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Quality Characteristics and Antioxidant Activity of the Korean Traditional Rice-Oat Wine "*Makgeolli*" Supplemented with *Codium fragile*, a Green Seaweed

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Quality Characteristics and Antioxidant Activity of the Korean Traditional Rice-Oat Wine "*Makgeolli*" Supplemented with *Codium fragile*, a Green Seaweed

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Abstract: This study developed *Makgeolli* (Korean traditional alcoholic beverage) by adding raw 20% *Codium fragile* (*Cf*), a green seaweed, to a mixed base consisting of rice and oats (1:1 ratio) (COM) and analyzed its quality characteristics and antioxidant activity. The rice based *Makgeolli* supplemented with raw 20% *Cf* was designated as control (CRM). The physicochemical (pH, acidity), microbiological (yeast, lactic acid bacteria), antioxidant (DPPH, ABTS), and sensory properties (7-henodonic scale) of CRM and COM were investigated during 7 days of fermentation. The pH/acidity levels of CRM and COM reached 3.66/0.68 and 3.69/0.95 after 7 days of fermentation, respectively. Compared to CRM, COM had lower alcohol and sugar contents ($P < 0.05$) (CRM: 14.5%/9.8 Brix, COM: 11.2%/7.4 Brix). However, COM contained more yeast and lactic acid bacteria ($P < 0.05$) (CRM: 1.30/4.28 log, COM: 6.04/6.09 log) compared to CRM. The antioxidant activity of DPPH/ABTS ($P < 0.05$) in COM was approximately 1.5 times that of CRM (CRM: 40.00/51.57%, COM: 56.76/70.91%). In addition, COM had an excellent sensory preference score ($P < 0.05$) due to its overall fresh flavor and savory taste (CRM: 4.93, COM: 6.06). Therefore, COM was shown to be *Makgeolli* with enhanced antioxidant function, with yeast and lactic acid bacteria, and improved flavor, suggesting the possibility of commercializing this *Makgeolli.*

Keywords: *Makgeolli*; rice-oat mixture; *Codium fragile;* antioxidant activity; sensory properties

1. Introduction

Makgeolli, also known as *Takju*, is a representative traditional Korean alcoholic beverage [1]. *Makgeolli* is a traditional fermented food that has been recognized for its nutritional value as a fermented liquor made through the parallel double-fermentation of saccharification and fermentation by adding *Nuruk* (a traditional Korean fermentation starter) and water to grains such as glutinous rice and barley [2]. The taste of fermented *Makgeolli* is such that the five tastes of sweet, sour, spicy, bitter, and astringent are in harmony [3]. It is a national alcoholic beverage that continues to receive public attention and devotion. In addition, *Makgeolli* is known to contain the essential amino acids of lysine, leucine, and arginine as well as acetylcholine, which supports liver function [4]. In recent years, the perception of drinking has changed, with healthier and more "light-hearted" drinking becoming increasingly popular. People are generally avoiding beverages that contain a high percentage of alcohol in favor of low-alcoholic beverages and non-alcoholic beverages. As a result, the steady growth in the market for traditional liquor has boosted consumer interest in unique beverages such as *Makgeolli* and has also prompted active preliminary research [5].

Codium fragile (*Cf*) belongs to the family of green algae, which is widely distributed in coastal areas around the world, including along the coasts of Korea, Japan, China, the Philippines, Hawaii, and Africa. Although *Cf* is mainly used as food, it is not as widely known as other seaweeds such as sea mustard, sea tangle, and laver [6]. In addition, it is often used as a supplementary ingredient in kimchi in the southern regions of Korea due to its distinctive sea flavor and odor neutralizing effect [2]. Extracts of *Cf* can be utilized not only as prebiotic materials but also as bioactive substances for health promotion purposes by increasing the antioxidant activity [7]. *Cf* extracts have also been reported to contain polyphenol and flavonoid compounds, which can be utilized as functional materials due to their antioxidant properties [8] and the presence of various bioactive substances [9]. *Cf* extracts were concluded to possess strong antioxidant power by inhibiting the synthesis of nitric oxide because of the large amount of phenolic compounds they contain [6]. In recent years, studies focusing on the development of products containing *Cf* have been reported. These products include *Cf Makgeolli* [2], *Cf* tofu [10], and *Cf* bread [11], all of which have enhanced antioxidant function as a result of the addition of sea staghorn.

Oats (*Avena sativa* L.: oats) is a winter crop that belongs to the rice family and is rich in protein and fat compared to other grains. In particular, about 70–80% of the protein is globulin, which has a high content of essential amino acids such as lysine [12]. In addition, *avenanthramide*, which specifically exists only in oats among all the grains, has been reported to have antioxidant and other effects [13]. The polyphenols contained in oats have also been confirmed to have excellent antioxidant properties [14]. In addition, unsaturated fatty acids and beta-glucan of dietary fiber are also contained in large amounts, and the University of Moscow reported that the consumption of oatmeal has the ability to detoxify the body of heavy metals [15]. After being named one of the world's top 10 health foods in 2002 and the only superfood among grains in 2009, oats have been recognized as a grain with excellent nutritional and health functional values. In addition, oats are being consumed as part of low-calorie diets because of changes in consumption patterns that favor grains rich in dietary fiber rather than simple sugars; consequently, the consumption and production of oats are increasing due to research and product launches of high-functional processed products based on oats [16].

