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Physicochemical characteristics of beer with rice *nuruk*

Kang Sun-a[1] · Kwon Ye-seul[1] · Jeong Seok-tae[1] · Choi Han-seok[1] · Im Bo-ra[1] · Yeo Su-hwan[1] · Kang Ji-eun[1]

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Abstract Beer production with rice or other malt substitutes suffers from a lack of suitable enzymes for saccharification. For this reason, rice *nuruk* (fermentation starter) was tested as a starch replacement for malt in the saccharification process of beer production. The results of this study show that the enzyme activities of rice *nuruk* made with brewing fungi were higher than those of malt. Saccharification and glucoamylase activities were high in *Aspergillus awamori* KCCM 30790 and α-amylase activity was high in *Aspergillus oryzae* CF1003. Overall, malt beer had significantly higher alcohol, pH, total acid, volatile acids, amino acids, free amino nitrogen, bitterness unit and ΔE than rice *nuruk* beer. Where as *Aspergillus awamori* KCCM 30790 beer had significantly higher soluble solids, reducing sugar than malt beer. According to a sensory evaluation, malt beer was better color, flavor and *Aspergillus oryzae* CF1003 beer was better taste, texture, overall acceptability than other beer. Therefore *Aspergillus awamori* KCCM 30790 beer was suitable considering enzyme activities (saccharification, glucoalmylase) and physicochemical characteristics (soluble solids, reducing sugar). And then *Aspergillus oryzae* CF1003 beer was suitable considering sensory evaluation (taste, texture, overall acceptability). Therefore rice *nuruk* like *Aspergillus awamori* KCCM 30790 and *Aspergillus oryzae* CF1003 were suitable as a substitute material that can replace for malt in beer proceccing.

Keywords Beer · Fungi · Malt · Rice *nuruk* · Saccharification

Kang Ji-eun (✉)
E-mail: kje0516@korea.kr

[1]National Institute of Agricultural Sciences, Nongsaengmyeong-ro 166, Wanju, Jeollabukdo, Republic of Korea

Introduction

Beer is one of the oldest beverages in recorded history, with the first records appearing from around 4000 BCE. The drink is a representative product based on a two-step fermentation process that involves the preparation of a saccharified solution using malts, hops, and water before fermenting the solution using yeast [1]. Koreans showed a significant amount of beer consumption; according to the National Tax Service statistics, the size of the domestic Korean beer market accounted for 51.4% of the total shipped volume of liquor in 2017 [2], representing the highest percentage of all shipped liquors.

Meanwhile, there has been a recent boom in beer importation in Korea, with an annual average rate of 23.2% [3]. Lager-type beers account for a vast majority of Korean beer sales at 95%, which is substantially higher than the 5% sales provided by ale-type beers. The recent increase in the demand for imported beer has resulted in a more diversified market. Moreover, the revision of the 2014 Liquor Tax Act enabled the sale of beer from the small-scale craft beer manufacturing industry. These circumstances have provided a wider variety of selection for consumers, accompanied by an increased interest in brewing beer, which can satisfy various personal preferences [1,4].

Beer is manufactured after saccharifying starch, the main component of grains. The saccharification process is one of the most important factors influencing the taste and quality of beer because the degree of saccharification determines the beer's sugar and alcohol content [5]. Malt, the main ingredient of beers, contains starch-hydrolyzing enzymes, thereby facilitating beer production through natural enzyme activities [4]. Rice is often used as a substitute for starch because of its soft texture and dry taste [2]. However, substituting starch with rice as a supplementary ingredient introduces several hurdles in the saccharification and filtration processes because rice lacks the starch-hydrolyzing enzymes that come from malts. As a result, it is difficult to obtain the required quality of wort, the saccharified solution made from adding water to malt, which is vital to the process. Therefore, the

addition of commercial enzyme formulations is required to compensate for the lack of enzymes used in preparing the wort with rice [6,7].

