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Extrusion of Ginseng Root in Twin Screw Extruder: Pretreatment for Hydrolysis and Saccharification of Ginseng Extrudate

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Abstract

The objective of this experiment was to investigate the effect of extrusion of ginseng roots in twin screw extruder on susceptibility of ginseng starch toward hydrolysis by α-amylase BAN 480L (Novozyme, Denmark) and cellulase Celluclast 150L and saccharification by amyloglucosidase AMG-E (Novozyme, Denmark). The extrusion was conducted at 22% and 30% moisture contents of feed at screw speed 300 rpm. Barrel temperature at zone 1 was adjusted at 100°C and 120°C. The results showed that extrusion process improved the ginseng α-amylase susceptibility as compared to traditionally dried ginseng (white and red ginseng). Reducing sugar of hydrolyzed extruded samples was 2,500% of its initial concentration, whereas the reducing sugar of hydrolyzed non-extruded sample was only 200% of its initial concentration. However, addition of cellulase during liquefaction lowered the saccharification yield of both non-extruded and extruded samples as well.

Key words: extrusion process, ginseng root, saccharification, susceptibility

INTRODUCTION

Korean ginseng roots come in forms of fresh, white and red ginseng. Fresh ginseng is root that is unprocessed and still in its original shape. White ginseng is peeled and sun-dried ginseng roots and red ginseng is steamed and dried ginseng roots. White and red ginseng roots are dried until moisture content is less than 14%. White and red ginseng roots are also processed into various products. Processed white ginseng includes ginseng powder, capsules, honeyed ginseng tea and some other ginseng drinks. Red ginseng is processed into sliced, extract, powder, tablets, capsules, tea, and some other ginseng beverages.

Ginseng root is also added into traditional rice wine during fermentation. Five year fresh ginseng root contains starch as high as 75.5% (dry basis) (1). Considering high content of starch, it is possible to use ginseng itself as a substrate for the fermentation of alcoholic beverage. Study on ginseng fermentation has been done by some researchers. Ahn and Lee (2) studied on utilization of ginseng meal for alcohol and yeast reproduction fermentation. The meal was enzymatic saccharified before using it as a fermentation substrate. The possibility of using ginseng root hair, white and red ginseng for making ginseng wine was carried out by Roh et al. (3). The study concluded that saponin of ginseng had to be removed up to 99.9% before using the ginseng as ethanol fermentation substrate since the substance suppressed yeast activity. Both studies involved enzymatic liquefaction and saccharification of the gelatinized ginseng starch without any other treatments.

Extrusion technology has been reported for starch pretreatment for further liquefaction and saccharification and as an enzyme bioreactor of various starches such as corn, cassava, barley, wheat, etc (4,5). However, it has little been tried to the processing of ginseng root.

Researches have been performed experiment on using extrusion technology as pretreatment of ginseng for further treatments such as release of ginseng active components and red ginseng production in Korea since 2003 (6-10).

The objective of this experiment was to study effect of moisture content of feed and extrusion temperature on susceptibility of dried ginseng extrudate on enzymatic hydrolysis and saccharification.

MATERIALS AND METHODS

Materials

Five year old fresh ginseng roots, white ginseng and red ginseng powder were bought from Dong-Jin Drug

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Table 1. Process variables for extruding ginseng root

Sample numbers	1	2	3	4
Moisture content (%)	22	30	22	30
Screw speed (rpm)	300	300	300	300
Barrel temperature (°C)				
zone 1	120	120	100	100
zone 2	130	130	100	100
zone 3	60	60	60	60
Die hole diameter (mm)	3	3	3	3

and Food Co. (Gumsan, Korea). Enzymes used for the experiment were α-amylase BAN 480L (Novozyme, Denmark) and amyloglucosidase AMG-E (Novozyme, Denmark) and cellulase Celluclast 1.5L FG (Novozyme, Denmark).

