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Novel Approach of Corn Fiber Utilization

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Abstract

The corn wet milling process produces a 10% (w/w of the processed corn) byproduct called corn fiber, which is utilized worldwide as a low-value feedstock for cattle. The aim of this study was to find a higher value use of corn fiber. The main fractions of corn fiber are: 20% starch, 40% hemicellulose, 14% cellulose, and 14% protein. Extraction of the highly valuable, cholesterol-lowering corn fiber oil is not feasible owing to its low (2% w/w) concentration in the fiber. The developed technology is based on simple and inexpensive procedures, like washing with hot water, dilute acid hydrolysis at 120°C, enzymatic hydrolysis of cellulose, screening, drying, and extraction. The main fractions are sharply separated in the order of starch, hemicellulose, cellulose, lipoprotein, and lignin). The lipoprotein fraction adds up to 10% of the original dry corn fiber, and contains 45% corn fiber oil, thus yielding more oil than direct extraction of the fiber. It is concluded that the defined method makes the extraction of the corn fiber oil economically feasible. The fractionation process also significantly increases the yield of cholesterol-lowering substances (sterols and sterol-esters). At the same time clear and utilizable fractions of monosaccharides, protein, and lignin are produced.

Index Entries: Bioethanol; corn fiber hydrolysis; corn fiber oil; phytosterol.

Introduction

Corn fiber is an abundant and inexpensive byproduct of the corn wet milling process, comprising about 10% of the processed dry corn. Corn fiber is a main ingredient of corn gluten feed (CGF), which is a low-value feed for cattle. Some countries, like the US export the corn fiber overseas, as the demand of the CGF is lower than the supply. The worldwide increasing demand for bioethanol results in more corn processing, but at the same time the demand for animal feed—depending on the country—has remained stagnant or is decreasing. The value of the fiber in the EU-25 fluctuates around 0.1 $/kg. The aim of this study was to find a more economic way of utilizing corn fiber.

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Opponents of bioethanol argue that its production is not economical. In fact, it is difficult to define the price of bioethanol because it significantly depends on the substrate from which it is made. It is necessary to define the raw material and the technology of production when economic feasibility of bioethanol is surveyed. Abundant and cheap substrates for bioethanol fermentation are still in demand. Such substrates could be agro-industrial byproducts like corn fiber or wheat bran. The American example shows that the significant increase in production of ethanol from corn leads to a surplus of corn fiber, so it is important to search for alternative options for utilizing the excess corn fiber.

Not only is the bioethanol production potential of the corn fiber valuable and noteworthy, but also the extractable lipid content of the fiber (called corn fiber oil) contains valuable cholesterol-lowering substances like phytosterols and phytosterol-esters (like ferulate-phytosterol-esters) *(1–15)*. According to our analysis, corn fiber oil contains phytosterols in the highest concentration (6–10%) ever reported in edible plant oils. Phytosterols are effective reducers of LDL-cholesterol levels in humans. A high LDL-cholesterol level is the main reason for heart and circulatory diseases. The oil also contains some tocopherols and tocotrienols, which are commonly known as vitamin-E. Ferulic acid, tocopherols, and tocotrienols are effective antioxidants. However, extraction of the highly valuable corn fiber oil is not economically feasible owing to its low (2–2.5% w/w) concentration in the fiber.

Dilute acid hydrolysis of lignocelluloses and corn residues has been widely discussed in the literature *(16–27)*. Dilute (0.5–5% w/w) acid pretreatment at the temperature of 120–180°C can effectively remove the hemicelluloses from the lignocellulose matrix. It has been shown that there is a direct relationship between dilute acid-mediated xylan removal and concomitant increased enzymatic breakdown of cellulose *(16)*. It seems clear that the efficiency of dilute acid hydrolysis—in terms of promoting enzymatic digestibility—depends on the hemicelluloses/cellulose ratio. Torget et al. *(16)* reported 90–100% digestibility of corn cob cellulose after dilute acid pretreatment, whereas corn stover, and wood samples did not reach this value. The hemicellulose/cellulose ratio of corn cob is 0.89, whereas at the other samples it varies between 0.43 and 0.64. Enzymatic hydrolysis of cellulose has been studied for a long time *(16,17)*. Pretreated celluloses usually need about 40 g FPU/g cellulose enzyme loading, plus 5–15 g IU/g cellulose β-glucosidase activity to achieve satisfactory glucose yield *(16)*.

