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Effects of Feed Moisture Content on Enzymatic Hydrolysis of Corn Starch in Twin-Screw Extruder and Saccharification of the Dried Extrudates

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Abstract The objective of this experiment was to study the influence of feed moisture content on the degree of enzymatic hydrolysis of corn starch in a twin screw extruder and the saccharification yield of the dried extrudate. The feed moisture content was set at 25, 30, and 35% and α-amylase solution was directly injected into the feed section at a barrel temperature of 95°C and screw speed of 250 rpm. Amyloglucosidase was used for the saccharification of the dried extrudate at a concentration of 0.055%(w/w). Expansion ratio and swelling factor of extrudates decreased with increasing the feed moisture content. Addition of α-amylase during extrusion process raised reducing sugar content of extrudates which also increased with the feed moisture content. The saccharification yield of dried extrudate was higher for the extrudate with lower feed moisture content.

Keywords: extrusion process, enzymatic hydrolysis, low moisture, sacharification

Introduction

Extrusion-cooking continues to be used by the food and feed industry. Recently the use of an extruder has been investigated as a continuous reactor for enzymatic modification of starches. Extrusion process has been applied for liquefying different kinds of starches to reduce saccharifying time for glucose syrup production or fermentation substrate preparation. Barley starch was liquefied using *Bacillus licheniformis* α-amylase in a twin screw extruder and then the liquefied syrup was saccharified using *Aspergillus niger* glucoamylase (1). The liquefaction of other cereal starches such as corn and wheat was performed by using a twin screw extruder with the addition of thermostable α-amylase Termamyl and 50-60% initial moisture content of the feed. After then liquefied syrup was saccharified with glucoamylase to produce high glucose syrup, which can be used as a substrate for ethanol fermentation (2).

Use of single and twin screw extruders for starch pretreatment followed by saccharification of dried extrudate was reported by Govindasamy *et al.* (3-5). Govindasamy *et al.* (6) investigated the effect of several extrusion variables on the extrusion of sago starch in a twin screw extruder and on saccharification of the dried extrudate by using *A. niger* amyloglucosidase AMG. It showed that there was no correlation between the yield of saccharification of the extudate and the extrusion variables applied with a high concentration of amyloglucosidase observed. However, at low concentration of the enzyme, the saccharification was related to the extrusion variables. Feed moisture content was a key factor as more susceptible towards the saccharification of extruded sago starch.

In addition, Govindasamy *et al.* (5) reported the effect of addition of α-amylase Termamyl 120 L into the extrusion of sago starch in a twin screw extruder. Using low concentration of the enzyme, raw sago starch was gelatinized and enzymatic liquefied in a twin screw extruder. Feed moisture, enzyme concentration, and barrel temperature were the most critical variables during the extrusion process. The study showed that initial and subsequent saccharifications were dependent of the amyloglucosidase concentration, moisture content, and the stage of the enzyme addition. Most of the study on enzymatic conversion by extrusion-cooking has been performed at relatively high feed moisture content.

Therefore the objective of this experiment was to study the effect of low moisture content of the feed on the degree of enzymatic hydrolysis of corn starch in a twin-screw extruder and the saccharification yield of dried extudate using amyloglucosidase AMG-E.

Materials and Methods

Materials Corn starch was provided by Samyang Genex Co. (Korea). The enzymes used were α-amylase (Termamyl-supra 120 L; Novozyme, Bagsvaerd, Denmark) and amyloglucosidase (AMG-E; Novozyme).

Extrusion process Extrusion was conducted in co-rotating intermeshing twin screw extruder (THK3IT; Inchen Machinary Co., Inchen, Korea) of which screw configuration with an L/D ratio is shown in Fig. 1. The

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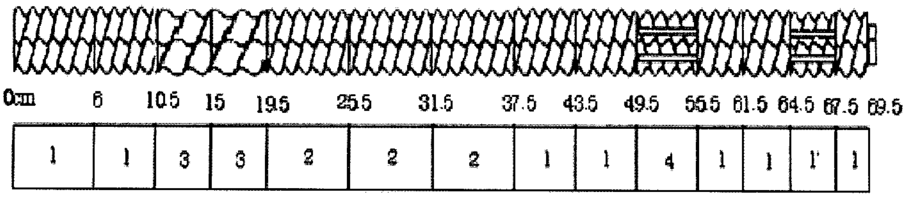