Since 2008, the market size of *Makgeolli* has grown globally due to the well-being trend and the spread of K-POP culture [17]. Preliminary research to investigate the production of *Makgeolli* with the addition of fruit and vegetables such as pears [18], cucumbers [19], blueberries [20], lotus leaves [21], quinoa [22], oats [23], black rice [24], brown rice [25], barley [26], red beans [27], millet [28], purple sweet potato [29], kelps [30], *Ecklonia cava* [31], sea staghorn [2], and green laver [32], has been conducted to determine the properties of these compositions, but the preparation of *Makgeolli* by mixing seaweed and oats has not yet been reported.

Against this background, this study aimed to test the possibility of manufacturing a new variation of *Makgeolli* with a strong *Cf* aroma and enhanced antioxidant properties by exploiting the health functional efficacy of oats. The physicochemical (pH, acidity, alcohol, and sugar content) and microbiological (yeast and lactic acid bacterial count) properties as well as the antioxidant activity (DPPH/ABTS) were analyzed by fermenting the rice-oat-based Korean wine "*Makgeolli*" supplemented with *Cf*.

2. Materials and Methods

2.1. Raw Materials

The *Codium fragile* (*Cf*) used in this experiment were collected from an oyster farm in Changho-ri, Sadeong-myeon, Geoje-si, Gyeongsangnam-do. The main ingredients of *Makgeolli* were purchased from a local market in Hadong-gun, Gyeongsangnam-do (rice), and from Hyundai Nongsusan in Pocheon-si, Gyeonggi-do (oats). The *Nuruk* (Korean-style bran *Koji*), made from Korean wheat, was purchased from Songhaggogja (Gwangju, Korea) and the yeast was purchased from Songcheon Yeast (Songcheon fermentation, Cheongyang-gun, Chungcheongnam-do).

2.2. Production of Rice-Oat Makgeolli with Cf

The manufacturing process of rice-oat *Makgeolli* is schematically shown in Fig. 1. Rice-oat *Makgeolli* was prepared by washing the rice and oats until the water remained clear. Subsequently, the rice and oats were soaked in water for about 12 hours, after which the rice and oats were placed on a sieve for 1 hour to allow the water to drain. The soaked rice and oats were placed in a cotton cloth, put in a steamer and steamed at 100 °C for one hour, and then allowed to cooling to room temperature before use. For the control group, the *Makgeolli* (CRM) to which *Cf* had been added was

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prepared as follows: 400 g of cooked rice and 100 mL of lukewarm water inoculated with 80 g of *Nuruk* at 40 °C the day before, 1 g of enzyme, 80 g of *Cf*, and 600 mL of water were homogenized in a sterilized glass bottle and fermented at 25 ± 1 °C for 7 days. For the experimental group, rice and oat *Makgeolli* (COM) supplemented with *Cf* was prepared as follows: 400 g of cooked rice and oats (1:1), 100 mL of lukewarm water inoculated with 80 g of *Nuruk*, at 40 °C the day before, 1 g of enzyme, 80 g of *Cf*, and 600 mL of water were homogenized in a sterilized glass bottle and fermented at 25 ± 1 °C with stirring once a day for 2 days. The *Cf* used in this study was prepared and added after grinding in a blender with 100 mL of water, and the total amount of water used for making *Makgeolli* was 800 mL. The top of the bottle was covered with a plastic wrap and a rubber band, placed in an incubator set at 25 °C, and allowed to ferment for 7 days. The samples were filtered (SUPRA22K, Hanil Science Industrial Co.) before testing. The physicochemical properties, microbiological properties, antioxidant activity, and sensory quality characteristics of the *Makgeolli* in the control (CRM) and experimental (COM) groups were investigated.

