



# Pretreatment of Wheat Bran for Suitable Reinforcement in Biocomposites

Atikur Rahman<sup>1,3</sup>, Chad A. Ulven<sup>2</sup>, Maren A. Johnson<sup>1</sup>, Cheyenne Durant<sup>1</sup>, and Khwaja G. Hossain<sup>\*1</sup>

<sup>1</sup>Division of Science and Mathematics, Mayville State University, Mayville, ND 58257, USA

<sup>2</sup>Department of Mechanical Engineering, North Dakota State University, 1340 Administrative Avenue, Fargo, ND 58102, USA

<sup>3</sup>Department of Irrigation and Water Management, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

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**ABSTRACT:** Wheat bran, abundant but underutilized, was investigated for its potential as a reinforcement in biocomposites through different pretreatment methods. Pretreatment methods included were dilute sodium hydroxide (NaOH), dilute sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), liquid hot water (LHW), calcium hydroxide (CaOH), organosolv such as aqueous ethanol (EtOH), and methyl isobutyl ketone (MIBK). Changes in chemical composition and fiber characteristics of the treated bran were studied using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Cellulose content increased to 35.1% and 29.6% in brans treated with H<sub>2</sub>SO<sub>4</sub> and NaOH, respectively. The SEM micrographs showed surface cleaning of treated bran while maintaining sufficient surface roughness for the H<sub>2</sub>SO<sub>4</sub>, NaOH, and MIBK treated brans. Crystallinity index increased slightly for all treatments except H<sub>2</sub>SO<sub>4</sub>. NaOH and H<sub>2</sub>SO<sub>4</sub> pretreated brans achieved important fiber characteristics, which could be useful for making thermoplastic biocomposites. Innovative use of bran in thermoplastic will create more opportunities for growers while enhancing biodegradability.

**KEYWORDS:** Biocomposites, fiber composition, pretreatment, surface treatment, wheat bran

## 1 INTRODUCTION

The use of petroleum-based plastics is thought to be the cause of widespread environmental issues because they do not decompose readily after their disposal. The use of biobased natural fibers as fillers or reinforcement agents in manufacturing biocomposites has received much attention recently. Several factors, such as their ability to degrade quickly, cheaper cost, light weight, high specific strength, and renewability, are favorable to applications for biocomposites [1–3]. The high relative advantages and diversified applications are reflected by the growth rate of biocomposite development. From 2003 to 2007, the average annual global growth rate of biocomposites was 38%. Globally, the volume of biobased plastics is likely to increase from 0.36 million metric tons in 2007 to 2.33 million metric tons by 2013 and to 3.45 million metric tons in 2020 [4]. However, biobased composites are still in their developmental stage, and, in combination with commodity synthetic polymers, they are an option for obtaining

overall cost and more environmentally favorable processing [1].

A large number of fiber sources were investigated, such as wood, hemp, feather, kraft pulp, and pineapple [5–11]. In addition, there is a continued search for new fiber sources for biocomposites. There are large amounts of grain by-products, such as straw, wheat bran, rice husk, and corn stalk, which can be used for producing biodegradable composites [12]. Biocomposites prepared from agricultural waste and macromolecular materials are more beneficial compared to other fiber materials due to their water absorption characteristics, workability, and superior mechanical properties [13]. For example, wheat kernel contains about 14.5% of bran, which is produced in huge amounts as a by-product every year from the milling of wheat [14]. Only 10% of this by-product is used in bakeries and in breakfast cereals as a dietary fiber supplement. The 90% of the remaining bran could be sold as animal feed, but due to high transportation costs, millers often dispose of the bran as waste, which causes environmental hazards.

Wheat bran contains phenolic compounds [15], starches [16], soluble and insoluble dietary fibers [17],

\*Corresponding author: k.hossain@mayvillestate.edu



and proteins [18]. The water insoluble component of bran consists of cellulose, hemicelluloses, and lignin, which offers advantages as reinforcing materials [19]. Cellulose, which consists of D-anhydroglucopyranose units joined together by  $\beta$ -1,4-glycosidic bonds, is the most profuse natural polymer on earth [20]. Microfibrils are formed by organizing stereoregular configurations of cellulose with the help of a regular network of inter- and intramolecular hydrogen bonds [21]. Biomass fibers derive their strength from hydrogen bonding in the microfibrils. However, little strength is derived from the hemicelluloses and lignin due to the amorphous nature of these two polymers [22]. Cellulose and hemicelluloses are confined by the lignin via hydrogen and covalent bonding [23]; they form agglomerations and, as a result, show inferior mechanical properties compared to pure cellulose. In general, increased cellulose fraction increases the strength of fibers [20]. Therefore, the search of cellulose-rich biomass or techniques to increase cellulose fraction is critical in the development of functional lignocellulosic fillers.

The performance of composites reinforced with natural fibers depends on many factors, including physical and chemical properties, cell dimensions, microfibrillar angle, defects, structure, mechanical properties, and the fiber-polymer matrix interactions [3]. The internal and external bodily structures of cellulose in the cell wall are complex and heterogeneous and intimately interact with other polysaccharide moieties, causing complex morphologies [24]. Pretreatment of lignocellulosic biomass changes the chemical composition and alters the surface conditions of fibers, which may improve composite performance. Chauvelon *et al.* [25] observed augmented cellulose content in wheat bran after removing heteroglycan and lignin through pretreatment with acids and alkali esterified with lauroyl chloride for cellulosic film preparation. In another study, surface treatment through mercerization changed the spiral angle and other mechanical properties of fibers [26]. Alkali treatment also removed lignin and hemicelluloses, which made the interfibrillar region less dense and less rigid, allowing the fibrils repositioning themselves along the direction of tensile deformation [20]. Alkali treatment removes the cementing materials, thus increasing percentage crystallinity index, which leads to improved sorting of cellulose chains [27]. Alkali treatment also disrupts hydrogen bonding, which makes the fiber surfaces rough [3]. Strong acid hydrolysis also removes the amorphous region of cellulose fibers and purifies cellulose microfibrils [28]. However, suitability of a treatment depends on the fiber source.

There are limited uses for wheat bran, including the manufacture of film for food preservation and as a

dietary fiber source. Hossain *et al.* [12] studied the lignocellulosic composition of wheat bran and Jiang and Guo [29] investigated the steam explosion pretreatment and enzymatic hydrolysis of wheat bran for evaluating saccharification performance. However, there has been no investigation so far on the potential of using wheat bran for making industrial material, such as biocomposites. Therefore, this study aimed to pretreat wheat bran through various thermo-physical and chemical methods, as well as to investigate its suitability for making thermoplastic biocomposites. Scanning electron microscope (SEM) and infrared spectroscopy analyses of treated and untreated brans were performed to characterize the fibers' surfaces. Use of wheat bran for making a value-added product could add an alternate use for wheat and attract wheat growers by offsetting their reduced profit margins incurred through low prices in the grain market.

