

# Pretreatment of Wet-Milled Corn Fiber to Improve Recovery of Corn Fiber Oil and Phytosterols

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## ABSTRACT

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The phytosterol-containing oil in the corn fiber (corn fiber oil) has potential use as a natural low-density lipoprotein (LDL) lowering nutraceutical but its low concentration (1–3%) makes it difficult and expensive to extract. Pretreatment of corn fiber with dilute acid or glucosidases removed nonlipid components of fiber, producing oil-enriched fractions that should be more amenable to efficient and inexpensive oil extraction. Acid, as well as enzymes, significantly increased the content of corn fiber oil and its phytosterol compounds by hydrolyzing (and removing)

the starch and nonstarch (cell wall) polysaccharides from the wet-milled corn fiber. Dual treatment of the fiber with acid and enzyme greatly increased the concentrations of corn fiber oil and its phytosterol components, compared with acid or enzyme treatments alone. Depending on the treatment, the oil concentration in the residual solids increased from 0.3 to 10.8% (21–771% increase in conc.) and the total phytosterol concentration increased from 19.8 to 1256.2 mg/g of fiber (11–710% increase in conc.) compared with untreated fiber.

Corn fiber has a potentially valuable oil that contains compounds shown to lower serum LDL-cholesterol levels (Moreau et al 1998; Hicks and Moreau 2001). These compounds are called phytosterols and there are three main classes of phytosterols in corn fiber oil: 1) ferulate phytosterol esters (FPE), 2) free phytosterols (St), and 3) fatty acyl phytosterol esters (St:E). Due to nutraceutical value, commercial products based on corn fiber oil can potentially sell for \$11.0 to 22.0/kg (\$5 to 10/lb), based on current market retail prices of products similar to corn fiber oil. The amount of oil in corn fiber is, however, very low,  $\approx$ 1.5 to 3.0% by weight. Due to the low oil concentration, the extraction efficiency is low (60–70%) (i.e., only 1.0–2.0% yield based on starting fiber). Moreover, extracting such a small amount of oil requires processing large amounts of fiber with long residence times in the extractors, and therefore can be expensive.

Increasing the concentration of corn fiber oil and its phytosterol compounds before extraction could significantly reduce the cost of extraction. Due to the potential high market value, a small increase in concentration of oil would have a significant economic impact.

Corn fiber is composed of 40% hemicellulose, 25% starch, 12% cellulose, 10% protein, 3% oil, and 10% other substances such as lignin and ash. Starch, hemicellulose, and cellulose can be removed from the fiber by dilute acid hydrolysis or using enzymes that break down hemicellulose and starch. Several methods, using dilute acid, have been developed to hydrolyze carbohydrate polymers of corn fiber into sugars for fermentation to ethanol (Grohmann and Bothast 1997; Dien et al 1999)

In this study, the effects of different enzymes, combination of enzymes, and dilute acid were evaluated for removing nonlipid components (polysaccharides) from the fiber, and thereby increasing the concentrations of corn fiber oil and its phytosterol compounds in the residue fiber.

## MATERIALS AND METHODS

A yellow dent corn hybrid (Pioneer 3394) grown during the 1999 crop season at the Agricultural Engineering Farm, University of Illinois at Urbana-Champaign, was field dried to  $\approx$ 16% moisture content and combine-harvested. Corn samples were hand-cleaned to remove broken corn and foreign material, packaged in plastic bags and stored at 4°C until wet-milled. The whole kernel moisture content of the samples was measured using the 103°C convection oven method (Approved Method 44-15A, AACC 2000).

Conventional corn wet-milling was done using the 1-kg laboratory corn wet-milling procedure as outlined by Eckhoff et al (1993). Four wet-milling runs were done in parallel and combined to obtain sufficient fiber for further experimentation. The moisture content of the mixed fiber was determined using the two-stage convection oven method (Approved Method 44-15A, AACC 2000).

