 nm using a UV-spectrophotometer. As a standard material, tannic acid purchased from Sigma (USA) was diluted and used.

2.6. Determination of total flavonoid content

0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminum nitrate, and 4.3 mL of 80% ethanol were added to 0.5 mL of the diluted sample at a constant rate, reacted at room temperature for 40 minutes, and then the O.D. value was measured at 415 nm using a UV-spectrophotometer.

2.7. Determination of total anthocyanin content

The total anthocyanin content was measured using the pH differential method [15]. After adding 4.5 mL of 0.025 M potassium chloride buffer (pH 1.0) and 4.5 mL of 0.4 M sodium acetate buffer (pH 4.5) to 0.5 mL of the diluted sample, respectively, the reaction solution was measured at 520 nm and 700 nm using a UV-spectrophotometer. The O.D value was measured in nm, and the measured value was calculated using the following equation.

$$\text{Anthocyanin content (cyanidin-3-glucoside equivalents, mg/100mL)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

A (absorbance)=(A520 nm-A700 nm) pH 1.0-(A520 nm-A700 nm) pH 4.5
MW (molecular weight of cyanidine-3-glucoside)=449.2 g/mol
DF (dilution factor)=dilution ratio of sample
1000=factor for conversion from g to mg
ε (cyanidin-3-glucoside molar absorptivity) =26,900 L × $mol^{-1} \times cm^{-1}$
1=path length in cm

2.8. Determination of total tannin content

The total tannin content was measured by modifying the method of Urve[16] *et al.*, 1 mL of 1% $K_3F_3(CN)_6$ and 1 mL of 1% $FeCl_3$ were added to 0.5 mL of the diluted sample at a constant rate, and the reaction was conducted at room temperature for 5 minutes, and the O.D. value was measured at 720 nm using a UV-spectrophotometer. As a standard, quercetin purchased from Sigma (USA) was diluted and used.

2.9. Statistical analysis

All experiments were repeated three times, and the results were expressed as mean values±standard deviation. To verify the significance ($p<0.05$) between each experimental group, statistical analysis was performed using SAS (Statistical Analysis System program, SAS Institute, Cary, NC, USA) and xlstat program, followed by analysis of variance (ANOVA), followed by Duncan's Multiple comparison was performed by multiple range test.

3. Results and discussion

3.1. Kinetics of alcohol fermentation

The results of alcohol fermentation are shown in Fig. 1. The final alcohol content of the strain *Saccharomyces cerevisiae* 5645, *Saccharomyces cerevisiae* KCTC 17798 and *Saccharomyces cerevisiae* KCTC 7942 was 7.8%, 7.6%, and 7.6%, respectively. Looking at the change in alcohol content by fermentation period, it was found that fermentation proceeded within 0.7% (v/v) without a significant difference in alcohol concentration during the first day of fermentation. However, from the 2nd day of fermentation, it was found that the three yeast strains proceeded with rapid alcohol fermentation and maintained it until the 5th day of fermentation. However, from the 6th day of fermentation, fermentation proceeded without a significant difference in the alcohol concentration of each yeast strain, and this fermentation pattern continued until the end of fermentation. It was similar to the results of this experiment that Woo *et al.*,[17] took about 7 to 8 days for alcohol fermentation even though the strains were different. In addition, in a study by Kim *et al.*,[18], it was found that even if alcohol fermentation was performed under the same conditions, it was greatly affected by the yeast involved in fermentation. Therefore, in this study, Saccharomyces cerevisiae 5645 strain, which showed the highest alcohol production, was established as the optimal condition for alcohol fermentation of aronia.

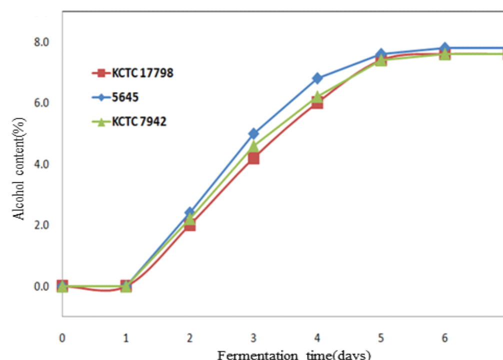

Fig. 1. The kinetics of alcohol fermentation of aronia using *Saccharomyces cerevisiae* 5645, *Saccharomyces cerevisiae* KCTC 17798 and *Saccharomyces cerevisiae* KCTC 7942. The fermentation was performed at 30℃, pH 4, 180 rpm. The extract of aronia 14%(v/w) was supplied at initial stage of fermentation.