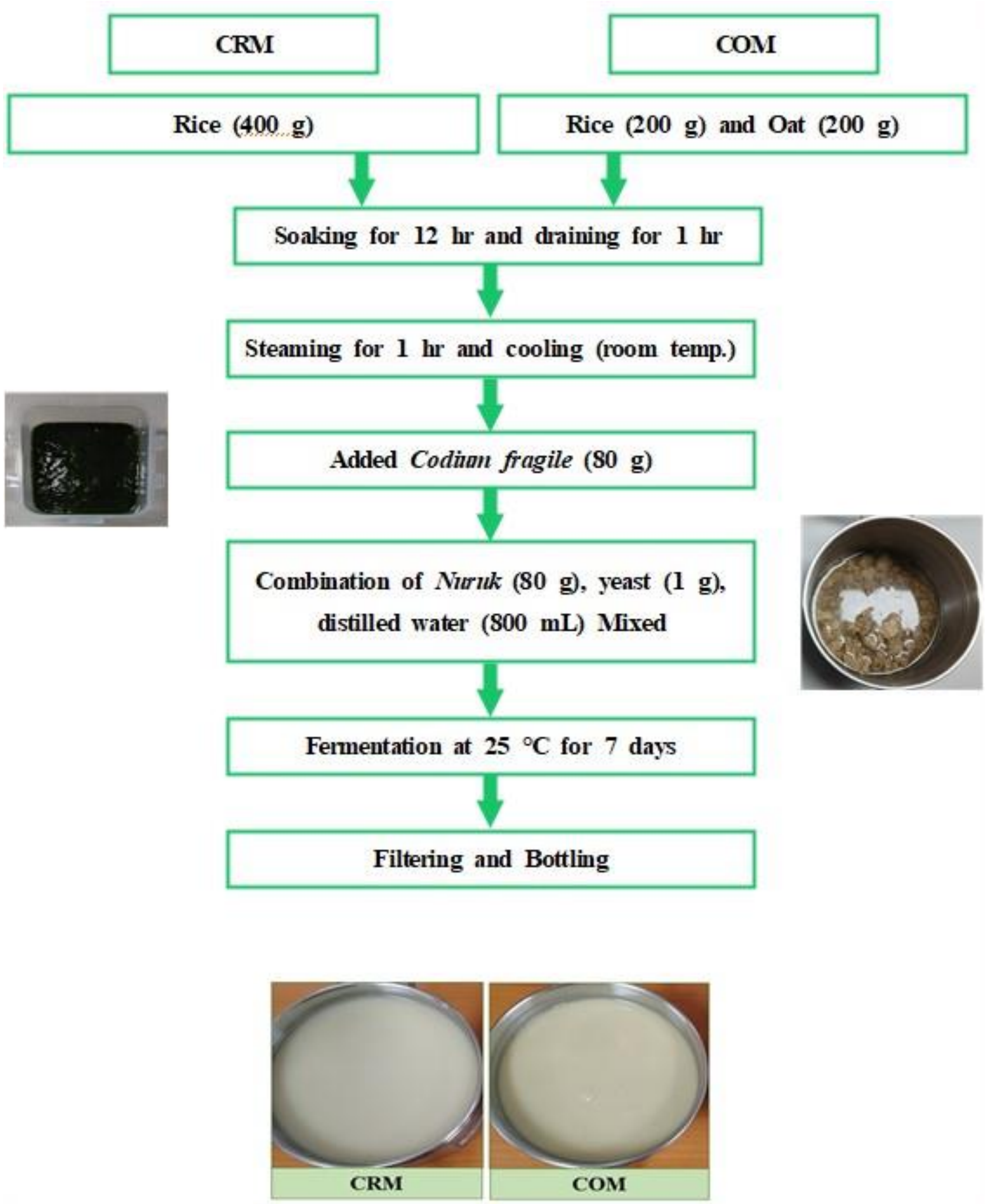

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Figure 1. A flow diagram for the preparation of *Makgeolli* added with *Codium fragile* + Oat. CRM: Codium fragile + Rice based Makgeolli; COM: Codium fragile + Oat based Makgeolli.

2.3. Analysis of Physicochemical Quality Characteristics

2.3.1. pH Value and Acidity

A *Makgeolli* sample (15 mL) was centrifuged (SUPRA22K, Hanil Science Industrial Co., Korea), after which the pH value of the supernatant was measured using a pH meter (A211, Thermo Orion, Benchtop, MI, USA). The acidity was determined by diluting 10 mL of the sample 10-fold. Then, 20 mL of the diluent was collected in a 50 mL Erlenmeyer flask, 2～3 drops of 1% phenolphthalein indicator were added, and the solution was titrated with 0.1 N NaOH until it turned pink. The acidity was calculated as the lactic acid content (%) by the following equation:

$$\text{Acidity (\%)} = (0.009 \times \text{NaOH titration volume (mL)} \times \text{NaOH titer} \times \text{dilution factor}) / (\text{sample volume (mL)}) \times 100$$

2.3.2. Brix and Alcohol Content

The Brix content was determined by analyzing the supernatant (obtained by centrifuging the sample with a centrifuge (SUPRA22K, Hanil Science Industrial Co., Korea)) using an electronic saccharimeter (PAL-1, ATAGO CO., Japan) during fermentation and expressed as Brix. The alcohol content was measured according to the National Tax Service's Liquor Analysis Regulations (2010). This was accomplished by quantifying 100 mL of the sample in a mass cylinder, after which it was transferred to a distillation flask and heated. Then, the flask was connected to a condenser and the liquid was distilled until the volume of distillate reached 70 mL. At this point, distillation was discontinued and the distillate was supplemented with 30 mL of distilled water. The alcohol content was measured using an alcoholmeter (211-DK-12; Daekwang). The alcohol content was expressed as a percentage (v/v).

2.4. Analysis of Microbiological Quality Characteristics

The yeast count and number of lactic acid bacteria were determined by diluting 1 mL of the sample in sterile physiological saline according to the decimal dilution method. Yeast counts were determined by mixing 1 mL of the diluted sample and potato dextrose agar (Difco Co., Detroit, MI, USA) evenly in a petri dish followed by incubation at 25 ± 1 °C for 5–7 days before counting. The lactic acid bacteria were counted by mixing 1 mL of the diluted sample and lactobacilli MRS (Difco Co., Detroit, MI, USA) thoroughly in petri dishes and counting the yellow colonies after 24–48 hours of incubation at 37 °C. The colonies that were produced were expressed as colony forming units (CFU/mL).

2.5. Analysis of Antioxidant Quality Characteristics

2.5.1. DPPH Radical Scavenging Ability

The DPPH [1-diphenyl-2-picrylhydrazyl] (Sigma-Aldrich Co., St. Louis, MO, USA) radical scavenging activity was measured by the method of Kang et al. (2016) [33]. A solution was prepared for measurement by adding 800 μL of a 1.5×10^{-4} M DPPH solution to 200 μL of the centrifuged fermentation supernatant (diluted fourfold), vortexed for 20 seconds, and stored in a refrigerator for 30 minutes. The absorbance was measured at 517 nm using a spectrophotometer (Spectronic2D, Thermo Electron Co., Waltham, MA, USA). The DPPH radical scavenging activity was expressed as a percentage (%) as shown below by calculating the absorbance of the CRM and COM.

$$\text{DPPH radical scavenging activity (\%)} = (A_{517} \text{ of control} - A_{517} \text{ of sample}) / (A_{517} \text{ of control}) \times 100$$

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2.5.2. ABTS Radical Scavenging Ability

