Cationic Surfactant-assisted Microwave-NaOH Pretreatment for Enhancing Enzymatic Hydrolysis and Fermentable Sugar Yield from Peanut Shells

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Production of bioethanol from lignocellulosic biomass is difficult due to differences in the feedstock. There is a need for an efficient pretreatment method that not only reduces the total process economy but also increases the total process efficiency. Following microwave-NaOH pretreatment of peanut shells in the presence of the cationic ionic surfactant cetyltrimethylammonium bromide (CTAB) and enzymatic hydrolysis, the pretreatment efficiency was significantly enhanced. The structural changes before and after pretreatment were detected by Fourier transform infrared (FTIR) analysis, X-ray diffraction (XRD), and scanning electron microscopy (SEM). FTIR and SEM showed the differences between the untreated and pretreated samples. The XRD profile showed that the degree of crystallinity was higher for pretreated materials than for untreated ones. These changes also verified the effect of CTAB during pretreatment of peanut shells.

Keywords: Peanut shells; Bioethanol production; Pretreatment; Cationic surfactant

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INTRODUCTION

Lignocellulosic biomass can be used as raw materials for the production of bioethanol (Alvira *et al*. 2010). Relevant to bio-energy, cellulosic bioethanol has attracted increasing attention due to the fact it is renewable and carbon neutral. In addition, it has the potential to reduce environmental pollution, stimulate economic development, and maintain energy security (Fang *et al*. 2010).

Lignocellulosic biomass contains two structural polysaccharides, cellulose and xylan, which are both primary sources of fermentable sugars. However, these polysaccharides are surrounded with lignin, forming a highly compact structure in the cell wall (Park *et al*. 2010). As one of many lignocellulosic biomasses, peanut shells are abundant and inexpensive byproducts of peanut processing operations. Every year, the yield of peanut shells reaches as high as 5 million tons in China alone (Qiu *et al*. 2012). Peanut shells also contain these polysaccharides, and they have great potential as feedstock for bioconversion of cellulosic ethanol. Bioethanol production from lignocellulosic biomass comprises the following main steps: feedstock pretreatment, hydrolysis of cellulose and hemicellulose, sugar fermentation, and recovery and purification of ethanol. At present, there are still a number of difficulties that impede the industrial-scale production of lignocellulosic ethanol from agricultural wastes.

It is vital to preprocess feedstock to destroy the structure between lignin and both cellulose and hemicellulose. The pretreatment leads to more exposed cellulose and hemicellulose for enzymatic saccharification. To solve this daunting pretreatment issue,

several methods of pretreatment of lignocellulosic biomass have been explored in a number of reports. Because of its processing time and efficacy, microwave pretreatment and microwave-assisted alkali treatment of lignocellulosic biomass has been widely studied (Zhu *et al*. 2005). This method can change the structure of cellulose, degrade hemicellulose and lignin in lignocellulosic biomass, and increase the enzymatic susceptibility of various materials (Azuma *et al.* 1985), including rice straw, cashew apple bagasse, and switchgrass (Jackowiak *et al*. 2011; Rodrigues *et al*. 2011; Zhu *et al*. 2005). Additionally, some groups have investigated the use of surfactants in pretreatment of lignocellulosic biomass (Kapu *et al*. 2012; Qi *et al*. 2010; Qing *et al*. 2010). These studies showed that surfactant-assisted pretreatment of lignocellulosic biomass improved lignin removal and reduced the required energy input.

We describe microwave-alkali pretreatment in the presence of a surfactant for the conversion of peanut shell into sugars and verify the effects of the surfactant on the efficacy of the pretreatment process. The structural features of the untreated and pretreated peanut shell were investigated by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) analysis.

EXPERIMENTAL

Materials

Luhua No.11 peanut biomass obtained from Shandong Academy of Agricultural Sciences (Qingdao, Shandong, China) was used in this study. Peanut shells were air-dried and milled to a size less than 1 mm for the pretreatment experiment. Their dry weight content was measured after heating to 105 °C for 24 h. The compositional analysis of untreated peanut shell was carried out using the analysis method of Van Soest (Biernacki *et al*. 2013; Van Soest 1963). The cellulase enzyme used in this study was a commercial *Aspergillus niger* cellulase from Shanghai Juncheng Biotech. Crop., China. The CMCase activity was 11.2 ± 0.02 IU/mg at pH 4.8 and 50 °C for 30 min, and filter paper activity 0.52 ± 0.01 IU/mg at pH 4.8 and 50 °C for 1 h.

