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Effect of Carbon Dioxide Injection on Physiochemical Properties and Saccharification of Extruded Corn Starch for Fermentation Substrate Preparation

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Abstract

The objective of this research was to investigate the effect of carbon dioxide injection during extrusion process at different melt temperatures 95, 115 and 135°C on physiochemical properties and enzymatic saccharification of extruded corn starch for fermentation substrate preparation. Carbon dioxide gas was injected during extrusion at the flow rate of 500 ml/min and pressure of 3 MPa. For enzymatic saccharification, 0.8% α-amylase enzyme was used at different saccharification periods. After extrusion, physiochemical properties and reducing sugar content were measured. Carbon dioxide injection had positive effects on breaking strength, elastic modulus, expansion ratio, specific length, piece density, water absorption index, water solubility index, free amino nitrogen and reducing sugar content (after saccharification) at melt temperatures 115 and 135°C. However, significant decrease ($p<0.05$) in specific mechanical energy input was obtained at only 135°C. The data clearly showed that carbon dioxide injection gave significantly increased ($p<0.05$) reducing sugar content at melt temperatures 115 and 135°C for fermentation substrate preparation.

Keywords: Extrusion process; Carbon dioxide injection; Corn starch; α-amylase; Saccharification

Introduction

Starch-containing crops form an important constituent of the human diet and a large proportion of food consumed by the world's population originates from them. The increasing number of commercially marketed products has created a demand for health-functional ingredients that are stable and convenient to use in formulation. Highly porous solids are easily dispersible in liquid formulations and can be used as a carrier for health-functional ingredients. The highly porous structure 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 the 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 able to produce starch, only a few plants are important for industrial starch processing. The major industrial sources are corn, tapioca, potato, and wheat.

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. A large-scale starch processing industry has emerged in the last century. Currently, we have seen a shift from conventional extrusion of starch to the use of CO_2 injection during extrusion in the production of starch hydrolysates, glucose and fructose syrups for fermentation. In conventional extrusion, liquid water has been used at high pressure in the melt to expand the products and it is converted to vapor on pressure reduction as the extrudate exits the die. In order to generate adequate steam to puff the extrudate, a high temperature and pressure is required at the entrance to the die so that flash evaporation will occur [1]. The conditions used in conventional extrusion of snack foods and, cereals would be detrimental to heat sensitive ingredients such as functional proteins, vitamins, flavors, and natural plant derived colors may be incorporated in the melt. In addition, steam-puffed extrudates have coarse and non-uniform cellular structures with cell sizes in the range of 1-3 mm, while expansion ratios in the range of 9-12. Moreover, steam-puffed extrusion is difficult to control pore size and product density of the extrudates [2]. Ferdinand et al. [3] studied structure formation in maize grits, wheat starch, and dehydrated potato granules in the absence of steam expansion by injecting low pressure CO_2 into the extruder. Cell size, cells per unit area, and expansion ratio of extrudates were more easily controlled in the supercritical fluid extrusion process as compared to traditional extrusion [4].

The main advantage of CO_2 injection in the barrel of an extruder is its function as plasticizer, which allows the processing of molecules which would otherwise be too fragile to withstand the mechanical stresses and the operating temperatures of a standard extrusion process. In addition, the dissolved CO_2 acts as foaming agent during expansion through the die. It is, therefore, to control pore generation and growth by controlling the operating conditions [5]. Therefore, this experiment was conducted to determine the effect of CO_2 injection on physiochemical properties and the saccharification yield of extruded corn starch using α-amylase enzyme for fermentation substrate preparation.

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Materials and Methods

Extrusion process

Corn starch provided by Samyang Genex Co. (Korea) was used for extrusion. Extrusion was conducted in twin-screw extruder (THK31T, Incheon Machinery Co., Incheon, Korea). All experiments were conducted at screw speed of 150 rpm, feed rate of 100 g/min, water injection rate of 24.73 g/min, die diameter of 3 mm and feed moisture content of 30%. Carbon dioxide injection flow rate was 500 ml/min and CO_2 pressure was 3 MPa. Melt temperature was controlled at 95, 115 and 135°C with and without CO_2 injection. The corn starch extrudates were dried and grinded into powder with a particle size of less than 0.5 mm and used as samples for physiochemical analysis and saccharification process.

