Research Article

Received: 15 January 2013 Revised: 23 May 2013 Accepted article published: 6 June 2013 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6252

Effect of thermostable α-amylase injection on mechanical and physiochemical properties for saccharification of extruded corn starch

Lin Myat and Gi-Hyung Ryu*

Abstract

BACKGROUND: In industry, a jet cooker is used to gelatinize starch by mixing the starch slurry with steam under pressure at 100–175 °C. A higher degree of starch hydrolysis in an extruder is possible with glucoamylase. Unfortunately, it is difficult to carry out liquefaction and saccharification in parallel, because the temperature of gelatinization will be too high and will inactivate glucoamylase. Since the temperature for liquefaction and saccharification is different, it is hard to change the temperature from high (required for liquefaction) to low (required for saccharification). The industrial gelatinization process is usually carried out with 30–35% (w/w) dry solids starch slurry. Conventional jet cookers cannot be used any more at high substrate concentrations owing to higher viscosity. In this study, therefore, corn starch was extruded at different melt temperatures to overcome these limitations and to produce the highest enzyme-accessible starch extrudates.

RESULTS: Significant effects on physical properties (water solubility index, water absorption index and color) and chemical properties (reducing sugar and % increase in reducing sugar after saccharification) were achieved by addition of thermostable α-amylase at melt temperatures of 115 and 135 °C. However, there was no significant effect on % increase in reducing sugar of extruded corn starch at 95 °C.

CONCLUSION: The results show the great potential of extrusion with thermostable α-amylase injection at 115 and 135 °C as an effective pretreatment for breaking down starch granules, because of the significant increase ($P < 0.05$) in % reducing sugar and enzyme-accessible extrudates for saccharification yield.

Keywords: extrusion process; thermostable α-amylase; corn starch; saccharification

INTRODUCTION

Starch-containing crops form an important constituent of the human diet and a large proportion of the food consumed by the world's population. The increasing number of commercially marketed products has created a demand for health-functional ingredients that are stable and convenient to use in formulations. Highly porous solids are easily dispersible in liquid formulations and can be used as a carrier for health-functional ingredients. The highly porous structures of extruded products make the process suitable for encapsulating enzymes, flavor and color additives and health-functional compounds in a carbohydrate matrix. Besides the use of starch-containing plant parts directly as a food source, starch is harvested and used in chemical or enzymatic processes for industrial purposes. Although there are a large number of plants that produce starch, only a few are important for industrial starch processing. The major industrial sources are corn, tapioca, potato and wheat.

Extrusion cooking has been used by the food and feed industry for some years. Recently, the use of an extruder as a continuous reactor for enzymatic modification of starches has been investigated. The extrusion process has been applied for liquefying different kinds of starch to reduce saccharification time for glucose syrup production or fermentation substrate preparation. A large-scale starch-processing industry has emerged in the last century. Owing to its flexibility, the twin-screw extruder is more commonly used for enzymatic liquefaction of starch.[1] 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.[2] The liquefaction of other cereal starches such as those of corn and wheat was performed using a twin-screw extruder with addition of thermostable α-amylase (Termamyl) and 50–60% (w/w) initial moisture content of the feed.[3]

The enzymatic hydrolysis of starch is an important industrial process that consists of three steps: gelatinization, liquefaction and saccharification. In industry, a jet cooker is used to gelatinize starch by mixing the starch slurry with steam under pressure at 100–175 °C.[4] The industrial gelatinization process is usually carried out with 30–35% (w/w) dry solids starch slurry. Increasing the substrate concentration during enzymatic hydrolysis can yield higher productivity and higher enzyme stability.[5,6] As the starch concentration increases, the temperature required to reach complete gelatinization increases rapidly.[7] The viscosity

* *Correspondence to: Department of Food Science and Technology, Kongju National University, Yesan, Choongnam 340-802, Korea.*
E-mail: ghryu@kongju.ac.kr

Department of Food Science and Technology, Kongju National University, Yesan, Choongnam 340-802, Korea

of the starch slurry increases with increasing starch content, and this complicates further processing. Moreover, a higher degree of starch hydrolysis in an extruder is possible with glucoamylase. Unfortunately, it is difficult to carry out liquefaction and saccharification in parallel, because the temperature of gelatinization will be too high and will inactivate glucoamylase.

The retention time is too short for reaction of starch in an extruder. As the temperature for liquefaction and saccharification is different, it is hard to change the temperature from high (required for liquefaction) to low (required for saccharification). According to the above observations, conventional jet cookers cannot be used any more at high substrate concentrations owing to higher viscosity. As the gelatinization temperature increases, addition of the enzyme during the gelatinization process is unfavorable, because it can lead to enzyme inactivation.[8] Furthermore, the efficiency of enzyme activity was found to decrease at temperatures below 80 °C owing to low energy of activation and above 150 °C owing to enzyme denaturation.[9] A different process is therefore needed to handle more concentrated starch slurries and to produce the highest enzyme-accessible starch extrudates. Extrusion of corn starch with thermostable α-amylase injection at different melt temperatures appears to be suitable to overcome these undesirable situations. Therefore the present experiment was conducted to investigate the effect of thermostable α-amylase injection on mechanical and physicochemical properties and saccharification of extruded corn starch at different melt temperatures.

