Effects of Thermomechanical Extrusion and Particle Size Reduction on Bioconversion Rate of Corn Fiber for Ethanol Production

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ABSTRACT

The objective of this research was to evaluate the effect of thermomechanical extrusion and particle size (PS) reduction on the bioconversion rate of corn fiber for ethanol production. Extrusion was conducted at a screw speed of 300 rpm, feed rate of 120 g/min, feed moisture content of 30%, melt temperature of 140°C, and die diameter of 3 mm. Raw and extruded corn fiber were separated into three different PSs ($1 > PS \geq 0.5$, $0.5 > PS \geq 0.3$, and $0.3 > PS \geq 0.15$ mm) with a wire sieve. Extrusion pretreatment and PS reduction resulted in a significant ($P < 0.05$) difference in physical properties and color values of extruded corn fiber as a result of accelerated degradation of corn fiber structure. Significant increase in water solubility index of extruded corn fiber at $0.3 > PS \geq 0.15$ mm was an indication of high degradation of starch during extrusion for higher release of polysaccharides. Moreover, extruded corn fiber at PS reduction $0.3 > PS \geq 0.15$ mm also significantly increased ($P < 0.05$) ethanol yield (69.11 g/L) and conversion (68.18%) by increasing protein digestibility and free amino nitrogen, which are essential for higher fermentation efficiency.

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Current bioethanol production relies on ethanol from starch and sugars, but there has been considerable debate about its sustainability. Bioethanol production from lignocellulosic biomass is an interesting alternative because lignocellulosic raw materials do not compete with food crops and are less expensive than conventional agricultural feedstock. Preferably, the feedstocks are by-products of existing industries that will not put extra pressure on land use. Corn fiber represents a renewable resource that is available in sufficient quantities from the corn wet-milling industry to serve as a low-cost feedstock for ethanol production. In the United States, the average productivity of corn grains is 7 metric tons/ha annually (Somerville et al 2010), and about 8% of the grain is fiber. The biological conversion of different lignocellulosic feedstocks dedicated to ethanol offers numerous benefits, but its development is still hampered by economic and technical obstacles (Sanchez and Cardona 2008). The ideal plant biomass for energy should have high yield, low energy input, low cost, low concentration of contaminants, and low nutrient requirements (McKendry 2002).

Corn fiber is a by-product of the corn wet-milling industry, and the bran is rich in hemicellulose; corn fiber contains approximately 17% residual starch, 18% cellulose, and 35% hemicelluloses. Because it is available in wet-milling ethanol plants, it is a logical lignocellulosic feedstock for conversion to ethanol. In their native conformation, cellulose and hemicelluloses are largely protected from enzymatic degradation through associations of these polymers with lignin and with each other, associations that act as a barrier and interfere with hydrolysis (Torget et al 1991; Moniruzzaman 1996). Moreover, lignin is composed of an irregular cross-linked network of polymers that gives the plant structural support, impermeability, and resistance against microbial attack and oxidative stress (Kilpenainen et al 2007; Hendriks and Zeeman 2009). Hence, lignin limits the accessibility of enzymes and the rate of hydrolysis by acting as a shield preventing the digestible part of the substrate from hydrolysis (Chang and Holtzapple 2000).

Moreover, the production of bioethanol from renewable biomass faces significant technical and economic challenges at present. A critical step in lignocellulosic biomass conversion to ethanol is hydrolysis of cellulose and hemicelluloses to fermentable sugars, which is affected by numerous factors including composition and structure of the feedstock, pretreatment method, type and loading of enzyme, cellulose crystallinity, and available surface area (Li et al 2004). The highly organized crystalline structure of cellulose poses another obstacle to enzymatic hydrolysis. This structure limits the available sites for enzymatic attack, because the average size of the capillaries in the biomass is too small to allow the entry of large enzyme molecules. Enzymatic action is confined to the external surfaces unless the feedstock structure is modified (Abraham and Kurup 1997). Therefore, the modification of the lignocellulosic substrate is achieved through various pretreatment strategies that disrupt the cell wall structure and make it accessible to enzymes.

Mechanical, chemical, thermomechanical, and biological methods have been used for pretreatment of lignocellulosic biomass (Sun and Cheng 2002). Among these methods, thermomechanical pretreatment (extrusion) has been a widely used method in which several unit operations are performed simultaneously during the extrusion process. In extrusion, the materials are subjected to heating, mixing, and shearing, resulting in physical and chemical changes during passage through the extruder. The major advantages of extrusion include increased digestible starch fraction, reduced molecular weight of biomolecules, creation of free sugars, changes in the native structure of biomolecules, and reduced viscosity of fermentation broth when using extruded products during fermentation (Zhan et al 2003). The extrusion process was first investigated as an alternative pretreatment method in the 1980s for pretreatment of crop residues, sawdust, and municipal waste in the presence of dilute sulfuric acid (Noon and Hochstetler 1982; Green et al 1988). The conversion rate from cellulose and hemicelluloses to sugars was found to be around 37% at a solid loading of 60% (Noon and Hochstetler 1982). A similar process that used twin-screw extrusion and concentrated sulfuric acid was described more recently for pretreatment of sawdust at a solid loading of around 40% (Miller and Hester 2007). However, no research has been conducted on extrusion of corn fiber as a pretreatment method for fermentation substrate preparation. Extrusion also changes the content, composition, and physiological effects of dietary fiber. Fiber content can be lowered through degradation of dietary fiber into lower-molecular-weight fragments. Moreover, macromolecular degradation of fiber by extrusion increases its solubility and changes its physiological effects (Lue et al 1991). Pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. Therefore, the objective of the present study was to

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determine the effect of extrusion pretreatment and particle size (PS) reduction on physiochemical properties and bioconversion of raw and extruded corn fiber for ethanol production.