Singh et al. studied the removal of nonlipid components of corn fiber, and thereby the increase of corn fiber oil and phytosterol concentration in the remaining fiber *(13–15)*. They examined the effect of dilute sulfuric acid and enzymatic pretreatments *(13)*. Depending on the treatment, the oil concentration of the fiber increased from 1.4% to 12.2% and the total phytosterol level increased from 2 to 14 mg/g fiber. This result was obtained

by dilute sulfuric acid pretreatment at the temperature of 121°C. In this case the weight loss was 76.1%. The authors also determined that the typical composition of the native corn fiber is: approx 40% hemicellulose, 12% cellulose, 25% starch, 10% protein, 10% lignin and ash, and approx 3% oil. With this background information a unique fractionation process was developed for corn fiber.

Materials and Methods

Analysis of the Raw Material

The raw material was kindly donated by Hungrana Rt. (Szabadegyháza, Hungary). The dry material content of the native fiber was approx 40%, so the fiber was immediately dried at room temperature to avoid biological decay. All analytical and technological processes were performed using the dry fiber, except when the catalytic effect of the sulfurous acid content of the native fiber was tested.

The polysaccharide content was measured as follows. The fiber was milled and dried at room temperature (less then 40°C). Dry material content of the milled fiber was then measured by drying at 105°C until weight stability. Five mililiter of sulfuric acid (72% w/w) was added to 1 g milled fiber. The suspension was incubated at room temperature for 1.5 h. Then it was diluted with 145 mL distilled water, and the suspension was stirred and incubated at 120°C for 1.5 h. The suspension was then chilled, and the supernatant was sampled. The sample was measured for monosaccharides by high-performance liquid chromatography (HPLC). The glucan, xylan, and arabinan content of the raw material were calculated according to Eq. 1:

$$C = \frac{c \times V}{f \times m} \tag{1}$$

where:
C is the polysaccharide content (mass fraction);
c the monosaccharide concentration measured by HPLC (g/L);
V the volume of the suspension (L);
F the hydration factor (glucan: 180/162; xylan: 150/132; arabinan: 150/132); and
m the dry weight of the analyzed raw material (g).

The starch content was measured as follows. Twenty milliliters of dilute hydrochloric acid ($\rho = 1.125$ g/mL) and 250 mL distilled water (DW) was added to 3 g milled fiber, then the suspension was incubated at 120°C for 1.5 h. Then the suspension was chilled, and sample was taken from the supernatant for HPLC analysis. Starch content of the raw material was calculated according to Eq. 1. Cellulose content was calculated as the difference of the glucan and the starch content. The protein content was measured according to Dumas' method *(28)*.

The oil content was measured as follows: 5–15 g milled fiber was placed into a paper vial, and extracted with hexane in a soxhlet-extractor until weight stability. Oil content was calculated as the yielded oil divided by the dry weight of the sample.

Ash content was measured by burning of 1–5 g dry fiber at 600°C for 6 h. Ash content was calculated as the yielded ash divided by the dry weight of the sample.

The HPLC analysis was performed using an Aminex HPX-87H column at 65°C. The eluent was 5 m*M* H_2SO_4 at a flow rate of 0.5 mL/min. Glucose, xylose, arabinose, cellobiose, acetic acid, and ethanol were detected and quantified by refractive index.

Fractionation

Step 1 (Destarching, Hydrolysis, and Fermentation of Supernatant)

Air-dried raw material was used except when the catalytic effect of the sulfurous acid content of the native fiber was tested. The dried fiber was diluted with DW so that the dry material concentration was 8% w/w. The suspension was incubated at 120°C for 2 h. At elevated temperatures the sulfurous acid catalyses the hydrolysis of starch by lowering the pH of the suspension. After cooling down the suspension was filtered through a 150 μm mesh nylon filter, thus fiber and supernatant were separated. The fiber was washed with 80°C DW three times to completely remove adsorbed substances. The fiber was dried at room temperature (less then 40°C). The supernatants were combined. The combined supernatant was diluted 1:1 with 8% (w/w) dilute sulfuric acid and then incubated at 120°C for 10 min to hydrolyze poly- and oligosaccharides. Then the solution was chilled and sample was taken for HPLC analysis.