MATERIALS AND METHODS

Materials and chemicals

Corn starch provided by Samyang Genex Co. (Seoul, Korea) was used for extrusion. Thermostable α-amylase (Termamyl-supra 120 L, Novozyme, Bagsværd, Denmark) was used for injection during extrusion. For saccharification, α-amylase (*Bacillus amyloliquefaciens*) was used. For amylose content determination, amyloglucosidase (*A. niger*) and pure amylose were purchased from Sigma Aldrich Co. Ltd (Steinheim, Germany).

Extrusion process

Extrusion was conducted in a twin-screw extruder (THK31T, Incheon Machinery Co., Incheon, Korea) with a die diameter of 3 mm. All experiments were performed at a screw speed of 150 rpm, a feed rate of 120 g min^{-1} and a water injection rate of 31.02 g min^{-1}. Thermostable α-amylase Termamyl-supra 120 L was used at a concentration of 0.675 g kg^{-1} dry corn starch. The feed moisture content was 30% (w/w). The melt temperature was controlled at 95, 115 and 135 °C with and without thermostable α-amylase injection. Corn starch extrudates were directly dried in an oven at 80 °C for 4 h and ground into powders of particle size less than 0.5 mm for the study of mechanical and physicochemical properties and saccharification.

Mechanical properties

Mechanical properties were determined using a texture analyzer (Compac-100, Sun Scientific Co., Ltd, Tokyo, Japan). The apparent elastic modulus (E_{app}) and breaking strength (F_{bs}/S) in bending tests of extruded corn starch were evaluated by the method described by Ryu and Ng.[10] E_{app} was determined by bending the extrudate between two supports until fracture occurred and was calculated as

$$E_{app} = (dF/dl)\left(64d^3/48\pi D^4\right)$$

where dF/dl is the slope of the linear section of the force–distance curve, d is the distance between the two supports (30 mm) and D is the diameter of the extrudate. F_{bs}/S in bending was calculated as the maximum peak (F_{bs}) divided by the cross-sectional area (S) of the extrudate. Each extrudate sample was approximately 42 mm in length and was placed on the two support bars perpendicular to the probe. Each value was reported as the mean of ten replications.

Physical properties

The expansion ratio was determined as the diameter of the extrudate divided by the diameter of the die exit (3 mm).[10] The specific length of extruded corn starch (L_{sp}) was evaluated as the straight length of the extrudate divided by the equivalent weight of the extrudate.[10] Each value was reported as the mean of ten replications.

The piece density (D_e) was determined as the weight of the extrudate (W_e) divided by the equivalent volume of the extrudate (V_e). The volume of the extrudate (V_e) was determined by substituting rapeseed weight (W_r) for extrudate volume and dividing it by rapeseed density (D_r). D_e of each sample was calculated as[10]

$$D_e = W_e \times D_r/W_r$$

and reported as the mean of ten replications

The water absorption index (WAI) was determined as the amount (g) of water absorbed by 1 g of sample as described by Lee *et al*.[11] Extruded powder (1 g) was mixed with distilled water (25 mL) in a 50 mL centrifuge tube. The tube was agitated in an incubator shaker (110 rpm) for 30 min at 30 °C and then centrifuged at 3000 × *g* for 20 min. The water solubility index (WSI) determines the amount of free molecules leached out from the starch granules in addition to excess water. WAI and WSI were evaluated according to Anderson *et al*.[12]

The specific mechanical energy (SME) input (kJ kg^{-1}) was calculated as[13]

$$\text{SME input} = (E - E_0)/P_r$$

where E is the electric power input to the material (J s^{-1}), E_0 is the electric power when idling (J s^{-1}) and P_r is the production rate (kg s^{-1}).

Color

A colorimeter (JP/CR-300, Minolta, Japan) was used to evaluate the color of raw and extruded corn starch. Color values L, a and b as measures of lightness, redness and yellowness respectively were recorded for each sample. The colorimeter was calibrated against a standard white tile ($L = 97.60$, $a = -0.19$, $b = 1.34$). The extrudates were ground in a laboratory grinder to a particle size of less than 0.5 mm prior to color analysis. Measurements were performed in triplicate.

Chemical properties

Extruded corn starch at different melt temperatures and raw corn starch were analyzed for their ash, lipid (ether extract) and crude protein contents (N × 6.25) according to AOAC standard methods.[14] Amylose content was determined by the simplified assay of Juliano.[15] A 0.2 g ground sample was suspended in 1 mL of 950 mL L^{-1} ethanol, mixed with 90 mL of 1 mol L^{-1} NaOH, incubated at room temperature for 10 min and heated in a boiling

water bath at 100 rpm for 30 min. The resulting suspension was allowed to cool, made up to exactly 100 mL with distilled water, mixed thoroughly and allowed to stand at room temperature for 2 h. A 5 mL aliquot of the vigorously resuspended sample was mixed with 1 mL of 1 mol L^{-1} acetic acid and 2 mL of iodine solution (prepared by dissolving 0.26 g of iodine in 10 mL of potassium iodide solution containing 2.6 g of potassium iodide) and the volume was adjusted to 100 mL with distilled water. The sample was incubated at room temperature for 20 min to develop a dark purple color. The sample absorbance at 620 nm was measured in a spectrophotometer against a blank consisting of 1 mL of 1 mol L^{-1} acetic acid and 2 mL of iodine solution and diluted to 100 mL with distilled water. The 620 nm absorbance was converted to amylose content (% of total starch) by means of a standard absorbance curve constructed using known concentrations of purified corn amylose (Sigma, St Louis, MO, USA). Amylopectin content was calculated as 100% − amylose content (%). Three replicates of each test were performed.