Fiber obtained from the wet-milling procedure was pretreated with four different enzymes (Table I) or combination of enzymes: 1) 2.5 mL each of xylanase, cellulase, and  $\beta$ -glucanase; 2) 2.5 mL each of xylanase, cellulase, and amylase; 3) 2.5 mL each of xylanase and cellulase; 4) 5.0 mL of  $\beta$ -glucanase and an acid (0.5%  $H_2SO_4/H_2O$  [v/v] at 100°C) before extracting the corn fiber oil and phytosterols. All enzymes used were supplied as gifts from commercial enzyme suppliers. The amylase (Termamyl 120L) was a gift from Novozymes. Relevant enzyme activities for the preparations were measured for xylanase, cellulase,  $\beta$ -glucanase, and amylase as an increase in reducing group equivalents according to the procedure of Johnston et al (1998), in acetate buffer, pH 4.5, at 40°C. Activity units were defined as the increase in reducing groups, equivalent to an increase of 1  $\mu$ g of sugar/min using barley  $\beta$ -glucan, carboxymethyl cellulose, oat spelt xylan, or corn starch as the substrate. Amylase activity was measured using gelatinized starch and the native starch activity used an ungelatinized starch suspension. Amylase units for Termamyl were defined and supplied by the manufacturer as 120 KNU/g of enzyme preparation using soluble starch. Relevant enzyme activities and protein content of the preparations are given in Table I.

For the enzyme treatments,  $\approx$ 5 g of ground fiber (ground to 20 mesh in a Wiley mill) was added to a 500-mL glass screw-top bottle to which 100 mL of 0.05M acetate buffer was added to maintain pH 4.5. The sample was equilibrated for 10 min in a water bath at 48°C before enzyme addition. After enzyme addition, the sample was incubated at 48°C for 4 hr, with intermittent gentle mixing every 30 min. After incubating, the fiber sample was screened using a 325-mesh screen and washed with 100 mL of distilled water. The screened fiber samples were dried and the moisture contents were determined using the two-stage convection oven method (Approved Method 44-15A, AACC 2000). For acid

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treatment of fiber at 100°C, the procedure described by Grohmann and Bothast (1997) was used. Oil and phytosterols were extracted from the dried fiber samples using an accelerated solvent extractor (Dionex ASE200). Ground fiber samples (1–2 g) were placed in 11-mL sample extraction cells. The extraction conditions in the cells were 1,000 psi pressure, 100°C, 5-min heat time, 10-min start time, three static cycles, 100% flush volume, 60-sec purge time.

For HPLC analysis, part of the sample was removed from the extract, as previously outlined by Moreau et al (1996). The lipid classes in the samples were separated and quantified by a modified version of an HPLC technique developed by Moreau et al (1996). The ternary-gradient HPLC modular system (model 1050 Hewlett Packard, Avondale PA) included two detectors connected in series. The first was a fixed-wavelength UV-visible detector (HP model 1050) set at 295 nm. The second was an evaporative light-scattering detector (Alltec-Varex Mark III, Alltech Associates, Deerfield, IL) operated at 40°C with nitrogen as a nebulizing gas at a flow rate of 1.60 L (STP)/min. The column was a Chromsep Cartridge LiChrosorb DiOL, 5 µm, 3 × 100 mm (Chrompack, Raritan, NJ). The mobile-phase gradient of hexane, 2-propanol, and acetic acid was the same as used by Moreau et al (1996) and the flow rate was constant at 0.5 mL/min. The remainder of the solvent was dried under nitrogen and heat (using an N-EVAP analytical evaporator, Organomation Associates, Berlin, MA) to determine the total extractable oil.

All of the pretreatments (enzyme and acid) were compared with a fiber sample with only buffer addition. The effects of adding 0.25–2.5 mL of enzyme for a combination of two enzymes (xylanase and cellulase) and 0.5–5.0 mL of β-glucanase were also evaluated on the oil content and total phytosterol yield of the fiber samples.

The neutral sugar composition of treated fiber samples was determined by HPLC after acid hydrolysis (Saulnier et al 1995) using 1N H<sub>2</sub>SO<sub>4</sub> at 100°C for 1.5 hr. Samples were neutralized by adding BaCO<sub>3</sub> and the reaction was monitored using pH paper. Samples were transferred to 50-mL centrifuge tubes and centrifuged to remove BaSO<sub>4</sub>. The supernatant was filtered through glass wool into glass vials. The sample was evaporated to dryness under a N<sub>2</sub> stream and then reconstituted in 0.5 mL of water. The samples were filtered through 0.2-µm PVDF Acrodisc filters. Samples were analyzed on a BioRad Aminex HPX-87P column

(300 × 7.8 mm) with de-ashing guard cartridges. The column temperature was controlled at 60°C using a BioRad column heater. Degassed deionized water was used as the eluent at 0.6 mL/min flow rate. Samples (10 µL) were injected using an Alcott auto injector. A refractive index detector (HP 1047A) measured eluted sugars. Data was collected and analyzed using ChromPerfect LE data software.