MATERIALS AND METHODS

Materials and Chemicals. Corn fiber, a by-product of corn wet-milling starch manufacture, was provided by Samyang Genex (Seoul, Korea). It contained approximately 17% residual starch, 18% cellulose, and 35% hemicelluloses. Cellulase (from *Trichoderma reesei* ATCC 26921) and β-glucosidase (from almonds) were purchased from Sigma (St. Louis, MO, U.S.A.). for saccharification. Phenol and sulfuric acid were purchased from Daejung Chemicals and Metals (Shiheung, Korea) for total sugar content determination. Thermostable α-amylase and amyloglucosidase (Megazyme International, Bray, Ireland) were used for determination of starch content. For determination of total, soluble, and insoluble dietary fiber (TDF, SDF, and IDF, respectively), thermostable α-amylase, protease, and amyloglucosidase (Megazyme) were used. The other necessary chemicals and reagents were purchased from Sigma-Aldrich (Steinheim, Germany).

Extrusion Process. Extrusion was conducted in a twin-screw extruder (THK31T, Incheon Machinery Co., Incheon, Korea). Based on a previous study (Lamsal et al 2010), extrusion conditions and PS reductions were selected. The experiment was conducted at a screw speed of 300 rpm, feed rate of 120 g/min, feed moisture content of 30%, melt temperature of 140°C, and die diameter of 3 mm. Before drying, the extruded corn fiber had approximately 28% moisture content, and fiber was directly dried in an oven at 80°C for 8 h until the moisture content was 3.5%. Raw and extruded corn fiber with the same moisture contents were separated into three different PSs ($1 > PS \geq 0.5$, $0.5 > PS \geq 0.3$, and $0.3 > PS \geq 0.15$ mm) with wire sieves. The separated samples were used for determination of cellulosic composition, physiochemical properties, cellulose crystallinity, and microstructure of raw and extruded corn fiber.

Proximate Composition. AOAC official methods (2000) were used for determination of crude ash (942.05, which corresponds to AACC International Approved Method 08-03.01), crude lipid (920.39, which corresponds to AACCI Approved Method 30-25.01), and crude protein contents (N × 6.25) (990.03) of raw and extruded corn fiber at tested PSs. Starch content was measured with a commercial kit (Megazyme) method based on the use of thermostable α-amylase and amyloglucosidase (McCleary et al 1994). TDF, IDF, and SDF contents were determined according to the method of Lee et al (1992) with a TDF assay kit (Megazyme). For IDF, the suspension was filtered through a crucible, and the residue was washed with preheated distilled water, 10 mL of 95% ethanol, and acetone. The crucible containing residue was dried overnight at 103°C and used for determination of protein and ash contents for IDF. For SDF, the residue was washed only with preheated distilled water, and the filtrate was precipitated with four volumes of preheated (70°C) 95% ethanol at room temperature for 1 h. The crucible containing residue was dried and used for determination of protein and ash contents for SDF.

Water Absorption Index (WAI) and Water Solubility Index (WSI). WAI was determined as the amount (g) of water absorbed by each gram of sample, as described by Lee et al (1999). Extruded powder (1 g) was mixed with 25 mL of distilled water in a 50 mL centrifuge tube. The tubes were agitated in an incubator shaker (110 rpm) for 30 min at 30°C. Then the tubes were centrifuged at 3,000 × *g* for 20 min. 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 the method of Anderson et al (1969).

Color. A colorimeter (JP/CR-300, Minolta, Osaka, Japan) was used to evaluate color value of raw and extruded corn fiber. 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 ($L = 97.60$, $a = -0.19$, and $b = 1.34$). Measurements were performed in triplicate.

Microstructures. Raw and extruded corn fibers of different PSs were examined with a field emission scanning electronic microscope (MIRA II LMH, Tescan USA, Cranberry Township, PA, U.S.A.). The samples were fixed in stubs containing a gold–palladium alloy before observation. All samples were examined at an accelerating voltage of 10 kV.

Crystallinity. X-ray diffraction of raw and extruded corn fiber at tested PSs was conducted according to the method of Jin et al (2007). The scan was carried out in an X-ray diffractometer (0/MAX-2500, Rigaku, Tokyo, Japan). The measurement conditions were as follows: CuKa (1.54 nm), 40 kV, 100 mA, $2\theta = 10$–$40°$, scan rate 10°/min, and reflection mode. Pure cellulose was used as a reference, with the diffraction peak at $2\theta = 23°$ corresponding region (Segal and Conrad 1957). The intensity of the diffraction peak at $2\theta = 23°$ for all samples was a measure of their relative crystallinity, which was used to compare raw and extruded corn fiber at different PS reductions for structural modification.

The crystallinity index (CI) was calculated with the following equation (Guthrie 1955):

$$CI = (I_p - I_{am})/I_p \times 100$$

where I_p is the peak intensity and I_{am} is the intensity attributed to amorphous cellulose.

Functional Properties. *Total Phenolics and Lignin Contents.* The total phenolics content of raw and extruded corn fiber at different PSs was determined according to the Folin–Ciocalteu colorimetric method (Slinkard and Singleton 1997). Sample (1 g) was extracted with 10 mL of 80% (v/v) methanol at room temperature for 2 h. The mixture was centrifuged at 3,000 × *g* for 30 min. Supernatant (300 µL) was mixed with 1.5 mL of 10% (v/v) Folin–Ciocalteu reagent, vortexed thoroughly, and allowed 5 min for reaction. Then the mixture was supplemented with 1.5 mL of 60 g/L sodium carbonate solution and incubated at room temperature for 2 h. The absorbance was measured at 765 nm against the blank containing 80% methanol. The concentration of total phenolics content in the extracts was determined as milligrams of gallic acid equivalent (GAE) per gram of dry sample by using an equation obtained from the standard gallic acid curve. Lignin content was determined as the residue remaining after total hydrolysis of cell wall polysaccharides by the method of Van Eylen et al (2011).