Fermentability of starch containing supernatant was tested. The combined supernatant was not suitable for ethanol fermentation because of its low starch concentration, so the supernatant of the first separation was tested for fermentation. Before fermentation the starch was hydrolyzed with 0.2 g α-amylase/g starch (Termamyl Supra, Novozymes, Denmark) and 0.1 g glucoamylase/g starch (AMG 300L, Novozymes). The first supernatant was incubated at 120°C for 10 min at the beginning of the hydrolysis (0.1 g α-amylase addition), later temperature was reduced to 90°C (+0.1 g α-amylase addition), and then to 60°C, when the glucoamylase was added. The pH of the hydrolysis was 4.6. Fermentations were conducted using commercial *Saccharomyces cerevisiae* at the temperature of 35°C, pH was adjusted to 4.8 using acetate buffer.

For inoculum preparation fresh commercial compressed baker's yeast (*S. cerevisiae*, Budafok Yeast and Spirit Factory Ltd., Budapest, Hungary) was used. The inoculum was prepared in a 750 mL-Erlenmeyer-flask containing 150 ml sterile solution in which the concentration of nutrients in g/L were 50 glucose, 2.5 yeast extract, 5 peptone, 1 KH_2PO_4, 0.3 $MgSO_4$,

and 2 NH_4Cl. The Erlenmeyer flask was incubated in a rotary shaker at 30°C and 300 rpm for 24 h.

To 90 mL of the first supernatant 5 mL of yeast inoculum and 5 mL acetate buffer (1*M*, pH = 5.5) were added and the flask was shaken at 300 rpm. Ethanol yield and glucose conversion were calculated in two different ways. Three flasks were sampled for HPLC analysis after 0, 3, 6, 24, and 48 h. Ethanol yield and glucose conversion were calculated using the concentration values provided by the HPLC before and after fermentation as:

$$Y = \frac{Y_{EtOH}}{Y_{EtOHmax}} \times 100\,(\%),$$

where:
Y is the ethanol yield;
Y_{EtOH} the yielded ethanol (g); and
$Y_{EtOH\ max}$ the maximal (theoretical) ethanol yield (grams), equals 0.51 times the consumed glucose.

$$C = \left(1 - \frac{C_{glucose}}{C_{glucose,\,start}}\right) \times 100\ (\%)$$

where:
C is the conversion
$C_{glucose}$ the concentration of glucose (g/L); and
$C_{glucose,\ start}$ the concentration of glucose (g/L) at the beginning of the fermentation.

Three other flasks were equipped with oneway valve plug. The weights of these flasks were measured before and after fermentation. Ethanol yield can be calculated from the weight difference, which is caused by carbon dioxide loss. The yielded ethanol is calculated as 92/88 times the weight of the carbon dioxide (weight difference).

Step 2 (Dilute Acid Hydrolysis)

After destarching, the fiber was suspended in 1% dilute sulfuric acid so that the dry material concentration of the suspension was set to 8% w/w. The suspension was incubated at 120°C for 2 h. Then the suspension was cooled down and filtered through a 150-μm mesh nylon filter, thus fiber and supernatant were separated. The fiber was washed with 80°C DW three times to completely remove adsorbed substances. The fiber was dried at room temperature (less then 40°C). The supernatants were combined. The combined supernatant was diluted 1:1 with 8% w/w dilute sulfuric acid and then incubated at 120°C for 10 min to hydrolyze poly- and oligosaccharides. After cooling down 3 mL of sample was taken for HPLC analysis.

Step 3 (Enzymatic Hydrolysis of Cellulose)

The dried fiber was suspended in 0.1*M* acetic acid/sodium-acetate buffer (pH = 4.8) so that the dry material concentration of the suspension was set to 3% w/w. The suspension was incubated at 50°C for 48 h and flasks were shaken at 200 rpm. Commercial enzymes, like Celluclast L 1.5 (Novozymes) and Novozyme 188 (Novozymes) were loaded. The total loaded enzyme was 5 FPU/g dry substrate plus 5 IU/g dry substrate β-glucosidase activity, added in four equal doses. These correspond to 10.2 IU/g cellulose enzymatic activity, respectively. After 48 h the fibrous material formed two sharply separating fractions: a white fine fiber with low-settling velocity and a brown coarse fiber with high-settling velocity. Thus the suspension was filtered in two steps. In the first step, the slowly settling white (fine) fiber fraction was decanted and filtered on a G3 glass funnel. In the second step, the quickly settling brown (coarse) fiber fraction was filtered through a 150-μm mesh nylon filter. Thus fine, coarse fibers and supernatant were separated. The fibers were dried at room temperature (less then 40°C). The supernatant was analyzed for carbohydrates by HPLC. Each fiber fractions of the above subscribed fractionation method were analyzed for dry material content, starch, cellulose, xylan, arabinan, acetate, protein, lipids, and ash using the defined methods.