Based on the results of the first experiment, another experiment was done with four different fiber pretreatments. Three treatments were done with dilute sulfuric acid at 25, 100, and 121°C. The fourth treatment was a combination of acid and enzyme treatments. The fiber was first treated with dilute acid at 100°C, rinsed with 250 mL of water, followed by adding acetate buffer, and then treating with 1.0 mL of cellulase enzyme. The fiber fraction for this experiment was obtained from wet-milling a different yellow dent corn hybrid (Pioneer 33A14) grown at the University of Illinois experiment station during the 2000 crop season. The protocol for the acid or the enzyme treatment followed for the second experiment was the same as described for the first. The experiment was maintained at 25°C using a recirculating water bath, and maintained at 100 and 121°C using an autoclave.

Based on the results of the second experiment, a third experiment was done in which the fiber fraction from a Midwestern wet-mill ethanol plant was treated with dilute sulfuric acid (0.75 H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O [v/v]) at 150°C for 5 and 15 min, using the procedure described by Dien et al (1999). The experiment was maintained at 150°C using a batch reactor equipped with an external hydro-mixer. For the second and third experiments, the weight percent difference was measured between the untreated and the treated fiber samples to determine the amount of nonlipid component removed from the fiber fraction.

All the fiber treatments (acid, enzyme, or combination) for the three experiments were done in duplicate. Fiber samples from both replicates were analyzed using HPLC at least twice. Results presented are the means of the multiple analyses.

## RESULTS AND DISCUSSION

The effects of different enzymes and acid treatment on removing carbohydrate polymers from corn fiber, and thereby increasing the concentration of corn fiber oil, were significant.

TABLE I  
Enzyme Activity Profiles Measured at pH 4.5 in Sodium Acetate Buffer

| Enzyme Preparation | Protein Content (mg/mL) | Cellulase Activity (U/mg) | β-Glucanase Activity (U/mg) | Xylanase Activity (U/mg) | Amylase Activity <sup>a</sup> (U/mg) | Native Starch (U/mg) |
|--------------------|-------------------------|---------------------------|-----------------------------|--------------------------|--------------------------------------|----------------------|
| Amylase            | nd                      | nd                        | nd                          | nd                       | 120                                  | nd                   |
| Cellulase          | 42.3                    | 1,862                     | 7,700                       | 626                      | 2,930                                | 200                  |
| Xylanase           | 13.6                    | trace                     | 842                         | 6,920                    | 2,355                                | 115                  |
| β-Glucanase        | 23.9                    | 594                       | 5,560                       | 926                      | 3,700                                | 2,677                |

<sup>a</sup> Activity units supplied by the manufacturer as per gram of preparation in KN units.

TABLE II  
Concentration of Corn Fiber Oil, Ferulate Phytosterol Esters (FPE), Free Phytosterols (St), and Fatty Acyl Phytosterol Esters (St:E) Extracted from Different Pretreated Fiber Samples<sup>a</sup>

| Pretreatment           | Oil (%)   | FPE (mg/100 g of fiber) | St (mg/100 g of fiber) | St:E (mg/100 g of fiber) | Total Phytosterols (mg/100 g of fiber) |
|------------------------|-----------|-------------------------|------------------------|--------------------------|--|
| Buffer                 | 1.4 ± 0.1 | 92.9 ± 2.6              | 35.3 ± 0.9             | 50.9 ± 2.2               | 179.1 ± 3.9                            |
| Sulfuric acid at 100°C | 3.1 ± 0.0 | 142.1 ± 8.4             | 56.2 ± 6.0             | 153.7 ± 0.7              | 351.9 ± 15.1                           |
| Enzyme 1 <sup>b</sup>  | 3.6 ± 0.4 | 138.7 ± 7.0             | 75.9 ± 9.6             | 187.0 ± 2.6              | 401.6 ± 19.2                           |
| Enzyme 2 <sup>c</sup>  | 2.7 ± 0.1 | 123.2 ± 4.4             | 62.6 ± 3.2             | 1,23.7 ± 13.6            | 309.4 ± 21.2                           |
| Enzyme 3 <sup>d</sup>  | 2.9 ± 0.1 | 122.3 ± 0.4             | 58.7 ± 0.3             | 153.0 ± 2.5              | 334.1 ± 2.5                            |
| Enzyme 4 <sup>e</sup>  | 2.8 ± 0.0 | 120.5 ± 5.8             | 62.8 ± 6.1             | 110.8 ± 5.6              | 294.1 ± 17.4                           |