Starch content was measured by a commercially available kit (Megazyme International Ltd, Bray, Ireland) method based on the use of thermostable α-amylase and amyloglucosidase.[16] A 100 mg ground sample was mixed with 0.2 mL of 800 mL L^{-1} ethanol in a test tube, 3 mL of thermostable α-amylase was added and the tube was incubated in a boiling water bath for 6 min. After addition of 4 mL of 200 mmol L^{-1} sodium acetate buffer (pH 4.5), the tube was incubated in a water bath at 50 °C for 30 min. The volume was adjusted to 10 mL with distilled water and the tube was centrifuged at 3000 × *g* for 10 min. A 1 mL aliquot of the solution was taken and again diluted to 10 mL with distilled water. Then a duplicate aliquot (0.1 mL) of the diluted solution was transferred to the test tube and 3 mL of GOPOD reagent was added, followed by incubation at 50 °C for 20 min. Finally, the absorbance of each sample and a D-glucose control was read against a reagent blank at 510 nm. Starch content (% dry weight basis) was calculated as [starch (%) × 100]/[100 − moisture content (% w/w)].

Saccharification

A 1 g ground dried sample was suspended in 43 mL of 20 mmol L^{-1} sodium acetate/acetic acid buffer (pH 5.6), then supplemented with 5 mL of 8 mL L^{-1} α-amylase solution[17] and incubated in rotary shaker (100 rpm) at 30 °C for 0, 2, 4, 6, 8 and 10 h. All experiments were replicated three times and the reducing sugar content of raw and extruded corn starch at specific time intervals was determined according to the DNS method[18] using 3,5-dinitrosalicylic acid. Glucose solution was used as a standard. The enzyme saccharification rate (% increase in reducing sugar) was calculated as (amount of glucose produced × 0.9/amount of starch in extruded corn starch) × 100.[19]

Experimental design and statistical analysis

A completely randomized design was performed to determine the effect of thermostable α-amylase injection during extrusion at different melt temperatures (95, 115 and 135 °C). Data were analyzed using SAS Version 6.12 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Mechanical properties

As shown in Fig. 1, thermostable α-amylase injection during extrusion significantly affected the breaking strength in bending ($P < 0.05$) at 135 °C. At this melt temperature, breaking strength increased significantly ($P < 0.05$) with thermostable α-amylase injection and had a positive correlation with expansion ratio. However, at 95 and 115 °C, there was no significant difference in the breaking strength of extruded corn starch with and without α-amylase injection. The breaking strength of extrudates with α-amylase injection at 95 °C had a positive correlation with expansion ratio, whereas that of extrudates with α-amylase injection at 115 °C had a negative correlation with expansion ratio. Therefore α-amylase injection at 115 °C had a positive effect on the breaking strength of extruded corn starch in comparison with α-amylase injection at 95 and 135 °C (Fig. 1). Several other researchers also demonstrated that breaking strength decreased with decreasing piece density.[20–25] Hsieh *et al*.[24] found that thinner cell walls with greater radial expansion were formed and extrudates became more breakable. The elastic modulus was also affected by α-amylase injection at 115 °C. As shown in Fig. 2, the elastic modulus of extruded corn starch with α-amylase injection at 115 °C decreased significantly compared with that of extrudates at 95 °C. However, it increased significantly ($P < 0.05$) in comparison with extrudates without α-amylase injection at 115 °C. The decrease in elastic modulus at 115 and 135 °C has a positive effect on the softness of the extrudates. The elastic modulus is the degree of stiffness of extrudates, which depends on the intrinsic rigidity of the expanded matrix and on the longitudinal expansion. The lower the apparent elastic modulus in bending, the crisper is the texture of the extrudates.[10]

Physical properties

The physical properties of extruded corn starch are shown in Table 1. The expansion ratio increased significantly ($P < 0.05$) with α-amylase injection at 95 and 135 °C, but there was no significant difference at 115 °C. This can be explained by the depolymerization of starch, since enzymatic hydrolysis leads to less ability of the starch melt to hold steam and prevent the collapse and shrinkage of air cells in the extrudate after exiting the die to form puffed extrudate.[26,27] On the other hand, the expansion ratio of extrudates with α-amylase injection at 115 °C was significantly higher ($P < 0.05$) than that of extrudates both with and without α-amylase injection at 95 and 135 °C. Enzymatic hydrolysis by addition of thermostable α-amylase would be responsible for increasing expansion ratio at 135 °C. The expansion ratio of extruded corn starch with and without α-amylase injection was positively correlated with specific length and negatively correlated with SME input at 135 °C (Table 1).

The specific length increased significantly ($P < 0.05$) with α-amylase injection at 115 and 135 °C. Higher melt temperature at α-amylase injection caused the feed material to melt fully and also reduced the melt viscosity, resulting in increased specific length of the extrudates. Our results at 95 and 135 °C are consistent with those of a previous study in which the specific length decreased with increasing SME input.[10] There was no significant difference in the piece density of extruded corn starch with and without α-amylase injection at the tested melt temperatures. However, α-amylase injection at 115 °C can decrease the piece density of extruded corn starch (Table 1).