Mechanical properties

Mechanical properties were determined by 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 [6]. The E_{app} in bending extrudates between two supports was determined as the extrudates strand deformed in bending until fracture occurred. E_{app} was calculated as equation:

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

Where dF/dI=the slope of the linear section of the force-distance curve; d=the distance between two supports (30 mm); D=the diameter of extrudates. Breaking strength (F_{bs}/S) in bending was calculated as maximum peak divided by the cross-sectional area of extrudates. Extrudate sample was cut at ~42 mm length. The single of extrudate was placed on the two support bars perpendicular to the probe. Each value was presented as an average of ten readings.

Physical properties

The expansion ratio was determined as the diameter of extrudates divided by the diameter of the die exit (3 mm) [6]. The specific length of extruded corn starch (L_{sp}) was evaluated as the straight length of extrudates divided by the equivalent weight of extrudates [7]. The values were reported as an average of ten readings.

The piece density (D_e) was determined as the weight of extrudates (W_e) divided by the equivalent volume of extrudates (V_e). The volume of extrudates (V_e) was determined by substituting rapeseed weight (W_r) for extrudates volume and dividing it by rapeseed density (D_r). The piece density of each sample was calculated according to the following equation and reported as an average of five readings [6].

$$D_{ex} = W_e \times \frac{D_r}{W_r}$$

The water absorption index (WAI) was determined as the amount (g) of water absorbed by each gram of sample as described by Lee et al. [7]. One gram of extruded powder was mixed with 25 ml of distilled water in 50 ml centrifuge tube. The tubes were agitated in incubator shaker (110 rpm) for 30 minutes at 30°C. Then, the tubes were centrifuged at 3000 rpm for 20 minutes. The water solubility index (WSI) determines the amount of free molecules leached out from the starch granule in addition to excess water. WAI and WSI were determined according to Anderson et al. [8].

$$\text{WAI}\ (\text{g}/\text{g}) = \frac{\text{Hydrated sample weight-Dry sample}}{\text{Dry sample weight}}$$

$$\text{WSI}\ (\%) = \frac{\text{Dry solid weight recovered by evaporation of the supernatant}}{\text{Dry sample weight}} \times 100$$

Specific mechanical energy input

The specific mechanical energy (SME) input was calculated using the following equation:

$$\text{SME} = \frac{E^V - E^0}{F^r}$$

Where E is electric current after feed material (amperes); E^0 is initial electric current (amperes); E^V is the voltage applied to the motor (volts) and F^r is the feed rate supplied to extruder (kg/s).

Chemical properties

Moisture content was analyzed by the standard method [9]. Total sugar content was measured using phenol-sulfuric acid method [10]. Two grams of sample was mixed with 20 ml of 70% ethanol solution and it was extracted at 80°C for 2 hr. Then, the extracted mixture was centrifuged at 3000 rpm for 20 minutes. The supernatant was decanted and it was volume made up to 40 ml with distilled water. The sample solution (1 ml) was mixed with 1 ml of 5% phenol solution and 5 ml of sulfuric acid. The mixture was left for 15 minutes for standing in the room temperature and the absorbance was read at 550 nm. Glucose was used for standard solution. Reducing sugar content was determined as glucose according to DNS method [11]. Free Amino Nitrogen (FAN) was analyzed according to the European Brewery Convention Method [12] with modification. Raw and extruded corn starch powder (150 mg) were mixed with 1.5 ml of deionized distilled water in a 1.5 ml microcentrifuge tube and vortexed and then centrifuged at 12,000 rpm for 20 minutes using a high speed microcentrifuge (Micro 17TR, Hani Science Industrial Co. Ltd. Korea). The supernatant (1 ml) was mixed with 1 ml of Ninhydrin color reagent and it was heated in boiling water bath for 16 min. The tubes were transferred to a cold water bath and 5 ml of dilution reagent was added, mixed and the absorbance was read at 575 nm against a blank containing 1 ml of water in place of sample.

Microstructures

Raw and extruded corn starch powders were examined with a field emission scanning electronic microscope (MIRA II LMH Tescan USA, Inc., Cranberry Township, PA). The samples were fixed in stubs containing a gold-palladium alloy before observation. All samples were examined at an accelerated voltage of 10 kV.

