An Efficient Method of Bio-Chemical Combined Treatment for Obtaining High-Quality Hemp Fiber

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This bio-chemical study focuses on obtaining high-quality hemp fiber. The effects of the structures and properties of hemp fibers in different treatment periods were studied. Moreover, the changes of the surface morphology, chemical composition, and breaking tenacity of hemp fibers were researched by scanning-electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), fluorescence microscopy, and fiber tensile testing. The results showed that by virtue of the enzyme scouring process, alkali refining process, and bleaching process, the pectin, lignin, and hemicellulose and other impurities were removed. Through the single factor experiment, the optimal process conditions for the bio-chemical combination of the degumming process were obtained. These conditions included 10 g of dried hemp fibers, 15% (v/v) pectinase solution, a temperature of 50 °C, a duration of 120 min, pH 8.0 (phosphate buffer), a liquor ratio (w/v) of 1:10, and 0.0625 mol/L NaOH. In these conditions, the residual gum content and breaking tenacity were 4.8% and 49.8 cN/tex, respectively, indicating that the treated hemp fibers met the requirements of the spinning process.

Keywords: Hemp; Degumming; Bio-chemical; Residual gum

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INTRODUCTION

Hemp fibers have relatively low fineness and no scratchiness, which promotes their high hygroscopicity, gas permeability, and other excellent properties. In addition, these fibers are resistant to bacteria, mold, and ultraviolet rays, and they can be used in healthcare applications (Mohanty *et al.* 2002; Keller 2003; Khan *et al*. 2014). With the low content of tetrahydrocannabinol (THC < 0.3%) that has been achieved by breeding of hemp varieties (Lachenmeier *et al.* 2004), hemp has gradually come to the forefront of textile applications. The hemp fiber can be blended and spun with cotton, silk, wool, and other chemical fibers, and it can also be spun into mono-fibers. However, the phloem of hemp contains hemicellulose, lignin, pectin, wax, and other chemical impurities that must be removed from the fiber surface in a key process called degumming (George *et al.* 2015). Degumming has significant effects on spinning quality, fabric style, and comfort. There is a direct relationship between the degumming quality and fiber properties (Sreenath *et al.* 1996; Nair *et al*. 2015).

Compared with ramie fiber, hemp fiber has higher hemicellulose and lignin contents, which directly affects its spinning performance. Commonly used degumming methods include chemical degumming (Di Candilo *et al.* 2000; Wang *et al.* 2003; Thomsen *et al.* 2006), physical degumming (Vignon *et al.* 1995; Dupeyre and Vignon 1998; Guo and Zhao 2010), and biological degumming (Nykter *et al.* 2008).

Because the water resistance of hemp is very strong, the fiber is difficult to ferment; chemical degumming is the primary method at this time. Chemical degumming consumes a great deal of energy and water and inflicts severe pollution on the environment, among other issues. The degumming of bast-fiber crops has been investigated at the molecular level, namely enzymatic degumming. Applying enzymes to facilitate hemp degumming effectively reduces the above problems, improves hemp quality, and reduces environmental pollution. Pectinase can reduce the residual gum content of hemp fibers, though it is difficult to achieve standard textile requirements. Thus, enzymatic degumming must be combined with a chemical treatment to further improve the degumming process of hemp.

Enzyme pre-treatment is the key step in the hemp bio-chemical degumming process. The main role of the pre-treatment is to remove parts of the pectin. Pectinase breaks down pectin into galacturonic acid (Fang *et al.* 2016) and reduces the burden on the subsequent degumming. Wetting allows the hemp fiber to swell and become loose, which improves the penetration of scouring agents. Under alkaline conditions, lignin reacts with alkali, and the ether bonds present in the compounds of α-phenyl ether, α-alkyl ether, β-aryl ether, and aryl ether linked with lignin would be attacked by nucleophile of OH^- and broken, In addition, the reaction degrades and dissolves large molecules such as lignin.

