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Effects of steaming and drying processing on Korean rice wine (*Makgeolli*) with deodeok (*Codonopsis lanceolate*)

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Abstract The physicochemical properties of deodeok (*Codonopsis lanceolate*) was investigated in relation to the different steaming time and cycles of steaming and drying (S/D). Additionally, the quality characteristics of *Makgeolli* with different amount (0-0.45%) of steaming and drying deodeok (SD) were measured comparison to non-steaming and drying deodeok (NSD). *L** values of deodeok tended to decrease as the number of S/D cycles and steaming times increased, while BI showed the opposite trend for *L** values. Reducing sugar increased significantly from 1 to 3 S/D cycles and decreased thereafter (p<0.05). Also, processed with steaming for 4 h and 5 S/D cycles had the highest antioxidant properties. Principal component analysis (PCA) revealed that the S/D process notably influenced the properties of deodeok. Quality characteristics of *Makgeolli* showed that 0.45% SD resulted in higher antioxidant properties than control or NSD.

Keywords: deodeok (*Codonopsis lanceolate*), steaming, drying, *makgeolli*, antioxidant properties

Introduction

Makgeolli has been a traditional and popular alcoholic beverage for centuries in Korea. The brewing process for *Makgeolli* includes the twice mashing step involving simultaneous saccharification and alcohol fermentation. Processing of *Makgeolli* usually starts with mixing nuruk or koji, yeast, and water from the first seed mash (Jung et al., 2014). *Makgeolli* contains 12-15% alcohol before dilution with water and the alcohol content can be affected by the fermentation conditions and mashing type. It also contains abundant vitamin B complex, organic acid, and protein (Lee et al., 2010). Currently, many manufacturers add fruits and vegetables to *Makgeolli* to produce differentiated varieties designed to meet diverse customer needs. Therefore, the effects of medicinal plants on the quality and antioxidant properties of *Makgeolli* have been investigated. Omija berries (*Schizandra Chinensis Baillon*), *Angelica gigas* Nakai water extracts, and the fruit of *Akebia quinata* have been used as minor ingredients in *Makgeolli* (Lee et al., 2013a; Lee et al., 2013b; Song et al., 2015). However, many of these previous studies did not evaluate the effects of pre-treatment of these additional ingredients in *Makgeolli* on bioactive substances related to antioxidant properties.

Deodeok (*Codonopsis lanceolate*), a member of the *campanulaceae* family known as “Sasam”, has traditionally been used in Chinese medicine along with ginseng, scrophularia root, pilose bellflower, and shrubby sophora. These five medicinal plants along with deodeok have been regarded as belonging to the same effective medicinal plants group due to their similarities in appearance and health benefits. Deodeok contains polyphenol, tannin, alkaloid and the triterpenoid form of saponin and has strong antioxidant, antimicrobial, antimutagenic activities, and anti-inflammatory effects (He et al., 2011; Ichikawa et al., 2009; Yongxu and Jicheng, 2008).

Recent studies have shown that thermal processing is an effective way to increase physiological active substances, thereby enhancing the antioxidant activity of food products due to the extraction of effective components (Kim et al., 2008). Moreover, some studies have proposed optimal processing conditions for obtaining the highest antioxidant activities (Vitali Čepo et al., 2014). The effects on antioxidants in heat treated licorice (*Glycyrrhiza uralensis Fisch*) extracts (Woo et al., 2007), garlic with different heat and pressure treatments (Lee et al., 2012), carob powder with different roasting treatments (Vitali Čepo et al., 2014), and heat treated deodeok and doragi-bellflower root with different temperatures (Hwang et al., 2011) have been reported. Steaming then drying, which is a well-known thermal processing method for ginseng, has been reported to improve the biological activities of ginseng. Changes in the ginsenosides and in ginseng quality resulting from different steaming and drying conditions have been reported (Jin et al., 2012; Kim et al., 2007). In particular, saponin is a major active component in ginseng that contains more of the saponin component and enhances activity such as antioxidation by 9 cycle steaming and drying (Kim, 2015). Thus, steaming and drying can increase saponin content of deodeok which might turn into more active saponins. However, there have been few studies regarding the effects on antioxidant and physicochemical properties of deodeok when processed under different steaming and drying conditions. Therefore, the objectives of this study were 1) to investigate changes in the physicochemical and antioxidant properties of deodeok associated with different steaming and drying conditions and 2) to determine the optimal amount of steaming and drying deodeok for *Makgeolli* preparation

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based on quality and antioxidant properties.

