Effect of heat-treated *Nuruk* on the quality characteristics of aged *Yakju*

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Abstract Long-term aging of *Yakju*, a traditional Korean liquor made of rice and *Nuruk* (a fermentation agent), causes browning and odor and flavor development. This study investigated the effects of heat-treated *Nuruk* (50–80 °C, 30 min) on *Yakju* quality. The saccharogenic powers and glucoamylase, α -amylase, and carboxypeptidase activities were similar in non-heat-treated *Nuruk* and that treated at 50 °C. However, acidic protease and alcohol dehydrogenase decreased above 50 °C. The content of nitrogen-containing compounds was inversely proportional to the heat-treatment temperature. Compounds that cause off-flavors decreased at 50–60 °C, but increased at 70–80 °C, whereas compounds that provide fragrance increased at 50–60 °C. Sensory evaluation indicated that bad taste attributes were higher in *Yakju* produced using non-heat-treated *Nuruk*. Therefore, heat treatment of *Nuruk* at 50 °C can be adopted as a method for improving *Yakju* quality, as enzymatic activities that affect color, aroma, and taste are regulated.

Keywords *Yakju* · *Nuruk* · Heat treatment · Flavor · Color

Introduction

Yakju is a traditional Korean liquor prepared by fermenting cooked rice with *Nuruk* [1]. The fermentation agent *Nuruk* is made of wheat, barley, or rice and water. During its preparation, numerous microorganisms from the surrounding environment grow in *Nuruk* [2], and the types of microorganisms in *Nuruk* affect the qualitative characteristics of *Yakju* [3].

Various chemical reactions occur during liquor aging. Sugars and amino acids produce carbonyl compounds and pyrazine through Maillard reactions, which cause off-flavors, burnt fragrances, and color changes in the liquor during aging. In addition, photo-oxidation of amino acids produces indol and harman compounds, which cause browning [4]. In sake, which is made with rice and rice *Nuruk*, isovaleric acid, ethyl isovaleric acid, and 1,1-diethoxy-3-butane have been identified to cause off-flavors during aging [5]. Isoamyl alcohol in sake is metabolized into isovaleraldehyde by alcohol dehydrogenase originating from *Nuruk* [5].

To suppress the development of off-flavors during aging of rice wine, methods in which rice is milled to remove precursor compounds and off-flavor-producing compounds are absorbed by activated charcoal or yeast are being developed in Japan. However, owing to the various food-stuffs used to produce Korean *Nuruk*, different types of microorganisms and enzymes are produced, which makes regulation difficult. In addition, as milling large amounts of rice is costly, new methods for inhibiting off-flavors through treatment of the fermentation agent are preferable for adoption by liquor companies.

In the case of wine, accelerated aging for 3 months can achieve changes similar to those observed with oxidative aging for 3 years because this process accelerates oxidation

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and Maillard reactions to induce flavor and color changes [6].

In this study, *Nuruk* was treated at different temperatures before brewing to determine the effects of heat treatment on enzymatic activities. Changes in color, taste, and flavor after aging *Yakju* for 1 year at 45 °C were investigated to identify an appropriate temperature range for heat treatment of *Nuruk* to produce aged *Yakju*.

Materials and methods

Materials

The rice cultivar Odaebyeo (92% whole brown rice, harvested in Cheolwon-gun, Gangwon-do in 2014), Fermivin (DSM Food Specialties, Delft, Netherlands), which is a widely used wine yeast, and *Nuruk* (saccharogenic power 1800, Korea Enzyme Agricultural Co., Hwaseong, Korea) were used in this study.

Yakju production

Nuruk (8.2 g) was ground and then mixed with water (300 mL) before being heat treated for 30 min at 50, 60, 70, or 80 °C. Rice was prepared by cleaning 1 kg of rice, soaking it in water for 1 h, drying for 1 h at room temperature, and then steaming (MS-30, Yaegaki Food and System Inc., Himeji, Japan) for 40 min. Subsequently, water (500 mL), steamed rice (492 g), and yeast (0.1% of total weight) were added to *Nuruk*. Fermentation was carried out in a water bath (DS-23SN, Dasol Scientific Co., Ltd., Hwaseong, Korea) at 30 °C until no change in weight was observed (14 days). The sample was then centrifuged (7000×g, 4 °C, 30 min) and the supernatant was filtered (Filter paper No. 2, Advantec Co., Tokyo, Japan) to prepare *Yakju*. The *Yakju* samples were then aged for 1 year at 45 °C [7].

Enzyme activity determination

Amylase and protease were activated using a Kikkoman brew analyzing kit (Kikkoman Co., Tokyo, Japan) according to the manufacturer's instructions. Absorbance measurements were carried out using a medical enzyme analyzer (Synergy MX, BioTek Instruments Inc., Winooski, VT, USA). Saccharogenic power was measured using a saccharogenic power measuring kit (Kikkoman). One unit of saccharogenic power was defined as the activity that released 1 μmol 4-nitrophenol (PNP) from 4-nitrophenyl β-D-glucoside per minute at 37 °C [8].