<sup>a</sup> Obtained from wet milling yellow dent corn hybrid Pioneer 3394. All yields are means of two values.

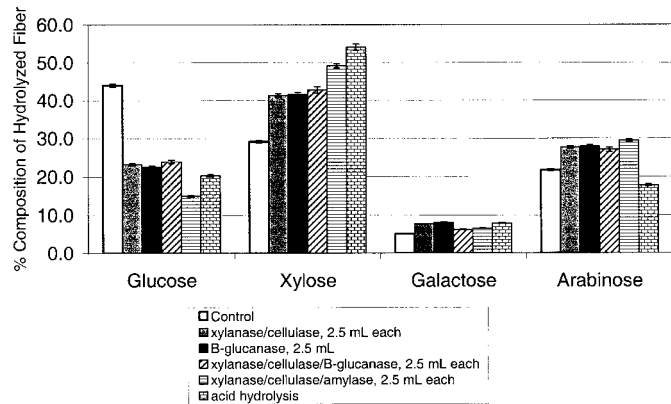
<sup>b</sup> 2.5 mL of xylanase + 2.5 mL of cellulase + 2.5 mL of β-glucanase.

<sup>c</sup> 2.5 mL of xylanase + 2.5 mL of cellulase + 2.5 mL of amylase.

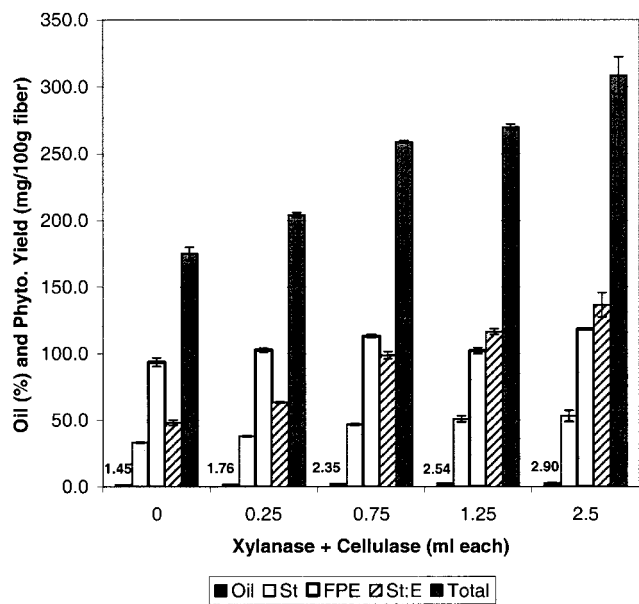
<sup>d</sup> 2.5 mL of xylanase + 2.5 mL of cellulase.

<sup>e</sup> 5.0 mL of β-glucanase.

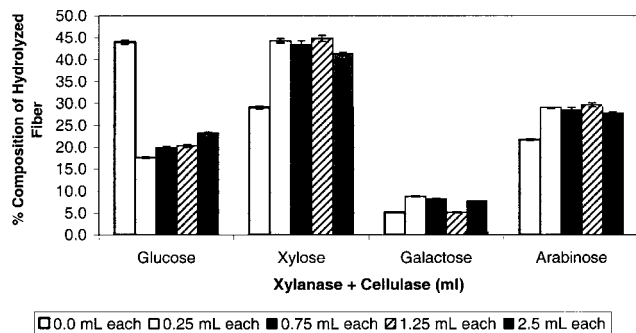
Depending on the treatment, the oil content of the pretreated fiber increased by  $\approx 1.3$  to 2.2% (93–157% increase in conc.) when compared with the control sample (Table II). Significant effects of the acid and the enzyme treatments were also observed on the individual and total phytosterol composition of the corn fiber oil. Depending on the treatment, the concentration of FPE in the corn fiber oil increased by  $\approx 27.6$  to 49.2 mg/100 g of fiber (30–53%



**Fig. 1.** Sugar composition (%) of residual solids of wet-milled corn fiber from a control, acid, and different enzyme treatments.



