Adding enzymes to improve the properties of the Korean barley Gwangmaek wort during mashing

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Abstract This study investigated the properties of the Korean barley Gwangmaek wort and compared them to those of the imported Pilsner. The reducing sugar content of Gwangmaek (52.13 mg/mL) was 13% lower than that of Pilsner. The filtration time for Gwangmaek (53 min) was 2.5 times longer than that of Pilsner. The α -amylase and amyloglucosidase treatments increased the reducing sugar content of Gwangmaek up to that of Pilsner. However, the filtration time (88 min) significantly increased after the enzyme treatment. In terms of β -glucan contents measured during the mashing process of Pilsner, Gwangmaek, and α -amylase and amyloglucosidase-treated Gwangmaek, the enzyme-treated Gwangmaek had the highest at 232.23 mg/L, followed by Gwangmaek (190.31 mg/L) and Pilsner (82.82 mg/L). When β -glucanase was added during mashing, there was no change in reducing sugar content. However, the filtration time significantly decreased from 88 to 29 min, and viscosity also declined from 1.78 to 1.42 cp.

Keywords: Gwangmaek, wort property, starch-degrading enzyme, β -glucanase

Introduction

South Korea's beer production reached 2 million kL in 2013 and it has recently grown by 8 percent annually (1). In Korea, beer is mostly made using imported malt. Lately, however, there have been a lot of studies on the development of different and diverse beers using local agricultural products (2-3). In particular, numerous varieties using Korean barley have been developed and their quality has been evaluated to brew beer (4). The Korean barley Gwangmaek is a two-row barley developed recently in Korea. It is resistant to pests and cold. In addition, it has been reported that it has great raw barley and malt qualities (5). However, its wort properties after mashing are little known.

The wort properties can have a large influence on the brewing process and the final product. In general, when the wort properties are evaluated after mashing, the following are analyzed: fermentable sugar, protein amount, viscosity, filtration time, and pH (4,6). The fermentable sugar has a significant effect on the growth of yeast cells and also affects the ethanol content in the final fermented product (7). If the fermentable sugar concentration is too high in the wort, residual sugar is generated, which in turn increases the calorie content in the final product and fermentation time (8). An appropriate amount of protein is helpful for the growth of yeast during fermentation, but excessive protein content could lower the quality

of beer by generating haze (9-11). pH is a critical factor that determines the quality of beer, having an effect on enzyme activity, colloidal stability, bitterness, and color during brewing (12-13). In addition, filtration time and wort viscosity can have an effect on beer production from economic aspects.

When the sugar content of wort is low because of low starch contents in barley or poorly modified malt, adjuncts or enzymes can be added (14). There have been attempts to increase the ethanol yield in beer by raising the wort sugar content by adding starch-degrading enzymes (8,15-17). Both α -amylase and amyloglucosidase generate glucose, maltose, or maltotriose by hydrolyzing the α -1,4 bond in starch. In particular, amyloglucosidase can further increase the fermentable sugar content by degrading the α -1,6 bond (15,18-19). In addition, the β -glucan content in barley can also affect beer brewing. Barley β -glucan is a glucose polymer with irregular structure consisting of β -1,4 and β -1,3 bonds and is associated with firmly linked peptide sequences in the barley endosperm cell wall (20). β -Glucan is degraded by intrinsic β -glucanase in barley during malting (21). However, insufficiently degraded β -glucan inhibits the wort separation by increasing its viscosity and thus increases the filtration time. In addition, it can form a chill haze after being precipitated during secondary fermentation, causing a decrease in the quality of the beer (22-23). This study compared Gwangmaek with Pilsner in terms of wort properties and investigated the use of α -amylase,

amyloglucosidase, and β -glucanase to improve the wort properties of Gwangmaek.

Materials and Methods

Materials Gwangmaek was provided by the National Institute of Crop Science in Korea while Pilsner malt was imported from Weyermann in Germany (Bamberg, Germany). Malting was carried out in the following three steps using an automatic micro-malting system (Phoenix Biosystems Co., Adelaide, Australia): steeping, germination, and kilning. The grist was prepared using a drum mill (Malt Drum Mill, Jeil Industry Co., Seoul, Korea) with a 1-mm gap. α -Amylase (60,000 bacterial amylase unit/g, Spezyme FRED), amyloglucosidase (or glucoamylase, 270 glucoamylase unit/g, BioWin AG), and β -glucanase (400 unit/g, BrewMax L) were provided from Vision Biochem Co. Ltd. (Seongnam, Korea). In this study, all enzymes were added to be prepared against the malt's weight.

