Effect of Millimeter Waves on the Microbiological and Physicochemical Properties of Korean Rice Wine *Makgeolli*

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Abstract Millimeter waves are electric waves between 30 and 300 GHz. They are known to affect microbial growth. Here, the effects of millimeter waves on the microbiological and physicochemical properties of *Makgeolli* irradiated during fermentation were investigated. Millimeter waves at 60 and 70 GHz were used, and the fermentation was carried out at 30°C. The numbers of both lactic acid bacteria and yeasts have changed with similar pattern each then during the fermentation, but the effect of millimeter wave treatment was not critical as growth patterns were independent of both irradiation period and wave frequency. Furthermore, pH and total acidity did not change during fermentation. Although irradiation drastically increased sugar production during the initial stage of fermentation, the change was not frequency dependent. At the end of fermentation, the alcohol content in irradiated *Makgeolli* was definitely higher than that in un-irradiated *Makgeolli*, and the difference was frequency dependent.

Keywords: *Makgeolli*, millimeter wave, fermentation, microbiological property, physicochemical property

Introduction

Makgeolli is a Korean traditional alcoholic beverage, which is prepared by saccharification of steamed rice, wheat, or barley by saccharogenic starter such as *nuruk* or *koji* and simultaneous fermentation to alcohol by yeast. *Makgeolli*, unlike other alcoholic beverages, is the only alcoholic beverage that is consumed along with all the contents of the fermentation mixture, including yeast and lactic acid bacteria. Fermented *Makgeolli* contains essential amino acids, sugars, organic acids, vitamins, and various organic compounds (1). Since these fermentation products positively affect health, people's interest in *Makgeolli* has increased, and several studies on *Makgeolli* are being conducted.

Previous studies on *Makgeolli* mainly focused on the quality characteristics (2-5); changes in the mash and flavor components (6,7) depending on the starch source, *nuruk*, and yeast; effect of various supplements on the quality characteristics (8,9); component analysis of fermentation products (10-12); and microbiological changes during fermentation (10,13,14). However, the effects of physical treatments such as ultrasound, UV, and radiation treatments during fermentation have rarely been investigated. Kim *et al.* (15) investigated the effect of UV-C and electron beam irradiation on the

Makgeolli quality. When the quality of the raw *Makgeolli* was measured after irradiation with UV-C and electron beam and stored at 4°C for 15 days, the microbial count was found to be under control and *Makgeolli* quality remained unchanged during the storage period.

In addition to UV-C and electron beam, electromagnetic waves are also known to affect the growth of microorganisms. The growth rate of *Saccharomyces cerevisiae* is affected by the frequency of the microwave (16). The stimulation *S. cerevisiae* with pulsed electric fields 1,000 V cm⁻¹ elevated its growth rate and cell number (17). The term "millimeter wave" collectively refers to all electric waves between 30 and 300 GHz (wavelength, 1-10 mm). These waves, unlike the microwave, do not generate heat and they have been reported to affect microbial growth. When *S. cerevisiae* and *S. carlsbergensis* were stimulated with millimeter waves, both cell growth and biomass increased (18,19). Exposure of *Candida albicans* to 1 kHz square-wave modulated millimeter waves at 72 GHz reduced colony formation by approximately 15% (20). Interestingly, irradiation with millimeter waves in the range of 41.8-42.0 GHz increased the growth rate of *S. cerevisiae* by 15% or decrease it by 29% depending on the external environment (21). Although several studies have reported the effects of millimeter waves on microbial

growth, the results were inconsistent and varied with the study conditions. Moreover, only few studies have investigated the quality characteristics of food products by direct irradiation with millimeter waves.

In this study, the effects of irradiation of *Makgeolli* with millimeter waves during the fermentation process on its microbiological and physicochemical properties were examined. We have used 60 and 70 GHz waves, the maximum absorption frequency range for food products, for irradiation, and rapid fermentation was performed for 5 days by controlling the amount of ferments. The total number of microorganisms, pH, acidity, and alcohol and sugar contents were measured during the fermentation.