A solution was prepared by dissolving 7 mM and 2.45 mM of potassium persulfate in distilled water. This solution was left to stand in the dark for 14–16 hours, during which time the ATBS cation radical ($ATBS^+$) [2'-Azino-bis diammonium (3-ethylbenzthiazoline-6-sulfonic acid)] (Sigma-Aldrich Co., St. Louis, MO, USA) was generated. The solution was diluted with ethanol and the absorbance was adjusted to 0.750 ± 0.002 at 415 nm by mixing with anhydrous ethanol in a 1:1 ratio. Thereafter, 3 mL of COM was added to 1 mL of the diluted $ATBS^+$ solution and allowed to react for 20 minutes, whereupon the absorbance at 415 nm was measured using a spectrophotometer (Spectronic2D, Thermo Electron Co., Waltham, MA, USA).

$$\text{ATBS radical scavenging activity (\%)} = (A_{415} \text{ of control} - A_{415} \text{ of sample}) / (A_{415} \text{ of control}) \times 100$$

2.6. Sensory Characteristics

A total of 16 adults (8 men and 8 women) were selected as panelists for the sensory test, including undergraduate and graduate students of the Department of Seafood Science and Technology at Gyeongsang National University. *Makgeolli* with a sugar content of 7~8 Brix was prepared using 2 parts water to 1 part filtered stock solution fermented for 7 days. After storing the *Makgeolli* at 4 °C for 24 hours, each panelist was provided with about 20 mL in a paper cup, and with water for rinsing their mouth after tasting each sample. The results of the sensory evaluation of *Makgeolli* were expressed by surveying the color, flavor, taste, appearance, and overall acceptability. Each item was evaluated on a 7-point scale (7 points for very good, 1 point for very bad).

2.7. Statistical Analysis

All the analyses were carried out in triplicate and the data were expressed as mean ± SD (standard deviation). The results were subjected to one-way analysis of variance (ANOVA) using SPSS software (SPSS Inc.). Duncan's multiple range test was used to compare the differences among mean values. One-way ANOVA was performed to evaluate the statistical significance of the differences between the CRM and COM samples. The statistical significance level was set to $P < 0.05$.

3. Results and Discussion

3.1. Changes in pH and Total Acid Content During Fermentation

The acidity and pH measurements that were made in daily intervals for 7 days while the *Makgeolli* (CRM, COM) was undergoing fermentation are provided in Table 1. The pH of *Makgeolli* is affected by the acidic substances that are produced during the fermentation period [34]. This factor is indicative of changes in the composition of *Makgeolli* and is an important indicator of the fermentation process, which is a complex process that involves the production of alcohol [35]. The pH of COM was 4.85 on day 0 of the fermentation, and was significantly lower at 3.63 on day 2 of the fermentation ($P < 0.05$). After this, the pH stabilized without any major changes until the final 7 day. Although on day 0 of the fermentation process, the pH of CRM was 5.28, which was higher than that of COM, the pH of CRM changed according to the same pattern to that observed for COM (Table 1). That is, the pH of both CRM and COM decreased rapidly on day 2 of the fermentation. This result was consistent with that of Kim et al. [36] and Park et al. [37] who reported that the pH decreases on day 2 of the fermentation during the production of *Makgeolli*. This occurs because organic acids and alcohol are produced by the action of various fermentation microorganisms (*Aspergillus*, *Rhizopus*, *Saccharomyces*, *Candida*, *Pichia*, *Bacillus*, *Psedomonas*, *Micrococcus*) as the fermentation progresses [38]. Additionally, the pH of 3.66 for CRM and 3.69 for COM on day 7 of the fermentation was similar to the pH range of 3.40–3.77 of commercially available unpasteurized *Makgeolli*. Kim et al. (2012) [24] noted that the pH of black rice *Makgeolli* fermented for 10 days was 4.03–4.23. The pH results of this study were within the standard range of 3.8–4.7, which is the pH range of rice wine according to the Liquor Tax Act.

Table 1. Changes of pH and acidity (%) in the CRM and COM during fermentation.

Fermentation Time (day)	pH		Acidity (%)	
	CRM	COM	CRM	COM
0	5.28 ± 0.00^{aA}	4.85 ± 0.00^{aB}	0.20 ± 0.00^{eA}	0.19 ± 0.00^{eB}
1	5.08 ± 0.01^{bA}	4.69 ± 0.00^{bB}	0.27 ± 0.03^{fA}	0.19 ± 0.05^{eB}
2	3.57 ± 0.01^{fB}	3.63 ± 0.01^{efA}	0.86 ± 0.01^{aA}	0.82 ± 0.02^{aB}
3	3.54 ± 0.02^{gB}	3.62 ± 0.01^{fA}	0.86 ± 0.01^{aA}	0.82 ± 0.02^{aB}
4	3.58 ± 0.01^{fB}	3.64 ± 0.01^{eA}	0.80 ± 0.02^{abA}	0.77 ± 0.01^{bB}
5	3.62 ± 0.02^{eB}	3.66 ± 0.00^{dA}	0.78 ± 0.01^{bcA}	0.71 ± 0.01^{cB}
6	3.69 ± 0.01^{cB}	3.67 ± 0.01^{dA}	0.72 ± 0.00^{cdA}	0.67 ± 0.02^{dB}
7	3.66 ± 0.03^{dB}	3.69 ± 0.01^{cA}	0.68 ± 0.02^{dA}	0.65 ± 0.01^{dB}

