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Research Note

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

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Table 1. Characteristics of produced *nuruks*

Nuruk No.	Strains	Raw materials	Saccharogenic power
1	*Aspergillus oryzae* N159-1	*Jokyoungmil*	359.98
2	*Aspergillus oryzae* N159-1	*Sinmichalmil*	364.19
3	*Aspergillus oryzae* N159-1	*Keumkangmil*	408.70
4	*Aspergillus oryzae* N159-1	*Sinmichal No.1*	323.23
5	*Aspergillus oryzae* N159-1	*Woorimil*	389.24
GRN159-1	*Aspergillus oryzae* N159-1	Glutinous rice	316
NGRN159-1	*Aspergillus oryzae* N159-1	Non-glutinous rice	255.1
CN[a]	*Aspergillus oryzae*: *Rhizopus oryzae* : *uncultured fungus* = 62.4 : 17.3 : 20.1	Wheat	300

[a]Commercial *nuruk*. Microbial community of CN was conducted by Macrogen (Seoul, Korea) with metagenomic analysis.

Table 2. Chemical contents of fermented alcoholic beverage prepared with different *nuruks*

Nuruk No.	Alcohol (%)	Soluble solid (sucrose, %)	pH	Total acid[a]	Amino acid[b]	Reducing sugar (mg/mL)	Ultraviolet light (OD_{280})
CN	16.0 ± 0.20	10.9 ± 0.34	4.15 ± 0.03	0.15 ± 0.02	0.21 ± 0.01	7.16 ± 0.62	7.38 ± 0.13
GRN159-1	17.1 ± 0.31	11.0 ± 0.09	4.50 ± 0.05	0.11 ± 0.01	0.17 ± 0.02	4.82 ± 0.28	8.56 ± 0.42
NGRN159-1	16.6 ± 0.26	11.1 ± 0.24	4.19 ± 0.09	0.16 ± 0.05	0.12 ± 0.01	6.52 ± 0.36	5.58 ± 0.22

Values are presented as mean ± SD (n = 3).
[a]Total acid content (%) measured as acetic acid.
[b]Amino acid content (%) measured as glycine.

Table 3. Organic acid contents of fermented alcoholic beverage prepared with different *nuruks* (mg/mL)

Nuruk No.	Lactic acid	Acetic acid	Citric acid	Succinic acid	Malic acid
CN	2.40 ± 0.17	0.42 ± 0.16	0.06 ± 0.02	1.4 ± 0.15	0.08 ± 0.00
GRN159-1	1.52 ± 0.23	0.23 ± 0.05	0.12 ± 0.01	1.30 ± 0.01	0.02 ± 0.00
NGRN159-1	1.90 ± 0.14	0.09 ± 0.01	0.06 ± 0.02	2.21 ± 0.12	0.02 ± 0.00

Values are presented as mean ± SD (n = 3).

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 *Sinmichal* 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

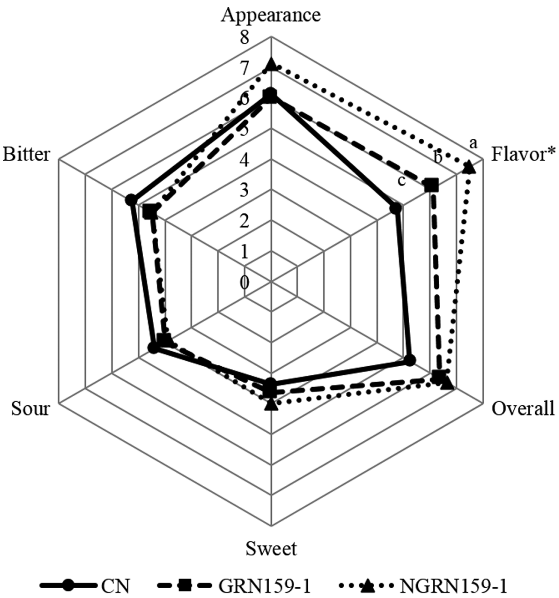

Fig. 1. Sensory evaluation of fermented alcoholic beverages prepared with different *nuruks*. 9, like extremely; 1, dislike extremely. Dots represent the mean ± SE (12 judges × 3 replications). [a-c]*Significant difference at the 5% level, as determined by Duncan's multiple-range test.

similar to that of the five wheat *nuruks* 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 *nuruks*, 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

Table 4. Volatile compounds of fermented alcoholic beverage prepared with different *nuruks*

No.	RT	RI	Compound	CN (%)[a]	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	5.775	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 pentanoater	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	0.028	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
			Total (%)	100	100	100

[a]Values are presented as % total peak area.

RT, retention time; RI, retention indices were determined using C10–C25 as external reference.

-: Not detected.

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 fresh sour taste (0.12 mg/mL). NGRN159-1 showed the highest succinic acid content 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 *nuruks* 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.

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