Materials and Methods

Materials

Deodeok was obtained from Ulmome Co. (Gumsan, Korea). For evaluation of antioxidant activity, 6-hydroxy-2,4,7,8-tetra-methylchromane-2-carboxyilic acid (trolox), $FeCl_3 \cdot 6H_2O$ (Iron(III) chloride hexahydrate), 2,4,6-Tris-2,4,6-tripyridyl-2-triazine (TPTZ), azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) purity of 98% minimum, potassium persulphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium sodium tartrate, 3,5-dinitrosalicylic acid were obtained from Sigma-Aldrich to analyze of reducing sugar. Rice (Incheon rice) was purchased from Nong-Hyup Market (Seoul, Korea). Yeast was purchased from Songcheon Yeast Ist. (Cheongju, Korea) and wheat bran was obtained from E-Dong Rice Wine Co., Ltd. (Pocheon, Korea). Puffed rice flour was obtained from Woori rice Co. (Namyangju, Korea) and purified enzyme was purchased from Wonderful Winery (Boeun-gun, Korea). To evaluate chemical properties during fermentation periods, bromothymol blue, neutral red, and sodium hydroxide solution were obtained from Sigma-Aldrich to measure acidity of *Makgeolli*.

Preparation of steaming and drying deodeok

Deodeok was washed and peeled, and then treated with different steaming times (2, 4, and 6 h) and different number of steaming and drying (S/D) cycles (1, 3, and 5). The steaming deodeok was dried at 60°C for 24 h after finishing every steaming process and the drying condition was constant. After S/D process, the sample was ground and passed through a 100 mesh sieve. Powdered samples were stored at 4°C prior to use. The powdered samples were extracted to analyze reducing sugar and protein contents, browning index (BI) and antioxidant properties. Extraction was conducted with distilled water using magnetic stirrer with stirring bar (550 rpm, Wisestir MS-MP8, Wise Laboratory Instruments, Wertheim, Germany) for 3 h. After extracting, the samples were passed through filter paper (#2, Advantec, Tokyo, Japan) prior to experiment.

Physicochemical properties of deodeok

The color value was measured using a Color Meter (DP-400, Minolta Co. Ltd. Japan) after calibrating using a Minolta calibration plate. Browning index (BI) was detected using the modified method of Kim et al. (2012). Each sample was diluted with distilled water to give absorbance signals on scale and measured absorbance at 420 nm using a spectrophotometer (Biomate 3S, Thermo scientific, Rockford, IL, USA). Reducing sugar measurement was followed by Kim et al. (2012), who used the modified method of DNS (Miller, 1959).

Manufacture of *Makgeolli*

Makgeolli was manufactured according the proportion of ingredients in Table 1. Seed mash and first mash were carried out at 24°C for 5 days to cultivate yeast. In the second mash, rice was soaked in water for 2 h, and was drained for 30 min. After that, rice was steamed for 40 min and cooled for 20 min at room temperature. After steaming and cooling the rice, all the materials, which were presented in Table 1, were mixed well and fermentation was conducted at 24°C for 5 days. Deodeok was prepared with the steaming for 4 h and 5 cycles of S/D deodeok (SD) and non-steaming and drying deodeok (NSD).

Physicochemical properties of *Makgeolli*

Alcohol contents measurement was followed: 100 mL of sample was put into the 100 mL measuring cylinder, and then it was transferred to boiling flask on the distillation apparatus. 30 mL of distilled water was added to the boiling flask. After that, distillation was carried out until 80 mL of distillate was obtained. The 80 mL of distillate was filled with distilled water up to the total volume

Table 1. Formulation of deodeok *Makgeolli*

Mashing step	Material	Sample (g)			
		Control	0.15%	0.30%	0.45%
Seed mash	Yeast	0.4	0.4	0.4	0.4
	Lactic acid	0.8	0.8	0.8	0.8
	Wheat barn *koji*	72	72	72	72
	Water	93.6	93.6	93.6	93.6
First stage mash	Wheat barn koji	497.8	497.8	497.8	497.8
	Water	796.4	796.4	796.4	796.4
Second stage mash	Rice	1,586	1,556	1,526	1,496
	Steaming and drying deodeok powder	0	30	60	90
	Puffed rice flour	712	712	712	712
	Purified enzyme	0.4	0.4	0.4	0.4
	Water	4,136	4,136	4,136	4,136
Filtration and immersion step	Aspartame	2.6	2.6	2.6	2.6
	Water	12,104	12,104	12,104	12,104

of 100 mL. The alcohol content was measured using alcoholometer (Deakwang Inc., Seoul, Korea). Reducing sugar measurement was previously described as above. The pH was measured using pH meter (Orion 3-Star, Thermo scientific, Rockford, IL, USA). The acidity was measured using titration method. Bromthymol blue and neural red were used as the indicator. 10 mL of sample was transferred into the beaker and then neutralization titration was conducted by 0.1 N NaOH. The determination of acidity was the amount of 0.1 N NaOH in mL used in titration.