Total and Reducing Sugars. Total sugar content was measured with the phenol–sulfuric acid method (Dubois 1956). Sample (2 g) was mixed with 20 mL of 70% ethanol solution and was extracted at 80°C for 2 h. Then the extracted mixture was centrifuged at 3,000 × *g* for 20 min. The supernatant was decanted and the 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 concentrated sulfuric acid. The mixture was left for 15 min standing at room temperature, and the absorbance was read at 550 nm. Glucose was used for the standard solution. Reducing sugars content was determined as glucose according to the dinitrosalicylic acid (DNS) method (Miller 1959).

Protein Digestibility. Protein digestibility (pepsin digestibility) was carried out as described by Mertz et al (1984). Raw and extruded corn fiber (200 mg) was suspended in 35 mL of pepsin solution (1.5 g of enzyme/1 L of 0.1M potassium phosphate buffer, pH 2) and incubated at 150 rpm and 37°C for 2 h. Pepsin (Samchun Pure Chemical Co., Seoul, Korea) digestion was stopped by addition of 2 mL of 2M NaOH at the end of the incubation period. The incubated slurry was centrifuged at 3,000 × *g* for 15 min, the supernatant was decanted, and the residues were washed in 10 mL of 0.1M potassium phosphate buffer (pH 2) and

centrifuged as before. Washing the residues with 10 mL of 0.1M potassium phosphate buffer was done twice, and the residue was freeze-dried. The freeze-dried samples were then weighed and analyzed for nitrogen content.

Free Amino Nitrogen (FAN). FAN was analyzed according to the European Brewery Convention (1987) method with modification. Raw and extruded corn fiber (150 mg) was mixed with 1.5 mL of deionized distilled water in a 2 mL microcentrifuge tube, vortexed, and then centrifuged at 12,000 × *g* for 20 min with a high-speed microcentrifuge (Micro 17TR, Hanil Science Industrial Co., Incheon, Korea). The supernatant (1 mL) was mixed with 1 mL of ninhydrin color reagent, and it was heated in a boiling water bath for 16 min. The tubes were transferred to a cold water bath, and 5 mL of dilution reagent was added and mixed, and the absorbance was read at 575 nm against a blank containing 1 mL of water in place of sample.

Semisimultaneous Saccharification and Fermentation. *Saccharomyces cerevisae* (ATCC 24858) was used for ethanol fermentation. Yeast cells were maintained on yeast malt (YM) agar medium with 21 g of YM powder and 20 g of agar per liter. Yeast cells were cultured in a rotary shaker at 200 rpm at 30°C for 48 h in a preculture medium (2% glucose, 0.5% peptone, 0.3% yeast extract, 0.1% KH_2PO_4, and 0.05% $MgSO_4 \cdot 7H_2O$, pH 5.5) (Zhan et al 2003). Sample (3 g) was suspended in 42 mL of 50mM sodium acetate–acetic acid buffer (pH 4.8) and 42 mL of fermentation medium containing (per liter) 3 g of peptone, 1 g of KH_2PO_4, and 1 g of $(NH_4)_2SO_4$ at pH 3.8, and it was sterilized in an autoclave at 121°C for 20 min. The sterilized medium was supplemented with 1.28 mg of cellulase and 0.89 mg of β-glucosidase per gram of sample, and it was saccharified in a rotary shaker at 150 rpm and 45°C for 48 h. The saccharified sample was inoculated with 1 mL of activated yeast broth. Before and after fermentation, the initial and residual reducing sugar contents were determined according to the DNS method (Miller 1959) with 3,5-dinitrosalicylic acid, and glucose was used as the standard solution. The flasks were incubated in a rotary shaker (200 rpm) for 72 h at 30°C. All experiments were conducted three times.

After fermentation, the samples (5 mL) were taken and centrifuged at 3,000 × *g* for 20 min to remove the cells, and the supernatants were used for determination of residual reducing sugar and ethanol contents. Ethanol content was determined with the redox titration method (www.outreach.canterbury.ac.nz). Ethanol content was calculated by subtracting the average volume of the sodium thiosulfate solution used for the sample from the average volume used for the blank titration. Conversion (%) was calculated as (initial reducing sugar concentration – residual reducing sugar concentration) / initial reducing sugar concentration × 100 (Dimitrellou et al 2008).

Statistical Analysis. Results were analyzed with SAS version 6.12 software (SAS Institute, Cary, NC, U.S.A.). When the effects of extrusion and PS reduction were shown, the results for each parameter of each PS reduction were evaluated with the least significant difference test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Proximate Composition. The proximate composition of raw and extruded corn fiber at different PSs is presented in Table I. No significant difference was observed in ash content of raw and extruded corn fiber at 1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm. However, 0.3 > PS ≥ 0.15 mm extruded corn fiber had a significant increase ($P < 0.05$) in ash content compared with that of raw corn fiber. In general, ash is mainly composed of phosphorus, sodium, potassium, and calcium. As shown in Table I, the increase in ash content may be because of the presence of phosphate groups after greater PS reduction (0.3 > PS ≥ 0.15 mm). There was no significant difference in protein content of raw and extruded corn fiber at tested PS reductions. However, a decrease in protein content was observed after extrusion because of an increase in protein digestibility (Tables I and II). Concerning the content of lipids, the extruded corn fiber at tested PSs exhibited a significant decrease ($P < 0.05$) in lipid content (Table I). This decrease may result from starch–lipid complexes. The formation of complexes with lipids modifies the properties of starch contained in corn fiber, for example, by reducing starch solubility in water, delaying starch retrogradation, and slowing starch hydrolysis by enzymes (Tang and Copeland 2006). Moreover, high temperature and screw speed during extrusion can cause lipid degradation (Asp and Björck 1989); our results are in agreement with this observation (Table I). However, there was no significant difference in lipid content between 1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm extruded corn fiber after extrusion. This result means that PS reduc-