Nevertheless, if beer makers use domestic surplus rice instead of imported malt as raw material, it will help boost domestic agricultural consumption. Rice beer also has the advantage of being able to produce beer products with various flavors in the unified malt beer industry. Previous studies have investigated the application of various supplementary ingredients in beer production, including the addition of various starch-hydrolyzing enzymes [8]. One study investigated the production of beers using *gokja*, a traditional type of *nuruk*, or fermentation starter [1].

The present study aimed to use enzymes produced by microorganisms with the addition of rice *nuruk* in beer production. In doing so, this research also aimed to simultaneously resolve the problem of low enzyme activities when substituting malts with rice and the problem of increasing surplus rice in Korea. Rice *nuruk* is widely used for fermentation and is made by cultivating and drying steamed rice with inoculated fungi for brewing [8,9]. Furthermore, rice *nuruk* is rich in various starch-hydrolyzing enzymes and has been shown to play a role in inducing efficient saccharification and fermenting alcohol, providing the primary source of flavor and taste to liquors [9]. Recent studies have attempted to produce a new type of product like syrup and salt, by combining rice *nuruk* as an enzymatic agent of the grain starch [8,9].

Moreover, rice *nuruk* as a supplementary ingredient and enzymatic agent of beers would resolve the challenge of consuming surplus domestic agricultural products while offering a wide variety of beer preferences to meet the increasing consumer demand [10]. Toward this end, a high-quality rice beer was prepared by adding rice *nuruk* with various fungal strains during the saccharification process. The characteristics that determine the taste and quality of the resulting rice beer were compared with those of traditional malt beer.

Materials and Methods

Raw materials and fungal strains

This study used rice *nuruk* from Samkwang rice harvested in 2017 at Yeongin Agricultural Cooperative Rice Processing Complex in Asan City, Chungcheongnam-do. The *nuruk* was prepared using two commercial fungal strains (*Aspergillus oryzae* CF1003 (*A. oryzae* CF1003) and *Aspergillus luchuensis* CF1005 (*A. luchuensis* CF1005)) obtained from Chungmoo Fermentation Co. Ltd., six strains (*Aspergillus usamii* KCTC 6954-6959 (*A. usamii* KCTC 6954-6959)) distributed from the Korea Collection for Type Cultures, and two strains (*Aspergillus awamori* KCCM 32790 and KCCM 60247 (*A. awamori* KCCM 32790 and KCCM 60247)) distributed from the Korean Culture Center of Microorganisms. For comparison, beers made with pilsner malt (Pilsner, Weyermann, Germany), hops (Cascade, Brewferm, Beverlo, Belgium), and ale-type yeast (Safale S-04, Bision Co., Sungnam, Korea) were also created for the study.

Preparation of the *nuruk* seed

The rice was washed thoroughly and immersed in water for 2 h, and then the water was slowly drained for 1 h to give it time to absorb moisture to the center of the rice. After placing the rice in a steamer (MS-30, Yaegaki Food & System Inc., Himeji, Japan), more steam was added to the rice for 60 min right after the first appearance of water vapor. The hard-steamed rice was left to cool, and then 200 g portions of rice were placed in conical flasks. The rice was then inoculated with the cultured strains, and cultivation was carried out in a *nuruk* fermentor (Mini 15, Yaegaki Co.) at 37 °C for 10 days until spore production.

Preparation of rice *nuruk*

For the preparation of the rice *nuruk,* 10 kg of rice was washed thoroughly and immersed in water for 2 h, and then the water was drained for 1 h. After placing the rice in a steamer (MS-30, Yaegaki Food & System Inc.), hard-steamed rice was prepared by adding more steam to the rice for 40 min right after the first appearance of water vapor. The hard-steamed rice was cooled to 40 °C, inoculated with the cultured strains, and cultivation was carried out in a *nuruk* fermentor (Mini 15, Yaegaki Co.) at 37 °C for 48 h. The finished *nuruk* was stored in a freezer for subsequent use.