Surfactants evaluated in this study were polyethylene glycol (PEG) 4000, PEG 6000, cetyltrimethylammonium bromide (CTAB), polyoxyethylene 20 sorbitan monolaurate (Tween® 20), and polysorbate 80 (Tween® 80).

Methods

Feedstock preparation

One gram of biomass was placed in a 100-mL stoppered conical flask and mixed with different surfactants and 30 mL of sodium hydroxide solution (1% w/v NaOH). The pretreatment was performed in a microwave oven (Guangdong Galanz Group Co. Ltd, Guangdong, China). The experiments on the effect of different process parameters on CTAB-assisted microwave-NaOH pretreatment of peanut shells were carried out at 480 W for 10 min with 1% (w/v) NaOH and 1% (w/w) CTAB. Neutralization of the pretreated sample was carried out by washing with tap water, and the sample was dried at 65 °C in a constant temperature oven. The pretreatment efficiency was determined by the hydrolysis efficiency, which was estimated by measuring the reducing sugar. After preliminary screening of various surfactants, the most effective surfactant was chosen for further research.

Enzymatic hydrolysis

Dried pretreated peanut shells were enzymatically hydrolyzed in a 100-mL stoppered conical flask by incubating 1.00 g of biomass with cellulase in 25 mL of 0.1 M sodium citrate buffer (pH 4.8). Samples were incubated at 45 °C and placed in a shaking water bath (SHZ-82, Changzhou Boyuan Instrument Plant, Changzhou, China) at 160 rpm for 48 h. Following the incubation, samples were centrifuged at 5000 rpm for 10 min to remove the unhydrolyzed residue. The reducing sugar contents of the supernatants were analyzed by employing the dinitrosalicylic acid method (Miller 1959).

FTIR analysis

FTIR spectroscopic analysis was performed to detect changes in functional groups that may have been caused by the pretreatment (Binod *et al*. 2012). The FTIR spectrum was recorded between 4000 and 400 cm^{-1} using a Rayleigh spectrometer (WQF-510, Beijing Analytical Instrument Co. Ltd, China) with a detector at 4 cm^{-1} resolution and 8 scans per sample. Discs were prepared by mixing 2 mg of dry sample with 400 mg of potassium bromide (KBr) in an agate mortar. The resulting mixture was successfully pressed at 30 MPa for 1 min.

XRD analysis

The crystallinity of both untreated and pretreated samples was measured by X-ray diffraction (D8, Bruker Optics Inc, Germany); radiation was Cu Kα (λ=1.54 Å). The samples were scanned in a 2θ range of 10 to 40°, and a step size of 0.02° was used for the analysis. The crystallinity index of each sample was expressed using the following equation (Bansal *et al*. 2010):

$$CrI\ (\%) = [(I_{002} - I_{am})/I_{002}] \times 100 \quad (1)$$

where *CrI* is the crystalline index, I_{002} is the maximum intensity of the (002) lattice diffraction, and the I_{am} is the intensity diffraction at 18.0°, 2θ degrees.

The degree of crystallinity was calculated as (Zhou *et al*. 2005):

$$\chi_c = F_c/(F_a + F_c) \times 100\% \quad (2)$$

where F_a and F_c are the area of the non-crystalline and crystalline regions, respectively.

The crystallite size was calculated from the Scherrer equation, with the method based on the width of the diffraction patterns. The crystallite sizes were determined by using the diffraction pattern obtained in the (002) orientation of sample:

$$D_{(hkl)} = K\lambda/\beta_0 \cos\theta \quad (3)$$

where $D_{(hkl)}$ is the crystallite size (nm), K is the Scherrer constant (0.94), and λ is the X-ray wavelength (0.15418 nm for Cu). β_0 is the full-width at half-maximum of the reflection hkl, and 2θ is the corresponding Bragg angle (Sun *et al*. 2008).

SEM analysis

Scanning electron micrographs were taken at a magnification of 800x for both untreated and pretreated peanut shells (particle size < 1 mm) using a JSM-6390LV

scanning electron microscope (JEOL USA, Peabody, MA) operating under high vacuum conditions at an accelerating voltage of 15 KV (Zhang *et al*. 2013). They were secured onto aluminum stubs with double-sided tape and coated with gold using the sputter coater supplied with the microscope.