Materials and Methods

Millimeter wave treatment *Makgeolli* samples were exposed to millimeter waves of a single frequency (Nizhniy Novgorod, Russia) from a system comprising a control unit, an oscillator, and a transit generator. The samples were irradiated at 60 and 70 GHz for all fermentation periods.

Production of *Makgeolli* For the *Makgeolli* used in this study, 438 g of rice was washed and immersed in water for 2 h and dried at 20°C on a sieve for 60 min. The rice was steamed in a steamer (slow cooker; Woosung Metal Co., Ltd., Seoul, Korea) at 100°C for 40 min and simmered for 10 min to make hard-boiled rice. For the *Makgeolli* starter for the rapid fermentation, the hard-boiled rice, *nuruk* (25.5 g), yeast (0.79 g), and water (0.87 L) were mixed and fermented at 25°C for 48 h. The *nuruk* was purchased from Korea Enzyme Co., Ltd. (Hwasung, Korea), and dried yeast was obtained from DSM Bakery Ingredient (Fermipan Instant Yeast Red, Dordrecht, Netherlands). For the main *Makgeolli* fermentation, rice (875 g) and water (1.75 L) were added to the *Makgeolli* starter, followed by rapid fermentation at 30°C for 5 days.

Total viable count of microorganisms in *Makgeolli* To determine the number of lactic acid bacteria (LAB), the *Makgeolli* was diluted by 10-fold serial dilutions and each dilution was spread on lactobacilli MRS agar (Difco, Becton Dickinson, Sparks, MD, USA) and the plates were incubated at 39°C for 48 h. Yeast were cultured in 3M™ Petrifilm™ Yeast and Mold Count Plates (3M, St Paul, MN, USA) at 25°C under aerobic conditions for 120 h. The viable cell counts of LAB and yeast were determined using the standard plate count method, and the results were expressed as log colony forming unit (CFU)/mL.

pH, titratable acidity, and soluble solids content The pH value was determined using a digital pH meter (Star A211; Thermo Orion, Beverly, MA, USA). Titratable acidity was expressed as % lactic acid and was evaluated by a previously described method (12,14). The content of total soluble solids was determined using a hand

refractometer (WM-7; Atago, Tokyo, Japan). The results were reported as Brix degrees (°Bx) at 25°C.

Total sugar and reducing sugar contents Total sugar concentration was measured by the phenol sulfuric acid method (12,22). Reducing sugar concentration was determined by the 3,5-dinitrosalicylic acid (DNS) method (23). Total sugar and reducing sugar contents were determined from a standard curve prepared against glucose concentrations.

Alcohol content For measurement of the alcohol content, 100 mL of the sample was collected in a mass cylinder and transferred to a 250-mL conical flask. The 100-mL mass cylinder used to collect the sample was rinsed 3 times with 10 mL distilled water, the water used for rinsing was combined with the sample in the 250-mL conical flask, and the alcohol was distilled. The alcohol content of the distilled liquid was measured using a Density meter (DMA 4500A; Anto Paar, Graz, Austria).

Statistical analysis The data were reported as the means±the standard deviation (SD) of experiments run in triplicate and were analyzed using SPSS 12.0 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA). A Pearson correlation test was performed to determine the correlation between variables. Significance levels were defined at $p < 0.05$.

Results and Discussion

Changes in the LAB and yeast counts following millimeter wave irradiation Organic acids are generated as *Makgeolli* fermentation progresses, which reduces the pH and produces flavor compounds such as esters, from the interaction of alcohol with amino acids and organic acids. Microorganisms play an important role in the production of alcohol, organic acids, and amino acids. The changes in LAB and yeast counts of *Makgeolli* following millimeter wave irradiation are presented in Fig. 1. The LAB counts in the irradiated and un-irradiated *Makgeolli* were 6.60–6.75 and 6.68–6.85 log CFU/mL, respectively. The pattern of changes was similar in both groups, and showed no significant differences based on millimeter wave frequency ($p < 0.05$).