The data indicates means with standard deviations (three samples/treatment). Within the same column, means with different letters (a-d for each fermentation time). Within the same row, means with different letters (A-B). The one-way ANOVA performed by t-test was carried out to evaluate the statistical significance of differences between CRM and COM samples. CRM: Codium fragile + Rice based Makgeolli. COM: *Codium fragile* + Rice / Oat based *Makgeolli.*

The acidity, which affects the pH of *Makgeolli*, is directly related to the taste and flavor of *Makgeolli* and can also affect its preservation [39]. On day 0 of the fermentation, the acidity of CRM and COM was 0.20% and 0.19%, respectively. Similar to the variation in the pH of CRM and COM, the acidity (CRM: 0.86, COM: 0.82) increased rapidly on the second day of fermentation. In a study on purple sweet potato rice malt *Makgeolli*, as fermentation progressed, the acidity increased because of the production of organic acids such as malic acid, succinic acid, and citric acid by the action of yeast and lactic acid bacteria [29]. The high acidity level on the 2 day of fermentation is considered to be due to the addition of water to the yeast the day before to speed up the initial fermentation as a way to activate the bacteria in the yeast. After the two days of fermentation, the acidity gradually decreased to 0.86, 0.80, 0.78, 0.72, and 0.68% for CRM and 0.82, 0.77, 0.71, 0.67, and 0.65% for COM from the third to the 7 day of the final fermentation. This mirrored the results of a study in which changes in the quality were observed during the fermentation of oat *Makgeolli* made with different amounts of water [40]. Meanwhile, a comparison of the change in the pH and acidity of COM and CRM revealed an ideal negative correlation, with increasing acidity and decreasing pH.

3.2. Changes in the Sugar Content during Fermentation

The measurements of the sugar content of CRM and COM are presented in Table 2. On day 0 of the fermentation, the sugar content of CRM and COM was the same at 1.6 Brix. On day 1 of the fermentation, this value increased rapidly to 8.5 and 7.2 for CRM and COM, respectively. The rapid increase in sugar content on the 1 day of fermentation is attributed to the method that was initially used to accelerate the fermentation by adding malt and yeast to water a day before soaking the starch in grains, which are ingredients of *Makgeolli*, to activate the bacteria in the yeast. On day 2 of the fermentation, the Brix of CRM and COM were 13.5 and 8.8, respectively. According to data from the National Institute of Agricultural Sciences, the carbohydrate content per 100 g is 78.74 g for rice and 66.66 g for oats. Therefore, COM has lower sugar content than CRM because rice contains more carbohydrates, which determine the sugar content [41]. Meanwhile, after the initial rapid increase in the sugar content, it gradually decreased to 10.6, 10.1, and 9.8 Brix for CRM and 7.7, 7.5, and 7.4 Brix for COM from the third to the final day (7 day) of the fermentation. This is the result of brown rice produced under different fermentation conditions. Another study on *Makgeolli* [25] also found the sugar content to increase on the 1 and 2 days of fermentation, after which it gradually decreased,

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which was consistent with the results of this study. The tendency for the sugar content to decrease is the result of the decomposition of starch into sugar due to the action of saccharification amylase and the nutrient source of yeast or fermentation. As the fermentation progresses with the substrate for a certain period of time, the sugar content decreases and the alcohol content increases [2].

Table 2. Changes of brix and alcohol (%) in the CRM and COM during fermentation.

Fermentation Time (day)	Brix (ºBx).		Alcohol (%)	
	CRM	COM	CRM	COM
0	1.6 ± 0.55^{fA}	1.6±0.00^{fA}	3.0 ± 0.00^{fA}	2.5 ± 0.00^{dB}
1	8.5 ± 0.12^{eA}	7.2 ± 0.00^{eB}	12.5 ± 0.00^{eA}	11.0 ± 0.00^{cB}
2	13.5 ± 0.06^{aA}	8.8 ± 0.00^{aB}	17.0 ± 0.00^{aA}	12.0 ± 0.00^{aB}
3	10.6 ± 0.06^{bA}	7.7 ± 0.10^{bB}	16.0 ± 0.00^{bA}	12.0 ± 0.00^{aB}
4	10.1 ± 0.12^{cA}	7.5 ± 0.06^{deB}	15.2 ± 0.29^{cA}	11.5 ± 0.00^{bB}
5	10.1 ± 0.00^{cA}	7.5 ± 0.01^{cB}	15.0 ± 0.00^{cA}	11.5 ± 0.00^{bB}
6	10.1 ± 0.00^{cA}	7.5 ± 0.00^{cB}	14.3 ± 0.29^{dA}	11.5 ± 0.50^{bB}
7	9.8 ± 0.29^{dA}	7.4 ± 0.10^{dB}	14.5 ± 0.00^{dA}	11.2 ± 0.29^{bcB}

The data indicates means with standard deviations (three samples/treatment). Within the same column, means with different letters (a-d for each fermentation time). Within the same row, means with different letters (A-B). The one-way ANOVA performed by t-test was carried out to evaluate the statistical significance of differences between CRM and COM samples. CRM: *Codium fragile* + Rice based *Makgeolli*. COM: *Codium fragile* + Rice / Oat based *Makgeolli.*