Statistical analysis

The content of the released reducing sugar was determined in triplicate, and data presented as the mean and standard deviation. Analysis of the one-way ANOVA was performed by SPSS software (version 16.0, IBM, US) in this study. Comparisons that yielded $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of Different Surfactants in Microwave-NaOH Pretreatment of Peanut Shells

To evaluate the potential of peanut shells as a raw material for sugar production, the structural polysaccharides and the lignin content were determined with the method described in the preceding part of this study. The cellulose, hemicellulose, and lignin contents in the peanut shells were 326.7 ± 5.2 g kg^{-1}, 200.1 ± 5.1 g kg^{-1}, and 221.5 ± 4.1 g kg^{-1}, respectively. Although the lignin content of peanut shells is high (Table 1), peanut shells included significant amounts of available sugars for conversion (Van Dyk *et al*. 2012; Kuprianov and Arromdee 2013).

Table 1. Approximate Composition (as a percentage) of Various Biomass Materials

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Corn cob	35-39	38-42	4.5-6.6
Rice straw	41	21.5	9.9
Corn stover	39	19.1	15.1
Wheat straw	36.6	24.8	14.5
Peanut shell[a]	46.5	9.7	41.3

[a] Ash-free basis

The presence of surfactants during acid pretreatment enhanced the enzymatic hydrolysis of recovered solids (Qi *et al*. 2010; Qing *et al*. 2010). The addition of surfactants during pretreatment led to increased removal of lignin and made the biomass surface more hydrophilic. We tested the effect of various surfactants on the efficiency of the microwave-alkali pretreatment reaction. PEG 4000 and PEG 6000 are uncharged polymers of ethylene oxide, polyoxyethylene 20 sorbitan monolaurate and polysorbate 80 are non-ionic detergents, and CTAB is a cationic surfactant.

In these experiments, peanut shells were pretreated in the presence of the surfactants, and the solids recovered were digested with cellulase. Reducing sugar content was used to assess the effect of the added surfactants in the pretreatment. As shown in Fig. 1, the presence of surfactants had a significant effect ($P < 0.05$) on the reducing sugar content during microwave-NaOH pretreatment. Compared to the control, PEG 4000, CTAB, and polysorbate 80 increased the enzymatic digestibility of the recovered solids. In fact, the statistical analysis show that PEG 4000 and polysorbate 80

did not play a positive role in improving the effect of pretreatment. However, Cao and Aita (2013) reported that dilute ammonia pretreatment in the presence of both PEG 4000 and polysorbate 80 enhanced the digestibility of sugarcane bagasse. Acid pretreatment with polyoxyethylene 20 sorbitan monolaurate has been shown to significantly improve the enzymatic hydrolysis of wheat straw (Qi *et al*. 2010).

Polyoxyethylene 20 sorbitan monolaurate failed to enhance the efficiency of microwave-NaOH pretreatment of peanut shells in the present study. Unlike other studies, we found that microwave-NaOH pretreatment with the cationic surfactant CTAB significantly improved the enzymatic hydrolysis of peanut shells. Sindhu *et al.* (2013) reported that CTAB-assisted ultrasound was used for pretreatment of sugarcane but it was not optimal. The discrepancy in the influence of surfactants on these substrates may be attributed to a variety of factors, including the compositional differences between peanut shells and other agriculture residues, differences in binding affinity between surfactants and the substrates, differences in the concentration of substrate and cellulase used in the enzymatic hydrolysis, and the difference in the source of cellulase.

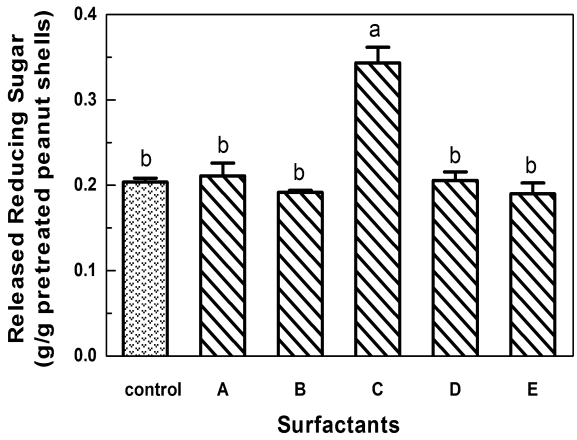

Fig. 1. Influence of different surfactants on microwave-NaOH pretreatment of peanut shells. A: PEG 4000; B: PEG 6000; C: CTAB; D: polyoxyethylene 20 sorbitan monolaurate; E: polysorbate 80. Results shown are means ± SD (n = 3), determined after 48 h of enzymatic hydrolysis. Values followed by the same letter are not significantly different ($P > 0.05$). Control refers to pretreatment in the absence of a surfactant.