Total polyphenol contents

Total polyphenol contents were determined according to the method of Singleton et al. (1999). 1 mL of sample was mixed with 1 mL of 10% Folin-Ciocalteu's phenol reagent and 1 mL of 10% Na_2CO_3. After 30 min reaction at room temperature in the dark, total polyphenol contents were evaluated by measuring absorbance at 750 nm. Gallic acid was used as the standard and total polyphenol contents of sample were expressed as mg of gallic acid equivalents (mg GAE/g).

Trolox equivalent antioxidant capacity (TEAC) using ABTS assay

Trolox equivalent antioxidant capacity (TEAC) was evaluated according to the method of Re et al. (1999). 7 mM ABTS solution and 2.45 mM potassium persulphate were mixed with in ratio of 1:1. Solution was kept in dark at room temperature for overnight prior to use. ABTS•+ solution was diluted with PBS (pH 7.4) to make an absorbance of 0.70±0.02 at 734 nm. 20 μL of sample was reacted with 980 μL ABTS•+ solution for 15 min at room temperature. ABTS value was evaluated by measuring absorbance at 734 nm using spectrophotometer. Trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid) used for calibration curve and activity was expressed as mmol Trolox equivalent antioxidant capacity (TEAC) per g of sample.

Ferric reducing antioxidant power (FRAP) value

FRAP (Ferric reducing antioxidant power) value was determined according to Benzie and Strain (1996) with slight modification. FRAP reagent was prepared freshly with 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM ferric chloride in proportion of 10:1:1 (v/v). The FRAP reagent was incubated at 37°C in a water bath prior to use. 50 mL of diluted sample was reacted with 150 μL of FRAP reagent for 4 min. FRAP value was evaluated by measuring the variation in absorbance at 595 nm using ELISA micro plate reader (Bio-Rad, Hercules, California, USA). Ferrous sulphate ($FeSO_4 \cdot 7H_2O$) was used as a standard and the results were expressed as μmol of $FeSO_4 \cdot 7H_2O$ equivalent per L of sample.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method of Brand-Williams et al., (1995) with some modifications. First, 0.2 mM DPPH solution was prepared fresh prior to use. Then, 40 μL of sample was allowed to react with 160 μL of 0.2 mM DPPH solution and was left in the dark for 30 min at room temperature. The antioxidant activity was evaluated by measuring the variation in absorbance at 517 nm using a Synergy HT Multi-microplate reader (BioTek Instruments, Winooski, VT, USA). DPPH radical scavenging activity was calculated using the following equation.

$$\text{DPPH radical scavenging activity (\%)} = 1 - \frac{\text{sample absorbance}}{\text{control absorbance}} \times 100$$

Statistical analysis

Statistical analysis was performed using a statistical analysis software system (SPSS software version 17.0 SPSS Inc., Chicago, IL, USA). All analyses were performed in triplicate. Analysis of variance (ANOVA) was applied to determine significant differences among samples, followed by Duncan's multiple range tests for mean comparison at $p<0.05$. All data were presented as mean± standard deviation. Principal component analysis (PCA) was additionally conducted using SPSS software version 17.0 to evaluate the influence of the steaming and drying conditions on the physiochemical and antioxidant properties of deodeok.

Results and Discussion

Physicochemical properties of deodeok with different steaming and drying conditions