Results and Discussion

Raw Material Analysis

Corn fibers of three seasons (2002–2004) and seven individual charges were analyzed. The starch content of the raw material was the most variable among the components. It varied from 14% to 26.3% w/w. This variability is caused by the quality of the raw corn, according to the processor (Hungrana Rt., Hungary). Average composition of the corn fiber can be seen in Table 1. It is noteworthy, that the 5% total lipid content is not directly extractable. Sixty percent of the lipids fraction is chemically bound as lipoproteins and lipopolisaccharides. These bonds must be cut in order to make total lipid content extractable. The high (37.1/15 = 2.5) hemicelluloses/cellulose ratio significantly differs from the value of most lignocelluloses (approx 25/50 = 0.5).

Composition of the dried fiber after destarching can be seen in Table 2. Components are indicated as g/100 g native (original) dry corn fiber. Table 2 shows that 100% of the starch could be extracted. It is noteworthy that no chemical reagents and no stirring was applied during extraction, thus this step might be an inexpensive, convenient process even at industrial scale. A significant part (23.6%) of the proteins was also dissolved, whereas only a slight (6.2%) loss of the hemicelluloses was detected. Cellulose and lipids were untouched during the first step of the fractionation. Therefore it can be concluded that this step can sharply separate the starch from other components.

Table 1
Composition of Corn Fiber

Component	%
Starch	21.3
Cellulose	15
Hemicellulose	37.1
Xylan	22
Arabinan	10.9
Glucan	2.2
Acetate	2
Protein	14
Total lipids	5
Extractable oil	2
Bound lipids	3
Ash	1
Sum	93.2

Table 2
Composition of the Fiber After Destarching (Step 1)

Component	g/100 g Native dry fiber	Change (%)
Dry weight	72.5	–27.5
Starch	n.d.[a]	–100
Cellulose	15	0
Hemicellulose	34.8	–6.2
Xylan	20.5	–6.8
Arabinan	10.1	–7.3
Glucan	2.2	0
Acetate	2	0
Protein	10.7	–23.6
Total lipids	5	0
Extractable oil	2	0
Bound lipids	3	0
Ash	1	0
Sum	66.3	–28.3

[a]n.d., not detectable.

Table 3 shows the composition of the liquid phase of the starch extraction. It can be seen that the starch was completely found in the liquid phase, whereas there is a slight difference between the extracted and the measured xylan and arabinan. This difference may be owing to the fact that the starch is extracted in the form of polysaccharides, whereas the extracted xylan and arabinan is probably in the form of mono- and oligosaccharides, which are subject to thermal destruction.

A slight change was made in the destarching process when the catalytic effect of the sulfuric dioxide absorbed in the native wet corn fiber

Table 3
Composition of the Liquid Phase After Destarching (Step 1)

Component	g/100 g Native dry fiber
Starch	21.3
Hemicellulose	1.9
Xylan	1.3
Arabinan	0.6
Glucan	n.d.
Acetate	n.d.
Sum	23.2

n.d., not detectable.

was tested. Fiber was not dried before starch extraction, and temperature was reduced from 120°C to 100°C, other parameters remained unchanged. pH of the suspension was 3.6 throughout the process. Results showed that only 55% of the starch was extracted, thus the reduction of the incubation temperature was not compensated with the catalytic effect of the sulfuric dioxide.

The supernatant was hydrolyzed with commercial amylases and then tested for ethanol fermentation. After 24 h of fermentation the glucose conversion was 100%, whereas the ethanol yield was 96.4–98.3%. Gravimetric method of tracing ethanol fermentation was also tested. However, gravimetric results were significantly different from the results given by HPLC. Ethanol yields measured by the gravimetric method were 6–16% lower, presumably because of the absorption of carbon dioxide in the fermentation liquor.