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). 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, 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, and one unit of α-amylase activity was defined as the activity that released 1 μmol 2-chloro-4-nitrophenol from 2-chloro-4-nitrophenyl 65-azido-65-deoxy-β-maltopentaoside per minute at 37 °C [9]. Carboxy peptidase activities were measured using an acidic carboxy peptidase measuring kit (Kikkoman). One unit of carboxy peptidase activity was defined as the activity that released 1 μmol carboxybenzomethyl-L-tyrosine-L-alanine per minute at 37 °C [10].

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 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 Na₂CO₃ (Sigma-Aldrich Co., St. Louis, MO, USA) and 1 mL of phenol (Sigma-Aldrich Co.). The resulting solution was maintained at 40 °C for 30 min and its absorbance at 660 nm was measured. Blanks 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, adding 3 mL of 0.4 M trichloroacetic acid, allowing to react for 60 min at 40 °C, and adding 0.5 mL of sample to remove the precipitate. Further treatments were the same as described above [11].

Acidic protease activity (U/mL)

$$= y \times 6/1(\text{amount of reaction solution}) \times 1/0.5 (V) \quad (1)$$

where $y = (E_s - E_b)$ is the tyrosine content based on a calibration curve. E_s : Absorbance of sample; E_b : Absorbance of blank; V : sample volume added to the reaction well (mL).

One unit of tyrosinase activity caused an increase in A 280 nm of 0.001 per minute at pH 6.5 at 25 °C in a 3 mL reaction mixture containing 10 mL of 50 mM potassium phosphate buffer (pH 6.5), 10 mL of 1 mM L-tyrosine solution (Sigma-Aldrich Co.), and 9 mL of distilled water. The reaction solution (2.9 mL) was mixed with 0.1 mL of sample, and then heated for 10 min at 25 °C. The absorbance of the resulting solution was measured at 280 nm. Blanks were prepared by mixing 2.9 mL of reaction

solution and 0.1 mL of 50 mM potassium phosphate buffer and subjecting the mixture to the same treatment as described above [12].

$$\text{Tyrosinase activity (mU/mL)} = \frac{[(\text{Es}-\text{Eb}) \times \text{Df}]}{\div (0.001 \times 0.1 (\text{V}))} \times 10(\text{extraction rate}) \quad (2)$$

Df: Dilution factor.

One unit of polyphenol oxidase activity was defined as the consumption of 1 mmol of oxygen per minute. Measurements were taken by heating a mixture of 2.8 mL of 0.1 M potassium phosphate buffer and 0.2 mL of sample for 5 min at 35 °C. The absorbance of the resulting solution was measured at 420 nm. Blanks were prepared using 3 mL of 0.1 M potassium phosphate buffer [13].

$$\text{Polyphenol oxidase activity (U/mL)} = (\text{Es}-\text{Eb}) / (0.01 \times 5 (\text{T})) \quad (3)$$

T: reaction time (min).

Alcohol dehydrogenase (ADH) activity was determined using an enzymatic activity assay (Sigma-Aldrich Co.). The activity was measured using a medical enzyme analyzer (Synergy MX, BioTek Instruments Inc.). The activity of the sample was analyzed by mixing 50 µL of protease, 82 µL of assay buffer, 8 µL of developer, and 10 µL of 2 M isopropanol. Blanks were prepared by mixing 50 µL of sample, 92 µL of assay buffer, and 8 µL of developer. The solutions were cultured for 2–3 min at 37 °C before measuring their absorbance at 450 nm.

$$\text{ADH activity (U/mL)} = (\text{B}/\Delta\text{T} \times \text{V}) \times \text{Df} \quad (4)$$

B: amount of NADH generated by ADH (nmol); T: reaction time (min); V: sample volume added to the reaction well (mL); Df: Dilution factor.

Physiochemical characteristics

Sample acidity was determined by measuring the amount of 0.1 M NaOH (Yakuri Pure Chemicals Co., Ltd., Kyoto, Japan) needed to neutralize 10 mL of sample. Alcohol content was measured using an alcohol analyzer (DA-155, Kyoto Electronics MFG. Co., Ltd., Kyoto, Japan). The amino acid value was determined by mixing the sample with known acidity with 5 mL of formalin (Yakuri Pure Chemicals Co., Ltd.) and 0.1 M NaOH [14]. The pH was measured using a pH meter (Thermo Scientific Co., Waltham, MA, USA) and the DNS method was used to analyze reducing sugars [15]. Total sugar content was analyzed

using the Somogyi method [16]. Volatile acid content was determined from the acetic acid content by titrating 30 mL of the sample with 0.01 M NaOH until pH 8.2 [14].

Nitrogen-containing compounds

Nitrogen-containing compounds were analyzed with an automatic amino acid analyzer (L-8900, Hitachi, Ltd., Tokyo, Japan). Sample (5 mL) and 5 mL of 5% trichloroacetic acid were mixed together and centrifuged (4 °C, 12,000×g, 15 min). The supernatant was removed and the pellet was analyzed (0.2 µm, Millipore Co., Cork, Ireland) using a PF #2622 (4.6 × 60 mm, Hitachi, Ltd.) column. The temperatures of the column oven and reactor were 57 and 136 °C, respectively. Ninhydrine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used for staining [17].