TABLE I
Proximate Composition (%, db) of Raw and Extruded Corn Fiber at Different Particle Sizes (PSs)[z]

Sample	PS (mm)	Crude Ash	Crude Lipid	Crude Protein	Starch	TDF	IDF	SDF
Raw	1 > PS ≥ 0.5	0.56 ± 0.07c	3.07 ± 0.18bc	10.11 ± 0.07c	12.67 ± 1.29c	79.69 ± 0.33a	60.09 ± 0.08a	19.60 ± 0.40c
	0.5 > PS ≥ 0.3	0.60 ± 0.06c	3.29 ± 0.15b	10.92 ± 0.04a	14.82 ± 2.68bc	75.35 ± 2.03b	55.03 ± 1.46b	20.32 ± 0.57c
	0.3 > PS ≥ 0.15	0.72 ± 0.04b	4.58 ± 0.05a	10.93 ± 0.01a	17.58 ± 1.06ab	60.70 ± 0.90e	36.80 ± 0.43e	23.90 ± 0.47c
Extruded	1 > PS ≥ 0.5	0.56 ± 0.02c	2.12 ± 0.10d	9.95 ± 0.10c	15.39 ± 1.38abc	71.52 ± 2.11c	36.80 ± 0.65e	32.21 ± 1.77ab
	0.5 > PS ≥ 0.3	0.63 ± 0.11bc	2.23 ± 0.18d	10.29 ± 0.09b	17.99 ± 1.22a	79.85 ± 0.37a	45.45 ± 0.56c	34.410 ± 0.19a
	0.3 > PS ≥ 0.15	0.85 ± 0.01a	2.89 ± 0.23c	10.09 ± 0.10c	17.27 ± 2.09a	69.22 ± 0.15d	41.62 ± 0.08d	27.60 ± 0.23b

[z] Means of three replicates based on least significant difference procedure at α = 0.05 level. Means with the same letter in the same column are not significantly different. TDF = total dietary fiber; IDF = insoluble dietary fiber; and SDF = soluble dietary fiber.

TABLE II
Functional Properties for Fermentation of Raw and Extruded Corn Fiber at Different Particle Sizes (PSs)[z]

Sample	PS (mm)	TPC (mg of GAE/g)	Lignin (%)	Total Sugar (mg/g)	Reducing Sugar (mg/g)	PD (% of Protein)	FAN (mg/mL)
Raw	1 > PS ≥ 0.5	23.75 ± 0.29a	10.15 ± 1.14b	8.95 ± 2.66e	2.40 ± 0.12cd	22.42 ± 0.64c	131.51 ± 1.32d
	0.5 > PS ≥ 0.3	24.07 ± 1.39a	10.63 ± 0.99ab	11.58 ± 0.78e	2.60 ± 0.02c	20.88 ± 0.09c	141.27 ± 1.54bc
	0.3 > PS ≥ 0.15	20.68 ± 1.18b	12.00 ± 0.67a	16.28 ± 2.15d	2.31 ± 0.11d	28.89 ± 1.46b	146.36 ± 1.89b
Extruded	1 > PS ≥ 0.5	14.65 ± 0.85d	10.63 ± 0.27ab	47.93 ± 1.54c	2.83 ± 0.14b	40.90 ± 1.02a	137.33 ± 1.85cd
	0.5 > PS ≥ 0.3	17.60 ± 1.65c	9.67 ± 0.38b	53.29 ± 0.03b	4.37 ± 0.04a	42.08 ± 0.92a	143.90 ± 1.29bc
	0.3 > PS ≥ 0.15	20.61 ± 1.17b	10.89 ± 0.16ab	58.39 ± 2.67a	4.22 ± 0.01a	42.31 ± 0.15a	157.75 ± 1.73a

[z] Means of three replicates based on least significant difference procedure at α = 0.05 level. Means with the same letter in the same column are not significantly different. TPC = total phenolics content; GAE = gallic acid equivalent; PD = protein digestibility; and FAN = free amino nitrogen.

tion (1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm) after extrusion could reduce the starch–lipid complexes compared with 0.3 > PS ≥ 0.15 mm. It was also reported that temperature above 100°C is preferred to ensure the removal of starch–lipid complexes (Van der Maarel et al 2002). In our experiment, the melt temperature was controlled at 140°C. Measurable starch content of extruded corn fiber was significantly increased ($P < 0.05$) compared with raw corn fiber at 0.5 > PS ≥ 0.3 mm (Table I), indicating that PS reduction 0.5 > PS ≥ 0.3 mm after extrusion did increase measurable starch. Zhan et al (2006) also reported that the extrusion process may break the intimate bonds between starch granules and the protein matrix of sorghum grain, and it resulted in an increase in starch availability for fermentation. However, there was no significant difference in starch content of raw and extruded corn fiber at 1 > PS ≥ 0.5 and 0.3 > PS ≥ 0.15 mm. TDF content of extruded corn fiber at 1 > PS ≥ 0.5 mm was significantly decreased ($P < 0.05$) compared with that of raw corn fiber. This modification probably occurred through degradation into low-molecular-weight fragments, because extrusion causes considerable solubilization of dietary fiber components, particularly pectic polymers and cellulose. Martín-Cabrejas et al (1997) stated that temperature causes degradation, which coincides with the present results in that the significant decrease in TDF content was observed in extruded corn fiber at 1 > PS ≥ 0.5 mm. However, there was no significant reduction in TDF content at 0.5 > PS ≥ 0.3 and 0.3 > PS ≥ 0.15 mm. These results are in agreement with those of Lue et al (1990), who reported decreased insoluble fiber with no net reduction in TDF content after extrusion of cornmeal and sugar beet fiber mixtures. Similar observations have been recorded for whole grain wheat flour (Siljestrom et al 1986) and extruded buckwheat and barley (Fornal et al 1987). TDF was not affected by these PS reductions. The increase in TDF of extruded corn fiber at 0.5 > PS ≥ 0.3 mm resulted primarily from increase in SDF. This increase in SDF could be partially because of the transformation of some IDF into SDF during extrusion. Bjorck et al (1984) reported a slight increase in TDF with a substantial shift from IDF to SDF in extruded white wheat flour. In the case of extruded corn fiber at 0.3 > PS ≥ 0.15 mm, the increase in IDF and TDF could result from the fiber–protein complexes present in the extruded corn fiber. The fiber–protein complexes present in extruded corn fiber at PS reduction 0.3 > PS ≥ 0.15 mm may resist digestion by enzymes. Therefore, the data given in Table I suggest that increases in SDF and fiber–protein complexes might have been responsible for the increased content of TDF at these