Enzyme activity determination

Amylase and protease were activated using a Kikkoman brew analysis kit (Kikkoman Co., Tokyo, Japan) according to the manufacturer's instructions. Absorbance (the degree to which an object absorbs light) measurements set at 400 nm were carried out using a medical enzyme analyzer (Synergy MX, BioTek Instruments Inc., Winooski, VT, USA). Saccharogenic power was measured using a saccharogenic power kit (Kikkoman). One unit of saccharogenic power was defined as an activity that released 1 μmol 4-nitrophenol (pNP) from 4-nitrophenyl β-D-glucoside per minute at 37 °C [11].

Glucoamylase and α-glucosidase activities were measured using a glucose-forming activity fractional quantification kit (Kikkoman), and α-amylase activity was determined using an α-amylase measuring kit (Kikkoman). First, one unit of glucoamylase activity was defined as the activity that released 1 μmol pNP from 4-nitrophenyl β-D-glucoside per minute at 37 °C. Second, one unit of α-glucosidase activity was defined as the activity that released 1 μmol pNP from 4-nitrophenyl α-glucoside per minute at 37 °C. Third, one unit of α-amylase activity was defined as the activity that released 1 μmol 2-chloro-4-nitrophenol from 2-chloro-4-nitrophenyl 6^5-azido-6^5-deoxy-β-maltopentaoside per minute at 37 °C [12].

Carboxy peptidase activities were measured using an acidic carboxy

peptidase measuring kit (Kikkoman). One unit of carboxypeptidase activity was defined as the activity that released 1 μmol carboxybenzomethyl-L-tyrosine-L-alanine per minute at 37 °C [13].

Acidic protease activity was determined by heating a mixture of 1.5 mL of casein (Junsei Chemical Co., Ltd., Tokyo, Japan) and 1.0 mL of pH 3.0 McIlvaine buffer for 5 min at 40 °C. Subsequently, 0.5 mL of the sample was added and allowed to react for 60 min at 40 °C. After 3 mL of 0.4 M trichloroacetic acid (Junsei Chemical Co., Ltd.) was added to remove the precipitate, 1 mL of this solution was mixed with 5 mL of 0.4 M Na_2CO_3 (Sigma-Aldrich Co., St. Louis, MO, USA) and 1 mL of phenol (Sigma-Aldrich Co.). The researchers maintained the temperature of the resulting solution at 40 °C for 30 min and measured its absorbance at 660 nm. Blanks (blank value measured with distilled water) were prepared by heating a mixture of 1.5 mL of casein and 1.0 mL of pH 3.0 McIlvaine buffer for 5 min at 40 °C. then added 3 mL of 0.4 M trichloroacetic acid, allowing it to react for 60 min at 40 °C, and adding 0.5 mL of the rice *nuruk* sample to remove the precipitate. Further treatments were conducted, as described above [14].

Acidic protease activity (U/mL) was calculated using the formula: $y \times 6/1$ (amount of reaction solution) $\times$ 1/0.5 (V). The $y = (Es - Eb)$ is the tyrosine content based on a calibration curve, Es is the absorbance of the sample, Eb is the absorbance of the blank, and V is the sample volume added to the reaction well (mL).

Preparation of beer

The researchers ground 500 g of malt and added it to a saccharification tank, along with 500 g of rice *nuruk* and 3 L of water. Saccharification was performed at 50 °C (15 min), at 62 °C (1 h), at 72 °C (15 min), and at 78 °C (15 min). After completing saccharification, 3 L of water at 78 °C was slowly poured into the mixture while filtering to recover the remaining sugar. The filtered wort was added into a brew kettle, and 0.1% (w/v) of hops was added when the solution started to boil. The mixture was allowed to boil for 1 h without closing the lid. The brew kettle was rapidly cooled to 38 °C, and the mixture was transferred into a sterilized fermenter to inoculate 0.05% (w/v) of dry yeast. The beer was fermented at 25 °C for eight days. At the end of fermentation, 800 mL of beer and 0.06% (w/v) of sugar (CJ Cheiljedang Co., Incheon, Korea) were added to a 1 L pressure-resistant beer bottle, and the solution was further aged in a room maintained at 4 °C for 10 days [1,7].