Effect of Different Process Parameters on CTAB-assisted Microwave-NaOH Pretreatment of Peanut Shells

Figure 2A depicts the effect of microwave power on the pretreatment of peanut shells. Microwave power had significant effects ($P < 0.05$) on the reducing sugar content in pretreatment, whether or not pretreatment was carried out in the presence of CTAB. At all microwave power tests, CTAB significantly increased the cellulosic digestibility of the pretreated solids. Microwave-NaOH pretreatment with CTAB produced more digestible solids than that of the control pretreatment (Fig. 2A). In the present study, 480 W of microwave power seemed to be optimal, as the reducing sugar yield decreased beyond this value. However, reducing sugar yields beyond 320 W revealed no significant differences according to analysis of the variance. Zhu *et al*. (2005) reported that rice straw pretreated by microwave/alkali at microwave power settings ranging from 300 to 700 W presented almost identical final compositions of cellulose when the irradiation was set at 300, 500, or 700 W for 15 min. The conclusion is consistent with our findings. At 320 W, the highest reducing sugar content was present after microwave-NaOH

pretreatment with CTAB. Microwave irradiation offers a fast process due to efficient and rapid heating and causes vibration of polar bonds within the biomass (Choudhary *et al*. 2012). The vibration of polar bonds leads to disruption and shock, which accelerates physical, chemical, and biological reactions. In addition, low microwave power heating requires less energy input than conventional heating to preprocess biomass, but higher microwave power may induce the generation of new hydrogen bonds which may impede pretreatment of peanut shells.

Figure 2B describes the effect of NaOH concentration on pretreatment of peanut shells. NaOH concentration had significant effects ($P < 0.05$) on the reducing sugar content in both microwave-NaOH with CTAB and control pretreatments. The microwave-NaOH pretreatment with CTAB improved the digestibility of the pretreated solids. Alkali pretreatment efficiently disrupts the ester bonds cross-linking lignin and xylan, leading to cellulose- and hemicellulose-enriched fractions and a more porous structure for enzyme access (Li 2012). However, an excess of NaOH solution results in high concentrations of nonreversible salt, which increases the interaction between ions and has an unfavorable impact on the pretreatment of peanut shells (Cheng 2011).

It can be seen in Fig. 2B that 1.5% NaOH caused a higher reducing sugar yield than other concentrations of NaOH. Increasing the NaOH concentration to 2.5% had almost no effect on the reducing sugar yield. According to the analysis of variance, reducing sugar yields beyond 1.5% NaOH (w/v, 0.45 $g \cdot g^{-1}$ dried solids) caused no significant differences. Investigators have reported that the yields of saccharides (sum of xylose, arabinose, and glucose) could not overcome 90 mg/g wheat straw dry matter at 2% NaOH, while increasing the NaOH concentration from 2% to both 3% and 5% provided much better yields (Janker-Obermeier *et al*. 2012). Liu *et al*. (2012) observed that the optimal conditions for microwave-NaOH pretreatment of vinasse were as follows: 0.06 $g \cdot g^{-1}$ NaOH, 523 W microwave power, 1:2 solid-to-liquid ratio, and 8 min pretreatment time. These results are different from the findings in the present study: 1% NaOH, 480 W microwave power and 10 min pretreatment time. Perhaps microwave heating of surfactants helps the dilute NaOH solution to remove uronic acids and acetyl groups from polysaccharides and increase the accessibility for enzymatic hydrolysis.

Figure 2C reveals the effect of CTAB loading on pretreatment. CTAB loading had a significant effect ($P < 0.05$) on the reducing sugar content in both microwave-NaOH with CTAB and control pretreatments. Compared to control pretreatment, the microwave-NaOH pretreatment with CTAB enhanced the digestibility of the pretreated solids. A CTAB loading of 0.4% resulted in the highest reducing sugar content after pretreatment. The minimum loading was suitable for pretreatment, according to the analysis of variance. CTAB has been used to help isolate plant DNA from lyophilized tissue (Murray and Thompson 1980). CTAB has the ability to dissolve the cell membrane of plants and precipitate nucleic acids and acidic polysaccharides in low-ionic strength solutions. Pectin and hemicellulose are acidic polysaccharides in cell walls. For this reason, we assume that CTAB may help to remove hemicellulose, which results in exposing more cellulose in microwave-NaOH pretreatment of peanut shells. In such cases, pretreatment increases the accessibility of cellulose to enzymes in the saccharification process. Further work is necessary to elucidate the mechanism behind this phenomenon.