Extrusion

Extrudate for the experiment was made from fresh ginseng only. Fresh ginseng was chopped using chopper (Hwajin Precision Co., Korea) and dried in oven at 90°C for 24 hours. The dried ginseng was extruded in co-rotating intermeshing twin screw extruder model THK3IT (Inchen Machinery Co., Korea). Variables applied in extrusion process are shown in Table 1. The extrudate was directly dried in an oven at 80°C for 4 hours. The dried extrudate was ground and sieved through 500 μm and kept in a glass jar at 4°C. Ground dried ginseng without extrusion treatment was used as a control sample.

Swelling factor

Swelling factor of ginseng starch was measured at different ratios of ginseng powder weight to water volume. The ratio of ginseng powder weight to water volume observed was: 100 mg to 250 μL, 100 mg to 375 μL and 100 mg to 500 μL. Swelling factor is defined as the amount of water absorbed by starch granules heated in excess water, based on the observation of blue dextran dye that dissolved in supernatant and interstitial water but not in intergranular water (11). The swelling factor was conducted as suggested by Tester and Sommerville (12) and calculated according to a formula suggested by Tester and Morrison (11).

Hydrolysis and saccharification

Several different enzyme treatments were observed (Table 2). Hydrolysis and saccharification were carried out in shaking water bath (Jeio Tech, SWB 10, Korea). Two grams of extruded ginseng or ginseng powder was suspended in 10 mL of 50 mM citrate-phosphate pH 6 which had been heated up to 60°C. α-Amylase only or α-amylase and cellulase were added prior to mixing the extrudate. Saccharification experiment was conducted by adding amyloglucosidase after hydrolysis process for 5 hours and adjusting pH of the reaction mixture to 4.5.

Table 2. Enzyme treatment for hydrolysis and saccharification

Treatment	Enzymes
Hydrolysis	α-amylase α-amylase and cellulase (added at the same time at 0 hour)
Hydrolysis and saccharification	α-amylase and amyloglucosidase (after hydrolysis for 5 hours and pH adjustment to 4.5) α-amylase, cellulase (added at the same time at 0 hour) and amyloglucosidase (after hydrolysis for 5 hours and pH adjustment to 4.5)

The pH of reaction mixture was adjusted with 50 mM citric acid. Extent of hydrolysis and saccharification was assayed every 30 minutes up to 6 hour-incubation by measuring reducing sugar content of the reaction mixture. The reducing sugar was determined as glucose according to Somogyi-Nelson method (13). The yields of extrudate hydrolysis and saccharification of control, white and red ginseng were compared.

RESULTS AND DISCUSSION

Swelling factor

Swelling process of starch granules is termed as gelatinization. Swelling factor was to observe the swelling characteristics of the extruded ginseng. Commercial liquefaction of starch is usually carried out at concentration of 20～40% of gelatinized starch. In this experiment, swelling of ginseng was observed at three different levels of ratio of ginseng to water. Fig. 1 shows the swelling factor of control (dried ginseng at 90°C for 24 hours), white and red ginseng. White ginseng is traditional product of air dried ginseng, whereas production

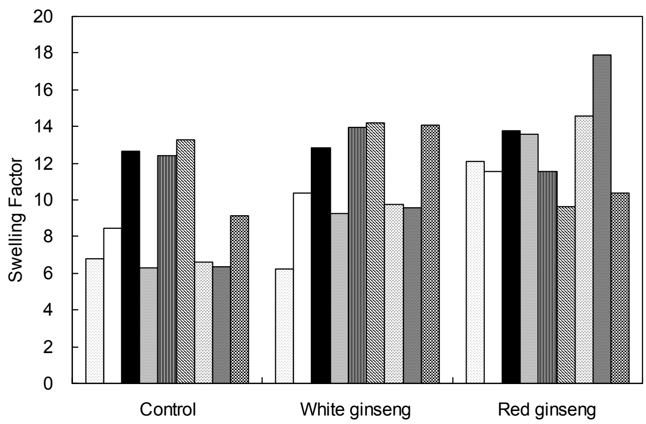

Fig. 1. Swelling factor of non-extruded ginseng.
▨ Temperature 50°C ginseng powder weight 100 mg and water volume 250 μL; □ 60°C/100 mg/250 μL; ■ 70°C/100 mg/250 μL; ▨ 50°C/100 mg/375 μL; ▥ 60°C/100 mg/375 μL; ▧ 70°C/100 mg/250 μL; ▤ 50°C/100 mg/500 μL; ▩ 60°C/100 mg/500 μL; ▦ 70°C/100 mg/500 μL.