TABLE III
Water Solubility Index (WSI), Water Absorption Index (WAI), and Color Values of Raw and Extruded Corn Fiber at Different Particle Sizes (PSs)[z]

Sample	PS (mm)	WSI (%)	WAI (g/g)	Color		
				L	*a*	*b*
Raw	1 > PS ≥ 0.5	6.05 ± 0.35c	10.55 ± 0.11ab	74.93 ± 0.36c	0.68 ± 0.06d	23.16 ± 0.47f
	0.5 > PS ≥ 0.3	3.94 ± 0.69d	10.57 ± 0.02ab	75.63 ± 0.14b	0.82 ± 0.12d	25.01 ± 0.47e
	0.3 > PS ≥ 0.15	3.82 ± 0.32d	10.82 ± 0.47a	77.39 ± 0.09a	0.10 ± 0.02e	25.74 ± 0.16d
Extruded	1 > PS ≥ 0.5	6.74 ± 0.21b	10.51 ± 0.04ab	56.25 ± 0.62f	5.81 ± 0.17a	27.25 ± 0.11c
	0.5 > PS ≥ 0.3	6.55 ± 0.05bc	10.43 ± 0.04b	60.81 ± 0.54e	4.85 ± 0.12b	29.04 ± 0.15b
	0.3 > PS ≥ 0.15	7.42 ± 0.16a	10.45 ± 0.09b	65.94 ± 0.27d	3.62 ± 0.08c	30.53 ± 0.42a

[z] Means of three replicates based on least significant difference procedure at α = 0.05 level. Means with the same letter in the same column are not significantly different. *L* = lightness; *a* = redness; and *b* = yellowness.

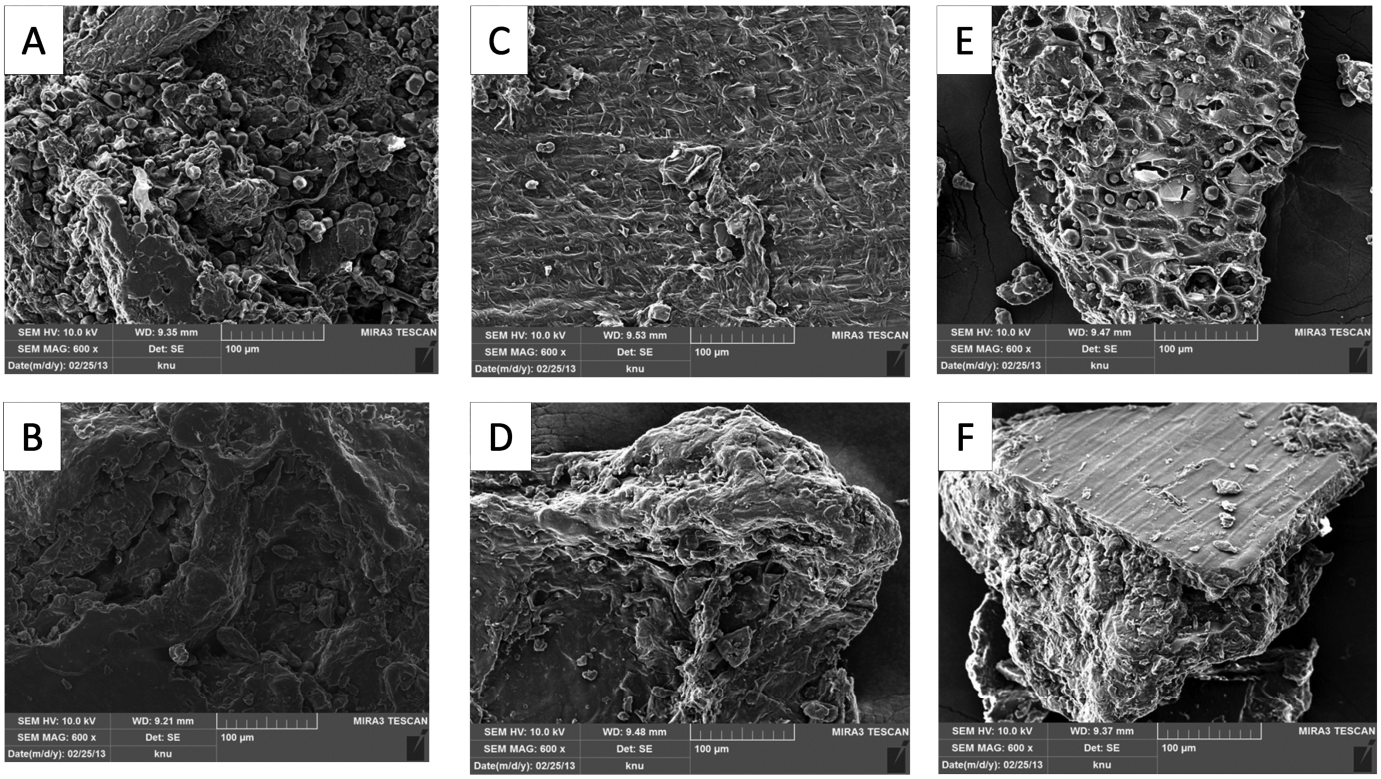

Fig. 1. Microstructures of raw and extruded corn fiber at different particle sizes (PSs). Raw corn fiber (**A**) and extruded corn fiber (**B**) at 1 > PS ≥ 0.5 mm. Raw corn fiber (**C**) and extruded corn fiber (**D**) at 0.5 > PS ≥ 0.3 mm. Raw corn fiber (**E**) and extruded corn fiber (**F**) at 0.3 > PS ≥ 0.15 mm.