1. 1/2 Pitch screw   1' CO_2 injection   L/D ratio : 25 :1
2. 2/3 Pitch screw   3. Forward paddle   ϕ : 2.9 cm
4. Reverse screw element

Fig. 1. Screw configuration for hydrolysis of corn starch.

process conditions applied were as follows: screw speed 250 rpm, feed rate 135.6 g/min, water/enzyme solution injection rate 15.5 mL/min, barrel temperature 95°C, screw diameter 29.0 mm, and die diameter 3 mm. The α-amylase Termamyl-supra 120 L was used at a concentration of 0.0675% (w/w), and added to 0.675 g/kg dry corn starch. Feed moisture content was controlled at three levels; 25, 30, and 35%. After extrusion, some of extrudates was directly dissolved in 50 mM phosphate-citrate buffer at pH 6.0 and assayed for residual enzyme activity. The rest of extrudate was directly dried in an oven at 80°C for 4 hr, ground, and then sieved through 150 mesh for other assays.

Reducing sugar and enzyme activity α-Amylase activity was assayed by measuring the reducing sugar released during the enzymatic reaction. Reducing sugar content in samples was determined according to Somogyi-Nelson method (7). One unit of enzyme activity was defined as the amount of enzyme which produced 1 mM glucose per min. Residual enzyme activity was measured by incubating the extrudate suspension directly dissolved in 50 mM phosphate-citrate buffer at 95°C, pH 6.0 for 10 min right after the extrusion process.

Saccharification of dried extrudate Two g of ground dried extrudate was suspended in 10 mL, 50 mM phosphate-citrate buffer at pH 4.3, was mixed with 0.055%(w/w) amyloglucosidae AMG-E, and incubated for 10 hr at 60°C in water bath (SWB 10; Jeio Tech, Seoul, Korea). Reducing sugar before and after incubation was assayed according to Somogyi-Nelson method (7).

Physical properties ***Swelling factor:*** 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 (8). Starch was weighed to 0.1 mg into triplicate 10-mL screw-cap tubes, 5 mL water added, and the tested temperature (40, 50, 60, 70°C) for 30 min. The tubes were then cooled rapidly to 20°C, 0.5 mL blue dextran (M_r 2×10^6, 5 mg/mL, Pharmacia, Piscataway, NJ, USA) was added, and the contents were mixed by gently inverting the closed tubes several times. After centrifuging at 1,500×g for 5 min, the absorbance of the supernatant (A_S) was measured at 620 nm. The absorbance of reference tubes (A_R) that contained no starch was also measured.

The swelling factor (SF) was measured according to the formula suggested by Tester and Morrison (8). Starch weight was corrected to 12% moisture, assuming a density of 1.4 g/mL. Free or interstitial-plus-supernatant water (FW) is given by

$$FW\ (mL) = 5.5\ (A_R / A_S) - 0.5 \quad (1)$$

The initial volume of the starch (V_0) of weight W (in mg) is

$$V_0\ (mL) = W/1400, \quad (2)$$

and the volume of absorbed intragranular water (V_1) is thus

$$V_1 = 5.0 - FW. \quad (3)$$

Hence the volume of the swollen starch granules (V_2) is

$$V_2 = V_0 + V_1 \quad (4)$$
$$\text{and } SF = V_2 / V_0 \quad (5)$$

This can also be expressed by the single equation.

$$SF = 1 + (7{,}700/W)[(A_S - A_R)/A_S] \quad (6)$$

Expansion ratio: Expansion ratio (ER) of the extrudate was calculated by using the following equation, as suggested by Lee *et al.* (9).

$$ER = D_E/D_D \quad (7)$$

Here D_E is the diameter of extrudate and D_D is the diameter of die.

Water absorption index: Water absorption index (WAI) was defined as the amount (g) of water absorbed by each gram of extrudate as described by Lee *et al.* (9). Extrudate powder 1 g (d.b.) was mixed with distilled water 25 mL in 50-mL centrifuge tube. The tube was agitated for 30 min in 30°C water bath (SWB 10; Jeio Tech). After then it was centrifuged at 1,118×g for 20 min the supernatant was removed carefully and the weight of the hydrated sample was measured. WAI was expressed by the equation.

$$WAI\ (\%) = (\text{Hydrated sample wt.} - \text{Dry sample wt.}) / \text{Dry sample wt.} \times 100 \quad (8)$$

Statistical analysis Statistical analysis was performed with a Statistical Analysis System (Cary, NC, USA). The mean and standard deviation were analyzed and Duncan's multiple range test was applied. The level of probability (p <0.05) indicated the statistical significance.