**Fig. 2.** Corn fiber oil (%) and individual and total phytosterols (mg/100 g of fiber) in residual fiber after treatment with different amounts of xylanase plus cellulase (mL).



**Fig. 3.** Sugar composition (%) of hydrolyzed residual wet-milled corn fiber after treatment with different amounts of xylanase and cellulase.

increase in conc.), the St increased by  $\approx 20.9$  to 40.6 mg/100 g of fiber (59–115% increase in conc.), and the St:E increased by  $\approx 59.9$  to 136.1 mg/100 g of fiber (118–267% increase in conc.) when compared with the control sample. Overall, depending on the treatment, the total phytosterol concentration in the corn fiber oil increased from 115 to 222.5 mg/100 g of fiber (64–124% increase in conc.), compared with the control sample (Table II).

The largest increases in the oil concentration in the fiber (157%) and the total phytosterol concentration in the corn fiber oil (124%) were observed with the enzyme treatment that included a combination of xylanase, cellulase, and  $\beta$ -glucanase enzymes (Table II). The second largest increase in the oil concentration in the fiber (121%) and its total phytosterol concentration (96%) was observed with acid hydrolysis. The significant increases in percentage of oil and phytosterol compounds in the pretreated fiber with the acid and different enzyme treatments were due to the breakdown and removal of nonlipid components of fiber (residual starch and cell wall material) by the different enzymes or by the dilute sulfuric acid. Analysis of the sugars in the residual solids after hydrolysis for pretreatment showed that different enzyme treatments reduced the ratio of glucose (starch and cellulose) to the total carbohydrate anywhere from 45 to 66% (Fig. 1). Acid treatment reduced ratio of glucose to the total carbohydrate by  $\approx 46\%$ .

The typical composition of wet-milled corn fiber is  $\approx 40\%$  hemicellulose, 12% cellulose, 25% starch, 10% protein, 10% lignin and ash, and  $\approx 3\%$  oil. Hemicellulose in corn fiber is primarily an arabinoxylan. Comparison of the sugars in the residual solids after acid or enzyme pretreatment and the control sample indicated that the enzyme treatments preferentially removed the xylan component and acid treatments preferentially removed the arabinose component.

The effects of the amount of enzyme used in the treatments were significant on corn fiber oil and its phytosterol concentration. As the amounts of xylanase and cellulase mixture increased from 0.0 to 2.5 mL, the concentrations of corn fiber oil and its total phytosterol increased by  $\approx 100$  and 76%, respectively (Fig. 2). An enzyme amount of 2.5 mL of the xylanase and cellulase mixture had the most significant effect in reducing the xylan component of the corn fiber compared with other amounts of enzyme (Fig. 3). Overall, all of the amounts of enzyme (of the xylanase and cellulase mixture) used (0.25–2.5 mL) had a comparable effect on hydrolyzing the starch and cellulose (glucose) from the fiber fraction. No significant differences in the arabinan content of the hydrolyzed fiber were observed between different amounts of enzyme (0.25–2.5 mL) for the xylanase and cellulase mixture.

A similar trend (as observed with the xylanase and cellulase mixture) of an increase in the corn fiber oil yield and its phytosterol yield was observed when the concentration of  $\beta$ -glucanase was varied from 0.0 to 5.0 mL (Fig. 4). As the concentration of  $\beta$ -glucanase increased from 0.0 to 5.0 mL, the corn fiber oil and its total phytosterol concentration increased by  $\approx 108$  and 79%, respectively. Analysis of the sugar composition of the hydrolyzed fiber showed small differences between different enzyme concentrations of  $\beta$ -glucanase. However, no conclusive effect of enzyme treatment on the sugar composition of hydrolyzed fiber could be ascertained (Fig. 5). In a previous study (Singh and Johnston 2002), this particular preparation digested ungelatinized starch, which may account for the significant decrease in the glucose content even with lowest level of addition.