Enzymatic hydrolysis and saccharification

One gram of ground dried sample was suspended in 43 ml of 20 mM sodium acetate-acetic acid buffer at pH 5.6. This mixture was supplemented with 5 ml of 0.8% α-amylase solution and incubated in rotary shaker (100 rpm) at 30°C for 0, 2, 4, 6, 8 and 10 hr. The reducing sugar content of raw and extruded corn starch at specific time internal was determined according to DNS method [11] using 3,5-dinitrosalicylic acid. Glucose solution was used as a standard.

Experimental design and statistical analysis

Completely randomized design (CRD) was performed to determine the effect of CO_2 injection during extrusion at different melt temperatures 95, 115 and 135°C. The data were analyzed by using the SAS program (Version 6.12, Statistical Analysis System).

Results and Discussion

Mechanical properties

As shown in table 1, CO_2 injection during extrusion significantly affected breaking strength in bending ($p<0.05$) at melt temperature 95°C and also numerically decreased at 115 and 135°C. Breaking strength decreased with CO_2 injection and had a negative correlation with expansion ratio ($p<0.05$). Martinez-Serna and Villota [13] reported that breaking force depended on degree of expansion and cell wall strength. Several other researchers also demonstrated that breaking strength decreased with increasing expansion ratio (i.e., a decrease in piece density) [14-19]. Hsieh et al. [18] found that thinner cell walls with greater radial expansion were formed, and extrudates became more breakable. Our results are consistent with those observations. However, there was no significant difference at 115 and 135°C. Elastic modulus was also affected by CO_2 injection ($p<0.05$). As shown in table 1, elastic modulus significantly increased at melt temperature 95°C and numerically decreased at 115 and 135°C with increasing melt temperature and was negatively correlated with expansion ratio. Decrease in elastic modulus at 115 and 135°C is a positive effect for extrudates. The elastic modulus is the degree of the 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 [6].

Physical properties

Expansion ratio was significantly increased by CO_2 injection ($p<0.05$) at tested melt temperatures 95, 115 and 135°C. The expansion ratio of corn starch extrudates was positively correlated with specific length at tested melt temperatures 95, 115 and 135°C. Jeong and Toledo [20] also reported that the expansion ratio of extruded rice flour with CO_2 injection increased with increasing CO_2 injection pressure and decreased with increasing piece density. Our results agreed with their observation (Table 1).

Specific length was significantly increased by CO_2 injection at 115 and 135°C ($p<0.05$). Higher melt temperature with CO_2 injection caused the feed material to melt fully and also reduced the melt viscosity, resulting in increased specific length of the extrudates. Present results are consistent with those of our previous study in which the specific length was decreased with increase in SME input [6]. Piece density of extrudates with CO_2 injection at 95°C was significantly ($p<0.05$) lower than those of extrudates without CO_2 injection. However, there was no significant difference ($p>0.05$) at 115 and 135°C.

The effect of CO_2 injection on water solubility index (WSI) and water absorption index (WAI) is shown in table 2. WSI of extruded corn starch with CO_2 injection was significantly increased ($p<0.05$) at melt temperature 135°C and also increased at both melt temperatures 95 and 115°C. Our results agree with those of Jeong and Toledo [20]. WSI of the rice flour significantly increased from 5.12% to 12.73% as CO_2 injection pressure increased from 0.1 to 0.6 MPa. WSI is related to the amount of low molecular weight products of starch degradation which are easily soluble because of reduced entanglement [8]. WSI values reflect the rate of penetration of water into the solid particles and diffusion of the soluble components outside of the particles. It is also a function of extent of dextrinization of starch. In general, WSI is often used as an indicator of degradation of molecular components because its measurement can be known by the conversion degree of starch during extrusion cooking. This conversion degree is amount of soluble polysaccharide released from the starch after extrusion. In this study, the increase in the melt temperature with CO_2 injection caused an increase in the macro-molecular degradation during extrusion process and resulting in an increase in WSI of extruded corn starch. WSI usually increases when starch chains degrade into smaller fragments. Low temperature and high moisture content might help reduce structural degradation during extrusion [3,21,22]. WSI had a tendency to increase as melt temperature increased with CO_2 injection. Therefore, it is reasonable that our extruded corn starch with CO_2 injection would be highly solubilized (dispersed) in water giving a high WSI (Table 2).