PS reductions. IDF of extruded corn fiber 1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm was also significantly decreased ($P < 0.05$) compared with that of raw corn fiber. This reduction was caused by the increased solubilization of insoluble fiber components (Martín-Cabrejas et al 1999). In contrast with TDF and IDF, a significant increase in SDF was observed in extruded corn fiber at tested PSs. Increased SDF can be caused by release of the soluble fraction from hemicelluloses as a result of heating (Table I).

WSI and WAI. WSI and WAI of raw and extruded corn fiber are shown in Table III. WSI of extruded corn fiber at tested PSs was significantly increased ($P < 0.05$) compared with that of raw corn fiber. 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 a function of the 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 the amount of soluble polysaccharide released from the starch after extrusion. WSI is also related to the amount of low-molecular-weight products of starch degradation that are easily soluble because of reduced entanglement. Thus, thermomechanical extrusion increased the WSI of extruded corn fiber.

WAI is a function of the internal voids in the milled sample powder and the thickness of the cell walls of the voids. The differences can be attributed primarily to physical effects related to the structure of voids in the solid. Significant decrease ($P < 0.05$) in the WAI of the extruded corn fiber was observed only at PS reduction 0.3 > PS ≥ 0.15 mm, which indicated the onset of dextrinization (Table III). Dextrinization was indicated as higher released reducing sugar and higher WSI. Compact granular structure was loosened to some extent as a consequence of PS reduction, facilitating development of WAI. However, there was no significant reduction in WAI of raw and extruded corn fiber at both PS reductions 1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm. The reduction in WAI (0.3 > PS ≥ 0.15 mm) and increase in WSI of extruded corn fiber of tested PSs are indications of extrusion that accelerated degradation of corn fiber granules and release of water-soluble compounds during extrusion. It can also be explained by color value of extruded corn fiber (Table III).

Color. Table III shows results for color values of raw and extruded corn fiber at different PSs with a colorimeter. Raw corn fiber had maximum lightness and minimum redness and yellowness. A significant decrease ($P < 0.05$) in lightness and increase in redness and yellowness were noted when PS was reduced after extrusion. This observation means that reducing sugars formed as a result of thermomechanical shear of corn fiber reacted with amino acids to form Maillard products (Table III). Redness and yellowness increased further when corn fiber PS was reduced after extrusion, which is an indication of greater susceptibility of the monosaccharide to form Maillard compounds (Onyango et al 2004). Only extruded corn fiber showed decreased lightness and increased redness and yellowness, which may be associated with caramelization or nonenzymatic Maillard reactions. Excessive Maillard reactions are, however, not desirable because they result in a decrease of protein value when glucose reacts with amino acids (Onyango et al 2004). Such a result was observed in 0.3 > PS ≥ 0.15 mm extruded corn fiber (Table I).

Microstructures. Microstructures of raw and extruded corn fiber at different PSs are shown in Figure 1. Scanning electron microscopy images clearly indicate that different degrees of structural disruption occurred in both extrusion and PS reduction of raw and extruded corn fiber. Raw corn fiber 1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm had some starch granules and compact structure without pores and cracks on the surface. A compact structure or fewer pores at the surface of the product can cause slower water penetration during extrusion. However, the surface of extruded corn fiber at tested PSs had many pores with cracks on the surface. Such porous structures would facilitate rapid water penetration. These pores and cracks make extruded corn fiber at tested PSs more susceptible to enzymatic digestion, because water and enzymes can easily penetrate the corn fiber through these pores, which was confirmed by significantly increased ($P < 0.05$) WSI and total sugar content. However, among the extruded corn fiber at tested PSs, more extensive serration, tunneling, and surface erosion were observed only at 0.3 > PS ≥ 0.15 mm, which was also confirmed by the highest WSI and total sugar content (Tables II and III). Therefore, in industrial bioethanol production, PS reduction of 0.3 > PS ≥ 0.15 mm after extrusion may be a better pretreatment for feedstock preparation because of its easy enzymatic digestibility for fermentation.

Crystallinity. X-ray diffraction results showed that extruded corn fiber at PS reduction 0.3 > PS ≥ 0.15 mm possessed the highest crystallinity, whereas a significant reduction ($P < 0.05$) in crystallinity was observed in extruded corn fiber 1 > PS ≥ 0.5 mm, which indicated that extrusion destroyed hydrogen bonds in the cellulose and suggested that extrusion plays a leading role in the dissolution of cellulose (Table IV). Degree of polymerization and cellulose crystallinity have been considered important factors in determining the hydrolysis rates of cellulosic substrates (Chang and Holtzapple 2000). In some studies in which crystallinity was suggested to be important, the lignocellulosic materials were mechanically pretreated; therefore, any decrease in crystallinity was accompanied by an alteration of other substrate characteristics such as PS reduction or increase in available surface area (Alvira et al 2010). It is possible that improvement of properties with extrusion in the collapsed state of fiber is accompanied by different effects on the structure of fiber (with different changes in the degree of crystallinity of cellulose in the fiber). However, the crystallinity of extruded corn fiber 0.3 > PS ≥ 0.15 mm was significantly higher than that of extruded corn fiber 1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm. This increase could be because of slower conversion of crystalline cellulose compared with amorphous cellulose, which would increase the percentage crystallinity of the hydrolyzed biomass (Zhang and Lynd 2004). Caulfield and Moore (1974) also mentioned that decrease in PS and increase in available surface area rather than high crystallinity affected the rate and extent of hydrolysis.