The number of yeast cells in the irradiated *Makgeolli* was 8.21 log CFU/mL at Day 0, which was significantly higher than that in the control (8.17 log CFU/mL). The number increased to 8.91–8.95 log CFU/mL in all *Makgeolli* samples from Day 1 of fermentation, but no significant differences based on irradiation and millimeter wave frequency were observed ($p < 0.05$). The numbers of yeast cells in all *Makgeolli* samples tended to decrease from Day 2 to the end of fermentation, and no significant differences based on irradiation and millimeter wave frequency were observed ($p < 0.05$).

In general, fungi are not detected during *Makgeolli* fermentation,

Fig. 1. Changes in microbial cell counts of *Makgeolli* irradiated with millimeter waves of different frequencies. (A) Lactic acid bacteria; (B) Yeast; Closed circle, Control (un-irradiated); Open circle, M60 (60 GHz wave); inverted triangle, M70 (70 GHz wave). Values are mean $\pm$ SD ($n=6$).

and the major microorganisms are known to be yeasts and LAB (14,24,25). In a *Makgeolli* produced using modified-*nuruk*, the numbers of LAB and yeast cells during fermentation were approximately 7 and 8 log CFU/mL, respectively (14). Consistent with the results of the present study, the numbers of LAB and yeast cells in raw *Makgeolli* from the market were 5-7 and 7-8 log CFU/mL, respectively (24,25). Although millimeter waves are known to affect microbial growth, both increased and decreased growth of the same microorganisms depending on the fermentation conditions have been reported (26). Therefore, here we investigated the growth patterns of *S. cerevisiae* used in *Makgeolli* fermentation and *Lactobacillus plantarum* from the prepared *Makgeolli* after irradiation with 60 and 70 GHz millimeter waves (data not shown). No difference was observed in the growth patterns of the two microorganisms, irrespective of millimeter wave irradiation. In general, an object needs to absorb electromagnetic waves for the waves to exert their effects, but millimeter waves at 60-70 GHz do not seem to be absorbed in sufficient quantities to have any effect on microbial growth (26).

Fig. 2. Changes to pH (A), titratable acidity (B), and soluble solid content (C) of *Makgeolli* irradiated with millimeter waves of different frequencies. Closed circle, Control (un-irradiated); Open circle, M60 (60 GHz wave); inverted triangle, M70 (70 GHz wave). Values are mean $\pm$ SD ($n=6$).

Changes in acidity, pH, and soluble solid content following millimeter wave irradiation pH is an important indicator not only of *Makgeolli* quality during fermentation, but also for determining the condition in the *Makgeolli* fermentation, such as the beginning of alcohol production. The pH of *Makgeolli* is affected by the type and concentration of various organic acids produced during the fermentation

Fig. 3. Changes to total sugar (A) and reducing sugar (B) content of *Makgeolli* irradiated with millimeter waves of different frequencies. Closed circle, Control (un-irradiated); Open circle, M60 (60 GHz wave); inverted triangle, M70 (70 GHz wave). Values are mean $\pm$ SD ($n=6$).

process and other acid-derived materials (27). Figure 2A shows the pH of *Makgeolli* produced following irradiation with millimeter waves of different frequencies. The pH of the un-irradiated *Makgeolli* during the fermentation period was 4.43-5.06, whereas the pH of *Makgeolli* irradiated with the 60 and 70 GHz waves was 4.51-5.13 and 4.44-5.15, respectively. The final pH of *Makgeolli* was 4.46-4.49. These values were within the normal pH range (4.0-4.6), showing that the *Makgeolli* used in this study seemed to have undergone normal fermentation (3,10,12,14). All *Makgeolli* samples showed significant pH reduction until Day 2 of fermentation, and the pH value remained consistent after Day 2. *Makgeolli* irradiated with 60 GHz millimeter waves tended to show significantly high pH during the fermentation period, whereas un-irradiated *Makgeolli* and those irradiated with 70GHz waves showed no significant pH changes from Day 3 of fermentation.