Color

The sample color was determined using Hunter values. To obtain the color difference between two samples, L (lightness), a (redness), and b (yellowness) values were determined using a colorimeter (Ultra Scan PRO, Hunter Lab Inc., Reston, VA, USA).

Volatile compounds

Volatile aromatic compounds were analyzed using a gas chromatography (GC) system (GC2010, Shimadzu Co., Kyoto, Japan) equipped with an HP-INNOWAX (60 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent Co., Santa Clara, CA, USA) column and a flame ionization detector. The initial temperature of the column oven was 45 °C (5 min), which was then increased at 5 °C/min to 100 °C (5 min), and further increased at 10 °C/min to 200 °C (10 min). N₂ was used as the carrier gas with a flow rate of 22.0 cm/s (linear velocity) and split ratio of 50:1. The temperatures of the injector and detector were 250 and 280 °C, respectively. The sample (1 mL) was injected after filtration (0.2 µm, Millipore Co.).

Sensory evaluation

Sensory evaluation was conducted by a panel of seven male and five female researchers (ages 27–60) with sensory evaluator experience. The fragrances and tastes of *Yakju* made with heat-treated *Nuruk* were given scores between 1 and 7. Chinese Shaoxing wine, aged for 3 years (Zhejiang Guyuelongshan Shaoxing Wine Co., Ltd., Zhejiang, China), which has a strong fragrance and flavor, was used as the reference. The evaluations were conducted three times [18]. 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.

Statistical analysis

All analyses were repeated three times. The differences in the various components in each sample were analyzed using Minitab 16 (Minitab Inc., State College, PA, USA). The significance level was set to 5% ($p < 0.05$) and ANOVA analysis was conducted. The sensory evaluation results were analyzed using principal component analysis (XLSTAT, version 2014, Addinsoft, Paris, France).

Results and discussion

Physiochemical characteristics

The fermentation rates (amount of CO₂ generated during the fermentation period) of *Yakju* produced using heat-treated *Nuruk* were 84.6 (control), 80.8 (50 °C), 66.7 (60 °C), 7.0 (70 °C), and 6.7 (80 °C) g CO₂/day (data not shown). The fermentation speed was similar in the control and 50 °C heat-treated sample, but decreased significantly above 60 °C. As shown in Table 1, the activities of α -amylase and glucoamylase, which break down starch, decrease significantly at temperatures above 60 °C. Therefore, alcohol production is decreased, as starch is not readily broken down into sugars.

The changes in the enzymatic activities of *Nuruk* treated at different temperatures are shown in Table 1. The glycation capacity, α -amylase activity, and glucoamylase activity of the non-heat-treated sample and the 50 °C heat-

treated sample were not significant but remained relatively stable. However, the acid protease activity at 50 °C or more was 3.2 times lower than that of the control, indicating that this reaction is heat sensitive. The carboxypeptidase activity at 50 °C was also similar to that of the control. The tyrosinase and polyphenol oxidase activities decreased significantly at 80 °C, while alcohol dehydrogenase activity decreased significantly above 50 °C ($p < 0.05$). The thermostabilities of tyrosinases produced by molds such as *Aspergillus* are low at 35 °C [19]. In contrast, the tyrosinase produced by *Bacillus thuringiensis*, which is widely distributed in soil, shows optimal activity at 75 °C and has very high thermal stability at 85 °C [20]. *B. thuringiensis* is also found in traditional Korean fermented foods containing yeast [20] and *Nuruk* [21]. The tyrosinase activity of heat-treated yeast does not decrease owing to the high heat stability of enzymes produced by microorganisms present in yeast. Therefore, the saccharogenic power and the activities of amylase and protease, which are enzymes involved in fermentation, are similar to or higher than those of the control when *Nuruk* is heat-treated at 50 °C; thus, normal fermentation is possible. However, alcohol dehydrogenase, which produces compounds with strong odors, can be reduced at 50 °C to improve the aroma. Kim et al. [22] also reported that, with the exception of some *Penicillium* sp. and *Rhizopus* sp., the optimal temperature for saccharification enzymes in *Aspergillus* sp. was 50 °C.