Physicochemical characteristics of beer

The pH was measured at room temperature through a pH meter (Orion 3 star, Thermo Fisher Scientific Inc., Waltham, MA, USA). Meanwhile, the sample acidity was determined by measuring the amount of 0.1 N NaOH (Yakuri Pure Chemicals Co., Ltd., Kyoto, Japan) needed to neutralize 10 mL of the sample. The amino acid value was determined by mixing a sample of known acidity with 5 mL of formalin (Yakuri Pure Chemicals Co., Ltd.) and 0.1 N NaOH [15]. Free amino nitrogen (FAN) was quantitated with glycine (Junsei Chemical Co., Ltd., Tokyo, Japan) using the ninhydrin method [16]. The soluble solids (Brix) were measured three times using a portable digital refractometer (PR101, ATAGO®, Tokyo, Japan) [4]. A 3,5-dinitrosalicylic acid method was used to analyze reducing sugars using glucose as its standard [17]. The alcohol content was measured using an alcohol analyzer (DA-155, Kyoto Electronics MFG. Co., Ltd., Kyoto, Japan). The volatile acid content was determined from the acetic acid content by titrating 30 mL of the sample with 0.01 N NaOH until reaching a pH of 8.2 [15].

The sample color was determined using Hunter values, scales for the accurate measurement of color. Lightness (L), redness (±a), and yellowness (±b) values were determined using a colorimeter (Ultra Scan PRO, Hunter Lab Inc., Reston, VA, USA). The bitterness unit of the beer was analyzed based on the National Tax Service Regulations on Liquor Analysis [6].

Sensory evaluation

Sensory evaluation was conducted by a panel of six male researchers and ten female researchers (aged 25-50 yr) with sensory evaluator experience. The fragrance and taste of beer made with rice *nuruk* were given scores between 1 and 15. The evaluations were conducted three times. The samples provided to the panel were labeled with random numbers according to a Williams Latin Square design, with intervals of 3-5 min between each sample evaluation [18].

Statistical analysis

The statistical significance of the experimental results was analyzed using Statistical Package for Social Sciences (SPSS Version 18.0, IBM, USA). All data were evaluated using a one-way analysis of variance (ANOVA) at a 5% significance level ($p < 0.05$).

Results and Discussion

Enzyme activity of rice *nuruk*

In beer processing, a saccharification solution is produced by converting starch into fermentable sugar by liquefying the enzymes produced by the malt, which is then subjected to an alcohol fermentation process [19]. During saccharification, α-amylase liquefies the starch, while glucoamylase breaks down the liquefied sugar and produces glucose [20].

In rice beer, however, the proportion of rice is increased instead of malt, which decreases the ratio of malt enzyme responsible for liquefaction and saccharification of starch. Rice beer uses commercial enzymes or *nuruk* microorganisms to compensate for the lack of malt enzymes [6,21]. Because this study aimed to produce beer using rice, rice nuruk made of *A. oryzae*, *A. luchuensis, A. usamii* and *A. awamori* were used instead of

Table 1 Enzyme activities (U/g, dry base) of malt and rice nuruk made with brewing fungi