## 2 EXPERIMENTAL

### 2.1 Milling and Bran Extraction

The bran used in this study was collected from hard red spring wheat. The wheat sample was milled using a Buhler MLU-202 laboratory mill. A sample of wheat was prepared for milling with a Carter-Day dockage tester (Minneapolis, MN, USA) with a number 8 sieve. The sample was then tempered in three stages: 1) pre-tempered to 12.5% moisture content (MC) for 72 h before milling if MC was below 11%; 2) tempered to 16% MC for 24 h before milling; and 3) finally tempered to 16.5% MC for 20 to 30 min before milling. The Buhler MLU-202 produced six flour products, one bran product, and one shorts product. The bran fraction was collected and used for experiment in this study.

### 2.2 Pretreatment Procedures

#### 2.2.1 NaOH Pretreatment

Milled wheat bran was loaded into a conical flask at 10 wt% solids in deionized water. The sodium hydroxide loading was 100 mg/g of dry bran. The flask was heated in a water bath at 80 °C for 3.5 h with occasional low speed shaking. After heating, the resulting slurry was removed from the flask and separated into solid and black liquors. To separate the black liquor, the slurry was first centrifuged for 10 min at 4000 rpm and the resulting caustic black liquor supernatant was decanted from the tube and discarded. The solids were washed three times through resuspension in 1 L of deionized water. The wash water was decanted from the solid fraction. Finally, the solid was vacuum

filtered on a 2  $\mu\text{m}$  pore size PTFE filter to remove the small remaining amount of wash water. The washed solid was dried at 60 °C and weighed periodically until a constant weight between two consecutive measurements was achieved. The dried bran sample was used for composition analysis, SEM imaging, and IR experiments.

### 2.2.2 Organosolv Pretreatment (EtOH)

Ethanol (90%) was mixed with bran at a ratio of 6:1 in a centrifuge tube. The centrifuge tube was placed in a beaker and heated for 4 h at 95 °C with periodic agitation. The samples were cooled to room temperature, and the pulp and liquor were separated by centrifuging for 10 min at 4000 rpm. The resulting black liquor was decanted. The pulp was resuspended and washed three times in 300 mL of aqueous ethanol with the same concentration as cooking liquor. The wash water was discarded, and the remaining solid fraction was vacuum filtered to remove remaining liquid. The washed solid was dried at 60 °C and weighed periodically until a constant weight between two consecutive measurements was achieved.

### 2.2.3 Liquid Hot Water (LHW) Pretreatment

Bran biomass was immersed in liquid water at 9 wt% solid loading. The LHW was carried out at 140 °C and 33 psi with a 1-h contact time in an autoclave. The treated sample was then centrifuged for 10 min at 4000 rpm. The resulting liquor from centrifugation was decanted from the top of the tube. The remaining solid was resuspended in 250 mL of deionized water for washing. Wash water was decanted, and solids with remaining water were vacuum filtered to remove excess water. After filtration, the solids were dried at 60 °C to a constant weight.

### 2.2.4 Lime (CaOH) Pretreatment

Lime (calcium hydroxide) was used as a pretreatment agent to dissolve lignin from wheat bran. Wheat bran was treated with lime at the ratio of 1 g of lime to 1 g of bran, and with water at a ratio of 7 mL of water to 1 g of bran. The bran was thoroughly mixed with the water and lime, and the mixture was heated for 2.5 h at 100 °C. After heating, the samples were centrifuged for 10 min at 4000 rpm. The liquor from the top of the centrifuge tube was decanted, and the solids were resuspended three times in 250 mL of deionized water for washing. Wash water was decanted, and the solids with remaining water were vacuum filtered to remove the excess water. After filtration, solids were dried at 60 °C to a constant weight.

### 2.2.5 H<sub>2</sub>SO<sub>4</sub> Pretreatment

Wheat bran was treated using diluted sulfuric acid at a concentration of 4% at 100 °C. The experiment was performed at a liquor/solid ratio of 10 g liquor to 1 g wheat bran (dry basis). The mixture of bran and acid was heated for 2.5 h. After heating, the mixture was taken from the reaction media and centrifuged for 10 min at 4000 rpm. The liquor from the top of the centrifuge tube was decanted, and the remaining solids were washed three times in 500 mL of deionized water and then vacuum filtered to separate the solids. After filtration, solids were dried at 60 °C to a constant weight.

### 2.2.6 Methyl Isobutyl Ketone (MIBK) Pretreatment

Organic solvents dissolve lignin, which may facilitate separation of lignocellulosic materials into their components. A modified method of Black *et al.* [30] was used in this study to treat bran. A single phase pulping liquor composed of 24% water, 44% methyl isobutyl ketone (MIBK), and 32% ethanol was prepared in a glass container. Bran was mixed with liquor at the ratio of 10 mL of liquor to 1 g of bran, and 2 mL of 0.05 M H<sub>2</sub>SO<sub>4</sub> catalyst was added. The sample mixture was heated at 100 °C for 2 h. The resulting pulp was washed with fresh neutral liquor, vacuum filtered, and dried at 60 °C to a constant weight.

## 2.3 Composition Analysis

The recovered solids were dried for several days in a vacuum oven at 60 °C until a constant value of the mass was obtained from two consecutive weighings. Analysis of the solids for composition was subsequently conducted. A total of seven components, including lignocellulosic fractions, were analyzed in triplicate. The analyses were performed at the Animal Sciences Department of North Dakota State University. The parameters analyzed included crude protein, neutral detergent fibers, acid detergent fibers, acid detergent lignin, fat, starch, and dry matter content. Dry matter was determined according to AOAC Method 967.03 [31], with few modifications. The samples were weighed at room temperature and then heated at 100 °C for 24 h. After heating, samples were conditioned in desiccators and weighed again. The percentages of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using an ANKOM-200/220 fiber analyzer (ANKOM Technology, Macedon, NY, USA), according to methods specified in the *USDA Agricultural Handbook* [32]. The percentage of starch was determined using an acid and enzymatic isolation

assay and microtiter reading with a SPECTRAMax<sup>®</sup> 340 microplate reader (Molecular Devices, Sunnyvale, USA). The cellulose and hemicellulose percentages were calculated using Equations 1 and 2.

$$\% \text{ Cellulose} = \% \text{ Acid Detergent Fiber} - \% \text{ Acid Detergent Lignin} \quad (1)$$

$$\% \text{ Hemicellulose} = \% \text{ Neutral Detergent Fiber} - \% \text{ Acid Detergent Fiber} \quad (2)$$

## 2.4 Scanning Electron Microscopy (SEM)

The morphology of treated and untreated brans was analyzed by SEM at the Electron Microscopy Center at North Dakota State University. Samples were attached to cylindrical aluminum mounts using double-stick carbon adhesive tabs (Ted Pella, Redding, CA, USA) and then sputter coated (Cressington 108 Auto, Ted Pella) with a conductive layer of gold. Images were obtained with a JEOL JSM-6490LV scanning electron microscope (JEOL USA, Inc., Peabody, MA, USA) at an accelerating voltage of 15 kV.