Functional Properties. *Total Phenolics and Lignin Contents.* Total phenolics content of raw and extruded corn fiber at different

TABLE IV
X-Ray Diffraction Maximum Intensity, Corresponding 2θ, and Crystallinity of Raw and Extruded Corn Fiber at Different Particle Sizes (PSs)[z]

Sample	PS (mm)	2θ	Maximum Intensity (Linear Count per Second)	Crystallinity (%)
Pure cellulose	…	23	1,159 ± 15.58a	83 ± 5.06a
Raw	1 > PS ≥ 0.5	20.48	189.87 ± 16.09ef	68.94 ± 5.84b
	0.5 > PS ≥ 0.3	20.48	191.14 ± 16.53de	67.03 ± 5.84bc
	0.3 > PS ≥ 0.15	17.48	212.86 ± 15.91d	62.54 ± 5.00d
Extruded	1 > PS ≥ 0.5	20.48	180.79 ± 16.55de	57.67 ± 4.69e
	0.5 > PS ≥ 0.3	21.48	237.20 ± 17.38c	65.22 ± 5.33cd
	0.3 > PS ≥ 0.15	21.48	251.27 ± 13.69b	69.06 ± 3.43b

[z] Means of 20 replicates based on least significant difference procedure at α = 0.05 level. Means with the same letter in the same column are not significantly different.

PSs is presented in Table II. Extruded corn fiber 1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm resulted in a significant decrease ($P < 0.05$) in total phenolics content. The reduction in total phenolics content may be attributed either to the decomposition of phenolic compounds under high extrusion temperature or to the alteration in molecular structure of phenolic compounds that may lead to reduction in the chemical reactivity of phenolic compounds (Sharma et al 2007). It was reported that more than 80% of the total phenolics in maize or other cereal grains are bound primarily to hemicelluloses in cell walls of the pericarp, aleurone layer, and germ (Mora-Rochin et al 2010). The minimum amount of toxic compounds after pretreatment is one of the key factors to take into consideration for an effective pretreatment for low-cost and advanced pretreatment processes (Yang and Wyman 2008), because pretreatment leads to generation of toxic compounds derived from sugar decomposition that could affect the subsequent hydrolysis and fermentation steps (Oliva et al 2003). It has also been reported that important changes in phenolics content by extrusion cooking might produce adverse effects for human and animal nutrition (Anton et al 2008). The removal of lignin showed no significant difference (at $P < 0.05$) between raw and extruded corn fiber at tested PSs. However, it was apparent that greater PS reduction (0.5 > PS ≥ 0.3 and 0.3 > PS ≥ 0.15 mm) led to decreased lignin content after extrusion. Lignin limits the rate of enzymatic hydrolysis by acting as a physical barrier, preventing the digestible parts of the substrate from being hydrolyzed (Chang and Holtzapple 2000). Esteghlalian et al (2001) stated that the presence of lignin reduced cellulose hydrolysis by binding cellulolytic enzymes. Yang and Wyman (2006) also reported that protein binding capacities contained in lignin reduced the total enzyme activity. Enzyme accessibility of corn fiber can be enhanced by thermomechanical extrusion and PS reduction.

Total Sugar and Reducing Sugar. Total sugar and reducing sugar contents of extruded corn fiber at tested PSs were significantly increased ($P < 0.05$) compared with those of raw corn fiber. Effective damage of native corn fiber during extrusion resulted in higher dextrinization and in an increased amount of soluble polysaccharides (Table II). There is some evidence to support that reduction of PS increases specific surface area and the probability of the cellulose becoming hydrolyzed (Chandra et al 2007).

Protein Digestibility. Protein digestibility has been used as a nutritional indicator for fermentation because yeast cannot produce any exoprotease like humans and animals during fermentation. Additionally, protein nutritional value is mainly dependent on digestibility. Wang et al (2008) reported a strong linear correlation between protein digestibility of grain sorghum and fermentation efficiency in ethanol production. The data clearly showed that extrusion significantly increased ($P < 0.05$) the protein digestibility of corn fiber compared with that of raw corn fiber at tested PS reductions (Table II). This increase may be because of two phenomena caused by extrusion: protein denaturation, which may increase exposure of sites susceptible to enzymatic activity (Camire 2002), and inactivation of trypsin and chymotrypsin inhibitors, leading to improved digestibility (Alonso et al 1998). The higher the susceptibility of protein to pepsin, the more antitrypsin activity in the substrate. Increase in extrusion temperature (100–140°C) enhances the degree of inactivation of protease inhibitors in wheat flour, and consequently the protein digestibility values are increased (Singh et al 2007).

FAN. The effect of extrusion significantly increased the amount of FAN only at 0.3 > PS ≥ 0.15 mm ($P < 0.05$) (Table II). One of the factors limiting the production of high ethanol content by brewing yeast is nutritional deficiency (Casey and Ingledew 1986). When the substrate for fermentation is supplemented with a nitrogen source, it can promote rapid fermentation to a higher ethanol content without using genetically modified yeast (Yan et al 2011). Therefore, FAN in the fermenting substrate is important for yeast performance. The higher the FAN content in the fermented slurry, the faster the fermentation process. Yan et al (2010) made the same conclusion about the effect of FAN on fermentation efficiency of field-sprouted sorghum and wheat. The assimilable nitrogen was found to be related to FAN, and the addition of diammonium phosphate increased the fermentation rate (Blateyron et al 2003).