3.3. Changes in Alcohol Content during Fermentation

The alcohol content of *Makgeolli* is one of the factors that greatly affects the quality of *Makgeolli* and is an important ingredient that affects the flavor and preservation [42]. The alcohol content is determined by the production of ethanol during the process in which yeast decomposes sugar saccharified by fermentation. As the fermentation progresses the alcohol content increases due to an increase in the ethanol content [1]. The variation in the alcohol content during the fermentation of COM is presented in Table 3. The production of alcohol started on day 0 of the fermentation at 3.0% for CRM and 2.5% for COM, whereupon it rapidly increased to 12.5–11.0% on the first day of fermentation to reach the highest level (17.0–12.0%) on the second day of fermentation. This is the result of a rapid increase in the sugar content and reducing sugar content on the 1 and 2 days of fermentation, resulting in the production of ethanol with the aid of the yeast [27]. In general, unlike the case of *Polygonatum odoratum* [1] and red beans [27], in which the alcohol content gradually increased as fermentation progressed beyond 2-3 days, in this study, it gradually decreased to reach 14.5% for CRM and 11.2% for COM after 7 days. Cho et al. (2012) reported that the amount of alcohol produced differed according to the saccharification power of yeast [29]. The results of this study showed that the alcohol content of COM was slightly lower at 14.5–11.2% compared to that of CRM, but methods were introduced to increase the initial fermentation speed and *Cf*. The sugar content of COM was 7.5 Brix and the alcohol content was 11.5% on days 4 to 6 of the fermentation, after which it decreased to 7.4 Brix and 11.2% on day 7. According to Kim et al. (1996), the point at which the sugar content no longer decreased signaled the end of fermentation [43]. In this study, the difference in the starch content of oats in the experimental group compared to the control group first depleted the sugar, a source of nutrients for the yeast, and the COM fermentation reached its end point after 4–6 days when the yeast was unable to ferment alcohol. The results obtained for the experimental group COM indicated the possibility of shortening the manufacturing period of the *Makgeolli*. Further analysis by way of additional research is expected to show that commercial production is a possibility.

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Table 3. Changes of yeast (CFU/mL) and lactic acid bacteria (CFU/mL) in the CRM and COM during fermentation.

Fermentation Time (day)	Yeast		Lactic Acid Bacteria	
	CRM	COM	CRM	COM
0	4.21 ± 0.59^{fB}	4.64 ± 0.84^{cA}	4.37 ± 0.00^{eA}	4.20± 1.35^{fB}
1	4.37 ± 0.14^{eB}	4.60 ± 0.07^{cA}	7.32 ± 0.02^{aA}	7.17 ± 0.03^{aB}
2	4.44 ± 0.05^{dB}	4.58 ± 0.06^{cA}	6.23 ± 0.35^{bB}	6.85 ± 0.16^{bA}
3	4.75 ± 0.12^{aB}	5.35 ± 0.92^{bA}	5.14 ± 1.39^{cB}	6.48 ± 0.01^{cA}
4	4.54 ± 0.01^{bB}	6.09 ± 0.71^{aA}	4.98 ± 0.46^{dB}	6.43 ± 0.16^{dA}
5	4.48 ± 0.01^{cB}	6.11 ± 0.05^{aA}	4.93 ± 0.09^{dB}	6.10 ± 0.02^{eA}
6	4.48 ± 0.01^{cB}	6.08 ± 0.07^{aA}	4.35 ± 0.04^{eB}	6.10 ± 0.02^{eA}
7	1.30 ± 0.01^{gB}	6.04 ± 0.05^{aA}	4.28 ± 0.02^{fB}	6.09 ± 0.18^{eA}

The data indicates means with standard deviations (three samples/treatment). Within the same column, means with different letters (a-d for each fermentation time). Within the same row, means with different letters (A-B). The one-way ANOVA performed by t-test was carried out to evaluate the statistical significance of differences between CRM and COM samples. CRM: *Codium fragile* + Rice based *Makgeolli*. COM: *Codium fragile* + Rice / Oat based *Makgeolli.*

3.4. Changes in the Number of Yeast and Lactic Acid Bacteria during Fermentation

The change in the yeast count during fermentation for 7 days is presented in Table 3. Immediately after soaking, the yeast count of CRM increased from 4.21 log_{10}CFU/mL on day 0 of the fermentation to the maximum of 4.75 log_{10}CFU/mL on day 3 of the fermentation, followed by a gradual decrease to 4.54–4.48 log_{10}CFU/mL on the 4 to 6 days of fermentation. In a study on changes in the yeast content during the fermentation period of *Makgeolli* [3], the yeast count increased rapidly from the third day, thereby following a trend similar to that in this study. However, according to a report by Park et al. (2015), the yeast count reached its maximum value on the fourth day of fermentation [44]. In our study, COM had a maximum value of 6.11 log_{10}CFU/mL on the 5 day of fermentation; thus, COM reached its maximum value one day later. In addition, unlike CRM, which rapidly decreased to 1.30 log_{10}CFU/mL on the 7 day of fermentation, COM gradually decreased to 6.04 log_{10}CFU/mL on the final day of fermentation. This is consistent with the study on *Makgeolli* by Jeon et al. (2023) [22] in which the decrease in the yeast count was attributed to the inhibitory effect of alcohol produced by fermentation. In our study, the effect of increasing the initial fermentation rate by adding water to the yeast a day before soaking is thought to be responsible for promoting the fermentation. A comparison between COM and CRM revealed that the former reached the maximum yeast value one day later than the latter, which is thought to be due to the differences in the supply of nutrient sources as well as the dependence of the fermentation ability on the type of grain used for making *Makgeolli* [1].