Figure 2D shows the effect of pretreatment time on pretreatment of peanut shells. Pretreatment time had a significant effect ($P < 0.05$) on the reducing sugar content in both microwave-NaOH with CTAB and control pretreatments. Along with the extension of

time, the reducing sugar content showed almost no change with microwave-NaOH pretreatment. At the same time, microwave-NaOH pretreatment in the presence of CTAB significantly increased the reducing sugar content. The highest reducing content of 0.328 g/g was received following 16 min of CTAB-assisted microwave-NaOH pretreatment. Zhu *et al*. (2006) reported that the process of wheat straw microwave-NaOH pretreatment was effective, and after 96 h enzymatic hydrolysis of the maximum reducing sugar content of 42.9±0.9 g/L was achieved after 25 min at 700 W of pretreatment. Although the reducing sugar content was not higher in the pretreatment of microwave-NaOH with CTAB, the pretreatment time was significantly shorter than the microwave-NaOH pretreatment time. This indicates that in the presence of CTAB, the required pretreatment time and heat input in microwave-NaOH pretreatment can be decreased.

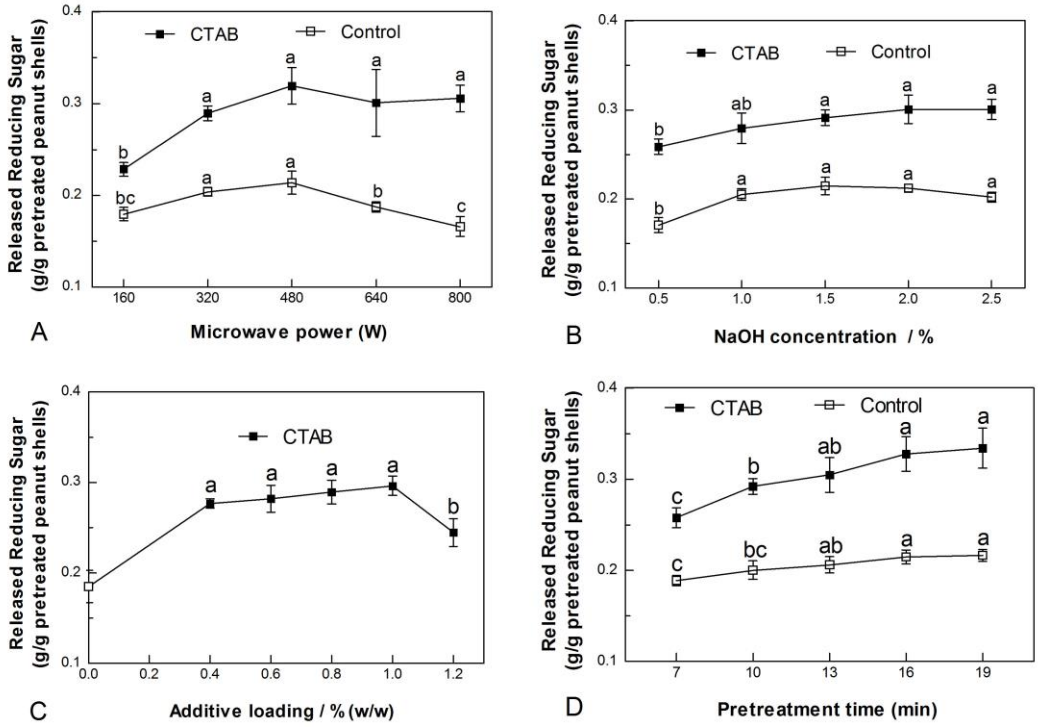

Fig. 2. Influence of different process parameters on CTAB-assisted microwave-NaOH pretreatment of peanut shells. Results shown are means ± SD (n = 3), determined after 48 h of enzymatic hydrolysis. Values followed by the same letter are not significantly different ($P > 0.05$). Control refers to pretreatment in the absence of a surfactant.

Characterization of Untreated and Pretreated Biomass

FTIR analysis

FTIR spectroscopy was used to investigate the changes to cellulose structures during pretreatment. There is a difference between untreated and pretreated samples. Figure 3 shows the FTIR spectra of untreated peanut shells, peanut shells pretreated with microwave-NaOH, and peanut shells pretreated with microwave-NaOH in the presence of CTAB. The most representative bands were summarized as follows.