After the lignin has been removed by alkali, hemicellulose depolymerization occurs; the reducing end-group of the hemicellulose chain is oxidized (Keller *et al.* 2001; Sinha and Rout 2008; Sawpan *et al.* 2011; Sharma *et al.* 2011). Cellulose is easily hydrolyzed by acid and easily oxidized. Therefore, the hemp chemical degumming process cannot employ inorganic acid and an oxidant as the main technology; instead, degumming can only incorporate alkali scouring (Kapoor *et al.* 2001; Adamsen *et al.* 2002; Dai and Fan 2010). To minimize damage to the fibers and their original mechanical properties, adding appropriate additives can improve the efficiency of degumming (Taha *et al.* 2007).

In the present work, the bio-chemical properties of high-quality hemp fibers were studied. First, the pectin on the surface of hemp fibers was treated with pectinase, and then the fibers were boiled in dilute NaOH. The morphology and structure of the hemp fibers in different degumming conditions were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), and fluorescence microscopy. The breaking tenacity and residual gum content were also investigated.

EXPERIMENTAL

Materials

Ying-kou City Xinyi Textile (Ying-kou, Liaoning province, China) supplied hemp. Alkaline pectinase (238.47 U/mL, optimal pH 8.0) was provided by the Engineering Research Center for Clean Production of Textile Dyeing and Printing, Ministry of Education, Wuhan Textile University. The alkaline pectinase was produced from *Bacillus subtilis* k30 that had been isolated from RAMCD407 by Yu *et al.* (2013). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent (Shanghai, China). Milli-Q (Advantage A10, Millipore, Billerica, MA, USA) water was used throughout the experiments.

Methods

Biochemistry degumming

The process began by a pretreatment water bath for the hemp fibers in 60 °C water. Tap water was used to wash away impurities on the surface of the hemp fibers, and then the fibers were dried and weighed. Alkaline pectinase was applied to a certain amount of dried hemp fibers, and the fibers were washed in tap water, dried, and weighed, followed by chemical degumming in the different concentrations of NaOH. Finally, to meet the requirements of hemp fiber whiteness in the industry, the fibers were bleached with hydrogen peroxide, washed with tap water, and air-dried. The step-by-step recipe details are given in Table 1.

Table 1. Fiber Processing Conditions

Process	Temperature (°C)	Time (h)	Liquor Ratio (w/v)	Other Conditions
Enzyme scouring	40-60	0.5-3.0	1:10	10 g of dried hemp fibers, 5%-25% (v/v) pectinase solution, pH 8.0 (phosphate buffer)
Alkali refining	100	1	1:10	0.0125-0.075 mol/L NaOH, 0.15% Na_2CO_3, 0.3% Na_4SiO_4, 0.3% Na_2SO_3, 0.05% primary alcohol ethoxylate (AEO)
Bleaching	80	1	1:10	0.5% H_2O_2, 0.3% Na_2CO_3, 0.5% Na_4SiO_4, 0.3% primary alcobol ethoxylate (AEO)

Breaking tenacity test

The hemp fibers were placed in an electrothermal dry box with a constant temperature between 45 °C and 50 °C. The system ensured that the moisture regain was less than 12.0%, After pretreatment, the temperature was between 20 ± 2 °C, the relative humidity was 65% ± 3% to reach moisture equilibrium, and the time was no less than 2 h. The breaking tenacity (cN/tex) was measured by a fiber strength tester (XQ-2, Shanghai New Fiber Instrument, Shanghai, China). The reported data corresponds to the mean ± SD of three replicates.

Residual gum content test

Three samples of hemp fibers (4 g each) were randomly chosen, dried to constant weight, and added to 150 mL of NaOH (0.5 mol/L). The solution was boiled for 3 h in a three-necked flask. Distilled water was used to wash away excess gum on the hemp fibers surface, and the fibers were dried to a constant weight. The residual gum content was calculated with Eq. 1,

$$W_c = (G_0 - G)/G_0 \times 100 \quad (1)$$

where W_c is the residual gum content (%), G_0 is dry weight of the sample (g), and G is the dry weight of the sample after extracting the residual gum (g).

Scanning electron microscopy

Scanning electron microscopy was used to observe the microstructure and surface morphology of crude hemp and hemp treated by enzymatic hydrolysis, alkali treatment, and bleaching. Hemp fiber bundles were 0.5 cm in length. The samples were coated with gold using a vacuum sputter and samples were observed under a Phenom pro microscope (Phenom-World, Eindhoven, Netherlands).