Table 2 shows the effect of steaming and drying conditions on the hunter color values of deodeok. *L** (lightness) values tended to decrease as the number of S/D cycles and steaming times increased. Ultimately, the 5-4 h conditions resulted in the lowest *L** value and a slight increase in *L** value was observed between the 5-4 h and 5-6 h conditions. The *a** (redness) values tended to increase significantly from 1 to 3 cycles of S/D, whereas no significant difference was observed after 3 S/D cycles ($p<0.05$). The *b** value increased significantly from 1 to 3 S/D cycles and decreased after 3 S/D cycles ($p<0.05$). The 5-4 h and 5-6 h conditions resulted in low *b** values of 9.55 and 10.26, respectively. Reducing sugar content was significantly affected by the number of S/D cycles ($p<0.05$) (Table 2). A dramatic increase in reducing sugar was observed from 1 to 3 S/D cycles. The highest content was observed in the 5-2 h sample. This might be due to hydrolyzed non-reducing sugar generating a lot of low molecular weight reducing sugar compound in the early phase, which is then eluted during the repeated steaming and drying process over a lengthy period of time (Lee et al., 2015). In some Maillard reaction studies, the BI has been used as an indicator of MRPs (Maillard reaction products) and it has been reported that BI is affected by reaction conditions or the intensity of thermal processing. A significant correlation with phenolic content and antioxidant activities has also been reported (Vitali Čepo et al., 2014). As shown in Table 2, BI was significantly affected by the number of S/D cycles and steaming times ($p<0.05$), such that it increased dramatically from 1 to 3 S/D cycles. BI increased significantly up to 0.83 in the 5-4 h sample ($p<0.05$), whereas no significant difference between the 5-4 h and 5-6 h samples was

Table 2. Physicochemical and antioxidant properties of deodeok with different steaming and drying conditions

Sample	Color values			Reducing sugar (mg/g)	BI (A_{420}/g)	Total polyphenols (mg GAE/g)	ABTS (mmol TE/g)	FRAP value (mM $FeSO_4$ eq./ g)
	*L**	*a**	*b**					
Control	87.71±5.55^{a}	0.43±0.11^{d}	14.10±4.45^{b}	124.17±1.01^{h}	0.054±0.007^{g}	2.39±0.06^{f}	23.36±1.43^{f}	17.49±0.52^{f}
1-2 h[1)]	76.93±8.10^{b}	1.07±0.07^{d}	16.44±1.78^{a}	159.05±2.35^{g}	0.104±0.000^{f}	2.03±0.07^{f}	17.72±1.81^{f}	10.64±0.52^{f}
1-4 h	72.74± 4.95^{bc}	2.41±0.36^{c}	17.12±1.52^{a}	170.47±1.59^{g}	0.135±0.000^{e}	2.48±0.03^{f}	20.04±0.22^{f}	12.33±0.20^{f}
1-6 h	69.19±6.20^{c}	3.08±0.40^{c}	17.86±2.16^{a}	198.04±1.48^{f}	0.131±0.003^{e}	2.57±0.02^{f}	21.997±0.72^{f}	10.98±0.39^{f}
3-2 h	53.49±1.85^{d}	7.99±1.37^{a}	18.19±1.74^{a}	414.41±6.20^{C}	0.342±0.000^{d}	15.09±0.33^{e}	125.04±0.93^{e}	70.94±3.32^{e}
3-4 h	43.25±3.89^{e}	7.89±1.31^{a}	14.18±2.83^{b}	425.23±11.79^{bc}	0.441±0.000^{c}	15.65±0.40^{e}	161.18±8.04^{d}	74.99±1.92^{d}
3-6 h	44.40±5.25^{e}	7.16±1.07^{ab}	12.84±2.67^{b}	437.94±14.46^{e}	0.532±0.011^{b}	17.50±0.14^{d}	184.95±1.15^{c}	79.48±1.97^{c}
5-2 h	42.95±1.85^{e}	7.52±1.05^{ab}	12.99±1.12^{b}	463.62±6.57^{a}	0.550±0.000^{b}	22.77±0.36^{c}	190.69±3.24^{b}	85.21±0.78^{b}
5-4 h	38.03±2.04^{ef}	7.16±0.93^{ab}	9.55±1.08^{c}	344.08±3.05^{e}	0.827±0.000^{a}	30.37±0.14^{a}	261.18±8.78^{a}	123.05±1.17^{a}
5-6 h	39.47±2.22^{e}	6.85±0.40^{b}	10.26±0.96^{c}	369.27±6.91^{d}	0.818±0.002^{a}	26.23±0.35^{b}	209.69±2.19^{b}	107.33±2.14^{b}

Means values marked with the different letter in the same column are significantly different at $p<0.05$.
[1)]Abbreviations were used for indication of steaming and drying condition, for example: 1-2 h refers to deodeok treated with steaming for 2 h and 1 cycle of S/D, etc.

observed. A similar pattern was observed in a study by Vitali Čepo et al. (2014) who reported that BI was affected by the intensity of thermal processing; however, BI did not continue to increase as the intensity of thermal processing increased.