Sample	Saccharification	α-Amylase	Glucoamylase	Carboxypeptidase
Malt	232.14±2.45[kl]	320.82±0.06[b]	143.18±6.78[k]	6,629.17±51.10[i]
A. oryzae CF1003	3,506.13±1.18[c]	401.45±1.14[a]	3,429.53±0.45[c]	72,392.62±15.04[g]
A. luchuensis CF1005	2,708.55±0.90[e]	65.72±42.59[h]	2,655.45±4.60[d]	2,4482.27±43.15[h]
A. awamori KCCM 30790	3,925.13±189.13[a]	281.39±2.22[c]	3,909.70±170.53[a]	10,6130.06±7.52[d]
A. awamori KCCM 60247	1,950.92±205.49[h]	81.18±2.04[g]	1772.57±203.84[h]	103,789.29±1,323.25[e]
A. usami KCTC 6954	2,744.58±475.99[d]	89.27±3.07[f]	2,524.83±448.70[e]	11,1895.11±455.83[b]
A. usami KCTC 6955	2,382.99±459.39[g]	46.03±1.21[k]	2,203.40±486.08[g]	74,288.53±125.05[g]
A. usami KCTC 6956	2,653.28±774.52[f]	109.32±3.85[d]	2,420.34±792.12[f]	99,906.36±1,148.34[f]
A. usami KCTC 6957	3,727.61±460.63[b]	92.90±0.85[e]	3,488.88±497.60[b]	106,201.44±895.04[d]
A. usami KCTC 6958	1,718.46±404.46[i]	55.42±3.36[j]	1,595.46±395.42[i]	112,461.69±1,289.28[c]
A. usami KCTC 6959	969.67±250.41[j]	60.79±4.19[i]	821.71±250.34[j]	125,095.66±752.26[a]

[1]Values are mean ± SD (n =3), different letters within the same column differ significantly (p <0.05)

commercially available enzymes.

The enzyme activities of malt and rice *nuruk* were first analyzed according to the different fungal strains used in the process. In terms of saccharogenic power and glucoamylase activity, the overall enzyme activity was significantly higher in rice *nuruk* compared to malt (p <0.05) (Table 1). The saccharogenic power of *A. awamori* KCCM 30790, possessing the highest enzyme activity at 3,925.13 U/g, dry base (the enzyme's strength per 1 g of nuruk) was approximately 16.9 times higher than that of malt, which had the lowest enzyme activity at 232.14 U/g, dry base. A similar trend was observed for glucoamylase activity, which was the highest for *A. awamori* KCCM 30790 at 3,909.70 U/g, dry base. It was approximately 27.3 times higher than malt's lowest activity of 143.18 U/g, dry base.

Glucoamylase can break down α-1,4-glucan bonds, but unlike α-amylase, it can hydrolyze liquefied starch from the non-reducing end to produce glucose and dextrin molecules of varying molecular weights. By contrast, α-amylase irregularly hydrolyzes the internal α-1,4-glucan bonds between amylose and amylopectin [20]. In other words, α-amylase produces dextrin with low molecular weight and oligosaccharides. The α-amylase does this by hydrolyzing starch during liquefying, resulting in a lower viscosity [7,22]. In the present study, the α-amylase activity of *A. usamii* KCTC 6955 was the lowest (46.03 U/g, dry base), whereas *A. oryzae* CF1003 showed the highest activity (401.45 U/g, dry base). *A. oryzae* CF1003's α-amylase activity is1.3times higher than malt's (320.82 U/g, dry base), suggesting that it is capable of rapidly liquefying starch. The enzyme activity of the proteolytic enzyme, carboxypeptidase, was significantly higher for the rice-based fermentation agents than for malt (p <0.05). *A. usamii* KCTC 6959 had the highest carboxypeptidase activity at 125,095.66 U/g, dry base, 18.9 times higher than malt, with the lowest carboxypeptidase activity at 6,629.17 U/g, dry base. Given that all of the isolated microorganisms showed higher enzyme activity than malt, rice *nuruk* appears to be an appropriate material as a partial substitute for malt.

Physicochemical characteristics of the beer

Table 2 shows the physicochemical compositions of different beers. The alcohol content of beer was determined to range from 5.40% (v/v) (*A. awamori* KCCM 30790) to 7.63% (v/v) (malts) and was thus significantly higher in malt beer (p <0.05). The pH ranged from *A. awamori* KCCM 30790's 4.41 to malt's 4.68. The beer manufactured using both malt and rice *nuruk* fell within the pH range of conventionally fermented beers [23,24]. The beer's sour taste is based on its total acidity and is derived from acids produced during the yeast proliferation and fermentation processes [1,23,25]. The total acidity was the lowest with *A. oryzae* CF1003 at 0.12% (w/v), but the difference from the others was not statistically significant (p <0.05). The volatile acids in the beers mainly include acetic acid (20-150 mg/L) and formic acid (20-40 mg/mL), which are produced by the degradation of glucose [3]. In this study, the volatile acid content ranged from 25.67% (w/v) (*A. awamori* KCCM 30790) to 35.20% (w/v) (malt), demonstrating significantly higher volatile acid levels in malt-based beer (p <0.05).