Water absorption index (WAI) measures the volume occupied by the starch after swelling in excess water, which maintains the integrity

Melt temp. (°C)	CO_2 injection	Breaking strength(N/m²)	Elastic modulus(N/m²)	Expansion ratio	Specific length (m/kg)	Piece density (g/cm³)
95	With CO_2 injection	3.14E+0.5^{b}	7.28E+05^{b}	1.69 ± 0.05^{a}	53.97 ± 1.88^{d}	0.63 ± 0.08^{b}
	Without CO_2 injection	5.71E+0.5^{a}	3.55E+06^{a}	1.54 ± 0.03^{b}	53.91 ± 1.95^{d}	0.85 ± 0.06^{a}
115	With CO_2 injection	1.76E+0.5^{c}	4.95E+05^{bc}	1.53 ± 0.07^{b}	57.91 ± 2.09^{c}	0.63 ± 0.04^{b}
	Without CO_2 injection	1.84E+0.5^{c}	6.06E+05^{bc}	1.4 ± 0.09^{c}	53.02 ± 0.98^{d}	0.66 ± 0.02^{b}
135	With CO_2 injection	6.24E+04^{d}	2.92E+05^{c}	1.71 ± 0.04^{a}	81.98 ± 3.52^{a}	0.35 ± 0.03^{c}
	Without CO_2 injection	6.50E+04^{d}	4.19E+05^{bc}	1.6 ± 0.15^{b}	70.46 ± 2.52^{b}	0.37 ± 0.02^{c}

Means of ten replications, based on least significant difference (LSD) procedure at α=0.05 level. Means with the same letter in the same column are not significantly different.

Table 1: The mechanical and physical properties of extruded corn starch at different melt temperatures.

Melt temperature (°C)	CO_2 injection	[1]WSI (%)	[2]WAI (g/g)	[3]SME(kJ/kg)
95	With CO_2 injection	3.60 ± 0.35^{de}	2.51 ± 0.13^{d}	534.91 ± 8.25^{a}
	Without CO_2 injection	3.23 ± 0.08^{e}	2.53 ± 0.06^{d}	542.81 ± 4.89^{a}
115	With CO_2 injection	4.90 ± 0.84^{c}	3.64 ± 0.22^{c}	306.98 ± 1.77^{b}
	Without CO_2 injection	4.10 ± 0.39^{dc}	4.55 ± 0.87^{b}	308.12 ± 6.56^{b}
135	With CO_2 injection	7.24 ± 0.24^{a}	5.03 ± 0.02^{ab}	169.55 ± 1.09^{d}
	Without CO_2 injection	6.51 ± 0.17^{b}	5.23 ± 0.31^{a}	181.29 ± 5.99^{c}
Raw corn starch	0.23 ± 0.02^{f}	0.75 ± 0.03^{e}	-	

[1]water solubility index, [2]water absorption index and [3]specific mechanical energy input
Means of three replications, based on least significant difference (LSD) procedure at α=0.05 level. Means with the same letter in the same column are not significantly different

Table 2: Water solubility index, water absorption index and specific mechanical energy input of extruded corn starch.

of starch in aqueous dispersion [23]. WAI of extruded corn starch with CO_2 injection was significantly decreased ($p<0.05$) at 115°C and also decreased at 95 and 135°C. 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 the extrusion was carried out, the raw material is a pre-gelatinized starch. The differences can be attributed primarily to physical effect related to the structure of voids in the solid. Thus, significantly decreased in WAI of extruded corn starch at 115°C can be attributed to CO_2 injection. The decrease in WAI and increase in WSI (Table 2) is an indication that extrusion with CO_2 injection may have accelerated degradation of starch granules and release of water-soluble compounds during extrusion. This change is more susceptible to enzymatic digestion. Our results agreed with Whalen [24] who reported that the WAI and WSI of extruded corn starch were also higher than those of raw corn starch due to swelling of highly degraded starch (Table 2).

The influence of CO_2 injection at different melt temperatures on specific mechanical energy (SME) input is shown in table 2. CO_2 injection during extrusion decreased SME input at tested melt temperatures. There was no significant difference in both melt temperatures 95 and 115°C. However, SME input was significantly decreased by CO_2 injection ($p<0.05$) at 135°C. The higher the melt temperature, the lower the SME input for both with and without CO_2 injection. In this experiment, changes in SME input caused by melt temperature had the same pattern as studied by other authors Ryu et al. [6] and Brent et al. [25] who reported that SME input was decreased with the increase in melt temperature. Therefore, SME input was decreased with increase in melt temperature with CO_2 injection. Higher SME input at lower melt temperature is explained by the higher viscosity of the melt at this temperature since SME input is the mechanical energy consumed to rotate the screws plus viscous melt [26].