Acidity is an important factor that affects not only the pH, but also the flavor, concentration of volatile components, and preservation of *Makgeolli*. During fermentation, pH is increased by organic acids generated by yeasts and LAB in the mash. High acidity indicates

Fig. 4. Changes to the alcohol content of *Makgeolli* irradiated with millimeter waves of different frequencies. Closed circle, Control (un-irradiated); Open circle, M60 (60 GHz wave); inverted triangle, M70 (70 GHz wave). Values are mean $\pm$ SD ($n=6$).

excessive fermentation, whereas too low acidity removes the unique acidic taste of *Makgeolli*. Therefore, it is important to maintain optimal acidity (12,27). Figure 2B shows the acidity of *Makgeolli* during the fermentation period. All *Makgeolli* samples showed 0.15-0.35% acidity from Day 0 to the end of fermentation, demonstrating a significant increase. There was no significant difference in acidity until Day 2 of fermentation, regardless of irradiation, whereas from Day 3, acidity of irradiated *Makgeolli* appeared to be lower or similar to that of un-irradiated samples. However, there was no difference based on irradiation or millimeter wave frequency. These findings are consistent with previous reports that the acidity of *Makgeolli* increased with from 0.1% at the beginning of fermentation to 0.30-0.38% at the end (14,28).

Figure 2C presents the soluble solid contents in irradiated *Makgeolli*. The soluble solid contents on Day 0 of fermentation was 7.27°Bx in un-irradiated *Makgeolli*, 6.63°Bx in *Makgeolli* irradiated with 60 GHz millimeter wave, and 5.83°Bx in *Makgeolli* irradiated with 70 GHz millimeter wave, demonstrating a significant increase. The soluble solid contents of all *Makgeolli* samples showed rapid increase on Day 1 of fermentation, and *Makgeolli* irradiated with 60 GHz waves showed the highest soluble solid content (10.47°Bx). The soluble solid contents in all *Makgeolli* samples tended to decrease from Day 2 of fermentation. These results indicate starch hydrolysis at the early stage of fermentation, which is consistent with the results of previous studies (2,28). Interestingly, the differences in the soluble solid contents in irradiated *Makgeolli* between Days 0 and 1 of fermentation were 3.84°Bx for the 60 GHz waves and 3.90°Bx for the 70 GHz waves. These values were higher than the value obtained for un-irradiated *Makgeolli* (2.6°Bx) during the same period. These findings indicate starch hydrolysis was more active in the irradiated samples, suggesting that millimeter waves have an effect on the starch hydrolysis enzymes. Although, it's not clear mechanism how

affect enzyme hydrolysis, similar phenomenon had been found in electromagnetic wave. Byeon *et al.* (29) reported that stimulation by ultrasonication at 20-35 kHz during fermentation of soybean *kaji* greatly increased the amylase activity. Irradiation of the seed embryos of *Isatis indigotica* Fort with He-Ne laser (5.23 mW/mm², 5 min) and microwave (1.26 mW/mm², 8 s) significantly increased the activities of α -amylase and the total amylase content in the seed embryos relative to that in un-irradiated controls (30). In conclusion, millimeter waves affect the starch hydrolysis enzymes during fermentation, although they had no observable effects on pH and acidity.

Changes in the total sugar and reducing sugar contents following millimeter wave irradiation

Total sugars comprise reducing sugars and non-reducing sugars. Slow reduction of the total sugar content during fermentation indicates that the sugar is being used by yeasts and sugar consumption rate might vary. Figure 3A shows the total sugar contents in irradiated *Makgeolli*. The total sugar contents were higher in un-irradiated *Makgeolli* (5.54%) on Day 0 than in irradiated *Makgeolli* (1.77 and 2.79% in samples irradiated with 60 and 70 GHz waves, respectively). Total sugar contents in all *Makgeolli* samples increased to the maximum on Day 1 of fermentation and decreased from Day 2. The content at the end of fermentation was 0.62% in un-irradiated *Makgeolli*, 0.17% in *Makgeolli* irradiated with 60 GHz waves, and 0.86% in *Makgeolli* irradiated with 70 GHz waves. In the early stages of *Makgeolli* fermentation, starch is hydrolyzed to sugar by the starch hydrolysis enzyme from the ferments, which greatly increases the total sugar contents. Since the hydrolyzed sugars are simultaneously used as nutrient sources or fermentation substrates of yeasts, the total sugar content decreases as fermentation proceeds (12,22). The total sugar contents were 5.76% in un-irradiated *Makgeolli*, 3.86% in *Makgeolli* irradiated with 60 GHz waves, and 2.79% in *Makgeolli* irradiated with 70 GHz waves on Day 1 of fermentation. The increase in total sugar contents from Day 0 to Day 1 was lower in un-irradiated *Makgeolli* (0.22%) than in irradiated samples (2.09% for 60 GHz and 2.37% for 70 GHz). This could be due to the increased starch hydrolysis enzyme activities following millimeter wave irradiation. In addition, un-irradiated *Makgeolli* showed a greater reduction in the total sugar contents from Day 1 to Day 2 of fermentation than irradiated *Makgeolli*, as the amount of total sugar contents produced by starch hydrolysis enzymes, which were affected by millimeter waves, was greater than that of total consumed sugars by yeasts during alcohol production.