Total polyphenol contents and antioxidant activities of deodeok with different steaming and drying conditions

As presented in Table 2, total polyphenols were significantly affected by the number of S/D cycles ($p<0.05$). No significant differences were observed among samples processed using 1 cycle of S/D. Total polyphenol content was increased significantly up to 5.8-fold in the 3-2 h sample compared to the control sample (2.4 →15.1 mg GAE/g), and the highest amount was observed in the 5-4 h sample which reached a level of 30.37 mg GAE/g. After peaking at the highest value, a significant decrease was observed from the 5-4 h sample to the 5-6 h sample ($p<0.05$). The ABTS value increased significantly up to 5.3-fold in the 3-2 h sample compared to the control (23.35→125.04 mmol TE/g) ($p<0.05$) (Table 2). The 5-4 h sample had the highest ABTS value (261.18 mmol TE/g) and a significant decrease was observed between the 5-4 h and 5-6 h samples ($p<0.05$). With respect to FRAP values, a 5.6-fold increase was observed in the 3-2 h sample compared to control (17.492→70.94 mmol $FeSO_4 \cdot 7H_2O$ eq/g) (Table 2). Consequently, the 5-4 h sample had the highest FRAP value (123.05 mmol $FeSO_4 \cdot 7H_2O$ eq/g) and a significant decrease was also observed between the 5-4 h to 5-6 h samples (123.05→ 107.332 mmol $FeSO_4 \cdot 7H_2O$ eq/g). Overall, the 5-4 h sample had the highest antioxidant properties with high total polyphenol content, ABTS and FRAP values. These data are consistent with the published results by Song et al. (2012) and Yonxu et al. (2008), who reported that increased total polyphenol content or antioxidant activities during the thermal process may has been due to the breakdown of cell walls, increased hydrolyzed polyphenol content and elution of water-soluble components.

Principal component analysis (PCA)

Principal component analysis (PCA) was conducted to evaluate the influence of the steaming and drying conditions on the physiochemical and antioxidant properties of deodeok. The resulting biplot, consisting of a loading plot and a score plot, is presented in Fig. 1. To determine the characteristics of each sample, samples were marked by triangles on the plot. PC1 (84.9%) was mainly determined by antioxidant activities and total polyphenol content. PC2 (12.0%) was primarily characterized by *b** (yellowness) and did not show any significant correlation with antioxidant properties. Chemical properties, such as reducing sugar, protein, and moisture content, were found on the positive side of PC2, whereas the FRAP value, ABTS value, total polyphenol content and BI were on the negative side of PC2. These results demonstrate that the intensity of the S/D process significantly influenced the changes in physicochemical and antioxidant properties of deodeok.

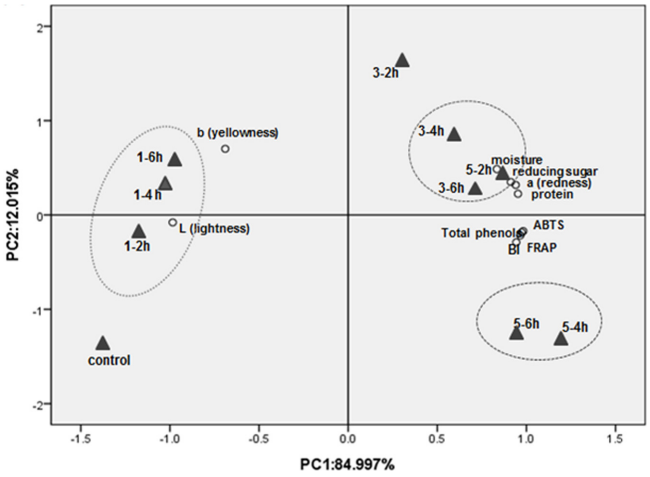

Fig. 1. Biplot obtained from principal component analysis of antioxidant properties and physicochemical properties on deodeok using different steaming and drying conditions. Abbreviations used to indicate the steaming and drying conditions, for example: 1-2 h refers to deodeok treated with steaming for 2 h and 1 cycle of S/D, etc.