Fluorescence microscopy

Fluorescence microscopy (Leica DM2500, Heidelberg, Germany) was used to observe the surface morphology of the treated and untreated hemp fibers. Four different filters were used: bright field, green (BP 515-560, dichromatic mirror: 580, suppression filter: LP 590, size K.), ultraviolet (BP 340-380, dichromatic mirror: 400, suppression filter: LP 425, size K.), and blue (BP 450-490, dichromatic mirror: 510, suppression filter: LP 515, size K.). The fiber samples were observed under 200× magnification.

Fourier-transform infrared spectroscopy

The chemical composition of hemp fibers was determined using a Tensor 27X infrared FT-IR spectrometer (Bruker Optics, Ettlingen, Germany). The spectra were recorded in the range of 400 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} and 64 scans. Before the fibers were tested, the hemp samples were dried, cut with scissors, and tableted with KBr.

Chemical components and degree of polymerization test

The main chemical constituents of hemp fiber, *viz*. fat and wax, water extractives, pectin, hemicellulose, lignin, and cellulose were determined by quantitative analysis of ramie chemical components (China standard GB 5889-86). The degree of polymerization (DP) was determined in cupriethylenediamine (CED) according to the testing method of degree of polymerization of ramie cellulose (China standard GB 5888-86).

RESULTS AND DISCUSSION

Optimization of the Degumming Process

Optimum enzyme content

During the alkaline pectinase pretreatment for hemp fibers, the pectinase penetrated the interior of the fiber and decomposed the pectin. This made the hemp fiber fluffy and soft, as well as created benefits for the next steps of the chemical degumming and bleaching process.

When determining the optimal content test of pectinase, the pectinase volume of solution volume ratio (V/V) was 5% to 25% at 50 °C, pH 8.0, and with a duration of 90 min.

Performance test results after the enzyme degumming are shown in Fig. 1. The residual gum content gradually decreased with increasing pectinase content and finally leveled off. This is because the pectin was decomposed further with the increased pectinase content; the residual gum no longer changed when pectin decomposition concluded. Because enzymatic reactions destroy hydrogen bonds between macromolecules, the fiber-breaking strength tended to decrease. To minimize fiber damage, the selected pectinase content was 15%.

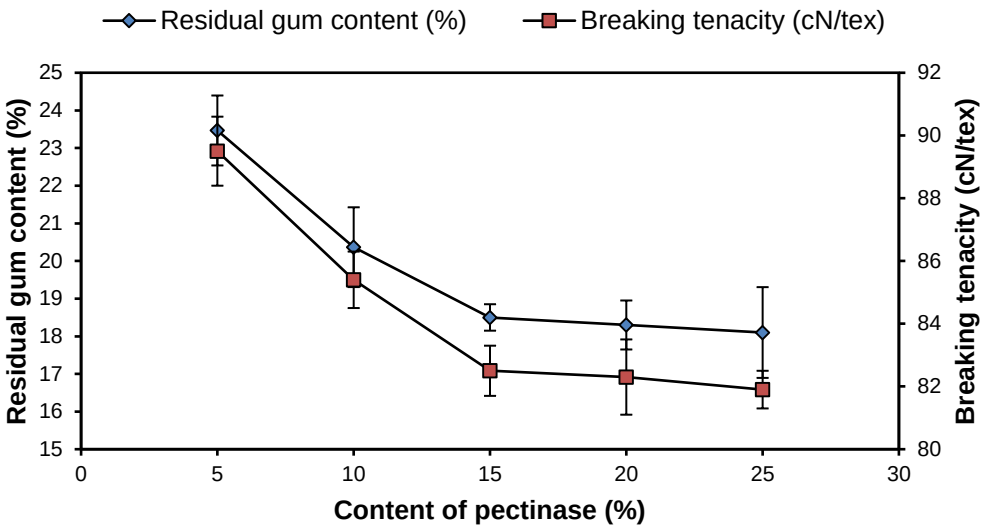

Fig. 1. Effect of pectinase content for residual gum content and breaking tenacity