The change in the lactic acid bacteria count during fermentation for 7 days is presented in Table 3. The difference between CRM (4.37 log_{10}CFU/mL) and COM (4.20 log_{10}CFU/mL) was insignificant in terms of the lactic acid bacteria counts on day 0 ($P > 0.05$). On day 1, the counts increased rapidly to 7.32 and 7.17 log_{10}CFU/mL for CRM and COM, respectively. On day 7, the final day of fermentation, the number of lactic acid bacteria in COM was about 1.5 times higher ($P < 0.05$) than in CRM (CRM; 4.28 log_{10}CFU/mL, COM: 6.09 log_{10}CFU/mL). According to food nutrition data from the National Institute of Agricultural Sciences, the total dietary fiber content per 100 g is 1.9 g for white rice and 18.8 g for oats [41]. The higher fiber content of oats is considered to be the reason for the higher bacterial count of COM than CRM, in that the dietary fiber becomes food for the lactic acid bacteria. In addition, Jeon et al. (2020) reported that the viscous polysaccharides, such as galacto arabinoxylan, in the COM enabled the lactic acid bacteria to proliferate because of their role as

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prebiotics, which constitute food for lactic acid bacteria, compared to the rice wine that is generally distributed in the market [2].

3.5. Changes in Antioxidant Activity (DPPH/ABTS Radical Scavenging Activity) during Fermentation

The results of measuring the DPPH radical scavenging activity on day 7, the final day of fermentation, of CRM and COM are shown in Fig. 2. The activity of COM was found to be approximately 1.5 times higher at 56.71% than that of CRM at 40.00%. This is consistent with a research report [45] that oats contain reductone, which stabilizes free radicals and terminates oxidation reactions in an antioxidant study of oat methanol extract. In a study [2] on *Makgeolli* prepared by adding 10 to 30% of *Codium fragile*, the DPPH activity of the group to which *Codium fragile* had been added was about 2.4 to 2.7 times higher than that of the *Codium fragile* free group.

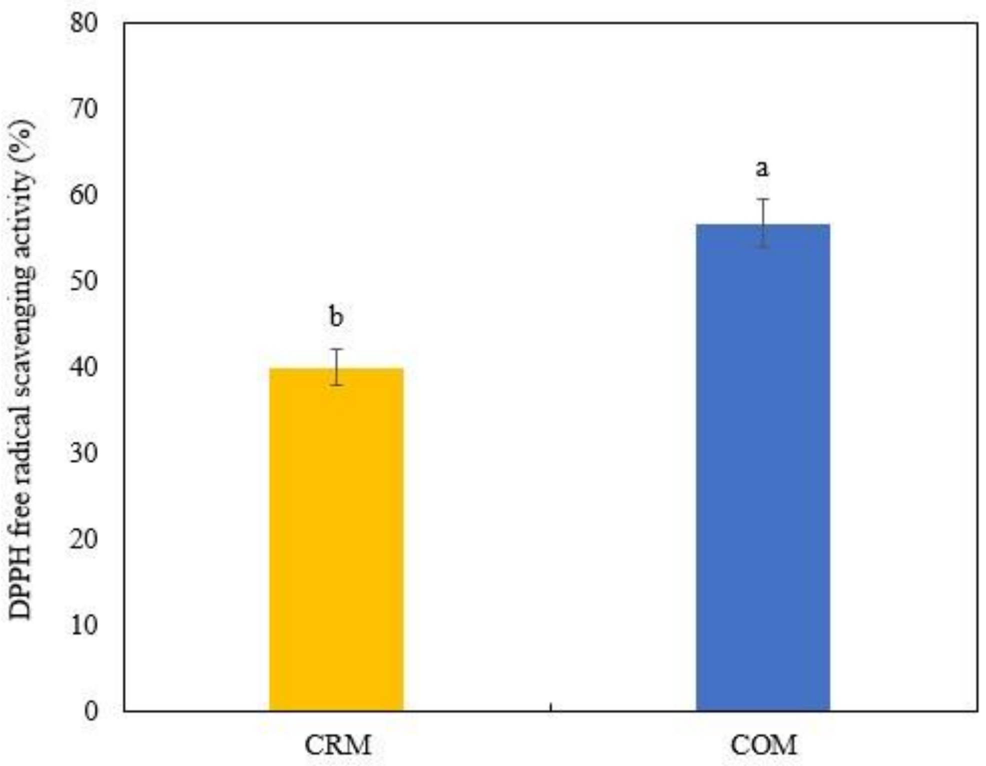

Figure 2. DPPH free radical scavenging activity in COM and CRM. The data indicates means with standard deviations (three samples/treatment). . CRM: Codium fragile + Rice based Makgeolli. COM: Codium fragile + Oat based Makgeolli.

The ABTS radical scavenging activity of CRM and COM are shown in Fig. 3. This activity was 51.57% for CRM and 70.91% for COM; that is, the activity of COM was about 1.5 times higher than that of CRM. According to reports of studies of *Makgeolli* [2], white bread [46], and tofu [47] to which *Codium fragile* as well as freeze dried *Codium fragile* had been added [48], the ABTS radical scavenging activity significantly increased as the added amount of *Codium fragile* increased. The high antioxidant activity of *Codium fragile* becomes evident from research results that show that the alcohol extract had an ABTS radical scavenging activity of 47% depending on the extraction method [49]. In a research paper on the antioxidant components and antioxidant activity of oats, Lee et al. (2018) reported the existence of the active substance *avenanthramide,* a phenol component [50], and Kim et al. (2012) reported that, among native and medicinal plant extracts, the total flavonoid content of oats was high [51]. Ham et al. (2015) reported that the ABTS radical scavenging ability of oats was 77.88–116.14 mg TEAC/100 g, and they detected polyphenol content higher than that of white rice, coix seed, and mung bean [45]. Although the amount of polyphenol and flavonoid, which are antioxidants, was not measured in this study, the COM *Makgeolli* is considered to have superior DPPH/ABTS radical

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scavenging activity because it contained more antioxidant components such as polyphenols and flavonoids than CRM *Makgeolli*.