The effects of α-amylase injection on the WSI and WAI are shown in Table 2. The WSI of extruded corn starch increased significantly ($P < 0.05$) with α-amylase injection at 95 and 115 °C, but there was no significant difference at 135 °C. The WSI reflects the rate of penetration of water into solid particles and diffusion of soluble components outside the particles. It is a function of

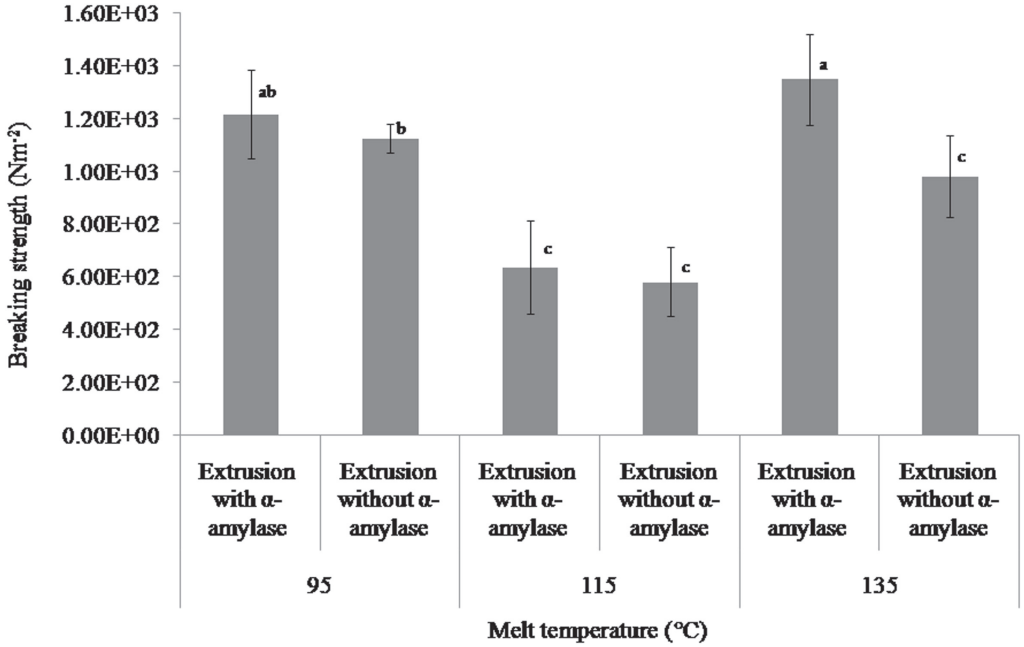

Figure 1. Breaking strength of extruded corn starch with and without α-amylase injection at different melt temperatures.

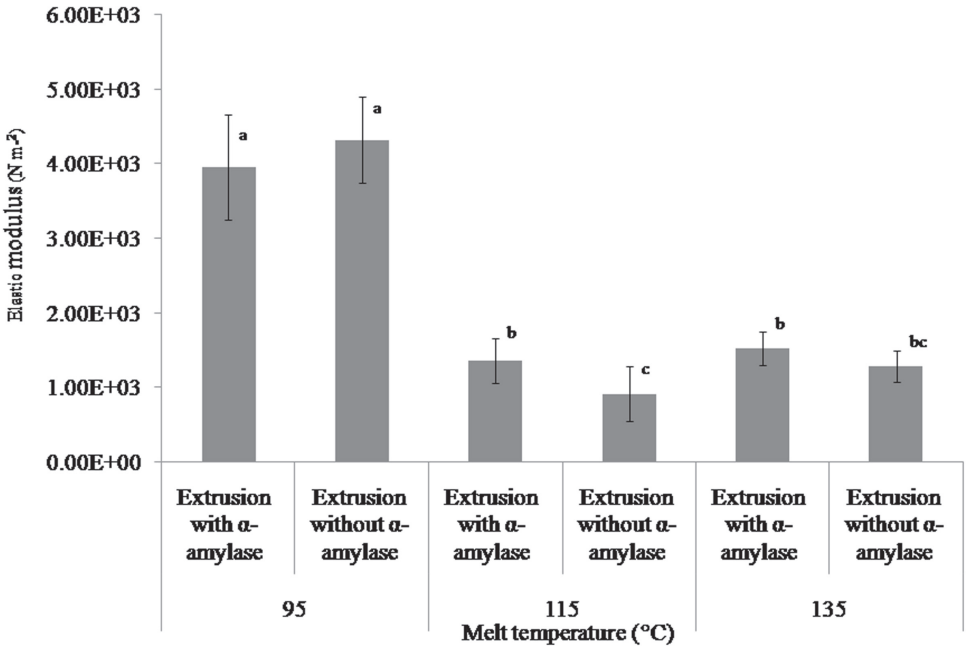

Figure 2. Elastic modulus of extruded corn starch with and without α-amylase injection at different melt temperatures.

the extent of dextrinization of starch. The WSI is often used as an indicator of degradation of molecular components because its value can be obtained from the conversion degree of starch during extrusion cooking. This conversion degree is the amount of soluble polysaccharide released from the starch after extrusion. The WSI is also related to the amount of low-molecular-weight products of starch degradation that are easily soluble because of reduced entanglement. Thus the increase in WSI with α-amylase injection at 95 and 115 °C was a sign of increased solubilization of starch (Table 2).