3.2. Alcohol fermentation characteristics depending on inoculation amount

To examine the effect of inoculation quantity on alcohol production using the selected strain, alcohol fermentation was conducted. The inoculation amount of the *Saccharomyces cerevisiae* 5645 yeast strain was adjusted from 1% to 7% at 2% intervals in order to measure changes in reducing sugar

levels and the quantity of alcohol produced (Fig. 2). The experimental results showed that the final alcohol content was the same at 7.8% for all four experimental groups. However, based on the main yeast inoculation amount, it was observed that the test group inoculated with 7% produced 5% or more alcohol the fastest, reaching 6.7% on the 3rd day of fermentation. In the case of the test group inoculated with 3% and 5%, alcohol was produced at a rate of 6.3% on the 4th day of fermentation, while the 1% test group generated more than 6% alcohol on the 5th day of fermentation. The change in reducing sugar also showed a rapid change, particularly with higher yeast inoculation amounts, starting as early as the 2nd day of fermentation. From the 5th day of fermentation, there was a gradual decrease in reducing sugar content. However, on the 6th day of fermentation, all inoculation amounts displayed reducing sugar contents of 1.45% or lower, indicating that alcohol fermentation was nearly complete. Overall, there was a tendency for higher yeast inoculation amounts to result in increased initial alcohol production during fermentation. As yeast fermentation progressed, the sugar content decreased while the alcohol content increased rapidly. Therefore, yeast inoculation amounts are considered to have an influence on both the initiation of initial fermentation and the production of alcohol in alcohol fermentation. In this study, considering industrial aspects, the yeast inoculation amount was set at 5% as the optimal condition for ensuring stable alcohol fermentation. This is because, in the case of facultative anaerobic yeast strains, such as *Saccharomyces cerevisiae,* oxygen presence leads to the complete decomposition of sugars into carbon dioxide, resulting in a significant reduction in ethanol production. Therefore, setting conditions for anaerobic state or minimizing oxygen exposure to facilitate favorable fermentation is not suitable, as it could lead to exceeding the reduction in alcohol production not only in laboratory-scale experiments but also in the context of industrialization due to cost considerations. This was confirmed to be consistent with the contents of an experiment conducted by Kim *et al.,* who set the inoculation amount to 5% during alcohol fermentation to establish the optimization of the alcohol fermentation process of concentrated grape juice[19].

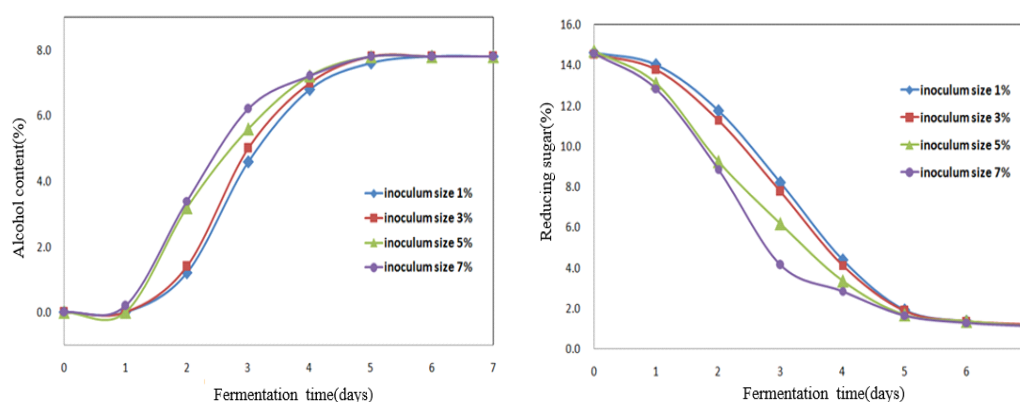

Fig. 2. Effects of inoculum size on alcohol fermentation of aronia using S*accharomyces cerevisiae* 5645. The fermentation was performed at 30℃, 180 rpm. The concentration of inoculum was 1, 3, 5, and 7%(v/v). The extract of aronia 14% (v/w) was supplied at initial stage of fermentation.

