Characteristics of Korean Alcoholic Beverages Produced by Using Rice Nuruks Containing Aspergillus oryzae N159-1

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Abstract Herein, nuruks derived from non-glutinous and glutinous rice inoculated with Aspergillus oryzae N159-1 (having high alpha-amylase and beta-glucosidase activities) were used to produce Korean alcoholic beverages. The resultant beverages had enhanced fruity (ethyl caproate and isoamyl alcohol) and rose (2-phenethyl acetate and phenethyl alcohol) flavors and high taste scores.

Keywords Aspergillus oryzae, Flavor, Nuruk, Rice

Aspergillus oryzae strains, known as useful fungal strains in the alcoholic beverage industry and considered part of a national industry in Japan, have been standardized through long-standing basic research to improve and maintain the high quality of sake. The sake industry has become established as a high value-added industry through standardization of the saccharification and fermentation conditions, and raw materials such as sugar and nitrogen sources. Since the 1990s, Japanese research teams have been conducting genomic and molecular biological studies on the genes involved in the saccharification and proteolytic processes of A. oryzae strains [1, 2]. Korean research teams have also isolated and identified fungi from nuruk—a fermentation starter in traditional Korean alcoholic beverage production—and analyzed their physiological characteristics, such as their saccharification and fermentation capabilities [3, 4]. The most common microorganisms isolated from nuruk include fungi, such as Aspergillus, Rhizopus, and Mucor; yeasts, such as Saccharomyces cerevisiae; and lactic acid bacteria [5]. Until recently, studies on nuruk have focused mainly on the distribution of microorganisms within the starter [6] and the separation of useful microorganisms therefrom [7]. The production of modified nuruk, using fungi that have been isolated from it, made use of wheat or bran as raw materials without going beyond the frame of conventional wheat nuruk [8]. The taste and flavor of Korean alcoholic beverages are determined mainly by the metabolic products (e.g., free sugars, amino acids, organic acids, and aromatic flavor compounds) that are produced during fermentation of the raw materials by the microorganisms present in the nuruk.

In this study, to improve the flavor of Korean alcoholic beverages, non-glutinous and glutinous rice were used as raw materials for producing various nuruks. Wines made from glutinous rice, black glutinous rice, non-glutinous rice, and naked barley had shown desirable sensory characteristics in a previous study [9]. Herein, new nuruks were produced by inoculating the raw materials with the fungus Aspergillus oryzae strain N159-1 (KCTC11927BP) [7] that was isolated from stocked nuruk, and the fermented alcoholic beverages were produced by using the yeast S. cerevisiae strain Y89-5-3 (KCTC11811BP) [10] that was also isolated from stocked nuruk. Solid-phase microextraction (SPME)-gas chromatography mass spectrometry (GC/MS) (Agilent Technologies, Santa Clara, CA, USA) analysis of the fermented beverages showed that the fruity and rose flavors were enhanced, and the sensory characteristics analysis showed a high flavor preference and overall preference.

In brief, 5 kg of each raw material grain was crushed, autoclaved, and cooled. Strain N159-1 was cultured on a potato dextrose agar plate at 30°C for 2 days, and on potato...
Kim et al. dextrose broth at 30°C for 2 days. Then, the mycelial cells were suspended in sterilized water (20%, 1 × 10^8 CFU/100 mL) in order to produce a disc-shaped nuruk (5 cm height × 25 cm width). The nuruk was incubated at 25°C for 20 days in an incubator, then dried naturally for 7 days, and finally stored in a room with a temperature of 10°C. The saccharogenic activity of the nuruk was measured using a 2% (w/v) soluble starch solution as the substrate, according to the methods of the National Tax Service Technical Service Institute [11]. The saccharogenic rate, which is the percentage of glucose formed by 1 g of nuruk acting on 1 g of soluble starch, was multiplied by the dilution-fold number to yield the saccharogenic power (SP).

For comparison with a commercial wheat nuruk (CN; Jinjugokja, Gyeongnam, Korea), experimental nuruks were prepared by inoculating the N159-1 strain into five kinds of wheat. The saccharogenic activity of the nuruk produced with Keumkangmil was the highest, at 408.7 SP, whereas that produced with Simmichal No. 1 was the lowest, at 323.23 SP. All of the nuruks showed higher saccharogenic activity than that of the CN. Two other nuruks, GRN159-1 and NGRN159-1, were produced by inoculating N159-1 into glutinous and non-glutinous rice, respectively. The saccharogenic activity of GRN159-1 was 316 SP, which was higher than that of the CN in Table 2. The characteristics of produced nuruks are shown in Table 1. The chemical contents and organic acid contents of the fermented alcoholic beverage prepared with different nuruks are shown in Tables 2 and 3, respectively.