Mashing Mashing was carried out using the traditional EBC (European Brewery Convention) Congress method according to EBC 4.5.1. The ground malt (50 g) was poured into a 2 L Duran laboratory bottle filled with 200 mL of 45°C distilled water and mixed well. After adding an appropriate amount of enzyme, the sample was incubated in a shaking water bath at 100 rpm and 45°C for 30 min. The temperature of the water bath was increased to 70°C at a rate of 1°C/min for 25 min. Then, 100 mL of distilled water (70°C) was added to the sample and incubated at 70°C for 1 hour. Once the mashing was completed, the mash was cooled to 20°C and distilled water was added to adjust to a final weight of 450 g. The mash was filtered through a glass funnel (20 cm in diameter) using Whatman filter paper (No. 597 1/2). The first 100 mL of filtrate was re-filtered and the collected filtrate was used as the final wort sample.

Analysis of wort properties and β -glucan content To evaluate the wort quality, reducing sugar, free amino nitrogen (FAN), pH, filtration time, and viscosity were measured. The amounts of reducing sugar and FAN were measured using the dinitrosalicylic acid (DNS) method (24) and a ninhydrin-based assay (25), respectively. Filtration time and viscosity were measured according to the ASBC (American Society of Brewing Chemists) method. In terms of filtration time, the time that the solution was no longer visible in a filter cake and started to split was taken as the end of the measurement. Viscosity was measured at 24°C using a Sine-Wave Vibro Viscometer (SV-10; AND, Tokyo, Japan). The β -glucan contents in the worts were measured, using a Megazyme β -glucan assay kit (K-BGLU; Megazyme International Ireland, Co. Wicklow, Ireland). All measurements were repeated three times and stated in average values. For the statistical analysis of all data measured in this study, SPSS (Statistics Package for the Social Science, ver. 21, SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2013 were used. Analysis of Variance (ANOVA) was used to

Table 1. Wort properties of Pilsner, Gwangmaek, and enzyme-treated Gwangmaek

	Pilsner	Gwangmaek	Gwangmaek treated with enzymes ¹⁾
Reducing sugar (mg/mL)	60.12 $\pm$ 1.22 ^a	52.13 $\pm$ 2.78 ^b	59.91 $\pm$ 2.68 ^a
Free amino nitrogen (mg/L)	151.79 $\pm$ 2.07 ^c	186.57 $\pm$ 2.9 ^b	195.55 $\pm$ 3.9 ^a
pH	5.68 $\pm$ 0.02 ^a	5.54 $\pm$ 0.01 ^b	5.55 $\pm$ 0.02 ^b
Viscosity (cp)	1.59 $\pm$ 0.01 ^c	1.71 $\pm$ 0.03 ^b	1.78 $\pm$ 0.01 ^a
Filtration time (min)	21.60 $\pm$ 2.35 ^c	53.33 $\pm$ 3.54 ^b	87.67 $\pm$ 4.16 ^a

¹⁾Gwangmaek malt was treated with α -amylase and amyloglucosidase (0.1% each of the malt weight) during the mashing. The data are expressed as the mean $\pm$ standard deviation ($n=3$). The mean values in the same row with different superscript letters are significantly different ($p<0.05$).

analyze the significant differences ($p<0.05$) between each sample.

Results and Discussion

Wort properties of Gwangmaek and Pilsner Pilsner is known as a typical beer barley for brewing lager beer. Because of its high quality, Pilsner was chosen for comparison to Gwangmaek. Table 1 shows the properties of the worts obtained from Gwangmaek and Pilsner malts. When the amount of reducing sugar in the worts from Gwangmaek and Pilsner was compared, the former (52.13 mg/mL) was lower than the latter (60.12 mg/mL) by about 15%. In terms of FAN level, Gwangmaek wort (186.57 mg/L) was higher than Pilsner wort (151.79 mg/mL). However, it has been reported that the desirable FAN level after saccharification is 200-250 mg/L (26). The pH of Gwangmaek and Pilsner were 5.54 and 5.68, respectively. They were within the general range for worts (pH 5.4-5.8) made with brewing water at neutral pH (13). In terms of filtration time for the mash, it should be short. In this experiment, however, Gwangmaek's filtration time was 53.33 minutes, 2.5 times longer than that for Pilsner (21.60 minutes). In terms of wort viscosity, Gwangmaek (1.71 cp) was higher than Pilsner (1.59 cp).