Reducing sugars are important components that are used as substrates for alcohol fermentation and their content affects sweetness. The reducing sugar contents in irradiated *Makgeolli* are shown in Fig. 3B. Reducing sugar contents on Day 0 of fermentation as 5.03% for un-irradiated *Makgeolli*, which was significantly higher than those of *Makgeolli* irradiated with 60 GHz (4.06%) and 70 GHz (2.83%) waves. Reducing sugar contents of un-irradiated *Makgeolli*

increased to 5.81% on Day 1 of fermentation, and those of irradiated *Makgeolli* increased to 5.05-5.06%, showing a much greater increase compared to un-irradiated *Makgeolli*. Reducing sugar content in all *Makgeolli* samples tended to decrease from Day 2 of fermentation until the end of fermentation, as yeasts used the reducing sugars produced by starch hydrolysis enzymes for alcohol fermentation (2,12,14). According to the results of investigation on the changes of total sugars and reducing sugars during *Makgeolli* fermentation, millimeter waves were found enhance the activities of starch hydrolysis enzymes.

Changes in alcohol content following millimeter wave irradiation

The hydrolytic enzymes in *nuruk* and *kaji* hydrolyze starch to sugars during the fermentation of *Makgeolli* and the resulting sugars are used as substrates for alcohol fermentation by yeasts. Therefore, alcohol content is a good indicator of the degree of fermentation. The alcohol contents of the *Makgeolli* produced in this study are shown in Fig. 4. Alcohol contents in all *Makgeolli* samples tended to increase significantly immediately after the beginning of fermentation (Day 0) and continued to increase until the end of fermentation. The alcohol contents in un-irradiated *Makgeolli* on Day 0 and Day 1 of fermentation were significantly higher than those in the irradiated samples. This is because the initial sugar contents of un-irradiated *Makgeolli* were higher than those of the irradiated samples, which would allow for earlier initiation of alcohol fermentation in the un-irradiated samples. The alcohol contents were higher in *Makgeolli* irradiated with 70 GHz millimeter waves on Day 2 and Day 3 of fermentation than other *Makgeolli*s. The alcohol contents of irradiated *Makgeolli* were higher than those in un-irradiated *Makgeolli* at the end of fermentation, and *Makgeolli* irradiated with 60 GHz millimeter waves had significantly higher alcohol content than that irradiated with 70 GHz waves ($p < 0.05$), suggesting that changes to alcohol content were frequency dependent. The alcohol content of *Makgeolli* depends on the amount of starch, fermentation conditions such as temperature and humidity, and the type of yeast (2,12,14,31). Alcohol contents of irradiated *Makgeolli* were higher than those of un-irradiated samples in the later stage of fermentation, which could be due to the upregulation of the activity of starch hydrolysis enzymes by the millimeter waves. Thus, the irradiated samples would have greater amounts of fermentable sugars available for alcohol fermentation than the un-irradiated control group would have.

Irradiation of *Makgeolli* with millimeter waves was found to have no effect on the growth of yeasts and LAB and on the quality characteristics of *Makgeolli*, such as pH and acidity. In contrast, the irradiation upregulated the activity of starch hydrolysis enzymes, thereby promoting alcohol fermentation. Our findings suggest that millimeter waves could be used to positively influence *Makgeolli* fermentation at the industrial scale, and highlight the need for further study on the use of millimeter wave irradiation of fermented foods.

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