of red ginseng involves steaming and drying of ginseng roots. It is possible that starch in white ginseng is still in a form of not gelatinized, but the starch in red ginseng could be in the form of gelatinized one. However, since red ginseng had been undergone drying process, it might be possible that the starch was undergone retrogradation process. Therefore, the starch in red ginseng might be less susceptible toward enzymatic hydrolysis.

Gelatinization process involves swelling of starch granules. During swelling process, amylose which is in amorphous region of starch granules is leached out. It makes the amylose more accessible for enzymatic hydrolysis. Since the sample was only heated for 30 minutes at the observed temperature during the experiment, it might be possible that the length of heating time was not enough to gelatinize control sample, and white ginseng. Therefore, the starch in the non-extruded samples was not swollen and gelatinized.

Fig. 2 shows the swelling factor of control sample and extruded ginseng samples. Most swelling factor of extruded ginsengs was higher than 20, whereas the swelling factor values of most unextruded samples were less than 14. This data showed that extrusion process altered the ginseng powder structure which caused the starch in ginseng to gelatinization during extrusion process and the extrudate was more easily to swell. Swelling factor was decreased with the increase in the barrel temperature.

Sample #1 was extruded at 22% moisture content of the feed at 130°C. Its swelling factor at lower ratio of ginseng to water (100 mg:250 μL and 100 mg:375 μL) was the highest at 60°C. At the highest ratio of ginseng to water, the sample's highest swelling factor was at 70°C. All other extrudate samples (#2, #3 and #4) had the highest swelling factor at 60°C at the lowest and highest ratios of ginseng to water which indicated that the sample could swell in limited amount of water. The results of this experiment indicated that the applied extrusion condition had caused the ginseng starch to be gelatinized during extrusion process.

Hydrolysis of ginseng starch

Fresh ginseng proximately contains 16.5% protein, 75.5% starch and 5.4% cellulose and red ginseng proximately contains 11.5% protein, 71.04% starch and 4.6% cellulose (1). This experiment was carried out to observe the effect of α-amylase from *Bacillus* and cellulase on hydrolysis of ginseng starch.

Figs. 3 and 4 show hydrolysis of non-extruded ginseng and extruded ginseng, respectively. The data indicated that the addition of cellulase increased the reducing sugar resulted from enzymatic hydrolysis of ginseng. Increase of reducing sugar in non-extruded ginseng hydrolysates was only 150~200% of the initial reducing sugar content. However, the increase of reducing sugar content in extruded ginsengs was much higher with the value of 1,500~2,500% of the initial concentration. Reducing sugar was decreased with the increase in the barrel temperature and moisture content. These data indicated that extrusion treatment improved α-amylase susceptibility of ginseng starch. Based on swelling factor data (Fig. 1), control, white and red ginseng were not well swollen or gelatinized at 60°C. Therefore, the starch in the samples was less susceptible to α-amylase action. All ex-