Effects of amylase and amyloglucosidase treatment on Gwangmaek wort properties The reducing sugar content in the Gwangmaek wort was comparatively lower than that in the Pilsner wort. To further extract fermentable sugar from Gwangmaek malt during mashing, α -amylase and amyloglucosidase were added. Figure 1 shows the amount of reducing sugar in wort that was obtained by adding enzymes at 0.1-4% of the malt weight. In fact, many studies have confirmed an increase in fermentable sugar when amyloglucosidase is added during saccharification (8,15). In this study, the amount of reducing sugar increased by 57.5% when amyloglucosidase was added (up to 4%), compared to the wort without amyloglucosidase treatment. When α -amylase was added, in contrast, the increase in the amount of reducing sugar was minor. Upon addition of amylase (up to 4%), the amount of reducing sugar

Fig. 1. Effect of α -amylase and/or amyloglucosidase treatments on the production of reducing sugar during the mashing of Gwangmaek malt. *means that the same amount of both enzymes was used.

just increased by 17.5%. It appears that these results were obtained owing to the difference in hydrolytic activity between the two enzymes. Johanan *et al.* reported that when amyloglucosidase was added during mashing, the total fermentable sugar content was increased by 20%, and the glucose level increased more than 90% in the wort (8). To get the reducing sugar level close to the amount of reducing sugar (60.12 mg/mL) of the control group (Pilsner), 4% α -amylase should be added to the Gwangmaek malt. When amyloglucosidase was used, 0.2% amyloglucosidase was enough. Furthermore, when both α -amylase and amyloglucosidase were added (0.1% each) to the Gwangmaek wort, Gwangmaek wort was similar to Pilsner wort with 59.91 mg/mL in terms of the amount of reducing sugar. It appears that the amount of reducing sugar was increased after small oligosaccharides created by α -amylase were degraded into glucose units by amyloglucosidase (19).

When the other properties of the Gwangmaek wort made by adding the two enzymes (0.1% each) were compared to those of the Gwangmaek wort without enzyme treatment, FAN and viscosity was increased by about 5% (Table 1). In particular, the filtration time was increased by about 65% compared to that for the Gwangmaek wort to which no enzymes were added. It appears that the increase of viscosity and filtration time after enzyme treatment is partly owing to the release of various polymers such as β -glucan (27). The viscosity of the wort is related to the content of β -glucan, which tends to form gels in the wort (28-29). The high viscosity of wort owing to the high content of β -glucan can increase the filtration time (30). Since barley with high β -glucan contents inhibits filtration by increasing wort viscosity, it is necessary to investigate and reduce the β -glucan content in the Gwangmaek wort to reduce the filtration time.

Measurement of β -glucan content in Gwangmaek wort In terms of the wort viscosity and filtration time in shown Table 1, the

Gwangmaek wort treated with amylase and amyloglucosidase was the highest, followed by the Gwangmaek wort to which no enzymes was added, and then the Pilsner wort. Since it was expected that the β -glucan content would be high in the Gwangmaek wort, the β -glucan content of the Gwangmaek wort was measured using a β -glucan assay kit (Table 2). The β -glucan content of the Gwangmaek wort (190.31 mg/L) was more than twice that of the Pilsner wort. In particular, the β -glucan content of the Gwangmaek wort to which starch-degrading enzymes were added (232.23 mg/L) was about 30% greater than the one without the enzyme. In general, the β -glucan content in barley significantly decreases because of degradation by β -glucanase during malting. However, the amount of β -glucan remaining after malting can differ depending on the malting conditions and barley variety (21,23,29). β -Glucan is one of the components of the barley endosperm cell walls (31-32). The starch granules are enclosed by the endosperm cell walls (33). The endosperm cell walls must be degraded during malting before enzymes can act on the starch granules. Therefore, it appears that the β -glucan content in the wort is increased by the extensive hydrolysis of starch by the added amylase and amyloglucosidase, which may affect the extraction of β -glucan from the cell wall (27).