## 2.5 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR was performed at the Materials Characterization and Analysis Laboratory at North Dakota State University and was used to observe the compositional change in the bran before and after treatment with different thermophysical and chemical methods. Bran specimens were prepared by mixing a small amount of bran with potassium bromide (KBr), followed by cold pressing to form discs. Infrared absorbance spectra of the bran specimens were recorded at ambient temperature and atmospheric pressure with a Nicolet 8700 FT-IR spectrometer (Thermo Electron Scientific Instruments LLC, Madison, WI, USA). The spectra were obtained by recording 32 scans, which were performed with a resolution of 4 cm<sup>-1</sup> between 400 and 4000 cm<sup>-1</sup>. The peak signals were recognized using software (OMNIC, Thermo Electron Scientific Instruments LLC, Madison, WI, USA). The crystallinity indices of the untreated and treated brans were calculated by infrared ratio, [I<sub>1472</sub> cm<sup>-1</sup>/I<sub>2900</sub> cm<sup>-1</sup>], as suggested by [33], which applies to both cellulose I and II and mixed lattices.

## 2.6 Data Analysis

Analysis of variance (ANOVA) of the different treatments was performed using GLM procedure of the Statistical Analysis System (version 8.0, SAS Institute Inc., Cary, NC, USA).

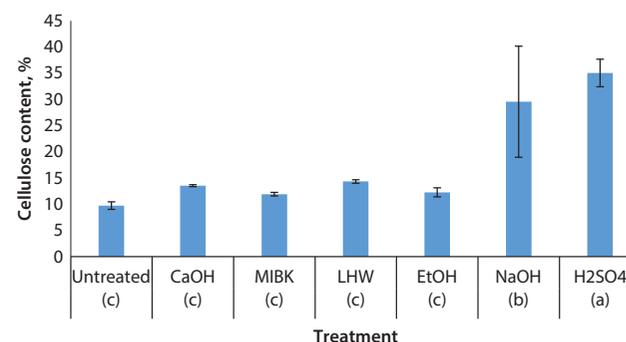
## 3 RESULTS AND DISCUSSION

The wheat bran was treated by several different methods. A number of treatment levels were investigated for each method. The best results from each method have been reported and discussed regarding their effectiveness for producing suitable fillers for thermoplastic biocomposites. Based on the treatment results, pretreatment methods have been suggested for bran because they might render superior biocomposite characteristics for large-scale uses.

### 3.1 Change in Compositions

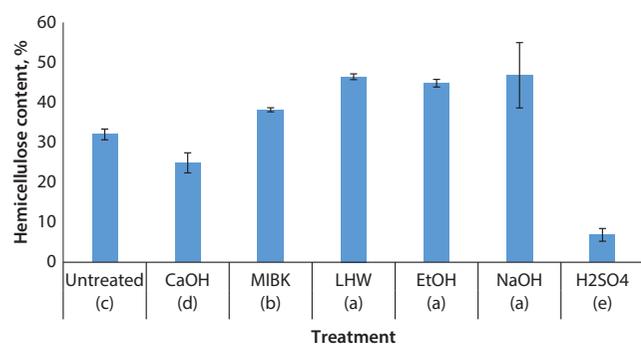
The cellulose fraction in bran increased after treatment with different methods, as shown in Figure 1. A significant increase in the cellulose content was observed for the NaOH and H<sub>2</sub>SO<sub>4</sub> treated brans. The highest increase of 35.0% was observed in the H<sub>2</sub>SO<sub>4</sub> treatment, followed by 29.6% in the NaOH treatment, compared with untreated bran, which contained 9.75% cellulose. Although CaOH, MIBK, LHW, and EtOH treatments increased the cellulose fraction, their cellulose contents were not significantly different than that of the untreated bran. Chauvelon *et al.* [25] observed a similar amount of cellulose enrichment in bran with H<sub>2</sub>SO<sub>4</sub> and KOH treatments, which were 38.3% and 31.7%, respectively. In another study, NaOH treated wheat straw under moderate temperature and pressure increased the cellulose fraction up to 63.1% [34].

The H<sub>2</sub>SO<sub>4</sub> treatment was effective in solubilizing hemicellulose and thereby reduced the fraction from bran, as shown in Figure 2. In H<sub>2</sub>SO<sub>4</sub> treated bran, a five-fold decrease in hemicellulose was observed. The hemicellulose content decreased from 32.0% in the untreated bran to 6.83% in the H<sub>2</sub>SO<sub>4</sub> treated bran. Compared with untreated bran, hemicellulose



**Figure 1** Cellulose content (%) in untreated and treated wheat brans. The error bar (N = 3) indicates standard deviation. Columns with different letters in parentheses are significantly different at the 95% confidence level.





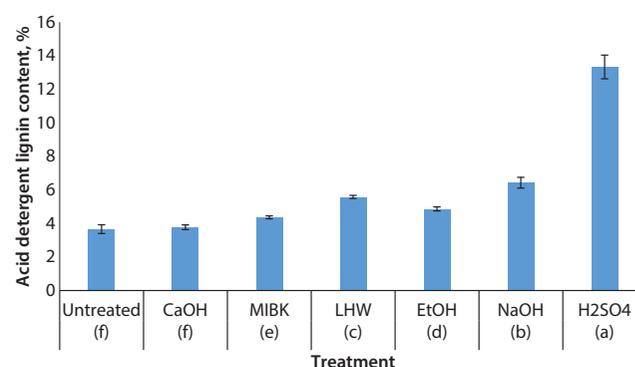
**Figure 2** Hemicellulose content (%) in untreated and treated wheat brans. The error bar (N = 3) indicates standard deviation. Columns with different letters in parentheses are significantly different at the 95% confidence level.

percentage increased with all other treatments except the CaOH treated bran, which had 24.9% hemicellulose and was higher than that of the H<sub>2</sub>SO<sub>4</sub> treated bran. However, among others which increased the hemicellulose content, NaOH treated bran had the highest content at 46.8% and was not significantly different from the LHW and EtOH treated brans. Lamsal *et al.* [35] found similar hemicellulose removal (~ 5%) from destarched bran with the same acid concentration but with a higher temperature and lower retention time.

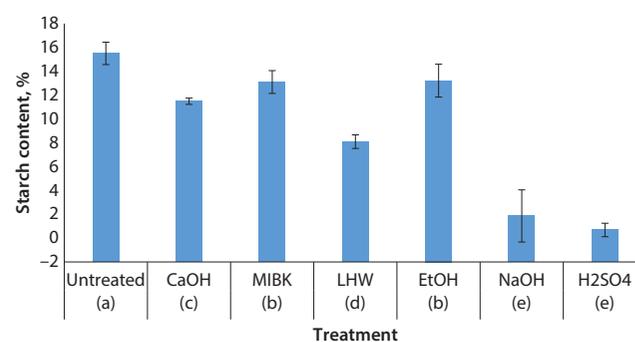
Acid detergent lignin or lignin content is usually lower in wheat bran [12], but none of the pretreatment methods used in the present study reduced the lignin content (Figure 3). Several studies have suggested that lignin removal from lignocellulosic materials depends on the part of plant materials targeted for lignin removal. Chemical pulping of wheat straw with NaOH removed 70% lignin [34]. However, a slight increase in lignin content was observed in wheat bran treated with diluted H<sub>2</sub>SO<sub>4</sub> [35], which supports the results of the present study.