3.3. Alcohol fermentation characteristics according to aronia concentration

To investigate the effect of concentration of aronia on alcohol fermentation, Aronia concentrate(65 brix) was diluted to levels of 6, 10, 14, and 18 brix, respectively. Subsequently, a 5% inoculation of main yeast was applied, followed by the progression of alcohol fermentation (Fig. 3). In the case of the experimental groups with Aronia concentrations of 10, 14, and 18 brix, the alcohol content showed a rapid increase until the 5th day of fermentation, followed by a gradual fermentation process. On the other hand, for the experimental group with an aronia concentration of 6 brix, alcohol fermentation continued until the 7th day, but the final alcohol content generated was 5.37%. In general, higher brix concentrations of aronia led to higher sugar content within aronia juice, which in turn resulted in an increased trend in final alcohol production. This observation is consistent with the findings of Hwang *et al.*,[20], indicating that as yeast fermentation progressed, sugar content decreased while alcohol content increased rapidly. Meanwhile, based on the results of this experiment, it can be concluded from an industrial standpoint that when using aronia for alcohol fermentation, setting the aronia's brix concentration at 14 brix, considering the natural brix level of aronia fruit, and supplementing with glucose, is the most reasonable approach in terms of industrial feasibility.

3.4. Alcohol fermentation characteristics according to glucose content

To examine the influence of glucose content on alcohol fermentation, a 14 brix diluted aronia solution was prepared and supplemented with 5% yeast inoculation. Subsequently, glucose concentrations of 0%, 4%, 7%, and 10% were individually added to the solution to analyze the characteristics of alcohol fermentation(Fig. 4). The final alcohol content in each experimental group was 3.2%, 5.4%, 7.2%, and 8.4%, respectively. It was observed that as the glucose content increased, the alcohol production also increased. This trend is consistent with the results mentioned in Huan *et al.*, study, which suggested that higher sugar content leads to higher alcohol production[21]. On the other hand, Son *et al.*,[22] reported that when the concentration of fermentable sugars increases beyond 8.0%,

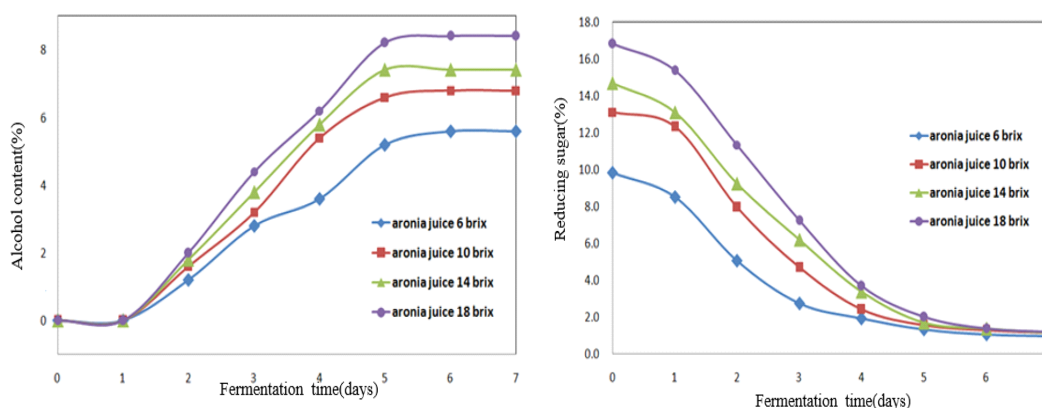

Fig. 3. Effects of aronia juice brix on alcohol fermentation of aronia using *Saccharomyces cerevisiae* 5645. The fermentation was performed at 30℃, 180 rpm. The concentration of inoculum was 5%(v/v). Aronia concentrations of 6, 10, 14, and 18 brix were supplied at the initial stage of fermentation.