Soluble solids were significantly higher in beer produced with *A. awamori* KCCM 30790 (8.97 °Bx) (p <0.05) compared to the others. The reducing sugar content, a monosaccharide with hemiacetal groups used as a nutrient source for microorganisms, was slightly higher in beers containing rice *nuruk* (0.24-0.29% (w/v)) than in those containing only malt (0.22% (w/v)). The reducing sugar of the completed beer typically ranges from approximately 0.5 to 0.8% (w/v), which was lower in this experiment. The high reducing sugar content in beers made using rice *nuruk* suggests that rice *nuruk* would be advantageous for yeast proliferation and alcohol production in beer manufacturing.

Approximately 50% (w/v) of the amino acids present in beer serves as the nutrient source of yeast. The amount is then reduced using proteins and peptides in metabolic processes, and the remaining amino acids are present as components of beers [16]. The amino acid (glycine) content ranged from 7.90 g/100 mL (*A. oryzae* CF1003) to 8.97 g/100 mL (*A. awamori* KCCM 30790).

Table 2 Comparison of physiochemical characteristics in beer with rice nuruk

	Malt	*A. oryzae* CF1003	*A. awamori* KCCM 30790
Alcohol (v/v, %)	7.63±0.06[a1]	6.77±0.06[b]	5.40±0.00[c]
pH	4.68±0.01[a]	4.48±0.02[b]	4.41±0.01[c]
Total acid (citric acid, %)	0.14±0.00[b]	0.12±0.00[c]	0.15±0.02[a]
Volatile acid (mg/L)	35.20±0.53[a]	33.53±0.61[b]	25.67±0.61[c]
Soluble solids (°Bx)	8.53±0.06[b]	7.90±0.00[c]	8.97±0.06[a]
Reducing sugar (%)	0.22±0.01[c]	0.24±0.01[b]	0.29±0.02[a]
Amino acid (Glycine, g/100 mL)	0.14±0.00[a]	0.11±0.00[b]	0.10±0.00[c]
FAN (mg/L)	362.06±3.45[a]	281.71±2.55[b]	265.54±1.40[c]
BU	8.78±0.32[a]	7.93±0.28[b]	2.80±0.09[c]
Lightness[2] (L*)	87.74±0.01[c]	92.95±0.02[a]	89.95±0.01[b]
Redness[3] (a*)	0.97±0.01[b]	-0.48±0.01[c]	1.45±0.02[a]
Yellowness[4] (b*)	27.94±0.02[a]	23.31±0.00[b]	20.38±0.01[c]
ΔE[5]	30.54±0.03[a]	24.37±0.01[b]	22.68±0.01[c]

[1)]Values are mean ± SD (n =3), different letters within the same column differ significantly (p <0.05)
[2)]Lightness (0 = white, 100 = black)
[3)]Redness (- = green, + = red)
[4)]Yellowness (- = blue, + = yellow)

[5)]$\Delta E = \sqrt{(L_{sample}-L_{standard})^2+(a_{sample}-a_{standard})^2+(b_{sample}-b_{standard})^2}$