The effectiveness of starch removal by various treatments used in the present study is presented in Figure 4. In all treatments, a significant removal of starch was observed. Almost all starch (0.68% in treated bran) was removed by H<sub>2</sub>SO<sub>4</sub> treatment, followed by NaOH (1.8% in treated bran) and LHW (8.09% in treated bran) compared with untreated bran (15.5%). Generally, biomass is treated with  $\alpha$ -amylase to remove starch. Lamsal *et al.* [35] observed starch removal in wheat bran from 20% to 9% by  $\alpha$ -amylase treatment. However, the present study effectively removed starch from bran using NaOH and H<sub>2</sub>SO<sub>4</sub> treatments.

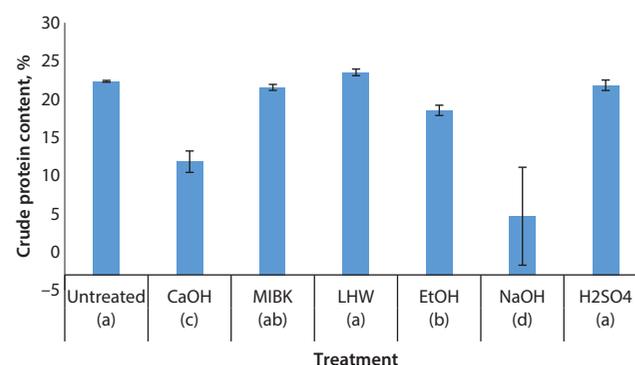
The crude protein fraction was significantly reduced by the NaOH, CaOH, and EtOH treatments, as shown in Figure 5. The highest removal was observed by



**Figure 3** Acid detergent lignin content (%) in untreated and treated wheat brans. The error bar (N = 3) indicates standard deviation. Columns with different letters in parentheses are significantly different at the 95% confidence level.



**Figure 4** Starch content (%) in untreated and treated wheat brans. The error bar (N = 3) indicates standard deviation. Columns with different letters in parentheses are significantly different at the 95% confidence level.

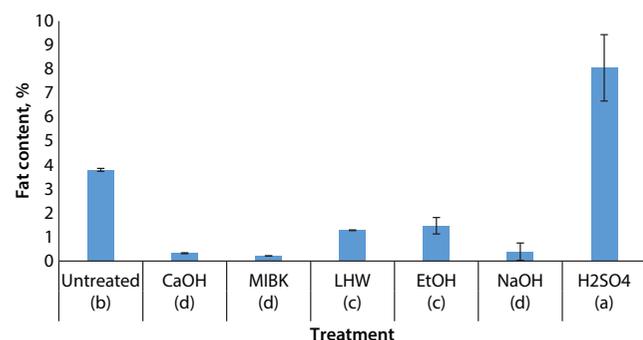


**Figure 5** Crude protein content (%) in untreated and treated wheat brans. Error bar (N = 3) indicates standard deviation. Columns with different letters in parentheses of abscissa are significantly different at 95% confidence level.

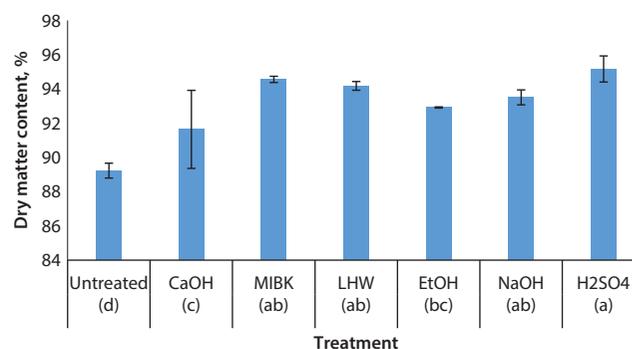
NaOH (4.67% in treated bran), followed by CaOH (11.8%) and EtOH (18.5%) compared with untreated bran (22.3%). However, LHW treatment increased the crude protein fraction, but the increase was not significantly different than that in untreated bran. Chauvelon *et al.* [25] treated wheat bran with alkali (KOH) and observed a decrease in protein fraction from 10.8% to 0.40% and 0.20% using hydrogen peroxide and sodium hypochlorite, respectively, which supports protein removal by alkali in the present study. A 27.9% crude protein removal by lime treatment was also observed in switchgrass [36].

Figure 6 shows the effect of pretreatment by different methods on the change in fat content in wheat bran. The fat was significantly removed by all treatments except H<sub>2</sub>SO<sub>4</sub>. The decreased fat contents ranged from 0.22% by MIBK to 1.47% by EtOH in treated bran compared with untreated bran (3.8%). However, compared with untreated bran, fat content increased more than two-fold (8.05%) with H<sub>2</sub>SO<sub>4</sub> treatment. In a different study, about a three-fold reduction of fat, from 0.60% to 0.20%, was observed in spelt treated with enzymes [37].

Pretreatment of wheat bran by different methods significantly increased the dry matter fraction, as shown in Figure 7. The average dry matter content in the untreated bran was 89.2%, while the dry matter content in the treated bran ranged from 91.6% (CaOH) to 95.2% (H<sub>2</sub>SO<sub>4</sub>). Increase of dry matter fraction by MIBK, LHW, and H<sub>2</sub>SO<sub>4</sub> was significantly different. Moisture uptake in biomass occurs mainly in hemicellulose, non-crystalline cellulose, accessible cellulose, starch, lignin, and the surface of cellulose [38]. Although moisture uptake by bran was not investigated, it is likely that pretreatment changed the chemical composition, texture, and structure, which influenced the water retention



**Figure 6** Fat content (%) in untreated and treated wheat brans. The error bar (N = 3) indicates standard deviation. Columns with different letters in parentheses are significantly different at the 95% confidence level.