Two adsorption bands, 1100 cm^{-1} and 900 cm^{-1}, arose from C-O-C stretching at the β-1,4-glycosidic linkages (Sindhu *et al*. 2012). The peak of 1030 cm^{-1} corresponds to C-O-C-O-C bonds in cellulose. Bands at 1000 to 1200 cm^{-1} are associated with structural changes of cellulose and hemicellulose. While the C-H bending occurs at 1281 cm^{-1} and 1373 cm^{-1} (Binod *et al*. 2012), the bands at 1316 cm^{-1} and 1431 cm^{-1} in the spectra can be assigned to symmetric CH_2 bending and wagging (Cao and Tan 2004). The peak of CH_2

stretching near the 2850-cm^{-1} region is a distinguishing feature of cellulose (Sun *et al*. 2008). The band adsorption in the 3250-cm^{-1} region is related to stretching of H-bonded OH groups (Sindhu *et al*. 2012).

The FTIR spectra were different for untreated and pretreated peanut shells, which demonstrates that there were structural changes to cellulose after pretreatment. The enhancement of adsorption peaks at 1000 to 1100 cm^{-1} after pretreatment indicates the increase in cellulose content in the recovered solids (Sun *et al*. 2008). The O-H stretching peak at 3300 cm^{-1} and the -CH_2 stretching peak at 2900 cm^{-1} are the distinguishing features of cellulose (Binod *et al*. 2012). The primary changes were broadening of the bond at 3200 to 3400 cm^{-1}, which was related to the O-H stretching of the hydrogen bonds (Hsu *et al*. 2010). The peak shifts to a higher wave number if the intensity of intermolecular hydrogen bond is weak (Sindhu *et al*. 2012). The FTIR spectra verified the stretching of hydrogen bonds of pretreated peanut shells arose at higher wave numbers, which signifies that the structure of pretreated peanut shells was looser than that of untreated ones.

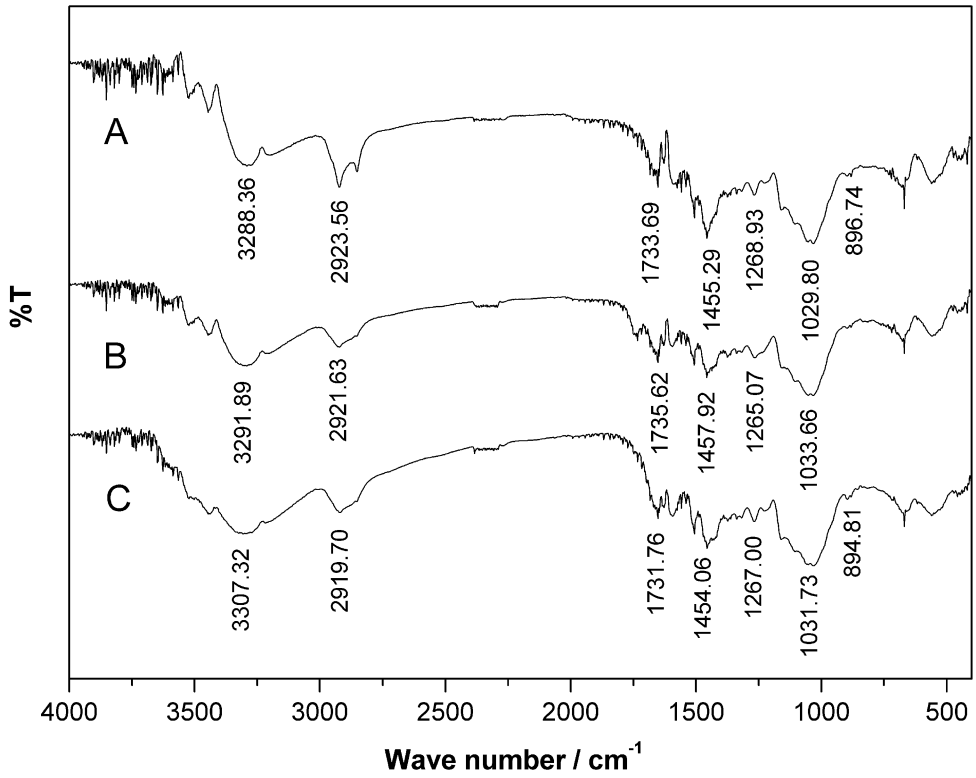

Fig. 3. FTIR spectrum of untreated and pretreated peanut shells: A: untreated, B: microwave-NaOH pretreated, C: microwave-NaOH pretreated in the presence of CTAB

XRD analysis

The X-ray diffraction profile of the untreated and pretreated peanut shells is shown in Fig. 4. The crystallinity index, crystallite size, and degree of crystallinity in the untreated as well as pretreated peanut shells are shown in Table 2. CTAB-assisted microwave-NaOH pretreatment gave the highest crystallinity index (43.32%), while the crystallinity index of untreated peanut shells was smaller (39.57%) than that of other samples. Cellulose crystallinity has been considered an important factor in determining

the hydrolysis rate. Some literature reported that pretreatment increases the crystallinity of the cellulose fraction (Alvira *et al*. 2010). This may be due to the removal or reduction of more easily available amorphous cellulose after pretreatment. The degree of crystallinity of the pretreated materials was greater than that of the materials that were not pretreated. This indicates that the pretreatment was effective. The pretreated materials showed a high crystallinity index, which indicated the removal of lignin by microwave-NaOH. It is possible that partial removal of lignin and hemicelluloses, and physical changes to the cellulose caused by pretreatment may be the reasons for the increased crystallinity index of pretreated biomass. We also found that the crystallite size was higher in the untreated peanut shells than in the pretreated ones.