Results from this study suggested that both enzyme and acid hydrolysis of corn fiber could effectively remove the nonlipid components of the fiber, increasing the corn fiber oil and its phytosterol content of the residual fiber. It appeared that the action of the acid on the fiber was predominantly on the residual starch and cellulose and the arabinose component of the fiber fraction, whereas the action of the enzymes used in this study was predominantly on the xylan component and the residual starch in the fiber fraction.

Based on the results of the first experiment, another experiment was done in which laboratory wet-milled fiber samples were treated with dilute sulfuric acid at three different temperatures and in combination with enzyme (Table III). These different treatments were compared with untreated wet-milled fiber (control sample).

A significant increase in the concentration of the corn fiber oil and two phytosterol classes (St and St:E) was observed with acid treatment at 25°C compared with the control sample (Table III). An order of magnitude increase in the corn fiber oil concentration and its phytosterol composition was observed as the temperature of acid was increased to 100 and 121°C. At 121°C, the dilute sulfuric acid treatment increased the concentration of corn fiber oil and its total phytosterol composition by  $\approx 771$  and 710%, respectively, when compared with the control sample. A significant increase in the corn fiber oil concentration and its phytosterol composition was observed with the combined dilute acid (at 100°C) and enzyme treatments when compared with the control sample and the dilute acid alone treatment at 100°C. However, the combined acid and enzyme treatment gave lower corn fiber oil concentration and phytosterol composition when compared with

the dilute acid alone treatment at 121°C. Combined enzyme and dilute acid treatment at 121°C was not evaluated. Based on the results with dilute acid at 100°C, it seems that the acid and enzyme treatment together might be more effective than acid or enzyme treatment alone in increasing the concentration of corn fiber oil and its phytosterol composition. Higher temperature acid treatment is critical in increasing in the corn fiber oil concentration and its phytosterol composition.

Increased concentrations of corn fiber oil and its phytosterol compounds due to the acid treatment alone or with both acid and enzyme treatments are partially due to the breakdown and removal of nonlipid components in the fiber fraction. As the temperature of acid treatment increased from 25 to 121°C, the percentage reduction in the weight of the treated fiber increased from  $\approx 44$  to 76% (Table III). With both acid (at 100°C) and enzyme treatment the reduction in weight percent of the treated fiber was  $\approx 80\%$ . The reduction in the weight percent cannot solely account for the increase in the oil yield and the yield of phytosterol compounds. The percentage weight reduction with both acid and enzyme treatment was higher compared with the acid alone treatment (at 121°C) but the oil and phytosterol yields were lower. This suggests that maybe acid treatment not only removes the nonlipid component of the fiber but also affects the extraction of the oil. More detailed study is required.

Based on the results from the second experiment, a third experiment (Table IV) was done to evaluate the effect of high temperature (150°C) dilute acid treatment for short reaction times

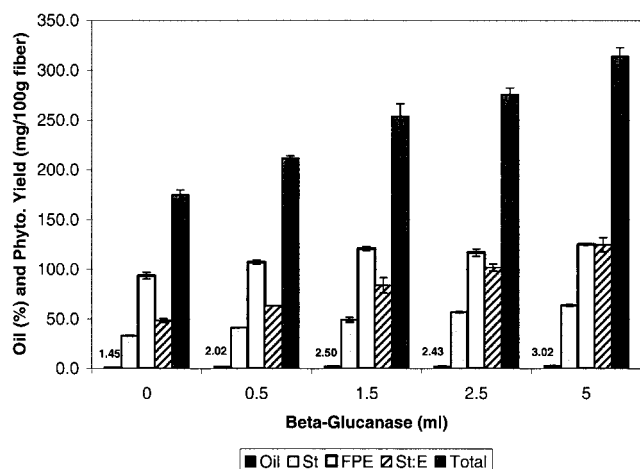


Fig. 4. Corn fiber oil (%) and individual and total phytosterol yields (mg/100 g of fiber) in residual fiber after treatment with different amounts of  $\beta$ -glucanase (mL).