Chemical properties

The moisture contents of raw and extruded corn starch with and without CO_2 injection ranged between 8.89% and 12.69%, those obtained were less than 20% and were acceptable [27]. Total sugar and reducing sugar contents of raw and extruded corn starch with and without CO_2 injection are shown in table 3. There was no significant difference in total sugar and reducing sugar contents of extruded corn starch with and without CO_2 injection. However, extruded corn starch with CO_2 injection can numerically increase total sugar and reducing sugar than those without CO_2 injection at each melt temperature.

CO_2 injection during extrusion can significantly increase ($p<0.05$) the amount of free amino nitrogen (FAN) at melt temperatures 95, 115 and 135°C (Table 3). Researchers have found that one of the factors limiting the production of high levels of ethanol by brewing yeast is of nutritional deficiency [28]. When a nitrogen source is supplemented in the fermentation system, the nutritional supplement can promote rapid fermentation to a higher ethanol level without the need to genetically modify yeast. Therefore, FAN in the sample is important for yeast performance. The higher the FAN in the fermented slurry, the faster is the fermentation process. Our results supported the effect of FAN on ethanol fermentation efficiency from field-sprouted sorghum as described by Yan et al. [29]. Casey et al. [30] made the same conclusion about the effect of FAN on fermentation efficiency of high gravity brewing from wheat. Therefore, FAN content in a sample could be a useful indicator of a sample's performance in ethanol fermentation. It was also showed that similar results agree with results reported by several other researchers [30-32]. Mullins and Nesmith [33] studied ethanol fermentation with high-tannin sorghum and revealed that the addition of nitrogen accelerated the ethanol fermentation rate. Moreover, Diammonium Phosphate (DAP) is widely used as Yeast Assimilable Nitrogen (YAN). The assimilable nitrogen was found to be related to FAN and the addition of DAP increased fermentation rate [34,35].

Microstructures

Microstructures of raw and extruded corn starch powder are shown

Melt temperature(°C)	CO_2 injection	[1]MC (%)	Total sugar (mg/g)	Reducing sugar (mg/g)	Free amino nitrogen (mg/ml)
95	With CO_2 injection	8.89 ± 0.09^{e}	11.00 ± 0.16^{a}	0.36 ± 0.03^{d}	254.10 ± 1.62^{c}
	Without CO_2 injection	9.02 ± 0.05^{e}	8.66 ± 1.03^{abc}	0.36 ± 0.01^{d}	202.46 ± 1.86^{d}
115	With CO_2 injection	11.12 ± 0.02^{b}	10.17 ± 1.62^{ab}	0.86 ± 0.03^{a}	202.68 ± 2.03^{d}
	Without CO_2 injection	10.12 ± 0.59^{d}	7.94 ± 2.29^{bc}	0.85 ± 0.07^{a}	164.11 ± 2.43^{e}
135	With CO_2 injection	11.49 ± 0.10^{b}	7.42 ± 1.54^{c}	0.81 ± 0.04^{ab}	313.03 ± 1.10^{a}
	Without CO_2 injection	10.65 ± 0.01^{c}	7.97 ± 1.34^{bc}	0.76 ± 0.03^{b}	128.59 ± 2.50^{f}
	Raw corn starch	12.69 ± 0.04^{a}	2.25 ± 0.25^{d}	0.50 ± 0.09^{c}	276.40 ± 1.6^{b}

[1]moisture content

Means of three replications, based on least significant difference (LSD) procedure at α=0.05 level. Means with the same letter in the same column are not significantly different