Table 3. Changes in alcohol content, reducing sugar content, pH and the acidity of *Makgeolli* with different amounts of deodeok during fermentation periods after the second mash

	Sample	Fermentation periods (after second mash)				
		1	2	3	4	5
Alcohol content (%)	Control[1)]	6.33±0.40^{NS}	10.17±0.06^{b}	12.30±0.35^{NS}	14.33±0.29^{NS}	15.53±0.12^{NS}
	SD 0.15%	7.73±1.55^{NS}	11.60±1.01^{ab}	13.23±0.59^{NS}	14.40±0.26^{NS}	15.37±0.25^{NS}
	SD 0.30%	7.73±0.68^{NS}	11.20±0.61^{ab}	12.90±0.46^{NS}	13.70±0.72^{NS}	14.87±0.81^{NS}
	SD 0.45%	7.93±1.69^{NS}	11.57±1.07^{ab}	13.43±0.40^{NS}	14.27±0.47^{NS}	15.43±0.40^{NS}
	NSD 0.15%	7.17±0.47^{NS}	11.20±0.87^{ab}	12.10±1.39^{NS}	13.93±1.36^{NS}	15.07±0.51^{NS}
	NSD 0.30%	7.90±0.80^{NS}	11.47±0.68^{ab}	12.60±1.30^{NS}	13.70±1.30^{NS}	14.90±0.85^{NS}
	NSD 0.45%	7.97±0.58^{NS}	11.80±0.40^{a}	13.20±0.70^{NS}	14.13±0.76^{NS}	15.27±0.80^{NS}
Reducing sugar (mg/mL)	Control	15.36±2.40^{a}	8.81±1.02^{NS}	4.99±0.37^{NS}	4.08±1.07^{NS}	3.76±0.13^{NS}
	SD 0.15%	12.37±2.13^{ab}	9.35±1.63^{NS}	6.52±1.97^{NS}	5.38±1.23^{NS}	3.94±0.67^{NS}
	SD 0.30%	11.72±0.76^{b}	10.39±1.91^{NS}	7.11±2.13^{NS}	5.63±1.32^{NS}	3.92±0.32^{NS}
	SD 0.45%	12.77±1.92^{ab}	10.72±1.49^{NS}	8.48±1.96^{NS}	6.07±1.78^{NS}	4.48±0.79^{NS}
	NSD 0.15%	10.67±2.48^{b}	8.34±2.61^{NS}	5.97±1.72^{NS}	4.84±1.86^{NS}	3.39±0.60^{NS}
	NSD 0.30%	12.33±1.06^{ab}	8.65±2.70^{NS}	6.38±1.48^{NS}	5.12±1.69^{NS}	3.94±1.00^{NS}
	NSD 0.45%	12.34±1.18^{ab}	8.60±2.73^{NS}	6.56±2.57^{NS}	5.08±2.07^{NS}	3.89±1.22^{NS}
pH	Control	3.56±0.08^{b}	3.68±0.06^{b}	3.58±0.02^{c}	3.83±0.04^{bc}	3.71±0.07^{b}
	SD 0.15%	3.81±0.05^{a}	3.71±0.12^{ab}	3.83±0.01^{ab}	3.86±0.07^{abc}	3.96±0.0^{a}
	SD 0.30%	3.80±0.10^{a}	3.79±0.08^{ab}	3.82±0.05^{ab}	3.90±0.05^{abc}	3.93±0.02^{a}
	SD 0.45%	3.88±0.12^{a}	3.88±0.13^{a}	3.90±0.12^{a}	3.97±0.06^{a}	3.98±0.09^{a}
	NSD 0.15%	3.78±0.07^{a}	3.73±0.07^{ab}	3.72±0.09^{b}	3.78±0.05^{c}	3.90±0.08^{a}
	NSD 0.30%	3.82±0.09^{a}	3.81±0.02^{ab}	3,83±0.02^{ab}	3.87±0.13^{abc}	3.94±0.05^{a}
	NSD 0.45%	3.91±0.10^{a}	3.88±0.14^{a}	3.87±0.02^{a}	3.94±0.05^{ab}	3.97±0.03^{a}
Acidity (mL)	Control	3.30±0.17^{NS}	3.93±0.23^{ab}	4.43±0.23^{NS}	4.80±0.17^{ab}	5.00±0.17^{NS}
	SD 0.15%	3.63±0.76^{NS}	3.97±0.67^{ab}	4.43±0.64^{NS}	4.67±0.58^{ab}	4.87±0.32^{NS}
	SD 0.30%	3.67±0.10^{NS}	4.37±0.25^{a}	4.57±0.29^{NS}	4.87±0.31^{a}	4.93±0.35^{NS}
	SD 0.45%	3.70±0.79^{NS}	4.30±0.61^{ab}	4.70±0.56^{NS}	4.87±0.49^{a}	5.10±0.44^{NS}
	NSD 0.15%	3.10±0.00^{NS}	3.77±0.40^{ab}	4.03±0.65^{NS}	4.50±0.36^{ab}	4.80±0.53^{NS}
	NSD 0.30%	3.07±0.25^{NS}	3.50±0.20^{b}	3.93±0.38^{NS}	4.13±0.21^{b}	4.57±0.57^{NS}
	NSD 0.45%	3.13±0.40^{NS}	3.70±0.46^{ab}	4.13±0.40^{NS}	4.37±0.25^{ab}	4.67±0.51^{NS}

Mean values in the same column marked with different letters are significantly different at $p<0.05$, ^{NS}: not significant
[1)]Control, without deodeok; SD, Steaming and drying deodeok; NSD, Non-steaming and drying deodeok.