Results and Discussion

Effect of α-amylase addition on physical properties of the extrudate ***Swelling factor:*** Swelling factor is used for measuring the extent of starch granule swelling. When water-starch slurry is heated, starch granules swell until they reach the point at which the swelling is irreversible. The process is called gelatinization (10). The swelling factor of the starch granules was observed to measure the extent of gelatinization. Figure 2 shows swelling factors of enzyme-treated samples with different feed moisture content. The swelling factor of extrudate with 25% feed moisture content was the highest over temperature. The higher the moisture content of the feed, the lower the swelling factor was. However, the swelling factor of enzyme treated extrudate with 30 and 35% feed moisture content was not significantly different at 60°C swelling temperature. Thus, it indicated that the starch in the extrudate with 25% moisture feed content was not severely degraded by α-amylase in the barrel as much as the extrudate made from the feed at moisture content of 30 and 35%.

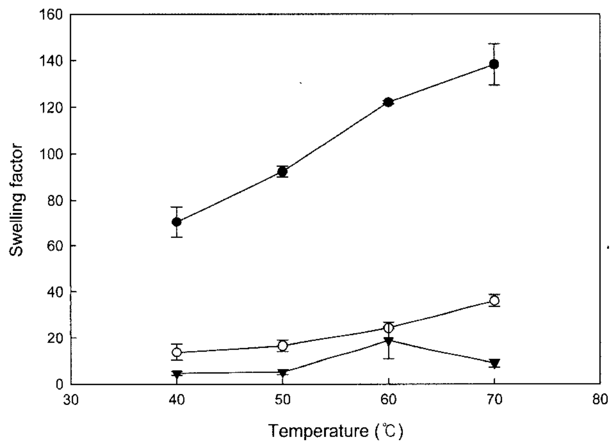

Fig. 2. Swelling factor of enzyme treated extrudate at different moisture content of the feed. –●– 25%, –○–30%, –▼– 35% feed moisture content.

Expansion ratio: Feed moisture content without α-amylase did not significantly influence the expansion ratio of the extrudate as shown in Fig. 3. Expansion ratio of all samples with α-amylase addition was less than that of the same moisture content samples without enzyme addition. Depolymerization of starch due to enzymatic hydrolysis leads to less ability of the starch melt to hold steam and to prevent the collapse and shrinkage of air cell in extrudate after exiting the die to form puffed extrudate (11, 12). Thus, the enzymatic hydrolysis by the addition of thermostable α-amylase would be responsible for the decreased expansion ratio.

Expansion ratio of extrudates both with and without enzyme addition was not significantly different at 25 and 30% feed moisture. However, it decreased with the increase in moisture content from 30 to 35%. The extrudate with enzyme addition was not fully expanded or puffed since barrel temperature was controlled to 95°C and starch melt was hydrolyzed by enzyme during extrusion. One of the reasons, it was not fully puffed can be found in the previous study, which reported that the expansion of extrudate started when the extrusion temperature reached to about 100°C (13) .

Water absorption of extrudate: Water absorption index of all samples was investigated and is shown in Fig. 4. Water absorption of all enzyme-treated extrudates increased, as compared to samples without enzyme treatment. Water absorption index of extrudates without enzyme addition was 408.7, 290.9, and 220.1% for 25, 30, and 35% feed moisture content, respectively. On the other hand, the value of water absorption index of the extrudate with enzyme addition was 454.4, 324.8, and 309.4%, respectively. In addition, water absorption index increased with the decrease in feed moisture. It showed that degradation of starch was more severe at higher feed

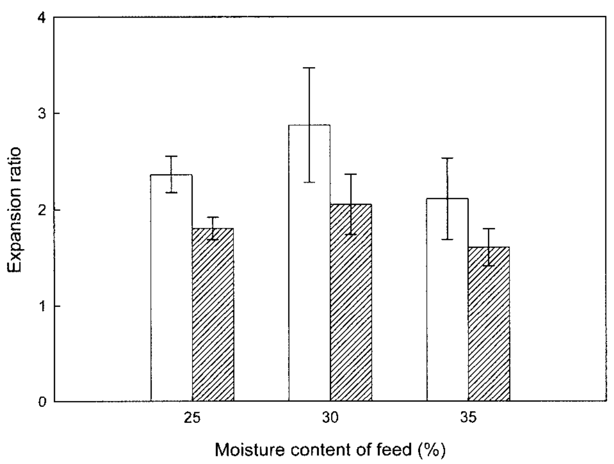

Fig. 3. Expansion ratio of corn starch extrudate. □ Without α-amylase addition, ▨ α-amylase addition.