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

Melt temperature (°C)	CO_2 injection	Saccharification period (hr)				
		2	4	6	8	10
95	With CO_2 injection	75.25^{c}	112.41^{d}	123.35^{c}	144.00^{dc}	147.31^{b}
	Without CO_2 injection	73.94^{c}	107.61^{d}	123.18^{c}	134.08^{d}	140.15^{b}
115	With CO_2 injection	137.62^{a}	145.48^{a}	158.30^{a}	186.99^{a}	174.54^{a}
	Without CO_2 injection	72.73^{c}	120.83^{c}	120.04^{c}	150.34^{c}	154.63^{b}
135	With CO_2 injection	113.77^{b}	137.28^{b}	150.51^{a}	172.78^{b}	146.69^{b}
	Without CO_2 injection	73.44^{c}	121.21^{c}	138.44^{b}	148.54^{c}	141.97^{b}
Raw corn starch	2.09^{d}	4.34^{f}	9.66^{d}	11.95^{e}	20.12^{c}	

Means of three replications, based on least significant difference (LSD) procedure at α=0.05 level. Means with the same letter in the same column are not significantly different

Table 4: Reducing sugar content (mg/g) of raw and extruded corn starch at different saccharification periods.

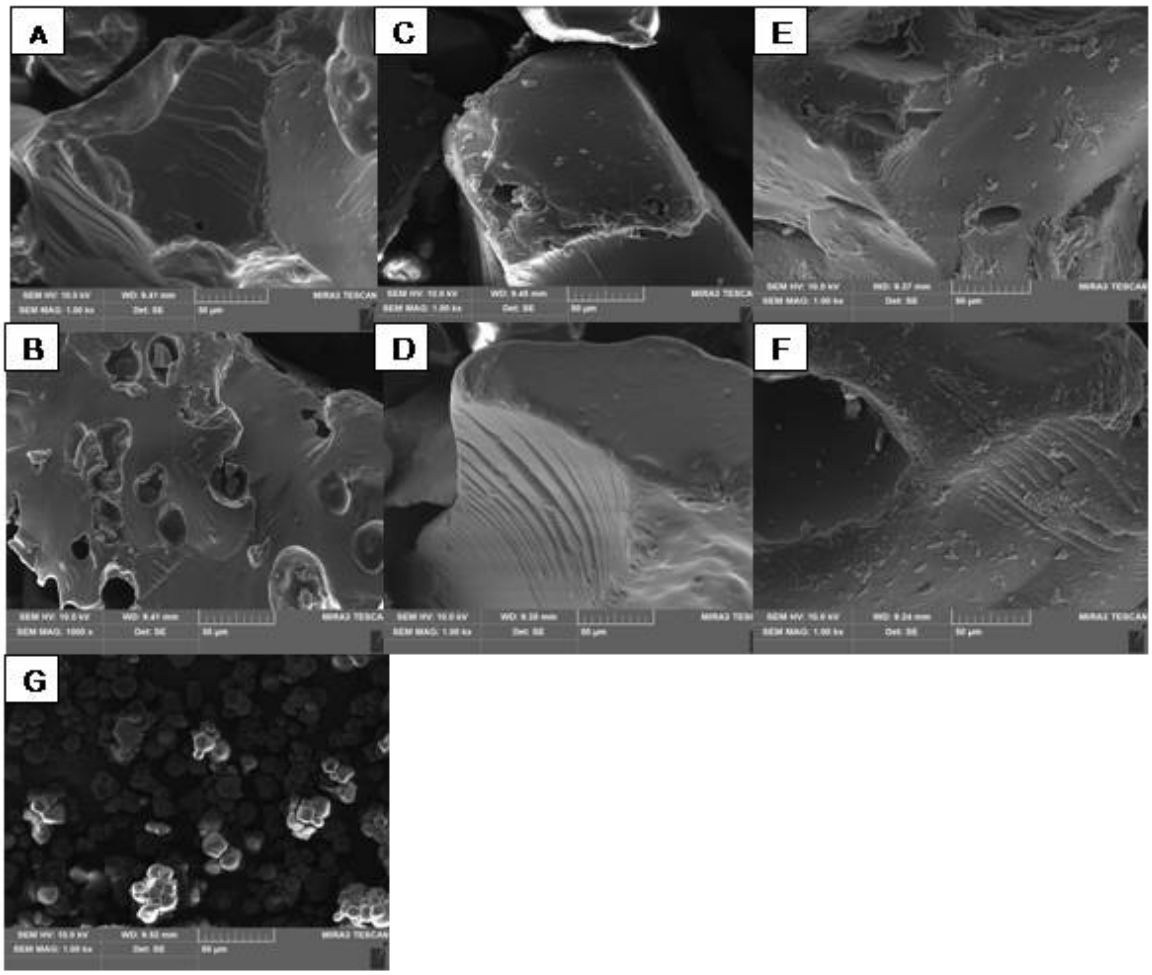

Figure 1: Microstructures of raw and extruded corn starch. **A)** 95°C with CO_2; **B)** 95°C without CO_2; **C)** 115°C with CO_2; **D)** 115°C without CO_2; **E)** 135°C with CO_2; **F)** 135°C without CO_2 and **G)** raw corn starch.