Optimum degumming temperature

In general, higher temperature activates enzymes, which is useful for the catalyzing reaction. However, the enzyme is protein, and when the temperature is higher than a certain range, it will denature and inactivate the protein, so that the reaction rate is reduced. To determine the optimal reaction temperature, the temperature range was 40 to 60 °C at pH 8.0, and the reaction time was 90 min, with 15% (v/v) pectinase solution. The changes in the properties of hemp fibers are shown in Fig. 2. The residual gum content gradually declined with increasing temperature, and there were sharp increases after 50 °C. This result suggested that the enzyme lost activity above 50 °C. Between 40 to 50 °C, the biological enzyme degumming ability was enhanced, and the breaking tenacity decreased noticeably. The breaking tenacity increased between 50 to 60 °C, which indicated that the enzyme lost activity above 50 °C, and the effect of the fiber degumming was decreased. Taken together, these results indicated that the best degumming temperature was 50 °C.

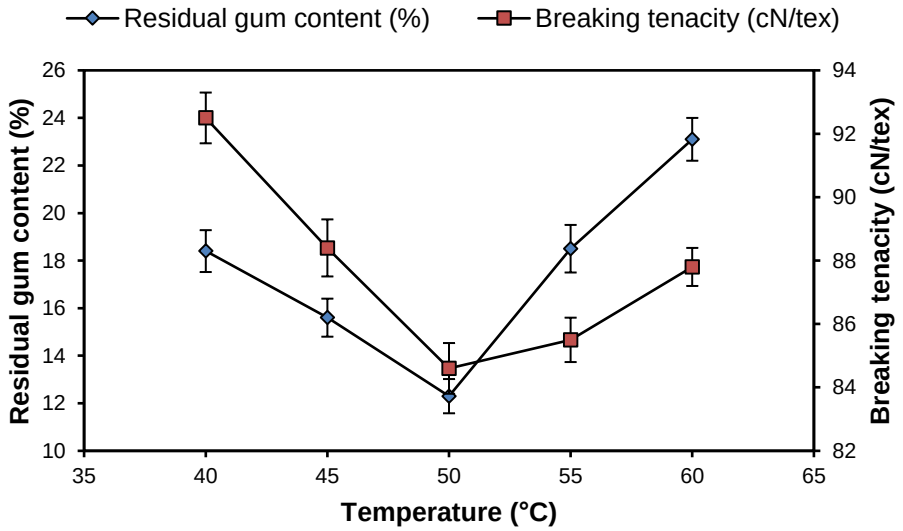

Fig. 2. Effect of temperature for residual gum content and breaking tenacity

Optimum degumming time

The optimal reaction time was determined in the time of 30 to 180 min at pH 8.0, 15% (v/v) pectinase, and 50 °C. The changes in the properties of hemp fibers are shown in Fig. 3. A prolonged treatment time decreased the residual gum content and breaking tenacity of hemp fiber, indicating that the longer treatment time removed more pectin. After 120 min, the residual gum was stable, but the fiber sustained a large amount of damage, such that the gum was likely to remain in the fiber. Therefore, the best time of treatment was 120 min.