The physiochemical properties of non-aged *Yakju* produced using *Nuruk* treated at various temperatures are shown in Table 2. Samples treated at 70 °C had significantly higher acidities ($p < 0.05$) than the other samples. The saccharification enzymes in samples treated at 70 °C and above were broken down, and metabolism of starch by

Table 1 Enzyme activity in heat-treated *Nuruk*

Enzyme activity	Heat-treated <i>Nuruk</i> extract				
	Control	50 °C	60 °C	70 °C	80 °C
Saccharogenic power (U/L)	370.61 $\pm$ 18.21 ^{b,1}	414.79 $\pm$ 5.00 ^a	76.82 $\pm$ 4.68 ^c	4.96 $\pm$ 3.12 ^d	1.12 $\pm$ 0.53 ^d
Amylase					
Glucoamylase (U/L)	350.12 $\pm$ 17.65 ^b	391.20 $\pm$ 4.71 ^a	82.02 $\pm$ 3.42 ^c	7.90 $\pm$ 3.22 ^d	ND ²
α -Amylase (U/mL)	10.72 $\pm$ 0.42 ^a	9.20 $\pm$ 0.32 ^b	2.71 $\pm$ 0.08 ^c	ND	ND
Protease					
Carboxypeptidase (U/L)	49.39 $\pm$ 3.41 ^a	52.45 $\pm$ 0.55 ^{ab}	39.33 $\pm$ 4.24 ^b	ND	ND
Acidic protease (U/mL)	402.47 $\pm$ 7.08 ^a	127.23 $\pm$ 10.81 ^b	8.18 $\pm$ 4.63 ^c	ND	ND
Tyrosinase (mU/mL)	18.82 $\pm$ 0.95 ^{bc}	22.95 $\pm$ 2.99 ^{ab}	25.41 $\pm$ 2.91 ^a	17.01 $\pm$ 0.76 ^{bc}	15.02 $\pm$ 2.91 ^c
Polyphenol oxidase (U/mL)	1.42 $\pm$ 0.04 ^c	1.71 $\pm$ 0.11 ^b	2.04 $\pm$ 0.14 ^a	1.32 $\pm$ 0.03 ^c	1.00 $\pm$ 0.05 ^d
Alcohol dehydrogenase (U/mL)	26.39 $\pm$ 1.08 ^a	24.60 $\pm$ 1.02 ^b	21.79 $\pm$ 0.85 ^c	19.45 $\pm$ 0.76 ^d	18.66 $\pm$ 0.74 ^e

¹Values are mean $\pm$ SD (n = 3), different letters within the same row differ significantly ($p < 0.05$)

²ND: not detected

Table 2 Physiochemical characteristics of *Yakju* produced using heat-treated *Nuruk*

Physiochemical characteristics	Non-aged <i>Yakju</i>				
	Control	50 °C	60 °C	70 °C	80 °C
Acidity (mL)	8.97 ± 0.11 ^{d,1}	10.36 ± 0.09 ^c	11.89 ± 0.13 ^b	15.04 ± 0.03 ^a	8.93 ± 0.09 ^d
Alcohol (%)	15.86 ± 0.06 ^a	15.66 ± 0.12 ^a	14.14 ± 0.81 ^a	ND ²	ND
Amino acid value (mL)	2.55 ± 0.07 ^a	2.12 ± 0.02 ^b	1.18 ± 0.02 ^c	0.97 ± 0.02 ^d	0.35 ± 0.07 ^e
Lactic acid (mg/100 mL)	501.97 ± 19.32 ^b	601.02 ± 39.21 ^b	800.37 ± 64.28 ^a	858.26 ± 67.95 ^a	563.80 ± 38.79 ^b
Mash recovery (%)	61.19	64.18	50.75	26.57	17.91
pH	4.19 ± 0.02 ^a	3.98 ± 0.02 ^b	3.85 ± 0.03 ^c	3.64 ± 0.03 ^d	4.01 ± 0.01 ^b
Reducing sugar (%)	0.52 ± 0.01 ^a	0.52 ± 0.02 ^a	0.35 ± 0.00 ^b	0.03 ± 0.00 ^d	0.14 ± 0.00 ^c
Total sugar (%)	6.27 ± 0.59 ^d	6.18 ± 0.16 ^d	10.91 ± 0.33 ^c	20.67 ± 0.87 ^b	36.97 ± 0.65 ^a
Volatile acid (mg/L)	275.93 ± 30.77 ^c	283.47 ± 2.08 ^c	394.67 ± 10.58 ^c	924.20 ± 15.65 ^b	1069.07 ± 26.29 ^a

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

²ND: not detected

yeast was not possible. Therefore, environmental bacteria could proliferate in the samples, resulting in slight acidification [23]. The lactic acid content was also highest in the sample treated at 70 °C (Table 2). Thus, it can be inferred that the acidity increase was caused by lactic acid bacteria ($p < 0.05$).

Samples treated at 50–60 °C and the control had similar alcohol contents, while no alcohol was detected in samples treated at 70 °C and above. Total sugar contents were similar in the control and the sample treated at 50 °C ($p < 0.05$), as were volatile acid contents. In contrast, the amino acid value, pH, and reducing sugar content significantly decreased with increasing temperature ($p < 0.05$). Similarly, Park et al. [23] reported significant decreases in the physiochemical properties of rice wine made with *Nuruk* as the heat-treatment temperature increased from 25 to 80 °C. As the activities of the enzymes that metabolize starch and peptides decrease with increasing temperature, normal alcohol fermentation does not occur above 50 °C.