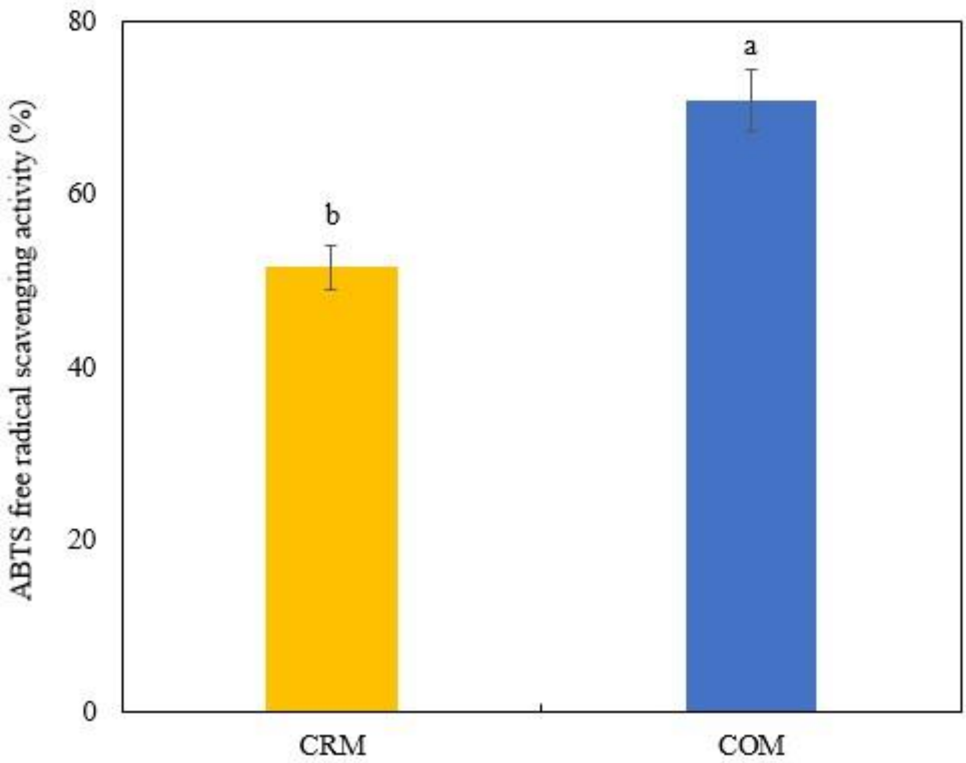

Figure 3. ABTS free radical scavenging activity in COM and CRM. The data indicates means with standard deviations (three samples/treatment). . CRM: Codium fragile + Rice based Makgeolli. COM: Codium fragile + Oat based Makgeolli.

3.6. Sensory Evaluation

The results of the sensory evaluation of CRM and COM are presented in Table 4. After 7 days of fermentation, the *Makgeolli* was stored at 4 °C for 24 hours, and the color, flavor, taste, appearance, and overall preference were assessed. Although the preference regarding the appearance did not differ significantly between the two groups ($P > 0.05$), CRM was preferred over COM in terms of the color, flavor, taste, and overall preference ($P < 0.05$). In the previous study (Jeon et al. 2020), rice-based *Makgeolli* to which *Codium fragile* had been added was reported to have sensory superiority [2]. In addition, the significant difference in the color and flavor of COM compared to those of CRM is considered to be due to the slightly darker color and savory taste resulting from the addition of oats. (which has a yellowish color) (Fig 1).

Table 4. Sensory tests in the CRM and COM during fermentation for 7 days.

Properties	Taste Tests				
	Color	Smell	Taste	Appearance	Overall Acceptability
CRM	3.81 ± 0.75[b]	4.31 ± 0.48[b]	5.06 ± 0.25[b]	4.94 ± 0.68[a]	4.93 ± 0.68[b]
COM	5.37 ± 0.50[a]	5.88 ± 0.50[a]	6.00 ± 0.52[a]	5.50 ± 0.89[a]	6.06 ± 0.44[a]

The data indicates means with standard deviations (three samples/treatment). Within the same column, means with different letters (a-d for each fermentation time). CRM: *Codium fragile* + Rice based *Makgeolli*. COM: *Codium fragile* + Rice / Oat based *Makgeolli*.

4. Conclusions

On the final day (day 7) of the fermentation in this study, both the sugar and alcohol content of COM were significantly lower ($P < 0.05$) than those of CRM. However, compared to CRM, COM contained 4.6 times more yeast and 1.4 times more lactic acid bacteria. Furthermore, in terms of the antioxidant properties of DPPH and the ABTS radical scavenging ability, the antioxidant activity of COM was excellent at about 1.5 times higher than that of CRM. In addition, COM achieved very good overall preference of 6.06 on the 7-point sensuality scale. In other words, based on our comprehensive evaluation of COM, it can be said to be a microbiologically excellent fermented beverage that contains low levels of sugar and alcohol and a large amount of yeast and lactic acid bacteria (> 6 log). At the same time, COM showed excellent antioxidant function and sensory preference. Therefore, based on these research results, the possibility of developing rice-oat mixed seaweed *Makgeolli* with added *Codium fragile* was shown to be realistic. However, the commercialization of this product is expected to require additional research, such as nutritional analysis and expiration date establishment.

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