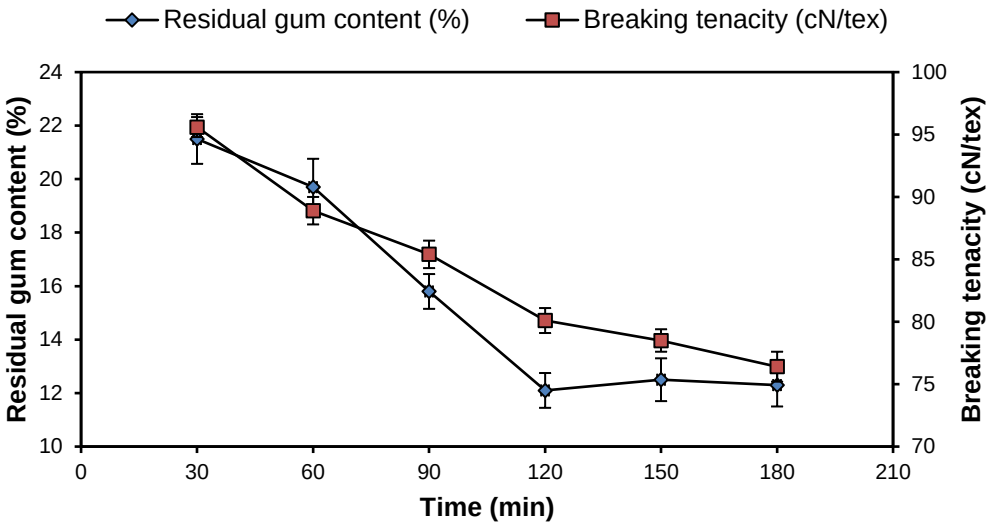

Fig. 3. Effect of react time for residual gum content and breaking tenacity

Optimum content of NaOH

Because the pectinase pretreatment only removes pectin, the optimum conditions of degumming experiment were determined, and the chemical degumming process of sodium hydroxide for hemp fiber was carried out. This method effectively removes lignin, hemicellulose, and other impurities.

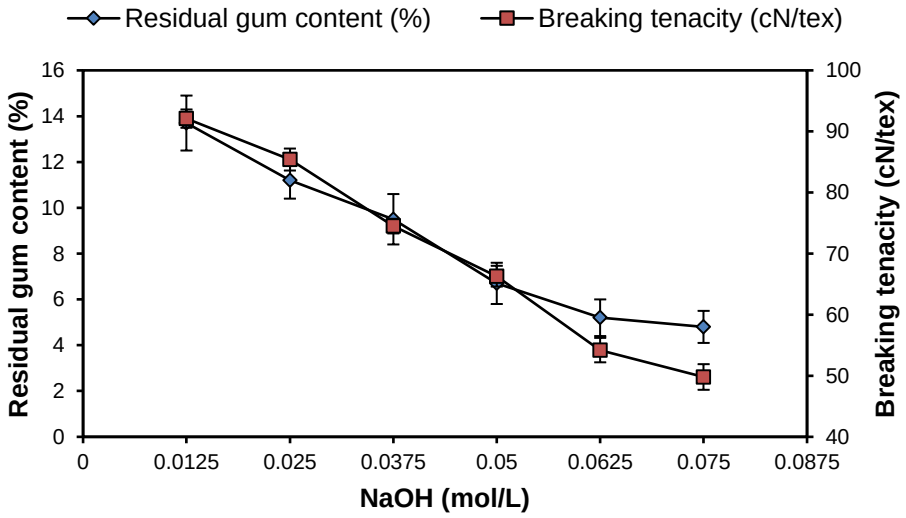

Fig. 4. Effect of NaOH content for residual gum content and breaking tenacity

The bio-chemical method uses a lower dosage of sodium hydroxide than the traditional chemical method. The dosage of sodium hydroxide was adjusted to the range 0.0125 to 0.075 mol/L in an alkali scouring process. As shown in Fig. 4, the increased sodium hydroxide content decreased the residual gum content, and the breaking tenacity of hemp fibers also decreased. However, the low degree of breaking tenacity affects the spinning performance. The sodium hydroxide concentration of 0.0625 mol/L was found to be the most suitable.

SEM Analyses

The morphology of the treated and crude hemp fiber surface was investigated by SEM, as illustrated in Fig. 5. The crude hemp fibers exhibited a continuous and irregular surface covered with impurities including lignin, pectin, waxes, and hemicelluloses (Fig. 5A).