in figure 1. The surface of the extruded corn starch powder without CO_2 injection at melt temperatures 115 and 135°C were very smooth without cracks. On the other hand, extruded corn starch with CO_2 injection had porous structures with crackers and holes on the particle surface (Figures 1C and 1E), which could be caused by water and CO_2 expansion and evaporation during extrusion. These holes make extruded corn starch granules more susceptible to enzymatic digestion because water and enzyme can more easily enter starch granules through these pores, which confirmed by the increase in reducing sugar content at saccharification periods (2-8 hr) at 115 and 135°C. Extruded corn starch with CO_2 injection has superior solvation power and could deeply penetrate and distribute corn starch particles during extrusion process. As a result molten corn starch exits the die; the flash of CO_2 produces expanded extrudates with porous structures. In conventional extrusion, water was normally used as physical blowing agent. CO_2 injection in the barrel of an extruder functions as plasticizer and allows the processing of molecules which would otherwise be too fragile to withstand the mechanical stress and the operating temperature of a standard extrusion process. Therefore, extrusion with CO_2 injection could effectively damage the native corn starch structures and increase their surface area. Zhan et al. [36] also reported that the injection of supercritical-fluid carbon dioxide during extrusion process increased the surface area of sorghum starch availability for fermentation.

Enzymatic hydrolysis and saccharification

Reducing sugar content of the raw and extruded corn starch after 10 hr-saccharification periods using 0.8% α-amylase was measured. As shown in table 4, CO_2 injection significantly increased ($p<0.05$) the production of reducing sugar by increasing the incubation period (2-8 hr) at 115 and 135°C. This can be explained by effective damage of native starch structures after extrusion which resulted in higher dextrinization and increased the amount of soluble polysaccharide. Reducing sugar content was increased with the decrease in the moisture content of the extruded corn starch (Tables 3 and 4). These data agreed with Shin et al. [37]. However, at 10 hr-saccharification periods, the production of reducing sugar decreased at these temperatures. Despite the higher initial reducing sugar content in the sample extruded with CO_2 injection at 115°C, more increase in the reducing sugar content after saccharification was observed in the samples extruded without CO_2 injection. Colona et al. [26] 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 the

easiness of dextrin structure which goes into solution as a substrate for enzymatic reaction. The higher the initial reducing sugar content, the lower increase of the reducing sugar content was observed after saccharification [38]. It is known that in the expense amount of glucose, α-amylase can easily form reversion products like maltose and isomaltose that would be counted as reducing sugar. Our results agreed with this observation (Table 3). The increase of the reducing sugar after saccharification was smaller for the extruded sample with CO_2 injection than those of extruded sample without CO_2 injection (Table 4). However, high reducing sugar content was produced by extruded corn starch with CO_2 injection during extrusion at 115 and 135°C. Based on our results and our previous observation, water solubility and absorption of extruded corn starch was higher than those of raw corn starch due to molecular fragmentation during the high shear and high temperature extrusion and due to swelling of highly degraded starch. Therefore, the amount of reducing sugar production from extruded corn starch with and without CO_2 injection was significantly ($p<0.05$) higher than those of raw corn starch at tested melt temperatures and saccharification periods. Extrusion with CO_2 injection can be used as a physical pretreatment method for fermentation substrate preparation.

Conclusion

Extrusion of corn starch with CO_2 injection at different melt temperatures was the most significant factor affecting all the product variables investigated. Low value of breaking strength, elastic modulus, piece density and water absorption index and high value of expansion ratio, specific length, water solubility index and reducing sugar production after saccharification were obtained by extrusion at melt temperatures 115 and 135°C. Finally, extrusion with CO_2 injection at melt temperatures 115 and 135°C could effectively gelatinize and degrade the native corn starch granules which resulted in higher dextrinization and subsequently increased the reducing sugar content after saccharification periods (2-8 hr) for fermentation substrate preparation.

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