The protein content of malts should be relatively low and homogeneous because the polymer protein has a high viscosity, which enhances the beer's texture and taste [2]. However, if the protein content is extremely low, the surface tension of beer foam cannot be maintained, and the foam would not last [1,3]. The FAN content is related to the degradation products of proteins. It needs to decrease during fermentation because higher FAN concentrations result in a more turbid beer, which also reduces the stability of the foam [16,26]. The FAN content necessary for the fermentation of beer yeast is approximately 20 mg per 100 mL of wort [3]. In this study, the FAN contents of all of the beers produced were at least 200 mg/L, ranging from 265.54 to 362.06 mg/L. Although the content of bitter substances is high in beer (Table 2), these substances also contribute to enhancing the beer's body feel (Table 3). However, the protein degradation of beers offsets the bitter taste [5]. Here, the bitter taste of malts (8.78 BU) was significantly higher (p <0.05) among all the beers tested, while *A. awamori* KCCM 30790 (2.80 BU) was determined to 3.13 times less bitter (p <0.001) compared to malt. Therefore, the enzymatic activity for protein degradation in rice *nuruk* (Table 1) was the highest with *A. awamori*, followed by *A. oryzae* CF1003, and then malts with the lowest. Based on a previous study demonstrating that the bitter taste is offset as protein degradation increases [5], the bitter taste was reduced by increasing the amount of rice *nuruk* added.

The color or chromaticity of beers is affected by phenolic compounds. Chromaticity is also affected by the Maillard reaction while brewing water and during the manufacturing, fermentation, and aging processes [16]. Thus, the color of beer becomes darker depending on the quality of added ingredients and brewing water, the delay in manufacturing, filtering, and fermentation processes; and excessive heating [3,17]. The ΔE values for comparing the chromatic difference between beer samples revealed that the color of the beers made with rice *nuruk* was significantly paler, and the yellowness value was lower compared to malt beer's (p <0.05).

Table 3 Comparison of sensory evaluation in beer with rice nuruk

	Malt	*A. oryzae* CF1003	*A. awamori* KCCM 30790
Color	10.69±3.09[a1]	7.44±3.14[b]	5.75±2.57[c]
Flavor	8.63±3.61[a]	6.88±3.61[c]	7.13±3.88[b]
Taste	6.56±3.93[c]	8.63±3.44[a]	7.31±4.29[b]
Texture	7.81±2.32[b]	8.25±3.49[a]	6.31±3.09[c]
Overall acceptability	7.38±4.40[b]	8.06±3.04[a]	7.31±3.68[c]

[1)]Values are mean ± SD (n =3), different letters within the same column differ significantly (p <0.05)

Sensory evaluation of beer

Table 3 shows the results of the sensory evaluation for all of the beers. The taste (8.63), texture (8.25), and overall balance (8.06) were significantly different in beers containing *A. oryzae* CF1003. Meanwhile, the color (10.69) and scent (8.63) were significantly different in malt beer (p <0.05). Thus, from a sensory perspective, beers containing *A. oryzae* CF1003 are the better option.

This study investigated the enzymatic activity of rice *nuruk*, prepared by selecting strains that would be suitable as a partial substitute for malts in beer production. The characteristics of beers using rice *nuruk* were compared with those of malt beer. Overall, the enzymatic activity of rice *nuruk* was significantly higher than

that of malt due to the brewing fungi. Saccharogenic power and glucoamylase activity were higher in *A. awamori* KCCM 30790, *A. usamii* KCTC 6957, and *A. oryzae* CF1003, while the α-amylase activity was higher in *A. oryzae* CF1003. The carboxypeptidase activity was extremely high in *A. usamii* KCTC 6959, 6954, and 6958, which suggests the possibility of an excess amino acid content in manufacturing beers. In the beers produced using only malts, the pH, acidity, alcohol content, amino acid content, FAN, chromaticity, and volatile acid content were significantly higher than those of the rice *nuruk* beers. However, beers containing *A. oryzae* CF1003 received the highest scores during sensory evaluation (taste, texture, and overall balance). They were further determined to be within the adequate range of the usual fermentation products [3]. These results show that rice *nuruk* using *A. awamori* KCCM 30790 and rice *nuruk* using *A. oryzae* CF1003 are adequate as partial substitutes for malts in beer production in terms of enzymatic activity and sensory terms, respectively. Therefore, rice *nuruk* can resolve the insufficient number of enzymes with other substitutes for malts during the saccharification process. As a result, a high-quality rice beer with differentiated taste and quality could be manufactured using rice *nuruk*.

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Conflict of interest The authors declare no conflict of interest.

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