The WAI was measured as the volume occupied by extruded starch after swelling in excess water, which maintains the integrity of starch in aqueous dispersion.[28] The WAI is a function of the internal voids in the milled sample powder and the thickness of the cell walls of the voids. Since extrusion was carried out, the raw material was a pre-gelatinized starch. Differences can be attributed primarily to physical effects related to the structure of voids in the solid. The WAI of extruded samples without α-amylase injection ranged from 4.39 to 6.46 g g^{-1}, with significant increases ($P < 0.05$) at 95 and 115 °C. Differences in the WAI of extrudates were positively correlated with the extent of enzyme activity, indicating the onset of dextrinization (Table 2). Dextrinization (indicated as higher released reducing sugar and WSI) was observed and also was dependent on melt

Table 1. Physical properties of extruded corn starch at different melt temperatures

Melt temp.(°C)	α-Amylase injection	Expansion ratio	Specific length (m kg^{-1})	Piece density (g cm^{-3})	SME[a] (kJ kg^{-1})
95	With α-amylase	1.55 ± 0.01d	52.53 ± 1.76e	0.80 ± 0.05a	445.62*a*
	Without α-amylase	1.47 ± 0.03de	52.69 ± 1.53e	0.76 ± 0.15a	396.15*b*
115	With α-amylase	2.05 ± 0.06b	72.87 ± 2.98c	0.34 ± 0.04b	324.34*c*
	Without α-amylase	2.14 ± 0.12a	68.58 ± 5.84d	0.43 ± 0.04b	305.06*c*
135	With α-amylase	1.73 ± 0.03c	126.84 ± 3a	0.40 ± 0.01b	79.36*e*
	Without α-amylase	1.49 ± 0.05de	91.31 ± 2.53b	0.33 ± 0.03b	159.21*d*

Means of ten replications based on least significant difference procedure at $\alpha = 0.05$ level. Means with the same letter in the same column are not significantly different.
[a] Specific mechanical energy input.

Table 2. Water solubility index (WSI), water absorption index (WAI) and color values (*L*, lightness; *a*, redness; *b*, yellowness) of extruded corn starch at different melt temperatures and of raw corn starch

				Color		
Melt temp. (°C)	α-Amylase injection	WSI (%)	WAI (g g^{-1})	*L*	*a*	*b*
95	With α-amylase	8.45 ± 0.43a	2.40 ± 0.06c	88.07c	−3.97e	11.09a
	Without α-amylase	2.59 ± 0.09d	4.39 ± 0.02b	92.71b	−3.27d	8.92b
115	With α-amylase	8.56 ± 0.79a	3.30 ± 0.56c	88.39c	−3.88e	11a
	Without α-amylase	2.81 ± 0.42 cd	6.46 ± 1a	92.96b	−3.04c	8.05c
135	With α-amylase	5.16 ± 2.14b	6.46 ± 1.74a	91.61b	−2.80b	8.93b
	Without α-amylase	4.36 ± 0.45bc	5.51 ± 0.48a	91.74b	−2.80b	7.56c
Raw corn starch		0.15 ± 0.03e	0.7 ± 0.01d	97.31a	−1.66a	2.82d

Means of three replications based on least significant difference procedure at $\alpha = 0.05$ level. Means with the same letter in the same column are not significantly different.

temperature. On increasing the melt temperature from 115 to 135 °C, extrudates with α-amylase injection showed enhanced WAI and decreased reducing sugar content (Tables 2 and 3). The compact granular structure was loosened to some extent as a consequence of increasing thermal energy input with minimal enzymatic degradation, but maintaining the integrity of hold water and facilitating development of the WAI. It has also been reported that greater enzymatic degradation at high feed moisture content produces starch with shorter chains and consequently results in decreased water absorption.[29] Budiasih *et al*.[30] also reported that the water absorption of corn starch extrudates with α-amylase injection decreased with increasing in feed moisture content from 25 to 35% (w/w). In the present extrusion conditions the feed moisture content was 30% (w/w). The reduction in WAI and increase in WSI of extruded corn starch with α-amylase injection at 95 and 115 °C (Table 2) are an indication that α-amylase injection may have accelerated the degradation of starch granules and the release of water-soluble compounds during extrusion. These changes are of nutritional importance, because insoluble native starch is more readily hydrated and solubilized after extrusion with α-amylase injection and is therefore more susceptible to enzymatic digestion.

The influence of α-amylase injection at different melt temperatures on SME input is shown in Table 1. α-Amylase injection during extrusion significantly decreased SME input at 135 °C, but there was no significant difference at 95 and 115 °C. The higher the melt temperature, the lower was the SME input both with and without α-amylase injection. In this experiment, the changes in SME input caused by melt temperature showed the same pattern as found by Ryu and Ng[10] and Brent *et al*.,[31] who reported that SME input decreased with increasing melt temperature. Therefore SME input decreased with α-amylase injection at a melt temperature of 135 °C. The higher SME input at lower melt temperature is explained by the higher viscosity of the melt at lower temperature, since SME input is the mechanical energy consumed to rotate the screws plus viscous melt.[32]

Color

Table 2 shows the color values measured with a colorimeter. Extruded corn starch without α-amylase injection at all tested melt temperatures had maximum lightness and minimum yellowness. A significant decrease ($P < 0.05$) in lightness and increase in yellowness were noted when corn starch was extruded with thermostable α-amylase injection. This means that reducing sugars formed as a result of mechanical shear of starch reacted with amino acids to form Maillard products (Tables 2 and 3). Yellowness increased further when corn starch was extruded with α-amylase injection at all tested melt temperatures. This is an indication of greater susceptibility of monosaccharides to form Maillard compounds.[33] Only extrudates with α-amylase injection showed the increase in yellowness (Table 2) that may be associated with caramelization or non-enzymatic Maillard reactions. Excessive Maillard reactions are not desirable, however, since they result in a decrease in protein value when glucose reacts with amino acids.[33]

Chemical properties

The proximate composition of extruded corn starch at different melt temperatures and of raw corn starch is presented in Table 3.