Nitrogen-containing compounds

As amino acids play an important role in liquor aging, the content of various nitrogen-containing compounds in *Yakju* was analyzed (Table 3). The total amount of nitrogen compounds increased after aging. During liquor production, 30–40% of the proteins in rice or *Nuruk* are metabolized by acidic protease or acidic carboxylase in *Nuruk* into amino acids or peptides, which are partially used by yeast, while the remainder become constituents of the liquor [18].

The contents of various amino acids were increased by the heat-treatment temperature, including sarcosine (17.1 times) and glutamic acid (4.6 times), as well as alanine, anserine, arginine, α -aminobutyric acid, histidine, methionine, ornithine, and valine (1.0–2.8 times). Although

the contents of the principal nitrogen-containing compounds decreased with increasing temperature, the constituents did not change significantly.

During liquor aging, methionine is converted into dimethyl disulfide (DMDS), which causes an off-flavor in the liquor [11]. Moreover, reducing sugars converted into furfural or aldehyde compounds through Maillard reactions have negative effects on the scent of *Yakju*. In particular, there is a very low odor threshold for volatile sulfur compounds (6.7, 7, and 0.18 $\mu\text{g/L}$ for dimethyl sulfide, DMDS, and dimethyl trisulfide (DMTS), respectively) [18]. An analysis of nitrogen-containing compounds in long-term aged sake showed a high correlation coefficient of 0.72 for DMTS content [24]. In this study, heat-treatment of *Nuruk* at 50 °C caused a decrease of cysteine (9.9 times) and methionine (615.8 times) in *Yakju*; therefore, it is assumed that the amount of compounds with strong odors produced from amino acids is reduced.

Color

Heat treatment of *Nuruk* causes a decrease in the a (redness), b (yellowness), and $\Delta E (\sqrt{L^2 + a^2 + b^2})$ of *Yakju* during aging (Table 4). Many components are responsible for the browning of *Yakju*. Most browning of *Yakju* during aging is caused by Maillard reactions of nitrogen-containing compounds with amino groups or sugars with carbonyl groups [25]. These reactions can proceed on exposure to sunlight to produce compounds such as melanoidin and harman. As shown in Table 3, the total amount of free amino acids in the Maillard reaction was decreased by 6.7–50.0 times and the tyrosine content of the tyrosinase activity was decreased by 30–90% (Table 3). Moreover, volatile compounds with low molecular weights, such as methylglyoxal, acetaldehyde, acetol, diacetyl, acetoin, glycoaldehyde, glyoxal, and dihydroxyacetone are

Table 3 Nitrogen-containing compounds in *Yakju* produced using heat-treated *Nuruk*

Compounds	Non-aged <i>Yakju</i>	Aged <i>Yakju</i>			
	Conc. (mg/L)	Conc. (mg/L)	Relative ratio ¹		
	Control	Control	50 °C	60 °C	70 °C
Alanine	296.77 ± 3.35 ^{a,2}	312.52 ± 6.75 ^a	0.13	0.03	0.02
Ammonia	48.20 ± 0.26 ^a	23.28 ± 0.82 ^b	0.32	0.21	0.15
Anserine	27.40 ± 0.15 ^b	40.40 ± 1.58 ^a	ND ³	ND	ND
Arginine	422.00 ± 5.37 ^b	646.26 ± 17.39 ^a	0.07	0.02	0.02
Aspartic acid	182.34 ± 45.74 ^a	186.08 ± 6.36 ^b	0.18	0.14	0.09
α.Aminoadipic acid	ND	21.67 ± 9.43 ^a	1.48	ND	ND
αDAminobutyric acid	22.16 ± 0.21 ^a	33.20 ± 6.81 ^a	0.27	ND	ND
βDAminoisobutyric acid	69.42 ± 0.15 ^a	34.26 ± 14.86 ^a	0.47	0.04	0.01
β.01.86is	44.23 ± 0.00 ^a	36.75 ± 7.57 ^a	0.21	0.01	0.01
γ.Aminobutyric acid	71.39 ± 0.25 ^a	58.07 ± 13.58 ^a	0.08	0.02	0.04
Carnosine	ND	11.56 ± 0.25 ^a	ND	ND	ND
Cysteine	ND	19.96 ± 2.39 ^a	2.02	ND	ND
Cystathionine	54.50 ± 0.52 ^a	42.63 ± 9.15 ^a	0.09	0.01	0.01
Ethanolamine	15.46 ± 1.30 ^a	13.71 ± 2.85 ^a	0.29	0.32	0.41
Glutamic acid	53.21 ± 0.69 ^b	244.21 ± 5.37 ^a	0.07	0.01	ND
Glycine	117.53 ± 1.27 ^a	109.00 ± 1.64 ^b	0.15	0.06	0.06
Histidine	48.03 ± 0.52 ^b	90.90 ± 1.23 ^a	0.11	0.03	0.02
Hydroxyproline	ND	ND	ND	ND	ND
Hydroxylysine	ND	126.75 ± 5.22 ^a	0.07	ND	ND
Isoleucine	128.20 ± 0.85 ^a	124.58 ± 6.60 ^a	0.21	0.03	0.02
Leucine	298.27 ± 1.76 ^a	289.15 ± 2.75 ^a	0.10	0.02	0.01
Lysine	211.52 ± 3.02 ^b	326.82 ± 9.00 ^a	0.13	0.04	0.03
Methionine	15.34 ± 0.02 ^b	43.11 ± 3.10 ^a	0.07	ND	ND
1-Methylhistidine	ND	ND	ND	ND	ND
3-Methylhistidine	ND	2.57 ± 0.92 ^a	1.84	ND	ND
Ornithine	24.81 ± 0.31 ^b	52.28 ± 1.32 ^a	0.15	0.02	ND
Phosphoethanolamine	23.27 ± 0.11 ^a	11.12 ± 0.29 ^b	0.38	ND	ND
Phenylalanine	195.10 ± 0.06 ^a	198.98 ± 27.88 ^a	0.06	0.02	0.01
Proline	335.22 ± 5.20 ^a	362.41 ± 10.53 ^a	0.07	0.03	0.03
Phosphoserine	32.32 ± 0.36 ^a	8.71 ± 12.32 ^a	3.66	0.64	0.53
Sarcosine	1.91 ± 0.01 ^b	32.76 ± 5.63 ^a	ND	ND	ND
Serine	148.39 ± 1.65 ^a	107.84 ± 3.29 ^b	0.17	0.05	0.03
Taurine	ND	ND	ND	ND	ND
Threonine	109.97 ± 1.15 ^a	81.32 ± 2.36 ^b	0.10	0.05	0.03
Tyrosine	178.64 ± 0.10 ^a	177.24 ± 18.66 ^a	0.30	0.02	0.01
Urea	ND	50.35 ± 71.21 ^a	ND	ND	ND
Valine	161.36 ± 1.42 ^a	165.69 ± 0.35 ^a	0.18	0.03	0.02
Total	3336.98	4086.12	0.15	0.03	0.02