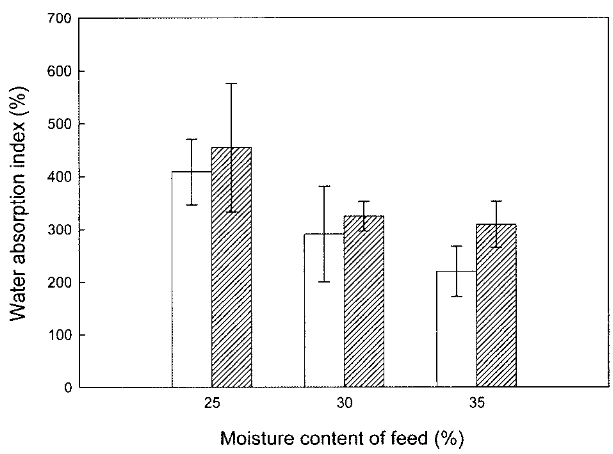

Fig. 4. Water absorption index of corn starch extrudate. □ Without α-amylase addition, ▨ α-amylase addition.

Table 1. Effect of an amylase addition during extrusion process on hydrolysis of corn starch

Feed moisture content[1)] (%)	Reducing sugar (mg glucose/g dried extudate)	
	Without α-amylase addition	α-Amylase addition
25	0.007±0.0005[b]	0.018±0.003[c]
30	0.026±0.0040[a]	0.305±0.009[b]
35	0.020±0.0090[a]	2,128±0.012[a]

[1)]Other extrusion conditions were screw speed 250 rpm, feed rate 135.6 g/min, water/enzyme solution injection rate 15.5 mL/min, barrel temperature 95°C, and die diameter 3 mm. Means with different letters are significantly different (p<0.05). Means±SD; n=3 for all treatment.

moisture content by the factor of more than 25%. Enzyme treatment increased the water absorption of extrudates with 25, 30, and 35% feed moisture content by 10.1, 10.4, and 28.9%, respectively.

Water absorption of extrudates with α-amylase addition was decreased with the increase in feed moisture. It is reported that more enzyme degradation in high feed moisture content produces starch with short chain, consequently decreasing water absorption (14). Water absorption index was related to the gel formed by amylopectin. However it is may have been understood due to the short time of enzymatic reaction and relatively low moisture content for enzymatic reaction, which allowed the enzyme to readily degrade amylose fraction rather than amylopectin.

In this experiment, enzyme treatment did not significantly affect the water absorption index of samples as much as feed moisture content. Thus water absorption capabilities were more affected by feed moisture content rather than enzyme addition.

Effect of α-amylase addition on reducing sugar increase of extrudate Feed moisture content affected the degree of hydrolysis of the starch during extrusion as shown in Table 1. The high feed moisture content resulted in higher degree of starch hydrolysis which was shown by high reducing sugar content in the extrudate (Table 1). With the same condition of other extrusion variables (barrel temperature and screw speed), the activity of the enzyme was higher at higher feed moisture content. The increase in reducing sugar content of the extrudate with enzyme addition was observed in the extrudate made from the highest moisture content (35%). Thus, it seems that high feed moisture content plays an important role in the hydrolytic activity of the enzyme. Adding enzyme during extrusion increased the reducing sugar content up to 257, 1,170, and 10,640% of the extrudate from 25, 30, and 35% moisture content of the feed, respectively.