Table 3. Chemical properties of extruded corn starch at different melt temperatures and of raw corn starch

Melt temp. (°C)	α-Amylase injection	Ash (g kg^{-1})	Lipid (g kg^{-1})	Protein (g kg^{-1})	Amylose (g kg^{-1})	Amylopectin (g kg^{-1})	Reducing sugar (g kg^{-1})	Starch (g kg^{-1})
95	With α-amylase	0.50c	4.31a	3.78b	246.53bc	753.47bc	4.65b	765.08bc
	Without α-amylase	0.52c	1.30c	3.74b	214.75cd	785.25ab	0.73d	796.72ab
115	With α-amylase	0.65c	3.38ab	3.80b	200.43d	799.57a	14.20a	593.42d
	Without α-amylase	0.56c	2.50abc	3.84b	212.24d	787.76a	0.91d	746.95bc
135	With α-amylase	1.26a	4.45a	3.74b	216.70cd	783.30ab	2.16c	613.77d
	Without α-amylase	0.94b	0.51c	3.33c	260.18b	739.82c	1.14d	719.67c
Raw corn starch		1.02b	2.20bc	5.59a	311.99a	688.01d	0.68d	843.54a

Means of three replications based on least significant difference procedure at $\alpha = 0.05$ level. Means with the same letter in the same column are not significantly different.

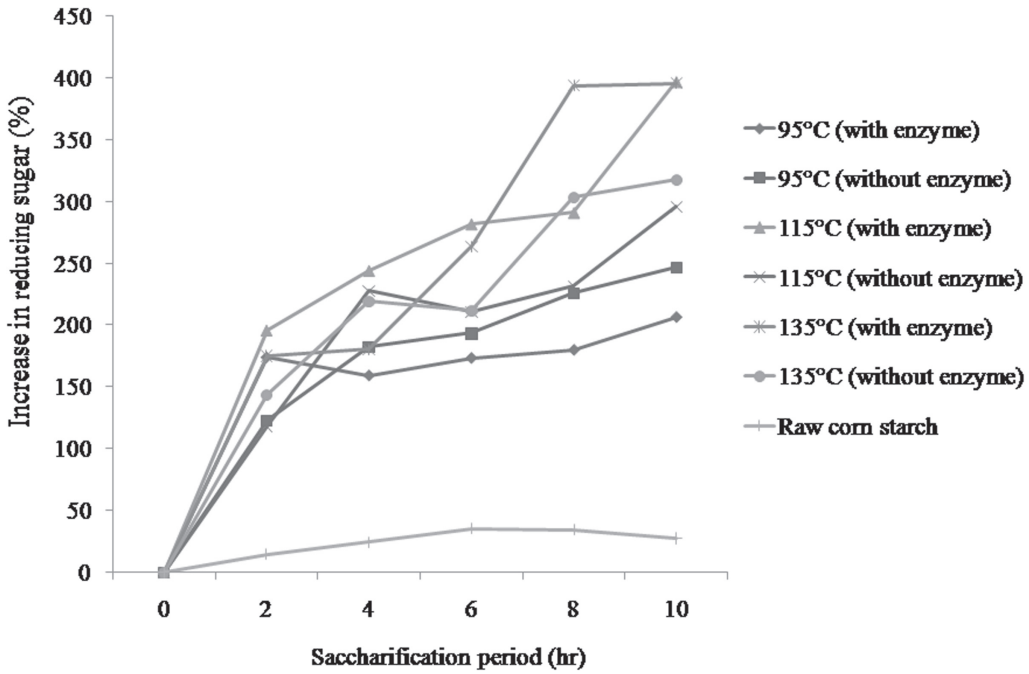

Figure 3. Saccharification rate (% increase in reducing sugar) of raw and extruded corn starch at different saccharification periods.

Raw corn starch had the highest protein content (5.59 g kg^{-1}), while extruded corn starch with α-amylase injection at 135 °C had the highest ash content (1.26 g kg^{-1}). There was a significant difference ($P < 0.05$) in lipid contents of extruded corn starch with and without α-amylase injection at 95 and 135 °C. This may be due to the formation of lipid–starch complexes. Amylose in cereal starches is complexed with lipids to form a weak crystalline structure and reinforce the granules. The formation of complexes with lipids modifies the properties of starch, e.g. reducing starch solubility in water, delaying starch retrogradation and slowing starch hydrolysis by enzymes (i.e. low reducing sugar content).[34] Our results (Table 3) are in agreement with those observations. However, there was no significant difference in lipid contents of extruded corn starch with and without α-amylase injection at 115 °C. This means that α-amylase injection at 115 °C could reduce starch–lipid complexes compared with injection at 95 and 135 °C. It is also reported that a temperature above 100 °C is preferred to ensure the removal of lipid–starch complexes.[4] However, the lipid content at 135 °C is higher than that at 115 °C, since an extrusion temperature of 135 °C is higher than the optimal range for enzyme activity. There was no significant difference in amylose and amylopectin contents of extruded corn starch with and without α-amylase injection at 95 and 115 °C. On the other hand, α-amylase injection at 135 °C significantly decreased ($P < 0.05$) the amylose content and increased the amylopectin content of extrudates. The starch content and quality factors (amylose and amylopectin) appear to affect expansion properties. The expansion ratio increased with increasing amylose content of extruded corn starch at both 95 and 115 °C. It is also reported that the expansion ratio of corn grits[35] and rice flour[36] increases with increasing amylose content. Our results (Tables 1 and 3) are in agreement with those of their observations.