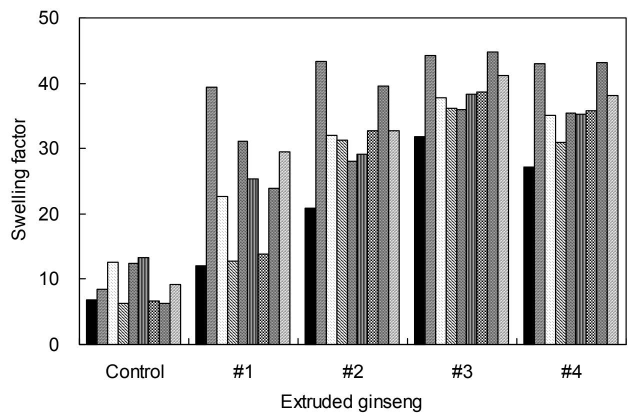

Fig. 2. Swelling factor of extruded ginseng.
■ Temperature 50°C ginseng powder weight 100 mg and water volume 250 μL; 60°C/100 mg/250 μL; 70°C/100 mg/250 μL; 50°C/100 mg/375 μL; 60°C/100 mg/375 μL; 70°C/100 mg/250 μL; 50°C/100 mg/500 μL; 60°C/100 mg/500 μL; 70°C/100 mg/500 μL.
Sample # refers to Table 1.

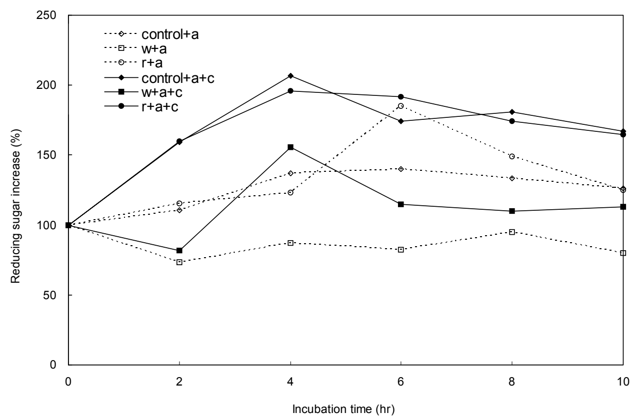

Fig. 3. Effect of cellulase (c) addition on hydrolysis of control, white (w) and red (r) ginseng by α-amylase (a).
control+a: Dried raw ginseng was treated with α-amylase.
w+a: White ginseng was treated with α-amylase.
r+a: Red ginseng was treated with α-amylase.
control+a+c: Dried raw ginseng was treated with α-amylase and cellulase.
w+a+c: White ginseng was treated with α-amylase and cellulase.
r+a+c: Red ginseng was treated with α-amylase and cellulase.

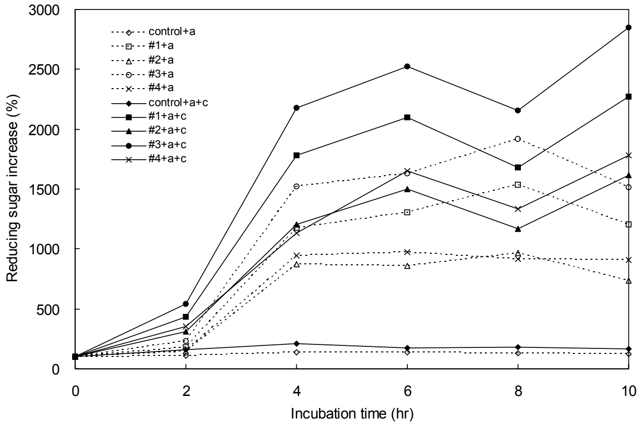

Fig. 4. Effect of cellulase (c) addition on hydrolysis of control, extruded ginseng (sample #1, 2, 3, and 4) by α-amylase (a). Sample # refers to Table 1.

truded samples were well gelatinized during extrusion process and more susceptible to α-amylase. Addition of cellulase hydrolyzed the cellulose in non-extruded samples as well as extruded ones as shown by the increase of reducing sugar content as compared to the sample without cellulase treatment.