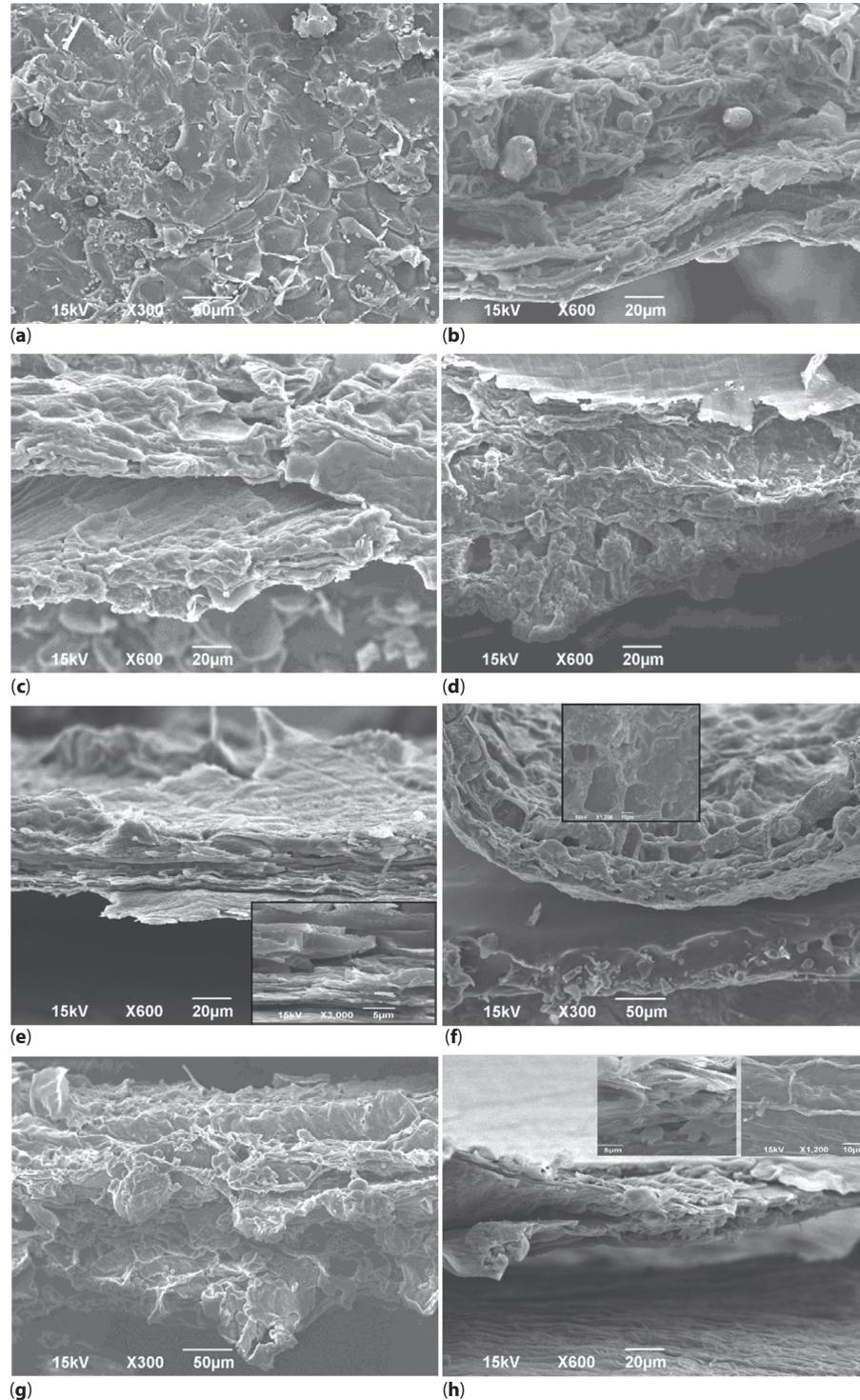
**Figure 7** Dry matter content (%) in untreated and treated wheat brans. The error bar (N = 3) indicates standard deviation. Columns with different letters in parentheses are significantly different at the 95% confidence level.

properties of the treated bran under ambient storage conditions.

### 3.2 Scanning Electron Microscopy (SEM)

Figure 8 shows the SEM micrographs of untreated and treated brans with different thermophysical and chemical methods. The surface morphology of untreated bran in Figure 8a shows the presence of protein, starch, fat, and globular particles. The presence of a smooth waxy surface over fibers called cuticle, which was identified as aliphatic wax [39], is more visible in the magnified view shown in Figure 8b. The fiber surface containing cellular materials, as shown in Figure 8b, was modified and became relatively cleaner through the treatments.

Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) treatment removed fat, starch, some hemicellulose, and waxy cuticles and exposed the fiber surfaces, as shown in Figure 8c. Although there was evidence of defibrillation, node-like cell materials held together adjacent fibers. Despite the cleaning of surfaces due to treatment, the microstructure of the surface shows roughness, which is advantageous for making biocomposites. Calcium hydroxide (CaOH) treatment did not solubilize cuticle layers, which were clearly visible in treated bran (Figure 8d). The observed terraces and pits in the treated bran were likely due to the removal of fat and globular materials. Similar to CaOH, LHW treatment also caused pits, but they were larger in size and number than those of CaOH treatment (Figure 8f). At a higher magnification (inset micrograph), the deposition of pseudo-lignin and/or protein on the surface of the holocellulose was observed [40]. Methyl isobutyl ketone (MIBK) treatment of bran removed most of the fat particles and resulted in a smoother surface (Figure 8e). At a higher magnification, the micrograph showed



**Figure 8** SEM micrographs of wheat bran. Untreated (a) and (b), and treated with (c)  $H_2SO_4$ , (d) CaOH, (e) Methyl isobutyl ketone (MIBK), (f) Liquid hot water (LHW), (g) Ethyl alcohol (EtOH), and (h) NaOH.

solid-like fibers, which were superimposed, one over another, and appeared as a sandwich-like structure, as evidenced from the inset micrograph. Treatment with EtOH did not remarkably change the surface

morphology from that of untreated bran, which is evidenced from the presence of cellular materials, including starch and protein (Figure 8g). The smooth waxy surface that resulted from EtOH treated bran might

not help with increasing fiber-matrix adhesion, which is essential for improving the mechanical strength of biocomposites. Alkali (NaOH) treatment removed all fat, starch, and protein particles from the bran, and the layer of cuticles was dissolved, resulting in a smoother and cleaner surface (Figure 8h). However, a closer look at the magnified micrograph revealed rough microstructures and some pits (inset micrograph). A rough surface could be advantageous for manufacturing biocomposites. Similar to  $H_2SO_4$ , NaOH treatment also shows node-like structures (inset micrograph), which bonds adjacent fibers together.

### 3.3 Structural Characteristics of Untreated and Treated Brans by FTIR

Figure 9 shows the spectra of untreated and treated brans with different thermophysical and chemical methods, in which the majority of peaks are labeled with the wave number in it. The functional groups of the characteristic peaks from the spectra are identified and presented in Table 1 [41–43]. The absorption peaks near  $3402$  to  $3423$   $cm^{-1}$  bands observed in different treatments were attributed to the stretching vibration of hydroxyl groups. The OH group may include absorbed water, aliphatic primary and secondary alcohols found in cellulose, hemicellulose, carboxylic acids, and phenolic compounds [44]. Intense bands of spectra observed in  $2923$  to  $2926$  and  $2855$   $cm^{-1}$  were attributed to the C-H stretching vibration of methyl, methylene, and methane groups, which are the moieties in

polysaccharides (cellulose and survived hemicelluloses) [44–46]. The band near  $1736$   $cm^{-1}$  appeared only in NaOH,  $H_2SO_4$ , and MIBK treated brans, suggesting the presence of carbonyl and unconjugated ketone and carboxyl group stretching. The absorption bands ranging from  $1660$  to  $1630$   $cm^{-1}$  are attributed to conjugated carbonyl stretching [45]. The absorption band near  $1539$   $cm^{-1}$  in untreated bran shifted to  $1518$ ,  $1530$ , and  $1519$   $cm^{-1}$  in LHW,  $H_2SO_4$ , and MIBK treated brans, respectively, but disappeared in NaOH and CaOH treated brans. The N-H vibration of amine indicated the presence of an amine group in protein, and the disappearance of this band in NaOH and CaOH treated brans could be indicative of the removal of protein. The range of absorption peaks from  $1027$  to  $1053$   $cm^{-1}$  is attributed to the lignin component, guaiacyl unit, and is an aromatic C-H plane deformation [44, 45]. The appearance of absorption peaks from  $1162$  to  $1170$   $cm^{-1}$  and near  $898$   $cm^{-1}$  for NaOH,  $H_2SO_4$ , and MIBK treated brans are seen in the spectra, which are typical of pure cellulose [46].