Some studies had been done on enzymatic liquefaction of starch in a twin screw extruder at 50-60% feed moisture content (2). The study indicated that using a twin screw extruder (Clextral BC 45; France) with 500-600 mm screws, dextrose equivalent (DE) of the extrudate in the presence of α-amylase Termamyl after exiting from the die remained low with the value of 2-4. However, using longer screw such as 1,222.5 mm barrel Werner & Pfleidere Continua 58 and the 1,000 mm barrel Clextral

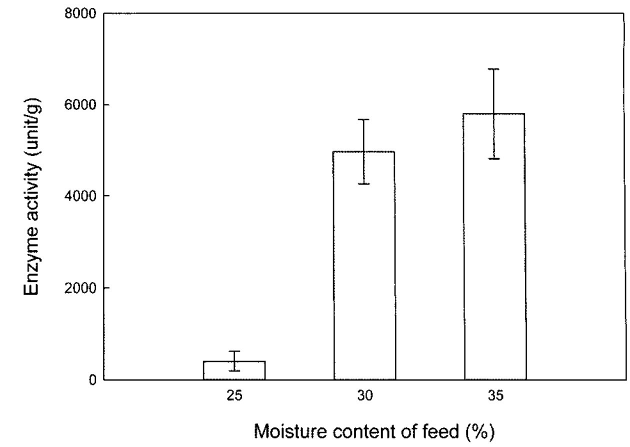

Fig. 5. Residual enzyme activity in corn starch extrudate.

BC 45 twin screw extruder, the DE of the extrudate was significantly higher with the value of 17-20. The initial moisture content of the feed was 55-60%. A more recent study by Govindasamy *et al.* (5) reported that the degree of hydrolysis of sago starch in a twin screw extruder was dependent on feed moisture content, enzyme concentration, and barrel temperature. The moisture content of the feed observed was 28.5-50.5%. Thus, the highest hydrolysis occurred at the highest moisture content of the feed.

Effect of feed moisture content on α-amylase stability during extrusion The residual enzyme activity of the extrudate of 25, 30, and 35% feed moisture content was 408, 4,960, and 5,793 unit/g enzyme (Fig. 5). The data show that the higher residual enzyme activity was observed with higher feed moisture, indicating that the enzyme was more active and more stable at higher moisture content.

Some other studies on the liquefaction of starch using an extruder mentioned that the enzyme in the extrudate was still active after the extrudate discharged from the die. The enzymatic reaction would be continued if the temperature was controlled to be favorable for the reaction of the enzyme (10). All studies on bioconversion at higher moisture were conducted at initial moisture content of around 50-60% (2). Our study on bioconversion at relatively low moisture content showed the same result as bioconversion at higher moisture.

Table 2. Increase of glucose content after 10 hr-saccharification of extruded corn starch using amyloglucosidase

Feed moisture content[1)](%)	Increase of glucose content (%)	
	Without α-amylase addition	α-Amylase addition
25	36,936±123[a]	16,314±45[a]
30	15,704±530[b]	401±12[b]
35	6,550±230[c]	3±0.02[c]

[1)]Other extrusion conditions were screw speed 250 rpm, feed rate 135.6 g/min, water/enzyme solution injection rate 15.5 mL/min, barrel temperature 95°C, and die diameter 3 mm. Means with different letters are significantly different (p<0.05). Means±SD; n=3 for all treatment.

Saccharification of dried extrudate Reducing sugar content of the extrudate after a 10 hr-saccharifcation using 0.055%(w/w) amyloglucosidase AMG-E was measured. As shown in Table 2, despite the higher initial reducing sugar content in the sample extruded at higher moisture content of the feed, more increase in the reducing sugar content after saccharification was observed in the samples extruded at lower feed moisture content. Colona *et al.* (13) stated that a low water absorption index indicated the restricted water accessibility of extruded starch to form suspension or solution. This condition might restrict to easiness of dextrin structure which goes into solution as a substrate for enzymatic reaction.

The extent of the reducing sugar increase after saccharification was smaller for the liquefied (Termamyl treated) extrudate than that of extrudate without an addition of Termamyl during extrusion (Table 2). The higher the initial reducing sugar content, the lower increase of the reducing sugar content after saccharification. It is known that in the expense amount of glucose, amyloglucosidase can easily form reversion products like maltose and isomaltose that would be counted as reducing sugar. Govindasamy *et al.* (3) also reported that the higher yield saccharification of sago starch pretreated with Termamyl 120 L in a single screw extruder was achieved at lower moisture content.

High reducing sugar was produced by adding α-amylase at sample extruded at high moisture content of the feed. On the other hand, the degree of reducing sugar increase after saccharification was greater for the sample with low moisture content of the feed.

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