Semisimultaneous Saccharification and Fermentation. Table V shows the amount of ethanol production (after 48 h of saccharification) from raw and extruded corn fiber at different PS reductions. The data clearly show that 0.3 > PS ≥ 0.15 mm reduction after extrusion gave significantly ($P < 0.05$) higher ethanol content. Significant reduction in WAI and increase in WSI of extruded corn fiber 0.3 > PS ≥ 0.15 mm are indications of high conversion degree of starch and of dextrinization during extrusion for a higher release of reducing sugar for fermentation. As a result, chances of fermentation-inhibiting compounds are lower, and there is a reduction in the total environmental impact of the whole process (Sun and Cheng 2002; Kim and Holtzapple 2006). Similar to the trend observed in ethanol content, conversion percent of extruded corn fiber 0.3 > PS ≥ 0.15 mm was significantly ($P < 0.05$) higher than those of 1 > PS ≥ 0.5 and 0.5 > PS ≥ 0.3 mm. It can be explained by higher initial reducing sugar, protein digestibility, and FAN content and lower residual sugar content of extruded corn fiber (Tables II and V). These levels may help in keeping the yeast cells viable for a longer duration and producing high ethanol content. Wang et al (2008) also reported that normal grain sorghum that exhibited higher protein digestibility gave higher fermentation efficiency in ethanol production. Many research groups have used a variety of supplements to counter the nutrient deficiency. During extrusion, hydrolysis of protein in corn fiber might help with the release of more starch granules from the protein matrix and increase FAN content in the extruded sample (Pérez-Carrillo et al 2008), which will facilitate yeast growth and increase the ethanol content and conversion percent. Reduction in the number of viable cells and nutrient depletion could cause a lower rate of ethanol fermentation. In industrial biofuel production, PS reduction following extrusion of corn fiber

TABLE V
Ethanol Content and Fermentation Parameters of Raw and Extruded Corn Fiber at Different Particle Sizes (PSs)[z]

Sample	PS (mm)	Initial Reducing Sugar (mg/g)	Residual Reducing Sugar (mg/g)	Ethanol Content (g/L)	Conversion (%)	Biomass Loss (%)	Yeast Growth (*A*)	Cell Viability (CFU/mL)
Raw	1 > PS ≥ 0.5	28.17 ± 1.56d	11.29 ± 0.10c	44.63 ± 0.81e	59.87 ± 1.88d	29.65 ± 0.80c	0.82 ± 0.02d	(69.5 ± 1.95) × 10^8b
	0.5 > PS ≥ 0.3	31.45 ± 0.66c	11.20 ± 0.01c	50.39 ± 0.81cd	64.37 ± 0.70b	32.03 ± 0.24c	0.55 ± 0.04e	(71.00 ± 1.31) × 10^8b
	0.3 > PS ≥ 0.15	35.72 ± 0.18b	13.62 ± 0.04a	57.59 ± 1.63b	61.87 ± 0.07cd	38.41 ± 0.66b	0.89 ± 0.02c	(70.5 ± 1.12) × 10^8b
Extruded	1 > PS ≥ 0.5	35.64 ± 0.07b	13.70 ± 0.14a	47.51 ± 1.63de	61.56 ± 0.30d	45.22 ± 1.05a	0.85 ± 0.01cd	(75.5 ± 1.61) × 10^8ab
	0.5 > PS ≥ 0.3	34.62 ± 0.07b	12.57 ± 0.16b	54.71 ± 1.63bc	63.70 ± 0.55bc	42.72 ± 0.17ab	1.23 ± 0.05b	(88.00 ± 1.41) × 10^8a
	0.3 > PS ≥ 0.15	39.00 ± 0.09a	12.41 ± 0.10b	69.11 ± 0.81a	68.18 ± 0.33a	45.80 ± 0.08a	1.36 ± 0.02a	(85.00 ± 1.41) × 10^8ab

[z] Means of three replicates based on least significant difference procedure at α = 0.05 level. Means with the same letter in the same column are not significantly different. *A* = absorbance at 600 nm, and CFU = colony forming unit.

may produce a better feedstock because of its higher enzyme accessibility and higher content of readily available reducing sugars for fermentation.

Thermomechanical extrusion and PS reduction ($1 > PS \geq 0.5$, $0.5 > PS \geq 0.3$, and $0.3 > PS \geq 0.15$ mm) significantly increased total sugars, reducing sugars, protein digestibility, and FAN contents ($0.3 > PS \geq 0.15$ mm) for yeast growth and fermentation ($P < 0.05$). In addition, extrusion and PS reduction ($1 > PS \geq 0.5$ and $0.5 > PS \geq 0.3$ mm) also significantly reduced total phenolics content degraded from pretreated lignocellulose corn fiber and led to decreasing the adverse conditions for fermentation ($P < 0.05$). Therefore, extrusion and PS reduction pretreatment have great potential to increase functional properties for fermentation, ethanol yield, and conversion percent of corn fiber.

CONCLUSIONS

Reduction in WAI and increase in WSI of extruded corn fiber at $0.3 > PS \geq 0.15$ mm are indications that extrusion accelerated the degradation of corn fiber structure and release of water-soluble compounds. Thermomechanical extrusion significantly increased total sugars, reducing sugars, protein digestibility, and FAN contents of $0.3 > PS \geq 0.15$ mm for yeast growth and fermentation ($P < 0.05$). In addition, SDF of the extruded corn fiber can be significantly increased by enhancing the release of the soluble fraction from hemicelluloses as a result of heating during extrusion. Therefore, extrusion pretreatment and PS reduction at $0.3 > PS \geq 0.15$ mm have great potential to modify physiochemical and functional properties for increased bioconversion rate of corn fiber in ethanol production.

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