### 3.4 Crystallinity Index (CI)

Table 2 shows the crystallinity index of untreated and treated brans with different thermophysical and chemical methods. A CI value of  $0.94$  was obtained for the untreated bran sample. An increase in CI was observed for CaOH, EtOH, MIBK, and NaOH treated brans, and a decrease was observed for  $H_2SO_4$  and LHW treatments. The highest increased CI was  $0.98$  for the NaOH and CaOH treated samples, and

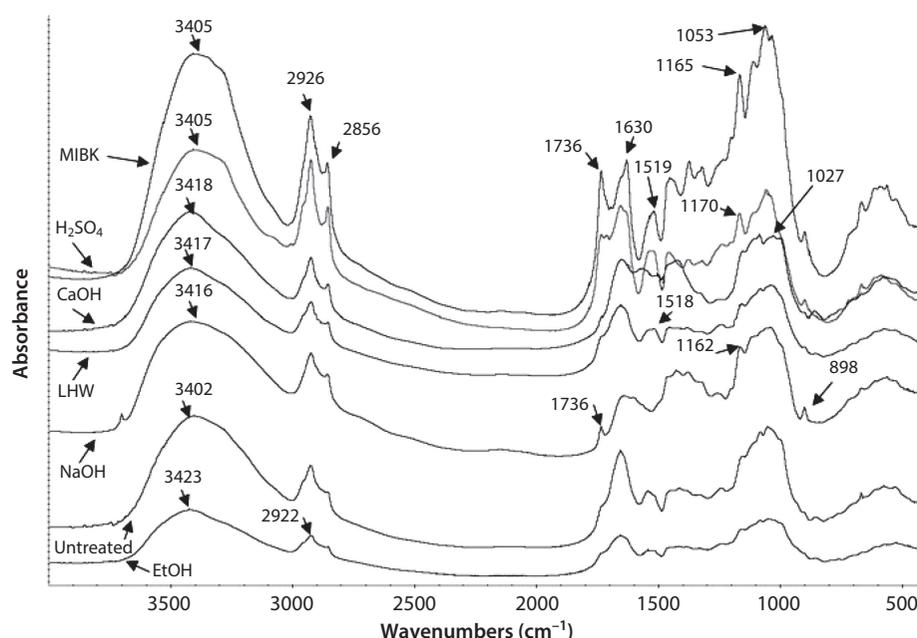


Figure 9 FTIR spectra of untreated and treated brans with different thermophysical and chemical methods.

Table 1 Characteristic functional groups of untreated and treated wheat bran with different methods in FTIR spectra.

Untreated		EtOH		NaOH		LHW		CaOH		H <sub>2</sub> SO <sub>4</sub>		MIBK	
Wave number (cm <sup>-1</sup> )	Characteristic group	Wave number (cm <sup>-1</sup> )	Characteristic group	Wave number (cm <sup>-1</sup> )	Characteristic group	Wave number (cm <sup>-1</sup> )	Characteristic group	Wave number (cm <sup>-1</sup> )	Characteristic group	Wave number (cm <sup>-1</sup> )	Characteristic group	Wave number (cm <sup>-1</sup> )	Characteristic group
3402	O-H	3423	O-H	3701	C-OH	3416	O-H	3418	O-H	3405	O-H	3405	O-H
2925	C-H	2923	C-H	3416	O-H	2924	C-H	2923	C-H	2924	C-H	2926	C-H
1655	C=O	1654	C=O	2924	C-H	1655	C=O	1655	C=O	2850	C-H	2856	C-H
1540	N-H	1539	N-H	1736	C=O	1518	N-H	1400	CH <sub>2</sub>	1736	C=O	1736	C=O
1411	CH <sub>2</sub>	1414	CH <sub>2</sub>	1641	C=O	1453	CH <sub>2</sub>	1083	C-O	1660	C=O	1630	C=O
1047	C-H	1045	C-H	1425	CH <sub>2</sub>	1036	C-H	1027	C-H	1525	N-H	1519	N-H
				1162	C-O					1460	CH <sub>2</sub>	1453	CH <sub>2</sub>
				1039	C-H					1170	C-O	1372	C-H
				898	C-H					1053	C-H	1319	C-O
										898	C-H	1165	C-O
												1045	C-H
												898	C-H

**Table 2** Crystallinity index of untreated and treated wheat brans samples.

	Untreated	CaOH	EtOH	H <sub>2</sub> SO <sub>4</sub>	LHW	MIBK	NaOH
CI	0.94	0.98	0.96	0.79	0.93	0.97	0.98

the lowest decreased CI was 0.79 in the H<sub>2</sub>SO<sub>4</sub> treated sample. The change in CI observed was relatively low (~5%) in this study, but could be advantageous for biocomposite manufacturing. Mwaikambo and Ansell [47] observed superior mechanical strength with low crystalline fibers compared to fibers with high crystallinity. The higher crystallinity may be obtained by the destruction of the primary cell wall, which may result in decreasing the mechanical properties.

### 3.5 Discussion

The performance of polymer composites reinforced with natural fibers depends on several factors such as fibers' chemical compositions, cell dimensions, microfibrillar angle, defects, structures, physical properties, chemical properties, and the interaction between the fiber and polymer matrix [3]. Pretreatment of fibers could modify many of the fiber properties, which may result in improving the performance of the resulting biocomposites from the pretreated fibers. A numbers of pretreatment methods have been proposed for fibers based on the differences in fiber properties and chemical compositions. The chemical composition varies from species to species as well as within the same species because of the differences in climate and environment. It also varies within different parts of the same plant because of the physiological functions. In this study, a broad range of treatment methods have been used to pretreat wheat bran for making a suitable reinforcing material for biocomposites.

The chemical composition of fibers is the most important characteristic of any lignocellulosic material. While cellulose fraction increases the mechanical strength of biocomposites, amorphous hemicelluloses, lignin, protein, and fat have little or no contribution to the resulting biocomposite strength. Sodium hydroxide (NaOH) and H<sub>2</sub>SO<sub>4</sub> treated brans showed higher fractions of cellulose content and lower fractions of hemicelluloses, fat, protein, and starch compared to all other treatments. Pretreatment of bran by these two methods could be considered as candidates in preparing wheat bran biocomposites. Sufficient surface roughness was observed in the SEM micrographs of the bran treated with MIBK, H<sub>2</sub>SO<sub>4</sub>, and NaOH, despite the cleaning of cellular materials from the bran surfaces. Alkali treatment disrupted the hydrogen bonding in the network structure, which increased the surface roughness [3]. Alkali treatment increases the interfacial adhesion,

which may increase compressive and tensile strength and lead to better moisture resistance [48,49]. Alkali treatment also repairs the defects in fibers, thus increasing the fracture strain [49]. Crystallinity increased in NaOH treated fiber, but decreased in H<sub>2</sub>SO<sub>4</sub> treated bran. Crystallinity has the effects of increasing mechanical strength and decreasing moisture absorption. Alkali treatment solubilizes some amorphous materials and allows the repacking of celluloses, which increases the crystallinity of bran. It also decreases the spiral angle and increases molecular orientation [50]. For better water resistance in H<sub>2</sub>SO<sub>4</sub> treated bran, surface modification, such as grafting, may be used.