¹Ratio of nitrogen-containing compounds in heat-treated and control samples²Values are mean ± SD (n = 3)³ND: not detected

produced in the intermediate stages of Maillard reactions. Polymerization and condensation of these molecules could produce unsaturated melanoidin, which is brownish [16]. In *Yakju* produced using heat-treated *Nuruk*, the activities of enzymes that break down protease and amylase are

inhibited, which decreases color changes owing to decreased production of browning compounds.

Table 4 Colour of *Yakju* produced using heat-treated *Nuruk*. (Color table online)

Hunter colour values	Non-aged <i>Yakju</i>	Aged <i>Yakju</i>			
	Control	Control	50 °C	60 °C	70 °C

L ¹	83.89 ± 0.00 ^{d,5}	44.32 ± 0.00 ^e	93.91 ± 0.00 ^b	93.99 ± 0.00 ^a	93.72 ± 0.02 ^c
a ²	0.58 ± 0.00 ^b	24.43 ± 0.00 ^a	− 2.14 ± 0.01 ^e	− 1.34 ± 0.01 ^c	− 1.68 ± 0.01 ^d
b ³	46.45 ± 0.02 ^b	70.07 ± 0.05 ^a	12.76 ± 0.01 ^c	8.40 ± 0.00 ^e	9.95 ± 0.00 ^d
ΔE ⁴	71.87 ± 0.03 ^b	116.08 ± 0.11 ^a	19.94 ± 0.02 ^c	13.10 ± 0.02 ^e	15.41 ± 0.21 ^d

¹Lightness (100 = white, 0 = black)²Redness (+ = red, − = green)³Yellowness (+ = yellow, − = blue)⁴ΔE = $\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$ ⁵Values are mean ± SD (n = 3), different letters within the same row differ significantly ($p < 0.05$)

Volatile flavor compounds

The contents of various volatile compounds in *Yakju* are shown in Table 5. Generally, the alcohol contents in the heat-treated samples were higher than those of the control sample, but the alcohol content decreased at 70 °C. Isoamyl alcohol (smells disagreeable, bitter) and 2-phenyl alcohol (smells like roses) increased at 50–60 °C, whereas isobutanol (bitter taste, strong odor) and n-propanol (unseasoned smell, spicy flavor) increased in all heat-treated samples. *n*-Butanol (smells like bananas, sweet flavor) was 1.85 times higher at 50 °C than in the control, but decreased at higher temperatures.

The contents of furfural (smoky scent) and isovaleraldehyde (soy sauce scent), which cause odors in aged liquor, decreased with heat-treatment temperature. Furfural is formed in *Yakju* by the Maillard reaction [26], but heat treatment of *Nuruk* lowers protease activity (Table 1), which could inhibit furfural formation. Similarly, isovaleraldehyde is formed by ADH activity [5], which is decreased by heat treatment of *Nuruk*.