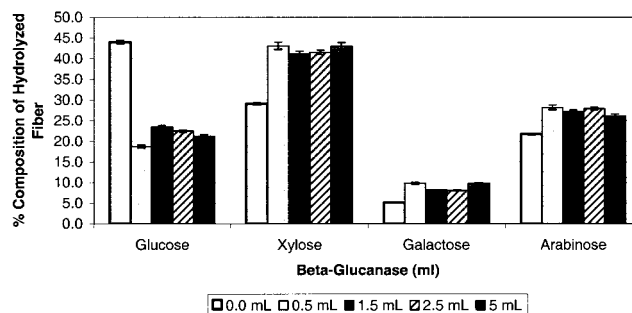


Fig. 5. Sugar composition (%) of hydrolyzed residual wet-milled corn fiber after treatment with different amounts of  $\beta$ -glucanase (mL).

TABLE III  
Concentrations of Corn Fiber Oil, Ferulate Phytosterol Esters (FPE), Free Phytosterols (St), and Fatty Acyl Phytosterol Esters (St:E) Extracted from Different Pretreated Fiber Samples<sup>a</sup>

| Pretreatment                                 | Weight Loss (%) | Oil (%)        | FPE (mg/100 g of fiber) | St (mg/100 g of fiber) | St:E (mg/100 g of fiber) | Total Phytosterols (mg/100 g of fiber) |
|--|-----------------|----------------|-------------------------|------------------------|--------------------------|--|
| Control                                      | 0               | 1.4 $\pm$ 0.0  | 88.4 $\pm$ 0.3          | 40.9 $\pm$ 1.2         | 47.6 $\pm$ 2.1           | 176.9 $\pm$ 3.6                        |
| Sulfuric acid at 25°C                        | 43.9            | 1.7 $\pm$ 0.0  | 84.3 $\pm$ 0.1          | 71.1 $\pm$ 2.8         | 41.3 $\pm$ 0.3           | 196.7 $\pm$ 3.2                        |
| Sulfuric acid at 100°C                       | 64.3            | 5.8 $\pm$ 0.0  | 224.9 $\pm$ 0.7         | 321.5 $\pm$ 8.1        | 120.4 $\pm$ 2.8          | 666.8 $\pm$ 6.1                        |
| Sulfuric acid at 121°C                       | 76.1            | 12.2 $\pm$ 0.0 | 386.6 $\pm$ 16.6        | 595.6 $\pm$ 10.6       | 450.9 $\pm$ 34.8         | 1,433.1 $\pm$ 62.0                     |
| Sulfuric acid at 100°C + enzyme <sup>b</sup> | 80.0            | 7.9 $\pm$ 0.1  | 327.1 $\pm$ 2.4         | 441.2 $\pm$ 10.1       | 259.0 $\pm$ 6.4          | 1,027.2 $\pm$ 1.3                      |

<sup>a</sup> Obtained from wet milling yellow dent corn hybrid Pioneer 33A14. All yields are means of two values.

<sup>b</sup> Sulfuric acid + rinse with water + 1.0 mL of cellulase.

TABLE IV  
Concentrations of Corn Fiber Oil, Ferulate Phytosterol Esters (FPE), Free Phytosterols (St), and Fatty Acyl Phytosterol Esters (St:E) Extracted from Dilute Sulfuric Acid Pretreated Fiber Samples<sup>a</sup>

| Pretreatment              | Weight Loss (%) | Oil (%) | FPE (mg/100 g of fiber) | St (mg/100 g of fiber) | St:E (mg/100 g of fiber) | Total Phytosterols (mg/100 g of fiber) |
|---------------------------|-----------------|---------|-------------------------|------------------------|--------------------------|--|
| Untreated fiber (control) | 0               | 1.6     | 79.9                    | 36.2                   | 129.0                    | 245.1                                  |
| Sulfuric acid at 150°C    |                 |         |                         |                        |                          |  |
| 5 min                     | 70              | 5.7     | 252.9                   | 361.0                  | 371.0                    | 984.9                                  |
| 15 min                    | 76              | 7.0     | 244.0                   | 306.0                  | 370.0                    | 920.0                                  |

<sup>a</sup> Obtained from a Midwestern U.S. corn wet-milling plant. All yields are means of two values

(5–15 min). Significant increases in the concentration of oil from 1.6 to 5.7% (256% increase in conc.) and total phytosterol compounds from 245.1 to 984.9 mg/100 g of fiber (302% increase in conc.) were observed for 5-min reaction time compared with the control sample. Treatment of fiber at 150°C for additional an 10 min further increased the oil content of the fiber by 1.3% (≈81%). However, no significant increase in the recovery of phytosterol compounds was observed.