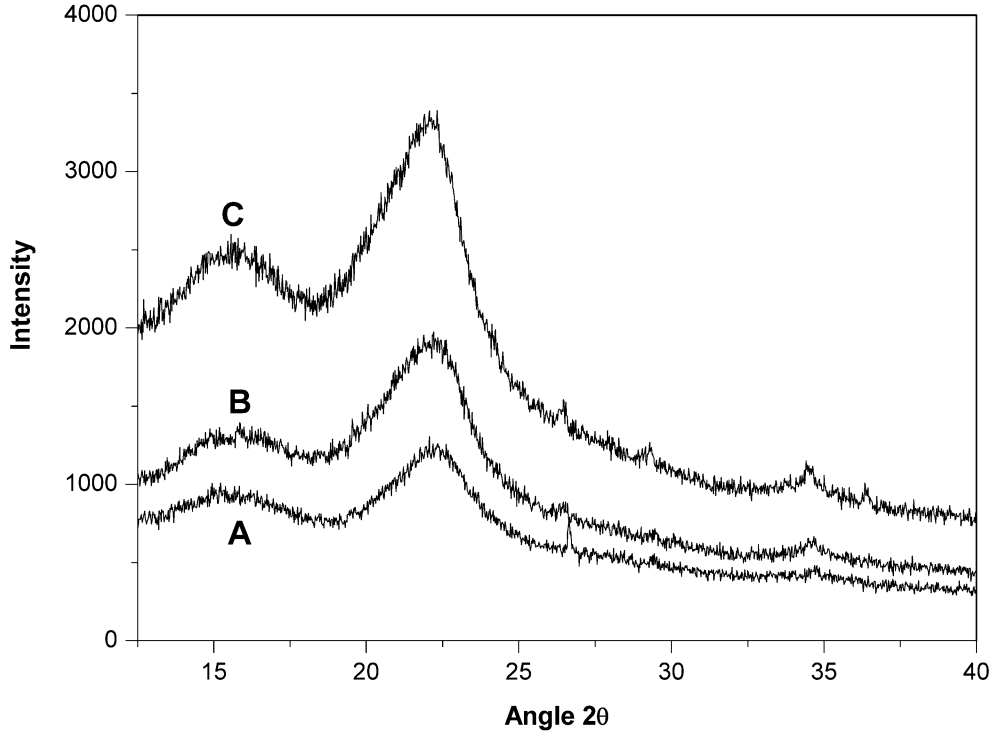

Fig. 4. X-ray diffraction profiles of the untreated and pretreated peanut shells: A: untreated, B: microwave-NaOH pretreated, C: microwave-NaOH pretreated in the presence of CTAB

Table 2. Crystallinity Index and Crystallite Size of Untreated and Pretreated Peanut Shells

Material	Crystallinity index (%)	Crystallite size (nm)	Crystallinity degree (%)
Untreated	39.57	0.170	53.86
Microwave-NaOH pretreated	42.49	0.166	61.82
CTAB+Microwave-NaOH pretreated	43.32	0.146	66.08

SEM analysis

SEM observations of peanut shells before and after pretreatment (Fig. 5) showed that pretreatment induced physical changes in the biomass. The untreated peanut shells have a compact and ordered surface, whereas the pretreated peanut shells have a rough

surface and crumbly texture. The cellulose of untreated peanut shells was difficult to hydrolyze to reducing sugar by cellulase. This also demonstrates that pretreatment removed external fibers, which increased the surface area of peanut shells, so that enzymes had more access to cellulose. Meanwhile, pretreatment contributes to enzymatic hydrolysis by breaking the highly compact structure in the cell wall of the biomass. Peanut shells pretreated by microwave-NaOH in the presence of a surfactant as the catalyst appeared rough, with numerous visible cracks.