Esters (fruity or flowery smells) increased in the heat-treated samples. Ethyl acetate (fruity fragrance) and ethyl laurate (violet fragrance) increased by 1.63 and 1.14 times, respectively, at 50 °C relative to the non-heat-treated control. Isoamyl acetate (banana smell) is an important flavor compound that depends on the concentration of isoamyl alcohol. As isoamyl alcohol is synthesized from L-leucine, using yeasts that are resistant to high

concentrations of L-leucine produces high concentrations of isoamyl acetate.

Notably, various off-flavors, including acetic acid (vinegar smell), butyric acid (buttery rancid smell), propionic acid (rancid smell), and vanillin (sulfur smell), are significantly inhibited in *Yakju* produced using heat-treated *Nuruk*. The contents of 5-hydroxy methylfurfural (precursor of furfural), tyrosol (bitter taste), which is synthesized by the metabolism of L-tyrosine by yeast, and benzaldehyde [27], which causes a soy sauce smell, are also decreased. Therefore, it is possible to control the odor generated during the fermentation process so that factors that are considered unpleasant in *Yakju* can be eliminated.

Sensory evaluation

The sensory evaluation results for the *Yakju* samples are shown in Table 6. Bitterness, saltiness, and sourness were highest in the control sample, while umami and sweetness were highest in the 50 °C heat-treated sample. The Chinese Shaoxing wine used as a reference was aged for 3 years or more. Moreover, this wine used rice and yeast as the main raw materials and was manufactured by the same method as Korean *Yakju* [28]. A previous study on Shaoxing wine revealed correlations between nitrogen-containing compounds and taste [28], with aspartic acid, threonine, serine, proline, glycine, and leucine having positive effects ($r = 0.42$ – 0.65), and methionine, histidine, and arginine having negative effects ($r = -0.75$ to -0.42). In the current study, the use of heat-treated *Nuruk* reduced the

Table 5 Volatile flavour compounds in *Yakju* produced using heat-treated *Nuruk*

Compounds	Aged <i>Yakju</i>				
	Conc. (mg/L)	Relative ratio ¹			
		Control	50 °C	60 °C	70 °C
Alcohol					
Active amyl alcohol	277.30 ± 33.18 ²	0.55	0.21	ND ³	
Benzyl alcohol	1.30 ± 0.05	1.28	0.05	ND	
Isoamyl alcohol	250.12 ± 6.52	1.49	1.88	0.02	
Isobutanol	223.02 ± 7.72	1.22	1.11	1.14	
Methanol	9.95 ± 0.46	0.62	1.16	1.10	
<i>n</i> -Butanol	2.99 ± 0.06	1.85	0.86	ND	
<i>n</i> -Propanol	71.07 ± 5.40	1.61	1.05	1.39	
2-Phenyl alcohol	73.43 ± 1.85	1.01	1.07	0.03	
Aldehyde					
Furfural	58.01 ± 7.42	0.50	0.44	0.30	
Isovaleraldehyde	7.13 ± 0.90	0.15	0.05	ND	
Ester					
Ethyl acetate	11.97 ± 0.06	1.63	2.09	0.31	
Ethyl butyrate	ND	ND	ND	ND	
Ethyl caprate	ND	ND	ND	ND	
Ethyl caproate	ND	ND	ND	ND	
Ethyl caprylate	ND	ND	ND	ND	
Ethyl lactate	41.92 ± 2.61	1.13	1.24	1.32	
Ethyl laurate	1.96 ± 0.27	1.14	1.53	1.54	
Isoamyl acetate	0.15 ± 0.00	0.33	ND	ND	
Organic acid					
Acetic acid	250.18 ± 2.68	0.38	0.56	0.30	
Isovaleric acid	5.27 ± 0.71	0.96	1.02	1.06	
<i>n</i> -Butyric acid	3.31 ± 0.17	0.68	0.95	ND	
Propionic acid	10.05 ± 0.79	0.42	0.28	ND	
Valeric acid	6.01 ± 0.73	0.59	ND	ND	
Sulfur					
Diacetyl	ND	ND	ND	ND	
Dimethyl disulfide	ND	ND	ND	ND	
Dimethyl trisulfide	ND	ND	ND	ND	
Vanillin	0.42 ± 0.06	0.64	0.26	ND	
Other					
Acetaldehyde diethyl acetal	192.94 ± 25.07	0.43	0.18	0.06	
Benzaldehyde	8.46 ± 0.11	0.48	0.18	ND	
2,3-Butanediol	104.34 ± 6.68	0.37	0.11	0.06	
Butyrolactone	137.64 ± 8.55	0.71	0.26	0.13	
Dehydromevalonic lactone	0.99 ± 0.10	0.99	0.45	ND	
Diethyl malate	2.65 ± 0.19	2.48	1.85	ND	
Diethyl succinate	234.34 ± 32.97	1.34	0.62	0.43	
4-Ethoxy-2-butanone	ND	ND	ND	ND	
Ethyl hydrogen succinate	519.79 ± 36.07	1.54	1.34	1.03	
Ethyl 2-hydroxyisovalerate	14.28 ± 0.47	0.89	0.25	ND	
Ethyl propanoate	4.48 ± 0.28	0.98	0.25	ND	
<i>p</i> -Hydroxybenzaldehyde acetate	5.67 ± 0.53	0.59	0.54	ND	
3-Hydroxy ethylbutyrate	ND	ND	ND	ND	
5-Hydroxy methylfurfural	114.07 ± 0.84	0.23	0.20	0.13	