New technologies, using dilute acid or enzymes, are being developed to hydrolyze corn fiber into fermentable sugars. These technologies would have no negative effects on the corn fiber oil and its phytosterol content. The residual mass left after acid or enzyme hydrolysis of corn fiber would have a higher concentration of the corn fiber oil. A higher concentration of oil in fiber would make its extraction more efficient and economical. Economically, acid treatment of fiber would be more feasible compared with enzyme treatment. However, use of acid could cause corrosion problems and also would pose environmental concerns. Use of enzymes may be expensive but may still be justified due to the high potential value of corn fiber oil and its phytosterol compounds.

### CONCLUSIONS

A significant effect of enzyme, combination of different glycosidase enzymes, as well as combination of dilute acid and glycosidase enzyme treatment, on removing nonlipid component and thereby increasing the concentration of oil and phytosterol composition in corn fiber was observed. Depending on the treatment, the oil concentration of the fiber increased anywhere from 0.3 to 10.8% (21–771% increase in conc.) and the total phytosterol level increased from 19.8 to 1,256.2 mg/g of fiber (11–710% increase in conc.) when compared with the untreated wet-milled fiber. A combination of dilute acid and enzyme treatment had the most significant effect on increasing the concentration of corn fiber oil and its phytosterol composition, when compared with dilute acid or enzyme treatment alone. The study also shows that elevated temperature for dilute acid treatment plays an important role in increasing corn fiber oil and its phytosterol composition. The higher the temperature, the greater the increase in concentration of oil and phytosterols. These increases in oil yield and phytosterol yield from the fiber were probably due to removal of cell walls and the residual starch in the fiber fraction. A detailed economic analysis of the process needs to be done to

evaluate the feasibility of using enzymes, acid, or a combination to increase the oil content of corn fiber.

New processes are being developed to convert corn fiber into ethanol. Efforts are being made to saccharify corn fiber to simple hexoses and pentoses for conversion to ethanol by recombinant organisms. The remaining corn fiber (resistant to hydrolysis) would be a convenient starting material for extracting corn fiber oil if the oil was not hydrolyzed or lost from residual fiber that survived the enzymatic and or acid saccharification process. This study shows that the oil and phytosterols remain intact in the residue left after the enzymatic and acid hydrolysis of fiber.

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### LITERATURE CITED

- American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Method 44-15A. The Association: St. Paul, MN.
- Dien, B. S., Iten, L. B., and Bothast, R. J. 1999. Conversion of corn fiber to ethanol by recombinant *Escherichia coli* strain FBR3. *J. Ind. Microbiol. Biotechnol.* 22:575-581.
- Eckhoff, S. R., Rausch, K. D., Fox, E. J., Tso, C. C., Wu, X., Pan, Z., and Buriak, P. 1993. A laboratory wet-milling procedure to increase reproducibility and accuracy of product yields. *Cereal Chem.* 70:723-727.
- Grohmann, K., and Bothast, R. J. 1997. Saccharification of corn fiber by combined treatment with dilute sulfuric acid and enzymes. *Process Biochem.* 32:405-415.
- Hicks, K. B., and Moreau, R. A. 2001. Phytosterols and phytostanols: Functional food cholesterol busters. *Food Technol.* 55:63-67.
- Johnston, D. B., Shoemaker, S. P., Smith, G. M., and Whitaker, J. R. 1998. Kinetic measurements of cellulase activity on insoluble substrates using disodium 2,2'-bichinchoninate. *J. Food Biochem.* 22:301-319.
- Moreau, R. A., Hicks, K. B., Nicolosi, R. J., and Norton, R. A. 1998. Corn fiber oil its preparation and use. US patent 5,843,499.
- Moreau, R. A., Powell, M. J., and Hicks, K. B. 1996. Extraction and quantitative analysis of oil from commercial corn. *J. Agric. Food Chem.* 44:2149-2154.
- Singh, V., and Johnston, D. B. 2002. Pasting properties and surface characteristics of starch obtained from an enzymatic corn wet milling process. *Cereal Chem.* 79:523-527.

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