Effect of SD on the chemical properties of *Makgeolli* during fermentation

Alcohol content, an indicator of the progression of fermentation, is one of the main factors that determine *Makgeolli* quality as it affects the preservation and flavor of *Makgeolli* (Lee et al., 2013a). The measured alcohol content during fermentation after the second mash is presented in Table 3. No significant differences among the samples were observed, except on the second day of fermentation. On the second day of fermentation, the control had a lower alcohol content (10.17%) compared to the others samples (11.20-11.80%). On the first day of fermentation, samples with SD ranged from 7.73 to 7.93% alcohol, whereas samples with NSD ranged from 7.16-7.96%. On the last day of fermentation, samples with SD had alcohol contents ranging from 14.90 to 15.26%, whereas samples with NSD had alcohol contents of 14.86, 15.06, and 15.43%, respectively. These results show that SD or NSD deodeok did not inhibit alcohol fermentation of *Makgeolli* in this study. Reducing sugar, which is used as a fermentation substrate, affects sweetness (Choi et al., 2013). Changes in reducing sugar during fermentation are shown in Table 3. No significant differences among the samples were found, except on the first day of fermentation. On the first day, control samples had the highest level of reducing sugar at 15.36 mg/mL, whereas NSD 0.15% had the lowest value of 10.67 mg/mL ($p<0.05$). The amounts of reducing sugar ranged from 10.67 to 15.36 mg/mL on the first day and decreased to 3.38-4.48 mg/mL on the last day of fermentation. On the last day of fermentation, no significant differences among the samples were observed. These results are in agreement with

earlier studies investigating changes in reducing sugar in *Makgeolli* with various added ingredients during fermentation (Choi et al., 2013; Song et al., 2015). Choi et al. (2013) and Song et al. (2015) reported that the reducing sugar content increased significantly during the early phases of brewing, such as the first mashing step, because carbohydrates in the ingredients were hydrolyzed by an amylase enzymatic reaction. However, reducing sugar tended to gradually decrease in the late phases of fermentation, such as after the second mash, because the sugar consumption of the yeast was higher than the amount of sugar produced by the fermentation starter. pH is used as an important indicator of the progress of the fermentation of *Makgeolli* and of quality during fermentation (Song et al., 2015). The pH of control samples remained low during fermentation (ranging from 3.56 to 3.78), whereas the SD 0.45% and NSD 0.45% samples had higher pH (Table 3). On the last day of fermentation, the pH of samples ranged from 3.7 to 3.98, and no significant differences were observed among the samples except the control (3.71) sample. Changes in acidity during fermentation are shown in Table 3. Acidity tended to increase as the fermentation period progressed. Acidity ranged from 3.07 to 3.70 mL on the first day and increased to 4.57-5.10 mL on the last day of fermentation. On the last day of fermentation, the SD 0.45% and control samples had high values of 5.1 mL and 5.0, respectively, whereas the NSD 0.30% sample had a low value of 4.56 mL with no significant difference. Similar patterns were observed by Choi et al. (2013) and Song et al. (2015), who investigated *Makgeolli* with fruits or vegetables added. They observed that acidity tended to increase as the fermentation period progressed due to the organic acids produced by the fermentation of yeast.

Effect of SD on the antioxidant properties of *Makgeolli*

As presented in Fig. 2(A), the total polyphenol content of *Makgeolli* increased significantly as the amount of SD increased. The samples of SD 0.15%, 0.30%, and 0.45% had values of 221.52, 250.49, and 286.40 mg GAE/g, respectively. No significant difference between the control (188.29 mg GAE/g) and NSD 0.15% (193.97 mg GAE/g) was observed. Levels measured in NSD 0.15, 0.30, and 0.45% were 193.97, 207.85, and 220.91 mg GAE/g, respectively. A slight increase was found when NSD was added in increasing amounts. The control samples had the lowest FRAP value, whereas SD 0.45% had the highest value, a 3-fold increase (Fig. 2(B)). A slight increase or no significant difference was observed between control and NSD samples. As shown Fig. 2(C), control samples had the lowest DPPH radical scavenging activity of 42.52%, whereas SD 0.45% had the highest value of 75.57%. However, no significant differences between control and NSD samples were observed. Overall, antioxidant properties gradually increased as the amount of SD increased, whereas a slight increase or no significant difference among the NSD samples was observed. This might be due to an enhancement of the antioxidant properties of deodeok depending on the conversion of the bioactive component facilitated during the steaming and drying process.