Wheat is one of the most important crop plants in the world. The utilization of wheat bran as biocomposites in plastic would make it more environmentally friendly and would find an alternative use for wheat bran in industrial products. This study would also encourage researchers to find other usages for wheat bran, such as isolation and characterization of nanoparticles, which could be used for drug and micronutrient delivery in animals and crop plants, respectively.

## 4 CONCLUSIONS

1. Cellulose content increased to 35.1% and 29.6% in treated bran with H<sub>2</sub>SO<sub>4</sub> and NaOH, respectively. Hemicelluloses content decreased to 6.83% and 24.9% for H<sub>2</sub>SO<sub>4</sub> and CaOH treated brans, respectively. No improvement in lignin fraction decrease was observed by any treatment.
2. Starch content decreased to 0.68% and 1.86% by H<sub>2</sub>SO<sub>4</sub> and NaOH treated brans, respectively. Fat content was removed effectively by all the pretreatment methods except H<sub>2</sub>SO<sub>4</sub>. NaOH treatment removed the crude protein effectively, and the crude protein fraction decreased to 4.67%.
3. SEM micrograph showed surface cleaning of treated bran while maintaining sufficient surface roughness for H<sub>2</sub>SO<sub>4</sub>, NaOH, and MIBK treated brans.
4. Presence of a pure cellulosic functional group was observed in the spectra of brans treated with H<sub>2</sub>SO<sub>4</sub>, NaOH, and MIBK.
5. Crystallinity index increased, though slightly, for all treatments except H<sub>2</sub>SO<sub>4</sub> treated bran.
6. A considerable amount of hemicellulose was not removed, and future studies should address this limitation to enhance the removal of more hemicelluloses.

In ongoing studies, the preparation of bran treated with NaOH is being pursued because of the reduction

in crude protein and fat content compared to H<sub>2</sub>SO<sub>4</sub> treated bran even though H<sub>2</sub>SO<sub>4</sub> treated bran has slightly higher cellulose content, which can be useful in plastic production as a reinforcing material. After analyzing the physico-mechanical properties of treated wheat bran biocomposites, the use of wheat bran as an effective filler will be proven.

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## REFERENCES

1. G. Scott and D. Wiles, Programmed-life plastics from polyolefins: A new look at sustainability. *Biomacromolecules* **2**(3), 615–622 (2001).
2. M. Fuqua and C. Ulven, Characterization of polypropylene/corn fiber composites with maleic anhydride grafted polypropylene. *J. Biobased Mater. Bio.* **2**(3), 258–263 (2008).
3. O. Faruk, A. Bledzki, H. Fink, and M. Sain, Biocomposites reinforced with natural fibers: 2000-2010. *Prog. Polym. Sci.* **37**(11), 1552–1596 (2012).
4. L. Shen, J. Haufe, and M.K. Patel, Product overview and market projection of emerging bio-based plastics PRO-BIP 2009, Report for European Polysaccharide Network of Excellence (EPNOE) and European Bioplastics, Universiteit Utrecht, Utrecht, The Netherlands, 2009.
5. J. Collier, M. Lu, M. Fahrurrozi, and B. Collier, Cellulosic reinforcement in reactive composite systems. *J. Appl. Polym. Sci.* **61**(8), 1423–1430 (1996).
6. M. Vignon, D. Dupeyre, and C. Garcia Jaldon, Morphological characterization of steam-exploded hemp fibers and their utilization in polypropylene-based composites. *Bioresour. Technol.* **58**(2), 203–215 (1996).
7. T. Bullions, R. Gillespie, J. Price-O'Brien, and A. Loos, The effect of maleic anhydride modified polypropylene on the mechanical properties of feather fiber, kraft pulp, polypropylene composites. *J. Appl. Polym. Sci.* **92**(6), 3771–3783 (2004).
8. A. Bledzki, M. Letman, A. Viksne, and L. Rence, A comparison of compounding processes and wood type for wood fibre-PP composites. *Compos. Part A Appl. Sci. Manuf.* **36**(6), 789–797 (2005).
9. R. Arib, S. Sapuan, M. Ahmad, M. Paridah, and H. Zaman, Mechanical properties of pineapple leaf fibre reinforced polypropylene composites. *Mater. Des.* **27**(5), 391–396 (2006).
10. A. Karmarkar, S. Chauhan, J. Modak, and M. Chanda, Mechanical properties of wood-fiber reinforced polypropylene composites: Effect of a novel compatibilizer with isocyanate functional group. *Compos. Part A Appl. Sci. Manuf.* **38**(2), 227–233 (2007).
11. K. Pickering, G. Beckermann, S. Alam, and N. Foreman, Optimising industrial hemp fibre for composites. *Compos. Part A Appl. Sci. Manuf.* **38**(2), 461–468 (2007).
12. K. Hossain, C. Ulven, K. Glover, F. Ghavami, S. Simsek, M.S. Alamri, A. Kumar, and M. Mergoum, Interdependence of cultivar and environment on fiber composition in wheat bran. *Aust. J. Crop Sci.* **7**(4), 525–531 (2013).
13. S. Panthapulakkal, S. Law, and M. Sain, Effect of water absorption, freezing and thawing, and photo-aging on flexural properties of extruded HDPE/rice husk composites. *J. Appl. Polym. Sci.* **100**(5), 3619–3625 (2006).
14. X. Xie, S. Cui, W. Li, and R. Tsao, Isolation and characterization of wheat bran starch. *Food Res. Int.* **41**(9), 882–887 (2008).
15. K. Kim, R. Tsao, R. Yang, and S. Cui, Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem.* **95**(3), 466–473 (2006).
16. A.D. Evers, C.T. Greenwood, D.D. Muir, and C. Venables, Studies on the biosynthesis of starch granules. Part 8. A comparison of the properties of the small and the large granules in mature cereal starches. *Starch - Stärke* **26**(2), 42–46 (1974).
17. W.S. Cui, P.J. Wood, J. Weisz, and M.U. Beer, Non-starch polysaccharides from pre-processed wheat bran: Chemical composition and novel rheological properties. *Cereal Chem.* **76**(1), 129–133 (2000).
18. D. Fellers, V. Sinkey, A. Shepherd, and J. Pence, Solubilization and recovery of protein from wheat mill-feeds. *Cereal Chem.* **43**(1), 1–7 (1966).
19. S. Taj, M.A. Munawar, and S. Khan, Natural fiber-reinforced polymer composites. *Proc. Pak. Acad. Sci.* **44**(2), 129–144 (2007).
20. A. Bledzki and J. Gassan, Composites reinforced with cellulose based fibres. *Prog. Polym. Sci.* **24**(2), 221–274 (1999).
21. C.Y. Liang and R.H. Marchessault, Infrared structure of crystalline polysaccharides. I. Hydrogen bonds in native celluloses. *J. Polym. Sci.* **37**(132), 385–395 (1959).
22. P. Tingaut, T. Zimmermann, and F. Lopez-Suevos, Synthesis and characterization of bionanocomposites with tunable properties from poly(lactic acid) and acetylated microfibrillated cellulose. *Biomacromolecules* **11**(2), 454–464 (2010).
23. I. Boukari, J. Putaux, B. Cathala, A. Barakat, B. Saake, C. Remond, M. O'Donohue, and B. Chabbert, In vitro model assemblies to study the impact of lignin-carbohydrate interactions on the enzymatic conversion of xylan. *Biomacromolecules* **10**(9), 2489–2498 (2009).
24. S. Janardhnan and M. Sain, Isolation of cellulose microfibrils—An enzymatic approach. *Bioresources* **1**(2), 176–188 (2006).
25. G. Chauvelon, N. Gergaud, L. Saulnier, D. Lourdin, A. Buleon, J. Thibault, and P. Krausz, Esterification of cellulose-enriched agricultural by-products and characterization of mechanical properties of cellulosic films. *Carbohydr. Polym.* **42**(4), 385–392 (2000).