Table 5 continued

Compounds	Aged <i>Yakju</i>			
	Conc. (mg/L)	Relative ratio ¹		
	Control	50 °C	60 °C	70 °C
Isovaleraldehyde diethyl acetal	2.28 ± 0.17	0.79	0.02	ND
2-Methyl butyraldehyde	ND	ND	ND	ND
(3 <i>E</i>)-3-methyl-3-hexen-2-one	9.93 ± 0.75	1.01	ND	ND
(4 <i>E</i>)-3-methyl-4-hexen-2-one	10.82 ± 0.40	0.33	ND	ND
2-Phenyl-2-butenal	ND	ND	ND	ND
Propyl methyl ketone	111.41 ± 8.61	0.40	0.12	0.07
2,4,5-Trimethyl-1,3-dioxolane	53.01 ± 4.56	0.24	0.09	0.02
Tyrosol	371.42 ± 17.04	0.47	0.37	ND

¹Ratio of flavour compounds in heat-treated and control samples²Values are mean ± SD (n = 3)³ND: not detected**Table 6** Sensory evaluation of *Yakju* produced using heat-treated *Nuruk*

Sensory evaluation	Non-aged <i>Yakju</i>	Aged <i>Yakju</i>				Chinese Shaoxing wine
	Control	Control	50 °C	60 °C	70 °C	
Odour attributes						
Caramel and burnt	2.75 ± 1.86 ^{c,1}	5.00 ± 1.04 ^{ab}	3.67 ± 1.13 ^{bc}	3.92 ± 1.83 ^{bc}	4.08 ± 1.83 ^{bc}	5.92 ± 0.90 ^a
Overall intensity of fragrance	3.67 ± 1.87 ^c	5.33 ± 0.98 ^{ab}	4.21 ± 0.72 ^{bc}	4.33 ± 1.44 ^{bc}	4.08 ± 1.88 ^{bc}	6.17 ± 0.83 ^a
Soy sauce	2.25 ± 1.82 ^c	4.67 ± 1.23 ^{ab}	3.88 ± 1.42 ^{bc}	3.42 ± 1.44 ^{bc}	2.79 ± 1.70 ^c	6.00 ± 1.13 ^a
Sulfur	1.75 ± 0.87 ^a	2.67 ± 0.98 ^a	2.17 ± 0.94 ^a	2.75 ± 1.48 ^a	2.33 ± 1.30 ^a	3.25 ± 1.91 ^a
Taste attributes						
Bitterness	3.25 ± 1.22 ^a	4.67 ± 1.67 ^a	4.58 ± 1.56 ^a	4.67 ± 1.67 ^a	3.17 ± 1.40 ^a	4.71 ± 1.86 ^a
Saltiness	2.75 ± 1.48 ^a	4.17 ± 1.70 ^a	3.00 ± 1.41 ^a	3.04 ± 1.45 ^a	4.08 ± 1.56 ^a	4.67 ± 2.15 ^a
Sourness	4.50 ± 1.57 ^a	3.92 ± 1.38 ^a	3.29 ± 1.21 ^a	3.58 ± 1.38 ^a	3.67 ± 0.78 ^a	4.42 ± 1.44 ^a
Sweetness	3.75 ± 1.54 ^a	2.75 ± 0.97 ^{ab}	2.92 ± 1.08 ^{ab}	2.50 ± 0.80 ^{ab}	2.25 ± 0.87 ^b	2.92 ± 1.16 ^{ab}
Umami	3.88 ± 1.54 ^a	4.04 ± 1.70 ^a	4.38 ± 1.07 ^a	4.00 ± 1.48 ^a	3.83 ± 1.11 ^a	4.50 ± 2.15 ^a

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

negative effects of amino acids on the taste of *Yakju*. The odor attributes were also highest in the control sample.

A comparison of flavor compounds in fresh sake and sake aged for 3 years showed that DMDS (sulfur smell) was 5 times, DMTS was 125 times, furfural and pyrazine (burnt smell) were 5 times, and methional was 25 times higher than in fresh sake [24]. Wine and sake that has been aged for 3 months at 45 °C is similar in terms of quality to sake aged for 3 years at 25 °C [29]. Similarly, it is expected that off-flavors and bad taste would be inhibited in *Yakju*, even if it were aged for over 10 years at 25 °C, as oxidases in *Nuruk* heat-treated at 50 °C are inactivated. In this study, heat treatment of *Nuruk* at 50 °C or higher was confirmed to decrease the protease activity decreased and

the content of nitrogen-containing compounds. The reduction of nitrogen-containing compounds inhibited color changes and the production of odors in aged *Yakju*.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest to declare.

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