After destarching, the resulting fiber was dried and suspended in dilute sulfuric acid to extract hemicelluloses. According to Table 4 hydrolysis with dilute acid impacted nearly every components: 64% of the dry weight of the fiber gained after destarching was extracted in step 2. Ninety seven percent of the hemicelluloses were extracted, and 56% of the proteins were also dissolved. Arabinan and acetate components of the hemicellulose seem to be easier to hydrolyze, presumably because of that they form side chains or substitutes, which are easier to hydrolyze than the xylan backbone. A significant part (15.3%) of the cellulose was also hydrolyzed, which was not the goal of this step. Yield of corn fiber oil extraction increased significantly (75%) owing to the tearing of lipopolysaccharide and lipoprotein bonds. Ash content of the corn fiber is 100% soluble in dilute sulfuric acid. Table 5 shows the composition of the liquid phase of the dilute acid hydrolysis.

Several dilute acid hydrolyses were performed to optimize the concentration of the sulfuric acid. It is noteworthy that only 0.5% dilute sulfuric acid at the relatively low temperature of 120°C can hydrolyze 97% of the hemicelluloses. We have previously reported *(17)* that 0.5% dilute sulfuric

Table 4
Composition of the Fiber Fraction After Dilute Acid Hydrolysis (Step 2)

Component	g/100 g Native dry fiber	Change (%)
Dry weight	26	–64.1
Starch	n.d.	0
Cellulose	12.7	–15.3
Hemicellulose	0.9	–97.4
Xylan	0.7	–96.6
Arabinan	0.2	–98
Glucan	n.d.	–100
Acetate	n.d.	–100
Protein	4.7	–56.1
Total lipids	5	0
Extractable oil	3.5	75
Bound lipids	1.5	–50
Ash	n.d.	–100
Sum	23.3	–64.9

n.d., not detectable.

Table 5
Composition of the Liquid Phase After Dilute Acid Hydrolysis (Step 2)

Component	g/100 g Native dry fiber
Hemicellulose	32.1
Xylan	19.2
Arabinan	9.9
Glucan	1
Acetate	2
Sum	32.1

acid at the same temperature (120°C) and residence time (2 h) can extract only 27.4% of corn-stalk hemicelluloses. Only 2% dilute sulfuric acid can extract 92–94% of corn-stalk hemicelluloses, which shows that corn fiber is a relatively easy-to-hydrolyze substrate compared to other lignocellulosic byproducts. Dilute acid hydrolyses were run to set the optimal concentration of the sulfuric acid. Acid concentrations, pH of the supernatant after hydrolysis, dry weight and hemicellulose content of the fiber after hydrolysis are shown in Table 6. The results show that it is not rational to decrease the concentration of sulfuric acid below 0.5% w/w, as the mass of the residual fiber and the amount of hemicellulose in the fiber increases, thus the sharpness of separation decreases.

After dilute acid treatment, the fiber was air-dried and subjected to enzymatic hydrolysis. After enzymatic hydrolysis, two solid fractions were obtained. There was a white fine, and a brown coarse fiber. The brown fiber

Table 6
Results of Different Dilute Acid Hydrolyses

Acid (%)	pH	Dry weight of the fiber	Hemicellulose in the fiber
0.5	1.02	26	0.9
0.7	0.85	27.1	0.7
0.9	0.72	24.6	0.3
1.1	0.67	23.8	0.4
1.3	0.56	23.4	0.4
1.5	0.50	25	0.3

Table 7
Composition of the Fine Fiber After Enzymatic Hydrolysis (Step 3)

Component	Fine fiber g/100 g native dry fiber	Coarse fiber g/100 g native dry fiber	Total change (%)
Dry weight	10.1	1.7	–54.6
Cellulose	0.4	0.7	–91.3
Hemicellulose	n.d.	n.d.	–100
Protein	4.7	n.d.	0
Total lipids	4.6	0.4	0
Extractable oil	4.6	0.4	42.9
Bound lipids	0	0	–100
Sum	9.7	1.4	–52.4

n.d., not detectable.