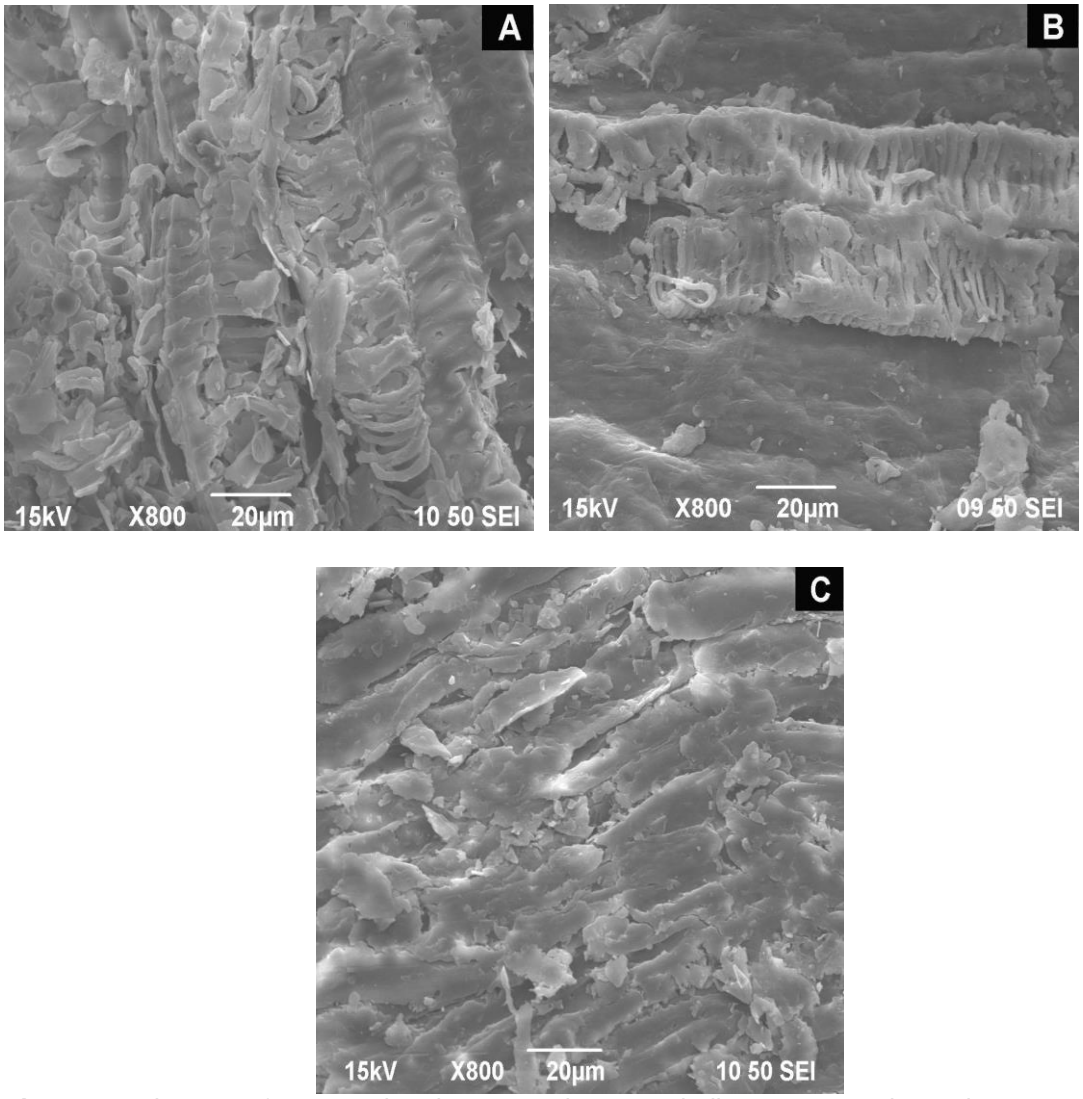

Fig. 5. SEM images of untreated and pretreated peanut shells: A: untreated, B: microwave-NaOH pretreated, C: CTAB-assisted microwave-NaOH pretreated

CONCLUSIONS

1. The findings of the current study demonstrate that sugar production from peanut shells using a surfactant-assisted microwave-NaOH pretreatment and enzymatic hydrolysis protocol presents a viable scheme for utilizing this agricultural waste.

2. The cationic surfactant CTAB, in addition to other widely researched non-ionic surfactants, substantially improves microwave-NaOH pretreatment and enzymatic hydrolysis of lignocellulosic biomass.
3. The cationic surfactant CTAB significantly enhanced the effect of microwave-NaOH pretreatment of peanut shells and reduced the required input energy during pretreatment.

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