Hydrolysis and saccharification of ginseng starch

Figs. 5 and 6 show hydrolysis and saccharification of non-extruded ginseng and extruded ginseng, respectively. Increase of reducing sugar in non-extruded ginseng hydrolysates was only 150～230% of the initial reducing sugar content. However, the increase of reducing sugar content in extruded ginsengs was much higher with the value of 2,000～3,000% of the initial concentration. Reducing sugar was increased with the decrease in the moisture content and barrel temperature. This data agreed with Shin et al. (14) and Kim et al. (15). Extrusion increased grain gelatinization, with degree of gelatinization in extruded barley by Shin et al. (14). The study included that extrusion improved digestibility of whole grains in comparison with those of raw grains. Degree of gelatinization was increased with the decrease in the moisture content by Kim et al. (15).

Addition of cellulase to both non-extruded and extruded samples lowered the saccharification yield. It has been known that activity of amyloglucosidase in the excess of glucose could alter the enzyme activity to form reversion products such as maltose and isomaltose (16). Such products affected the measurement of reducing sugar which was calculated as glucose. Govidasamy et al. (17) suggested that the problem could be solved by balancing the amount of enzyme, temperature and time of incubation.

CONCLUSION

Extrusion process improved the susceptibility of ginseng on α-amylase hydrolysis and saccharification using amyloglucosidase. Addition of cellulase increased the hydrolysis yield of ginseng, but the enzyme lowered the saccharification yield. In order to improve saccharification yield, it is necessary to carry out further investigation to optimize saccharification condition including enzyme concentration, temperature and time of reaction.

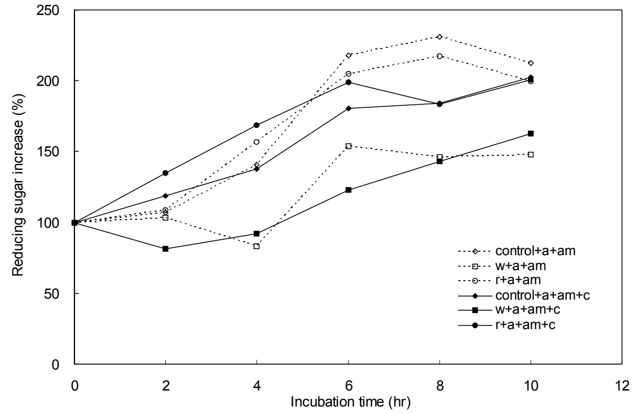

Fig. 5. Effect of cellulase (c) addition on hydrolysis of control, white (w) and red (r) ginseng by α-amylase (a) and amyloglucosidase (am).
control＋a＋am: Dried raw ginseng was treated with α-amylase and amyloglucosidase.
w＋a＋am: White ginseng was treated with α-amylase and amyloglucosidase.
r＋a＋am: Red ginseng was treated with α-amylase and amyloglucosidase.
control＋a＋am＋c: Dried raw ginseng was treated with α-amylase, cellulase and amyloglucosidase.
w＋a＋am＋c: White ginseng was treated with α-amylase, cellulase and amyloglucosidase.
r＋a＋am＋c: Red ginseng was treated with α-amylase and cellulase and amyloglucosidase.

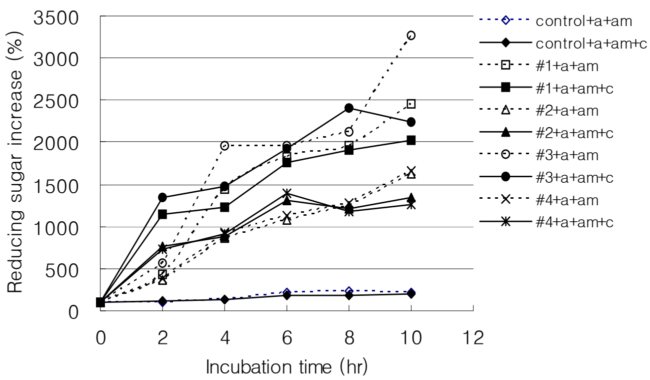

Fig. 6. Effect of cellulase (c) addition on hydrolysis of control, extruded ginseng (sample #1, 2, 3, and 4) by α-amylase (a) and amyloglucosidase (am).
Sample # refers to Table 1.

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