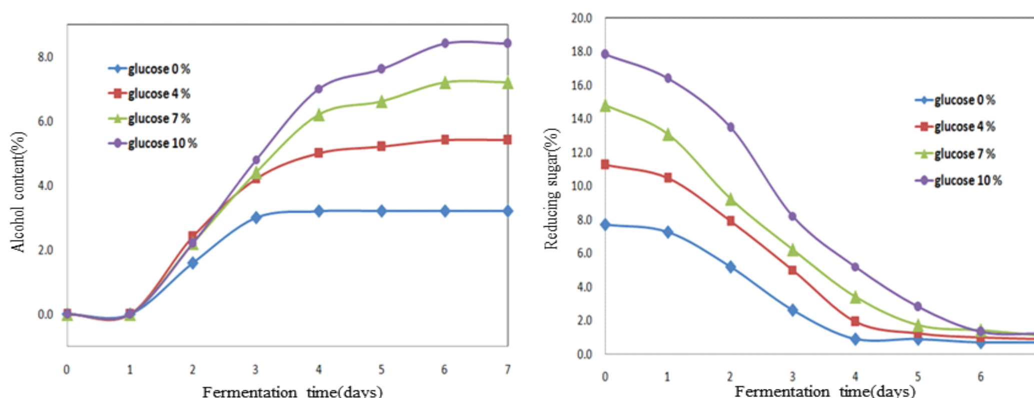

Fig. 4. Effect of glucose contents on alcohol fermentation of aronia using S*accharomyces cerevisiae* 5645. The fermentation was performed at 30℃, 180 rpm. The concentration of inoculum was 5%(v/v). Glucose concentrations of 0, 4, 7, and 10 % were supplied at the initial stage of fermentation.

alcohol production decreases. This contrasts with the findings of this study, and it is believed that this difference could be attributed to variations in the concentration of fermentable sugars in the raw materials used and the yeast strains used.

Therefore, in this study, in order to analyze the industrialization potential of aronia alcohol fermentation, the yeast strain *Saccharomyces cerevisiae* 5645 was used, with an inoculation rate of 5%, aronia brix concentration of 14 brix, and glucose content of 7%, for conducting scale-up fermentation experiments.

3.5. Scale up of alcohol fermentation

In order to analyze the industrial potential of aronia vinegar production, a scale-up was performed using a 100 L fermentation tank with a 14 brix diluted aronia solution for alcohol fermentation under optimized conditions(Table 1, Fig. 5.). The total fermentation time for alcohol fermentation was 8 days (192 hours), and the final alcohol content was measured at 7.4%. The recovery amount was approximately 36.08 L, showing a recovery rate of about 90.2%, and the amount of loss due to evaporation was 1.11 L, showing a loss rate of about 2.8%. In the 100L fermentation tank, the characteristics of alcohol fermentation showed that the alcohol production initially formed a lag phase during the first 40 hours, followed by continuous increase through the logarithmic growth phase, reaching a final alcohol content of 7.4% at around 168 hours. As fermentation progressed, the reducing sugar content showed an inversely proportional trend to alcohol production. After 136 hours, there was a decrease in the rate of alcohol production due to the depletion of fermentable sugars. However, the alcohol content continued to increase until 168 hours.

Table 1. Comparison of fermentation yield on the alcohol fermentation in 100 L fermentation tank

	Aronia alcohol fermentation
Start volume	40.00 L (100 %)
Harvest volume	36.08 L (90.2 %)
Sampling	2.81 L (7.0 %)
Loss	1.11 L (2.8 %)
Fermentation time	8 days (192 hr)
Alcohol / Acidity	7.40 %

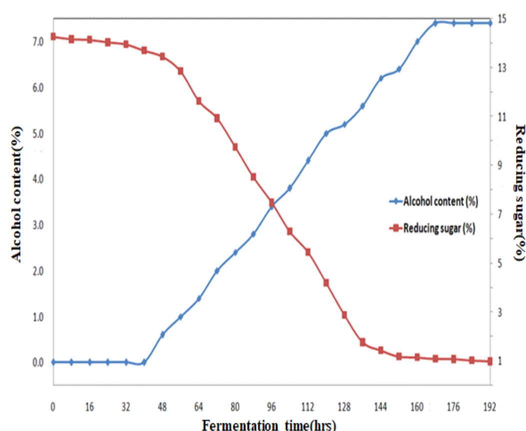

Fig. 5. Pattern of aronia alcohol fermentation using a 100 L fermenter.
The fermentation conditions were carried out with the following parameters:yeast strain used was *Saccharomyces cerevisiae* 5645, fermenter capacity was 100 L, aronia concentration was 14 brix, inoculation amount was 5%, glucose content was 7%, fermentation temperature was 30℃, and fermentation time was 192 hours.