26. S. Aboulfadl, S. Zeronian, M. Kamal, M. Kim, and M. Ellison, Effect of mercerization on the relation between single fiber mechanical-properties and fine-structure for different cotton species. *Text. Res. J.* **55**(8), 461–469 (1985).
27. D. Varma, M. Varma, and I. Varma, Coir fibers. 1. Effect of physical and chemical treatments on properties. *Text. Res. J.* **54**(12), 827–832 (1984).
28. S. Wang, Q. Cheng, T.G. Rials, and S.H. Lee, Cellulose microfibril/nanofibril and its nanocomposites, in *8th Pacific Rim Bio-based Composites Symposium*, Knoxville, TN, pp. 301–308 (2006).
29. S-T. Jiang and N. Guo, The steam explosion pretreatment and enzymatic hydrolysis of wheat bran. *Energ. Source. Part A* **38**(2), 295–299 (2016).
30. S.K. Black, B.R. Hames, and M.D. Myers, Method of separating lignocellulosic material into lignin, cellulose and dissolved sugars, US Patent 5730837, assigned to Midwest Research Institute (1998).
31. AOAC, Moisture in animal feed, Method 967.03, AOAC International, Gaithersburg, MD, USA (2005).
32. H.K. Goering and P.J. Van Soest, Forage fiber analyses (apparatus, reagents, procedures, and some applications), *ARS/USDA Handbook No. 379*, U.S. Department of Agriculture, Washington, DC (1970).
33. M.L. Nelson and R.T. O'Connor, Relation of certain infrared bands to cellulose crystallinity and crystal lattice type. Part II. A new infrared ratio for estimation of crystallinity in celluloses I and II. *J. Appl. Polym. Sci.* **8**(3), 1325–1341 (1964).
34. S. Panthapulakkal, A. Zereshkian, and M. Sain, Preparation and characterization of wheat straw fibers for reinforcing application in injection molded thermoplastic composites. *Bioresour. Technol.* **97**(2), 265–272 (2006).
35. B. Lamsal, R. Madl, and K. Tsakpundis, Comparison of feedstock pretreatment performance and its effect on soluble sugar availability. *Bioenergy Res.* **4**(3), 193–200 (2011).
36. V. Chang, B. Burr, and M. Holtzapple, Lime pretreatment of switchgrass. *Appl. Biochem. Biotechnol.* **63**, 3–19 (1997).
37. A. Bledzki, P. Franciszczak, and A. Mamun, The utilization of biochemically modified microfibrils from grain by-products as reinforcement for polypropylene biocomposite. *Express Polym. Lett.* **8**(10), 767–778 (2014).
38. A. Bledzki, A. Mamun, N. Bonnia, and S. Ahmad, Basic properties of grain by-products and their viability in polypropylene composites. *Ind. Crops Prod.* **37**(1), 427–434 (2012).
39. K.G. Satyanarayana, K. Sukumaran, P.S. Mukherjee, C. Pavithran, and S.G.K. Pillai, Natural fiber-polymer composites. *Cement and Concrete Comp.* **12**(2), 117–136 (1990).
40. Y. Pu, F. Hu, F. Huang, B. Davison, and A. Ragauskas, Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. *Biotechnol. Biofuels* **6**(15), 1–13 (2013).
41. N. Kobayashi, N. Okada, A. Hirakawa, T. Sato, J. Kobayashi, S. Hatano, Y. Itaya, and S. Mori, Characteristics of solid residues obtained from hot-compressed-water treatment of woody biomass. *Ind. Eng. Chem. Res.* **48**(1), 373–379 (2009).
42. R. Kumar, G. Mago, V. Balan, and C. Wyman, Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresour. Technol.* **100**(17), 3948–3962 (2009).
43. Ö.P. Çetinkol, D.C. Dibble, G. Cheng, M. Kent, S.B. Knierim, M. Auer, D.E. Wemmer, J.G. Pelton, Y.B. Melnichenko, J. Ralph, and B.A. Simmons, Understanding the impact of ionic liquid pretreatment on eucalyptus. *Biofuels* **1**(1), 33–46 (2010).
44. J. Bouanda, L. Dupont, J. Dumonceau, and M. Aplincourt, Use of a NICA-Donnan approach for analysis of proton binding to a lignocellulosic substrate extracted from wheat bran. *Anal. Bioanal. Chem.* **373**(3), 174–182 (2002).
45. F. Xu, J. Sun, R. Sun, P. Fowler, and M. Baird, Comparative study of organosolv lignins from wheat straw. *Ind. Crops Prod.* **23**(2), 180–193 (2006).
46. M. Ibrahim, F. Agblevor, and W. El-Zawawy, Isolation and characterization of cellulose and lignin from steam-exploded lignocellulosic biomass. *Bioresources* **5**(1), 397–418 (2010).
47. L. Mwaikambo and M. Ansell, Mechanical properties of alkali treated plant fibres and their potential as reinforcement materials II. Sisal fibres. *J. Mater. Sci.* **41**(8), 2497–2508 (2006).
48. E. Bisanda, The effect of alkali treatment on the adhesion characteristics of sisal fibres. *Appl. Compos. Mater.* **7**(5–6), 331–339 (2000).
49. K. Goda, M. Sreekala, A. Gomes, T. Kaji, and J. Ohgi, Improvement of plant based natural fibers for toughening green composites – Effect of load application during mercerization of ramie fibers. *Compos. Part A Appl. Sci. Manuf.* **37**(12), 2213–2220 (2006).
50. S. Sreenivasan, P. Iyer, and K. Iyer, Influence of delignification and alkali treatment on the fine structure of coir fibres (*Cocos Nucifera*). *J. Mater. Sci.* **31**(3), 721–726 (1996).