Effects of β -glucanase treatment on Gwangmaek wort properties

To investigate the effects of β -glucan content on filtration time and viscosity in the worts, β -glucanase was added during the mashing. Various amounts of β -glucanase (0.1, 0.2, or 0.4% of the malt weight) were added to the mash to which 0.1% each α -amylase and amyloglucosidase was added previously. After the mashing process, changes in filtration time and viscosity in the worts were measured (Fig. 2). As the amount of β -glucanase increased, wort viscosity and filtration time decreased. When 0.4% β -glucanase was added, the filtration time was 29 minutes. It declined by 70% compared to the

Fig. 2. Effect of β -glucanase treatment on the filtration time and viscosity after mashing of Gwangmaek. Gwangmaek malt was treated with 0, 0.1, 0.2, and 0.4% β -glucanase. All samples were also simultaneously treated with α -amylase and amyloglucosidase (0.1% each of the malt weight).

Table 2. Comparison of the β -glucan concentration in mash

	Pilsner	Gwangmaek	Gwangmaek treated with enzymes ¹⁾
β -Glucan (mg/L)	82.82 $\pm$ 6.96 ^c	190.31 $\pm$ 8.55 ^b	232.23 $\pm$ 7.97 ^a

¹⁾Gwangmaek malt was treated with α -amylase and amyloglucosidase (0.1% each of the malt weight) during the mashing. The data are expressed as the mean $\pm$ standard deviation ($n=3$). Values with different superscript letters in the same row represent significant differences ($p<0.05$).

Table 3. Measurement of reducing sugar, free amino nitrogen, and pH of Gwangmaek wort after treatment of β -glucanase

	Amount of β -glucanase used		
	0.1%	0.2%	0.4%
Reducing sugar (mg/mL)	60.25 $\pm$ 2.03 ^a	61.62 $\pm$ 1.01 ^a	62.85 $\pm$ 1.00 ^a
Free amino nitrogen (mg/L)	196.97 $\pm$ 4.64 ^b	199.93 $\pm$ 3.44 ^b	209.29 $\pm$ 3.4 ^a
pH	5.54 $\pm$ 0.02 ^a	5.54 $\pm$ 0.01 ^a	5.53 $\pm$ 0.01 ^a

Gwangmaek malt was treated with α -amylase (0.1% of the malt weight), amyloglucosidase (0.1% of the malt weight) and the increasing amount of β -glucanase (0.1-0.4% of the malt weight) during the mashing. The data are expressed as the mean $\pm$ standard deviation ($n=3$). Means sharing the same superscript are not significantly different from each other ($p<0.05$).

wort without β -glucanase addition while the viscosity decreased by 20% to 1.42 cp. Therefore, the addition of 0.4% β -glucanase to Gwangmaek malt during the mashing was useful to reduce the filtration time and viscosity of the wort as much as those of Pilsner (Table 1). These results matched the previous results showing that the β -glucan content of malt is highly correlated with wort viscosity, and high β -glucan content in wort makes filtration difficult. In the case of wine, a decrease in filtering rates was reported despite the existence of a small amount (10 mg/L) of β -glucan, and this problem was solved by adding β -glucanase (34). In Gwangmaek wort,

therefore, it appears that the β -glucan content also had a considerable effect on filtration time. Meanwhile, the β -glucan content in the wort treated with β -glucanase was less than 10 mg/L, and it must have been mostly degraded by β -glucanase (data not shown).

Table 3 shows the changes in the Gwangmaek wort properties after β -glucanase was added. The amounts of reducing sugar slightly increased as the addition of β -glucanase increased, but no significant difference was observed ($p<0.05$). β -Glucanase degrades β -glucan into small fragments, such as tri- and tetra-saccharides as the major products (35). The amount of reducing sugar generated by the β -glucan degradation is far smaller than the amount produced by starch degradation. Therefore, a slight increase in the amount of reducing sugar in the total wort was insignificant. In addition, there were no significant changes in pH ($p<0.05$), which means that β -glucan degradation had no effect on wort pH.

According to comparison of wort properties between Korean Gwangmaek and imported Pilsner, the former was lower in the amount of reducing sugar but greater in β -glucan content and filtration time than the latter. The treatment of α -amylase and amyloglucosidase increased the amount of reducing sugar during mashing of Gwangmaek, but the filtration time increased significantly. The addition of β -glucanase significantly reduced the filtration time and viscosity of the Gwangmaek wort. There were no significant changes in reducing sugar content, FAN, and pH after enzyme treatment. This study suggested that the addition of diverse enzymes during the mashing process for Gwangmaek would be helpful in brewing beer using Gwangmaek through the improvement of its wort properties.

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