The starch content of extruded corn starch decreased significantly ($P < 0.05$) with α-amylase injection at the tested melt temperatures. α-Amylase injection resulted in a higher degree of starch hydrolysis, as shown by the high reducing sugar content in extrudates (Table 3). It is possible thermomechanically to 'open up' or 'denature' the starch structure under extrusion cooking

conditions, which would allow simultaneous initiation of starch breakdown in the presence of thermostable α-amylase.[37–39]

Saccharification

Figure 3 shows the time course of % increase in reducing sugar using 8 mL L^{-1} α-amylase enzyme saccharification at different melt temperatures. After a 10 h saccharification period, the highest saccharification rate of approximately 397% was observed in samples extruded with α-amylase injection at 115 and 135 °C. A higher % increase in reducing sugar after saccharification was observed in samples extruded with α-amylase injection on increasing the melt temperature. It is also reported that greater enzymatic degradation produces starch with shorter chains, consequently decreasing water absorption and increasing reducing sugar at 95 and 115 °C (Tables 2 and 3). Research also indicated that an increase in temperature led to an increase in starch fragmentation.[40] Such fragmentation would render the starch content more accessible to α-amylase, which would enhance enzymatic hydrolysis and % increase in reducing sugar at 135 °C. However, since this extrusion temperature is higher than the optimal range for enzyme activity, the degree of hydrolysis decreased owing to enzyme inactivation. Partial inactivation of α-amylase at 135 °C may have been the cause of low reducing sugar (Table 3). Yeung[9] also reported that the dextrose equivalent of extruded barley flour with thermostable α-amylase injection decreased at 120 and 140 °C owing to inactivation of α-amylase at these temperatures. However, the % increase in reducing sugar of extruded corn starch with α-amylase injection at 95 °C after a 2 h saccharification period was lower than that of extruded corn starch without α-amylase injection (Fig. 3). The higher the initial reducing sugar content of extrudates, the lower was the % increase in reducing sugar obtained after saccharification[30] (Table 3 and Fig. 3). Extruded corn starch without α-amylase injection showed a higher % increase in reducing sugar with an increase in melt temperature from 115 to 135 °C (Fig. 3). This results from fragmentation of starch due to mechanical shear. According to our results, a significant beneficial effect on physicochemical properties of corn starch extrudates for enzyme accessibility and the highest saccharification yields were achieved by extrusion with thermostable α-amylase injection at melt temperatures of 115 and 135 °C and subsequent saccharification using 8 mL L^{-1} α-amylase for 8 h.

CONCLUSION

Extrusion with thermostable α-amylase injection at different melt temperatures was the most significant factor affecting the physicochemical properties of extruded corn starch for saccharification. Enzyme-accessible extrudates were achieved by injection of thermostable α-amylase at melt temperatures of 115 and 135 °C. Finally, extrusion with thermostable α-amylase injection at 115 and 135 °C could effectively gelatinize and degrade native corn starch structures and resulted in the highest % increase in reducing sugar after 10 h of saccharification.

ACKNOWLEDGEMENT

This research was supported by the Training Program of Graduate Students in Regional Innovation through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012307KJ).