settled faster then the white fiber, thus they could be separated via decantation. Results are seen in Table 7. It can be seen that even 4 IU/g DW enzymatic activity could hydrolyze more than 91% of the cellulose present in the fiber after dilute acid hydrolysis. This proves the superior digestibility of the corn fiber cellulose after pretreatment. As previously reported *(17)*, dilute (2%) acid hydrolysis at the temperature of 120°C resulted in only 56% cellulose digestibility in corn stalk. We draw attention to the fact that we produced a solid fraction of corn fiber, which contains 46% corn fiber oil. This is significantly more than that reached by Singh et al. *(13)* (12.2%) through dilute sulfuric acid pretreatment. This novel technology yielded 5 g corn fiber oil/100 g dry corn fiber. In fact, this is the highest yield of corn fiber oil ever reported. During the sequential hydrolyzes lipopolysaccharides and lipoproteins degraded, thus bound lipids became extractable. Concentration of phytosterols and phytosterol-esters in the oil contents of each fiber fractions was also determined (Table 8). The results show that the yield of phytosterol-esters slightly decreased—presumably because of acid catalyzed hydrolysis—but the yield of phytosterols significantly increased. The total phytosterol yield increased from 1.45 to 1.84 g/kg CF, which corresponds to 27% increase.

Table 8
Yields of Phytosterols and Phytosterol-Esters

	Direct extraction	Fractionation
Free phytosterol yield (g/kg CF)	0.46	1.24
Phytosterol-ester yield (g/kg CF)	0.99	0.60
Sum	1.45	1.84

Table 9
Composition of the Liquid Phase After Enzymatic Hydrolysis (Step 3)

Component	g/100 g native dry fiber
Cellulose	11.6
Hemicellulose	0.7
Xylan	0.7
Arabinan	n.d.
Sum	12.3

n.d., not detectable.

Through the fractionation process it is proven that corn fiber contains no significant amounts of lignin. The fine and coarse fibers contain only 0.7 g undetermined ("others") fraction/100 g processed native fiber altogether, which means that this is the maximum (theoretical) amount of acid insoluble lignin present in the native fiber. Those results from Singh et al. *(13–15)* and other previous authors are inconsistent with these results. The applied Hägglund's method *(29)* does not seem to be suitable when the raw material contains significant amounts of unhydrolizable protein and lipids, which is the case at corn fiber. Those high values of lignin (7–12%) given by previous authors include protein and lipid fractions (and probably nucleic acids, as well). The real amount of acid insoluble lignin present in native corn fiber is less than 0.7 g/g fiber, but the exact determination of this value was not the goal of this study.

Table 9 shows the composition of the liquid phase after enzymatic hydrolysis. The cellulose fraction can be found in the liquid phase in the form of glucose monosaccharides. These conditions of enzymatic hydrolysis yielded no byproducts, so the glucose content of this solution is fermentable to ethanol at theoretical yields.

Conclusions

The novel technology described in this article implements the simultaneous, optimized production of bioethanol and corn fiber oil, which we consider the two most valuable products that can be made from corn fiber. This process is able to cleanly separate the fractions of native corn fiber. The first step removed all the starch content of the native corn fiber. The

liquid phase of the first step—containing the extracted starch—was 96.4–98.3% fermentable to ethanol after enzymatic hydrolysis by commercial amylases. Only 0.5% w/w dilute sulfuric acid and the temperature of 120°C was needed to remove 97.4% of the hemicellulose and also to make remaining cellulose 91.3% enzymatically digestible.

The third step hydrolyzed the cellulose, which resulted in two solid fractions: a white, fine, and a brown, coarse fiber. These solid fractions could be separated via decantation or flotation. The fine fiber added up to 10% of the original CF, and contained 46% w/w corn fiber oil. This means that we produced 5 g corn fiber oil/100 g CF, which is the highest yield of CFO reported. It is noteworthy that only 40% of the lipid content of corn fiber is directly extractable, since 60% of the lipids are in the bound forms of lipoproteins and lipopolysaccharides. The original concentration of corn fiber oil in the fiber (2%) was 23 times increased in the residue. The process described herein liberated all of the lipids present in the corn fiber.

Another important observation of this study is that corn fiber contains no significant amount of lignin. It is likely that the values (7–12%) of lignin content given by previous papers may not be correct. The applied Hägglund's method *(29)* is not adequate for determining the lignin content of corn fiber owing to its significant fraction of unhydrolysable proteins and lipids. Our mass balances indicate that lignin can only be present in traces in the native corn fiber.