![Fig. 1. Sensory evaluation of fermented alcoholic beverages prepared with different nuruks. 9, like extremely; 1, dislike extremely. Dots represent the mean ± SE (12 judges × 3 replications). *Significant difference at the 5% level, as determined by Duncan's multiple-range test.](image_url)
similar to that of the five wheat nurucks inoculated N159-1. However, the saccharogenic activity of NGRN159-1 was 255.1 SP, which was slightly lower but did not result in any problems during the alcoholic beverage production (Table 1).

Using the commercial, GRN159-1, and NGRN159-1 nurucks, three Korean alcoholic beverages (hereafter named CN, GRN159-1, and NGRN159-1, respectively, for simplicity) were then produced. The alcohol content of CN was 16%, whereas that of GRN159-1 and NGRN159-1 was comparatively higher at 17.1% and 16.6%, respectively. All three alcoholic beverages showed similar soluble-solids contents, ranging from 10.9% to 11.1%. The pH of GRN159-1 was the highest at 4.5, whereas the values for CN and NGRN159-1 were similar at 4.15 and 4.19, respectively. GRN159-1 showed the lowest total acid at 0.11%, whereas the values for CN and NGRN159-1 were similar at 0.15% and 0.16%, respectively. In terms of the amino acid level, NGRN159-1 showed the lowest value at 0.12%, which was also the lowest [12]. Whereas the ultraviolet absorption of GRN159-1, which is an index of aromatic amino acids that can cause a bitter taste, was 8.56. For the reducing sugar content, GRN159-1 showed the lowest value at 4.82 mg/mL (Table 2). For the

| No. | RT  | RI  | Compound             | CN (%) | GRN159-1 | NGRN159-1 |
|-----|-----|-----|----------------------|--------|----------|-----------|
| 1   | 2.455 | <1,000 | Ethyl acetate       | 1.141  | 1.157    | 0.688     |
| 2   | 2.874 | <1,000 | Ethyl alcohol        | 40.332 | 35.619   | 40.061    |
| 3   | 4.192 | 1.021 | Ethyl butanoate      | 0.058  | 0.221    | 0.049     |
| 4   | 4.27  | 1.031 | Ethoxyacetic acid    | 0.024  | 0.15     | -         |
| 5   | 5.319 | 1.092 | Isobutyl alcohol     | 0.021  | 0.016    | 0.018     |
| 6   | 5.443 | 1.096 | Propanoic acid       | 0.116  | 0.354    | 0.157     |
| 7   | 7.775 | 1.110 | Isoamyl acetate      | 0.217  | 3.177    | 2.542     |
| 8   | 8.307 | 1.216 | Isoamyl alcohol      | 5.262  | 10.916   | 6.35      |
| 9   | 8.823 | 1.227 | Ethyl caproate       | 0.478  | 0.784    | 0.495     |
| 10  | 12.61 | 1.352 | 1-Hexanol            | 0.028  | -        | -         |
| 11  | 15.15 | 1.428 | Ethyl caprylate      | 1.542  | 2.767    | 1.888     |
| 12  | 15.88 | 1.457 | Isoamyl caproate     | 0.08   | 0.1      | 0.13      |
| 13  | 18.29 | 1.533 | Ethyl nonanoate      | 0.056  | 0.118    | 0.083     |
| 14  | 21.35 | 1.635 | Ethyl caprate        | 3.842  | 4.897    | 2.965     |
| 15  | 21.95 | 1.653 | Isoamyl caprylate    | 0.057  | 0.1      | 0.064     |
| 16  | 22.56 | 1.679 | Diethyl succinate    | 0.023  | 0.022    | 0.047     |
| 17  | 26.43 | 1.819 | 2-Phenethyl acetate  | 0.098  | 0.147    | 0.519     |
| 18  | 27.07 | 1.842 | Ethyl dodecanoate    | 2.169  | 1.894    | 1.102     |
| 19  | 27.53 | 1.862 | Isopentyl decanoate  | 0.069  | 0.076    | 0.06      |
| 20  | 28.87 | 1.913 | Phenylethyl alcohol  | 1.002  | 2.074    | 1.309     |
| 21  | 31.94 | 2.036 | Isopropyl tetradecanoate | - | 0.026 | 0.02 |
| 22  | 32.26 | 2.051 | Ethyl tetradecanoate | 3.973  | 2.343    | 1.938     |
| 23  | 32.52 | 2.060 | n-Caprylic acid      | 0.066  | 0.065    | 0.047     |
| 24  | 32.67 | 2.061 | Isoamyl laurate      | 0.027  | 0.027    | 0.026     |
| 25  | 34.3  | 2.135 | Hexadecanal          | 0.023  | 0.057    | -         |
| 26  | 34.68 | 2.152 | Ethyl pentanoate     | 0.081  | 0.081    | 0.065     |
| 27  | 34.95 | 2.163 | 9-Hexadecenyl myristate | 0.046 | 0.023 | 0.025 |
| 28  | 36.17 | 2.217 | Butyl dodecanoate    | 0.024  | 0.192    | 0.222     |
| 29  | 37.11 | 2.262 | Ethyl hexadecanoate  | 20.985 | 18.279   | 21.252    |
| 30  | 37.42 | 2.274 | n-Decanoic acid      | 0.249  | 0.154    | 0.179     |
| 31  | 37.62 | 2.282 | Ethyl 9-hexadecenoate | 0.224 | 0.153 | 0.189 |
| 32  | 39.35 | 2.354 | Ethyl heptadecanoate | 0.028  | 0.02     | 0.034     |
| 33  | 39.62 | 2.365 | 2-Methyl propyl hexadecanoate | 0.045 | 0.083 | 0.114 |
| 34  | 39.86 | 2.375 | 1-Hexadecanol        | 0.051  | 0.108    | 0.105     |
| 35  | 40.15 | 2.390 | 4-Octadecylmorpholine | 0.233 | 0.262    | 0.267     |
| 36  | 41.1  | 2.422 | n-Hexadecanoic acid  | 0.293  | 0.757    | 0.082     |
| 37  | 42.25 | 2.458 | Ethyl octadecanoate  | 0.975  | 1.349    | 1.424     |
| 38  | 42.93 | 2.480 | Ethyl oleate         | 6.825  | 5.732    | 7.181     |
| 39  | 44.67 | 2.488 | Ethyl linoleate      | 9.119  | 5.626    | 8.2       |
| 40  | 47.38 | >2.500 | Ethyl linolenate     | 0.118  | 0.066    | 0.103     |