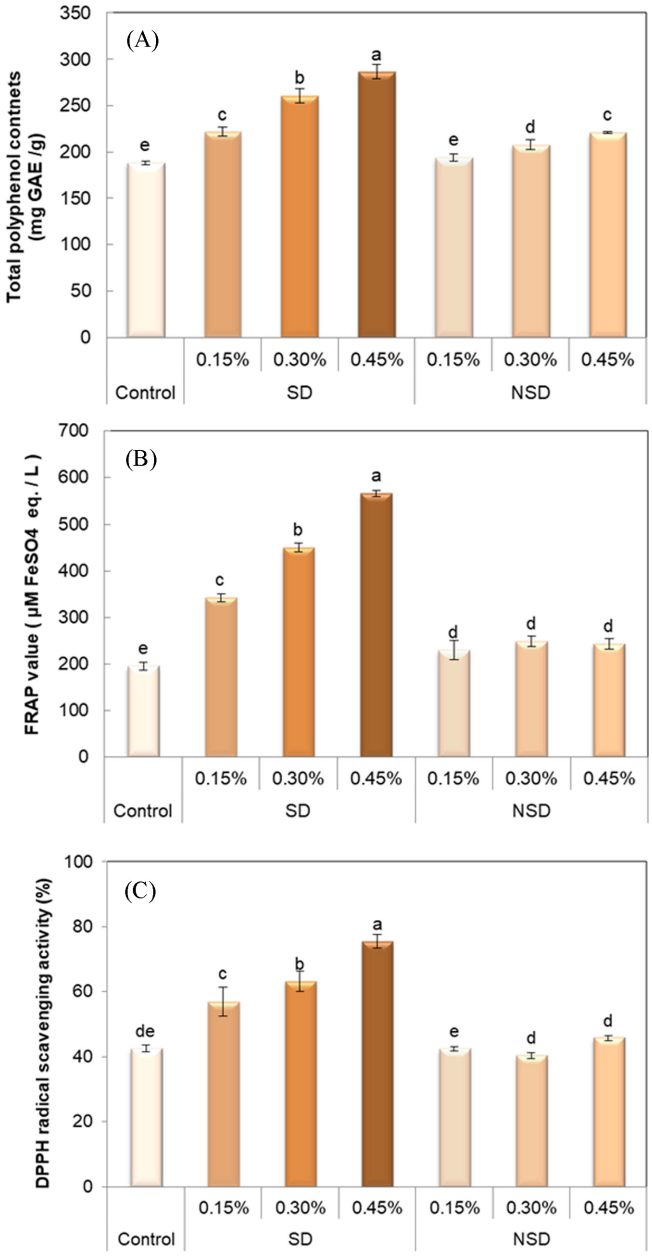

Fig. 2. Antioxidant p of *Makgeolli* with different level of deodeok. (A) Total polyphenol content; (B) FRAP value; (C) DPPH radical scavenging activity. Different letters above the bars indicate values that are significantly different from each other ($p<0.05$). Control, without deodeok; SD, Steaming and drying deodeok; NSD, Non-steaming and drying deodeok.

Conclusion

The intensity of the S/D process significantly influences physicochemical and antioxidant properties of deodeok and the pattern of the samples depended on the S/D conditions. Overall, deodeok processed using the 5-4 h conditions had the highest level of antioxidant properties. Therefore, the 5-4 h condition was chosen as the proper steaming and drying conditions for deodeok to be used for *Makgeolli*. Based on the results of fermentation

characteristics of *Makgeolli* with different level (0, 0.15, 0.30, and 0.45%) of steaming and drying deodeok (SD) at the 5-4 h conditions, control samples had higher alcohol contents and acidity than other samples. The data also revealed that SD 0.45% had higher antioxidant properties than other samples. Considering the antioxidant properties, these results suggest that 0.45% SD is the optimal addition amount for *Makgeolli*.

Conflict of interest statement

All authors declare there are no conflicts of interest.

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