REFERENCES

1 Harper JM, Food extruders and their applications, in *Extrusion Cooking*, ed. by Mercier C, Linko P and Harper JM. American Association of Cereal Chemists, St Paul, MN, pp. 1–15 (1989).
2 Linko P, Hakulin S and Linko YY, Extrusion cooking of barley starch for the production of glucose syrup and ethanol. *J Cereal Sci* **1**:275–284 (1983).
3 Linko P, Extrusion cooking in bioconversion, in *Extrusion Cooking*, ed. by Mercier C, Linko P and Harper JM. American Association of Cereal Chemists, St Paul, MN, pp. 235–245 (1989).
4 Van der Maarel MJEC, Van der Veen B, Uitdehaag JCM, Leemhuis H and Dijkhuizen L, Properties and applications of starch-converting enzymes of the α-amylase family. *J Biotechnol* **94**:137–155 (2002).
5 De Cordt S, Hendrickx M, Maesmans G and Tobback P, The influence of polyalcohols and carbohydrates on the thermostability of α-amylase. *Biotechnol Bioeng* **43**:107–114 (1994).
6 Klibanov AM, Stabilization of enzymes against thermal inactivation, in *Advances in Applied Microbiology*, Vol. 29, ed. by Laskin AI. Elsevier, Amsterdam, pp. 1–28 (1983).
7 Donovan JW, Phase transitions of the starch–water system. *Biopolymers* **18**:263–275 (1979).
8 Baks T, Kappen FHJ, Janssen AEM and Boom RM, Towards an optimal process for gelatinization and hydrolysis of highly concentrated starch–water mixtures with *alpha*-amylase from *B. licheniformis*. *J Cereal Sci* **47**:214–225 (2008).
9 Yeung JS, Extrusion cooking of barley flour with and without thermostable α-amylase. *Master Thesis*, University of Alberta (2001).
10 Ryu GH and Ng PKW, Effects of selected process parameters on expansion and mechanical properties of wheat flour and whole cornmeal extrudates. *Starch/Stärke* **53**:147–154 (2001).
11 Lee EY, Ryu GH and Lim ST, Effect of processing parameters on physical properties of corn starch extrudates expanded using supercritical CO_2 injection. *Cereal Chem* **76**:63–69 (1999).
12 Anderson RA, Conway HF, Pfeifer VF and Griffen JEJ, Gelatinization of corn grits by roll and extrusion cooking. *Cereal Sci Today* **14**:1–4, 11–12 (1969).
13 Gu BJ and Ryu GH, Effect of die geometry on expansion of corn flour extrudate. *Food Eng Prog* **15**:148–154 (2011).
14 AOAC, *Official Methods of Analysis* (16th edn). Association of Official Analytical Chemists, Washington, DC (1995).
15 Juliano BO, A simplified assay for milled-rice amylose. *Cereal Sci Today* **16**:334–340 (1971).
16 McCleary BV, Solah V and Gibson TS, Quantitative measurement of total starch in cereal flours and products. *J Cereal Sci* **20**:51–58 (1994).
17 Zhan X, Wang D, Tuinstra MR, Bean S, Seib PA and Sun XS, Ethanol and lactic acid production as affected by sorghum genotype and location. *Ind Crops Prod* **18**:245–255 (2003).
18 Miller GC, Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* **31**:426–428 (1959).
19 Sharma SK, Kalra KL and Kocher GS, Fermentation of enzyme hydrolysis of sunflower hulls for ethanol production and its scale up. *Biomass Bioenergy* **27**:399–402 (2004).
20 Mercier C and Feillet P, Modification of carbohydrate components by extrusion-cooking of cereal products. *Cereal Chem* **52**:283–297 (1975).
21 Bhattacharya M and Hanna MA, Textural properties of extrusion cooked corn starch. *Lebensm Wiss Technol* **20**:195–201 (1987).
22 Hutchinson RJ, Siodlak GDE and Smith AC, Influence of processing variables on the mechanical properties of extruded maize. *J Mater Sci* **22**:3956–3962 (1987).
23 Halek GW and Chang KLB, Effect of extrusion operation variables on functionality of extrudates, in *Food Extrusion Science and Technology*, ed. by Kokini JL, Ho CT and Karwe MV. Marcel Dekker, New York, NY, pp. 677–691 (1992).
24 Hsieh F, Grenus KM, Hu L and Huff HE, Twin-screw extrusion of rice flour with salt and sugar. *Cereal Chem* **70**:493–498 (1993).
25 Ryu GH and Walker CE, The effect of extrusion conditions on the physical properties of wheat flour extrudates. *Starch/Stärke* **47**:33–36 (1995).
26 Ryu GH, Kim BS and Mulvaney SJ, Optimization of extrusion process for diary ingredient fortification of cornmeal puffed via CO_2 gas injection. *Food Sci Biotechnol* **11**:552–556 (2002).
27 Ha DC, Lee JW and Ryu GH, Change in ginsenosides and maltol in dried raw ginseng during extrusion process. *Food Sci Biotechnol* **14**:363–367 (2005).

28 Yang SH, Peng J, Lui WB and Lin J, Effects of adlay species and rice flour ratio on the physiochemical properties and texture characteristic of adlay-based extrudates. *J Food Eng* **84**:489–494 (2008).
29 Ofoli RY, Komolprasert V, Saha BC and Berglund KA, Production of maltose by reactive extrusion of carbohydrates. *Lebensm Wiss Technol* **23**:262–268 (1990).
30 Budiasih W, Solihin Kim BS, Jae YC and Ryu GH, Effect of feed moisture content on enzymatic hydrolysis of corn starch in twin-screw extruder and saccharification of the dried extrudates. *Food Sci Biotechnol* **16**:381–385 (2007).
31 Brent JL, Mulvaney SJ, Cohen C and Baftsch JA, Thermomechanical glass transition of extruded cereal melts. *J Cereal Chem* **26**:301–312 (1997).
32 Colonna P, Tayeb J and Meuser F, Extrusion cooking of starch and starchy products, in *Extrusion Cooking*, ed. by Mercier C, Linko P and Harper JM. American Association of Cereal Chemists, St Paul, MN, pp. 295–471 (1989).
33 Onyango C, Henle T, Ziems A, Hofmann T and Bley T, Effect of extrusion variables on fermented maize–finger millet blend in the production of uji. *LWT – Food Sci Technol* **37**:409–415 (2004).
34 Tang MC and Copeland L, *Effect of Lipid Concentration on the Formation of Starch–Lipid Complexes*. Value Added Wheat CRC, North Ryde (2006).
35 Bhuiyan MZ and Blanshard JMV, The behavior of maize flours and grits in relation to extrusion quality. II. Gelatinization characteristics of extruded corn starches. *Stärke* **34**:262–266 (1982).
36 De Mosqueda MB, Perez CM and Juliano BO, Varietal differences in properties of extrusion-cooked rice flour. *Food Chem* **19**:173–187 (1986).
37 Linko P, Linko YY and Hakulin S, Continuous extrusion processing of starchy materials for the production of syrups and ethanol, in *Thermal Processing and Quality of Foods*, ed. by Zeuthen P. Elsevier Applied Science, London, pp. 122–126 (1984).
38 Komolprasert V and Ofoli RY, A dispersion model for predicting the extent of starch liquefaction by *Bacillus licheniformis* α-amylase during reactive extrusion. *Biotechnol Bioeng* **37**:681–690 (1991).
39 Roussel L, Vieille A, Billet l and Cheftel JC, Sequential heat gelatinization and enzymatic hydrolysis of corn starch in an extrusion reactor: optimization for a maximum dextrose equivalent. *Lebensm Wiss Technol* **24**:449–458 (1991).
40 Thaender O and Westerlund E, Studies on chemical modifications in heat-processed starch and wheat flour. *Starch* **39**:88–93 (1987).