3.6. Content analysis of total polyphenols, total flavonoids, total anthocyanins and tannins

The contents of total polyphenols, total flavonoids, total anthocyanins and tannins in aronia alcohol fermentation products were analyzed(Table 2). As observed in Table 2, the content of total polyphenols, total flavonoids, total anthocyanins, and tannins in aronia alcohol fermentation significantly decreased as the fermentation progressed ($p<0.05$). The content of total phenolic compounds was 412.45±6.75mg/100 mL in aronia juice, while in the alcohol-fermented product, it was 93.98±2.03 mg/100 mL. The total flavonoid content in aronia juice was 477.65±4.08 mg/100 mL, whereas in the alcohol-fermented product, it was 105.88±2.19 mg/100 mL. The total anthocyanin content in aronia juice was 389.75±6.77 mg/100 mL, and 253.49±5.27 mg/100 mL for the alcohol-fermented product. The content of total polyphenols, total flavonoids, and total anthocyanins in aronia alcohol fermentation products was observed to be lower compared to that in aronia juice.

Table 2. Total polyphenol, flavonoid, anthocyanin and tannic acid contents of aronia fermented products

	Aronia juice	Alcohol fermentation
Total polyphenol(mg GAE[1]/100 mL)	412.45±6.75[a]	93.98±2.03[b]
Total flavonoid(mg QE[2]/100 mL)	477.65±4.08[a]	105.88±2.19[b]
Total anthocyanin(mg C3G[3]/100g)	389.75±6.77[a]	253.49±5.27[b]
Total tannic acid(g/100 mL)	5.56±±0.05[a]	2.30±0.03[b]

1) GAE: gallic acid equivalent.
2) QE: Quercetin equivalent.
3) C3G: cyanidin-3-glucoside equivalent
Data were the mean±SD of triplicate experiment
Means with different small letters(a-b) within the same row significantly different at $p<0.05$.

This is attributed to factors such as fermentation period, fermentation temperature, oxidative reactions during fermentation, as well as pretreatment of raw materials, extraction conditions, and the amount of added extract.

On the other hand, the content of total tannins was measured to be 5.56±0.05g/100 mL in aronia juice, whereas in the Aronia alcohol fermentation product, it was observed to be 2.30±0.03g/100 mL. This reduction in the total tannin content can be attributed to oxidation and enzyme activity during the fermentation process. Shin reported that the tannin component of aronia can be reduced using yeast isolated from salted fish[23]. In this experiment, it was found to be consistent with the research result that the tannin component is reduced during alcohol fermentation of aronia.

4. Conclusion

In this study, in order to analyze the acidic potential in vinegar production using aronia, we first established process optimization for alcohol fermentation characteristics of aronia.

1. As the conditions for optimizing alcohol fermentation using aronia, the yeast strain *Saccharomyces cerevisiae* 5645, the main seed inoculum was set to 5%, the concentration of aronia brix was set to 14 brix and the glucose content was set to 7%.

2. As a result of scale-up using optimal conditions for aronia alcohol fermentation, the total alcohol fermentation time was 8 days(192 hours), and the final alcohol content was measured as 7.4%. The recovery volume of the aronia alcohol fermented solution was approximately 36.08 L, representing around 90.2% recovery efficiency. This result can be attributed to losses arising from evaporation or the release of CO_2 gas during alcohol fermentation. The observed loss amount was approximately 1.11 L, which accounts for roughly 2.8% of the total volume.

3. The alcohol-fermented solution using aronia showed lower antioxidant activity compared to the undiluted aronia solution. This is thought to be primarily attributed to the decrease in antioxidants resulting from oxidation during the fermentation process. However, the decrease in tannin content due to the fermentation process is considered to potentially enhance overall palatability by reducing the characteristic astringent taste of aronia.

4. It is expected that industrial-scale alcohol fermentation utilizing aronia can be achieved through the optimal conditions for aronia alcohol fermentation established in this study. Moreover, this approach could potentially be extended to natural fermentation processes involving other berries.

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