Values are presented as % total peak area.
RT, retention time; RI, retention indices were determined using C10–C25 as external reference.
-: Not detected.

Table 4. Volatile compounds of fermented alcoholic beverage prepared with different nurucks
organic acids, CN showed the highest lactic acid and acetic acid contents at 2.4 and 0.42 mg/mL, respectively. GRN159-1 showed the highest amount of citric acid with a palatable taste (2.21 mg/mL) (Table 3).

With regard to the sensory evaluation, CN scored lower than average for flavor preference, whereas NGRN159-1 had an excellent score of 7.5 ± 0.76 and GRN159-1 had a good score of 6.13 ± 1.46 (p < 0.05). CN had strong sour and bitter tastes but a weak sweetness taste. GRN159-1 and NGRN159-1 had adequate sour and sweet tastes. The overall preference was the highest for NGRN159-1 at 6.63 ± 1.92, followed by GRN159-1 with a similar score of 6.38 ± 1.06. CN showed average preference at 5.25 ± 1.49 (Fig. 1). Results of the SPME-GC/MS-analyzed volatile flavor compounds and their relative peak area values are presented in Table 4. GRN159-1 and NGRN159-1 showed the higher ester compounds with fruity and flower flavor characteristics (e.g., isoamyl acetate, ethyl caproate, ethyl caprylate, and 2-phenethyl acetate), and higher alcohol compounds with banana and rose flavors (e.g., isoamyl alcohol and phenethyl alcohol), as compared with the levels in CN [13]. These results show improved fruity and floral characteristics of the two new beverages over those of CN using the Y89-5-3 strain [10].

In conclusion, Korean alcoholic beverages, brewed with different types of nuruk that had been produced by inoculating the nuruk-derived fungus A. oryzae N159-1 into non-glutinous and glutinous rice, showed enhanced fruity (ethyl caproate and isoamyl alcohol) and rose (2-phenethyl acetate and phenethyl alcohol) flavors as well as higher taste preference scores relative to the beverage brewed with a commercial nuruk. This is due to the fact that the starch component of the non-glutinous and glutinous rice had been well decomposed by A. oryzae N159-1, thereby increasing the amount of sugar available to S. cerevisiae to easily produce the flavor components.

**ACKNOWLEDGEMENTS**

This research was supported by the Main Research Program (E0170701-01) of the Korea Food Research Institute (KFRI) funded by the Ministry of Science, ICT & Future Planning, and by the Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (No. 914003-4).

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