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References

1 Hicks, K. B. and Moreau, R. A. (2001), *Food Technol.* **55,** 63–67.
2 Vanhanen, H. T., Kajander, J., Lehtovirta, H., and Miettinen, T. A. (1994), *Clin. Sci.* **87,** 61–67.
3 Singh, V., Moreau, R. A., and Cooke, P. H. (2001), *Cereal Chem.* **78,** 436–441.
4 Moreau, R. A., Hicks, K. B., and Powell, M. J. (1999), *J. Agric. Food Chem.* **47,** 2869–2871.
5 Moreau, R. A., Singh, V., Eckhoff, S. R., Powell, M. J., Hicks, K. B., and Norton, R. A. (1999), *Cereal Chem.* **76,** 449–451.
6 Singh, V., Moreau, R. A., Doner, L. W., Eckhoff, S. R., and Hicks, K. B. (1999), *Cereal Chem.* **76,** 868–872.
7 Ramjiganesh, T., Roy, S., Vega-Lopez, S., McIntyre, J., and Fernandez, M. L. (1999), *FASEB J.* **13,** 561.
8 Moreau, R. A., Powell, M. J., and Hicks, K. B. (1996), *J. Agric. Food Chem.* **44,** 2149–2154.
9 Singh, V., Moreau, R. A., Haken, A. E., Hicks, K. B., and Eckhoff, S. R. (2000), *Cereal Chem.* **77,** 665–668.
10 Taylor, S. L. and King, J. W. (2000), *J. Am. Oil Chem. Soc.* **77,** 687–688.
11 Wilson, T. A., DeSimone, A. P., Romano, C. A., and Nicolosi, R. J. (2000), *J. Nutr. Biochem.* **11,** 443–449.

12 Singh, V., Moreau, R. A., Haken, A. E., Eckhoff, S. R., and Hicks, K. B. (2000), *Cereal Chem*. **77,** 692–695.
13 Singh, V., Johnston, D. B., Moreau, R. A., Hicks, K. B., Dien, B. S., and Bothast, R. J. (2003), *Cereal Chem*. **80,** 118–122.
14 Singh, V. and Moreau, R. A. (2003), *Cereal Chem*. **80,** 123–125.
15 Singh, V., Moreau, R. A., and Hicks, K. B. (2003), *Cereal Chem*. **80,** 126–129.
16 Torget, R., Walter, P., and Himmel, M. (1991), *Appl. Biochem. Biotech.* **28–29,** 75–86.
17 Kálmán, G., Varga, E., and Réczey, K. (2001), *Chem. Biochem. Eng. Quart* **16,** 151–157.
18 Esteghlalian, A. and Hashimoto, A. G. (1997), *Bioresour. Technol*. **59,** 129–136.
19 Schell, D. J., Walter, P. J., and Johnson, D. K. (1992), *Appl. Biochem. Biotech*. **34–35,** 659–665.
20 Lee, Y. Y., Iyer, P., and Torget, R. W. (1999), *Adv. Biochem. Eng. Biotechnol*. **65,** 93–115.
21 Chen, R., Lee, Y. Y., and Torget, R. (1999), *Appl. Biochem. Biotech.* **57–58,** 133–146.
22 Torget, R. and Hsu, T. (1994), *Appl. Biochem. Biotech.* **45–46,** 5–21.
23 Torget, R., Himmel, M. E., and Grohmann, K. (1991), *Biores. Technol*. **35,** 239–246.
24 Chen, R., Wu, Z., and Lee, Y. Y. (1998), *Appl. Biochem. Biotech*. **70–72,** 37–49.
25 Hsu, T. A. and Himmel, M. (1996), *Appl. Biochem. Biotech.* **57–58,** 3–18.
26 Hsu, T. and Nguyen, Q. (1995), *Biotechnol. Tech*. **9,** 25–29.
27 Schell, D. J., Torget, R., Power, A., Walter, P. J., Grohmann, K., and Hinman, N. D. (1991), *Appl. Biochem. Biotech*. **28–29,** 87–97.
28 AOCS Official Method Ba 4e-93: Generic Combustion Method for Determination of Crude Protein.
29 Hägglund, E. (1951), *Chemistry of Wood*, Academic Press, New York.