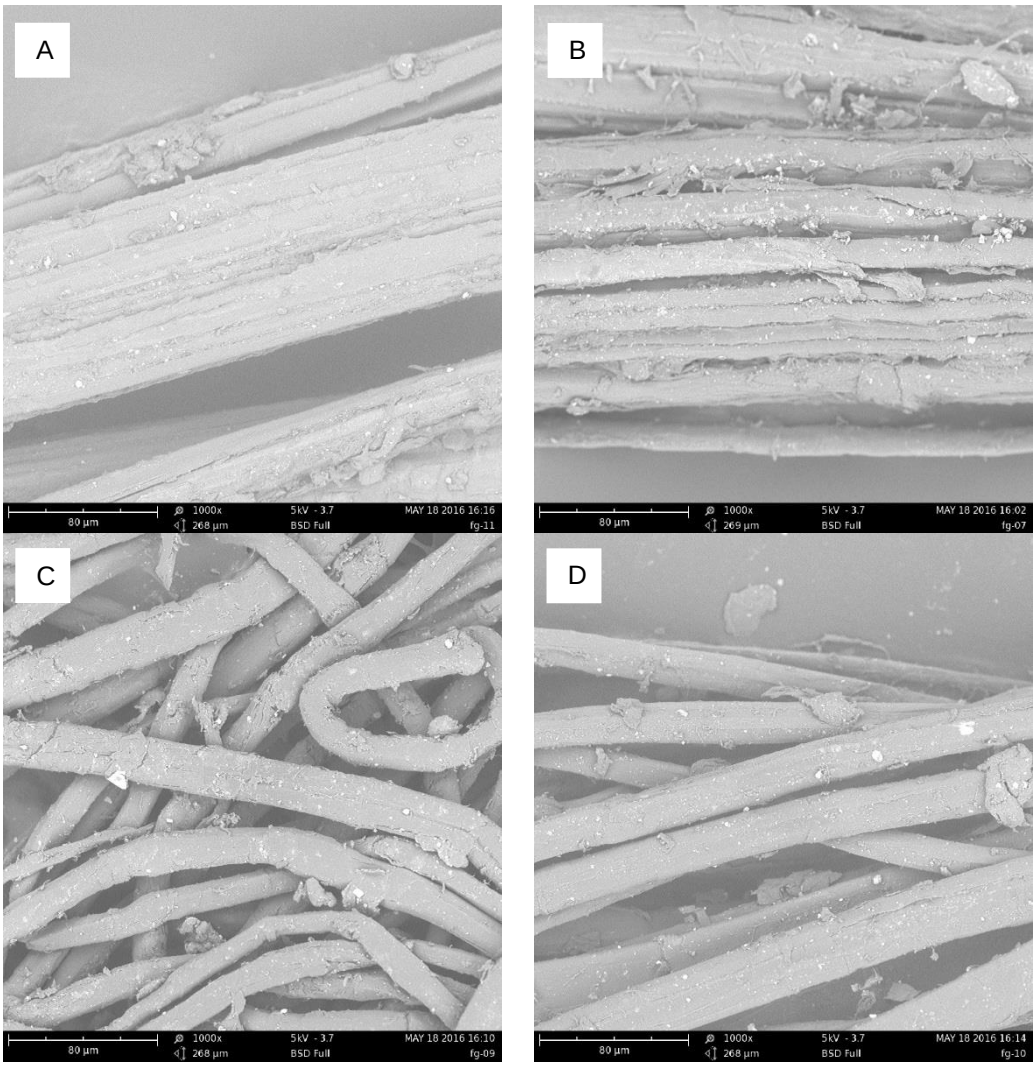

Fig. 5. Scanning electron micrograph of longitudinal view of (A) crude hemp (1000x), (B) enzymatic hydrolysis (1000x), (C) enzyme followed by alkali treated (1000x), and (D) bleached hemp fibers (1000x). Scale bars = 80 μm in each micrograph

These impurities are localized on the surfaces of crude hemp fibers (Liu *et al*. 2013). When the pectin was removed by enzymatic hydrolysis (Fig. 5B), the fibers were dispersed, and the fiber bundles were clearly visible. The lignin, waxes, and hemicelluloses were almost completely removed after enzymatic and alkali treatment (Fig. 5C). After this process, the fibers were cleaner and smoother, and the fiber bundles were more detached and different from the crude hemp fibers. To meet industrial requirements, the hemp fibers were bleached (Fig. 5D). After the H_2O_2 treatment, the surfaces were smoother, and the fibers were finer, suggesting that the majority of the non-cellulosic materials were removed.

Fluorescence Microscopy Analyses

To further analyze the structure of fiber surfaces, fluorescence microscopy was used with four filters: bright field (BF), green (N2.1), UV (A), and blue (I3) (Fig. 6). Under the four different filters, fibers (treated and crude) showed different surface structures. This effect was not due to the fluorescence properties of the hemp fiber, but rather due to light scattering. Light scattering intensity was increased with increasing roughness on the hemp fibers. Therefore, untreated hemp fibers (Fig. 6A-6D) showed higher color intensity than degummed hemp fibers (Fig. 6E-6H). After bleaching, no light scattering or color intensity was observed due to the maximum smoothness of the fiber. Thus, after the pectinase treatment, alkali treatment, and bleaching, the gum content was removed to a sufficient extent to make the hemp fibers smoother (Basu *et al*. 2009).

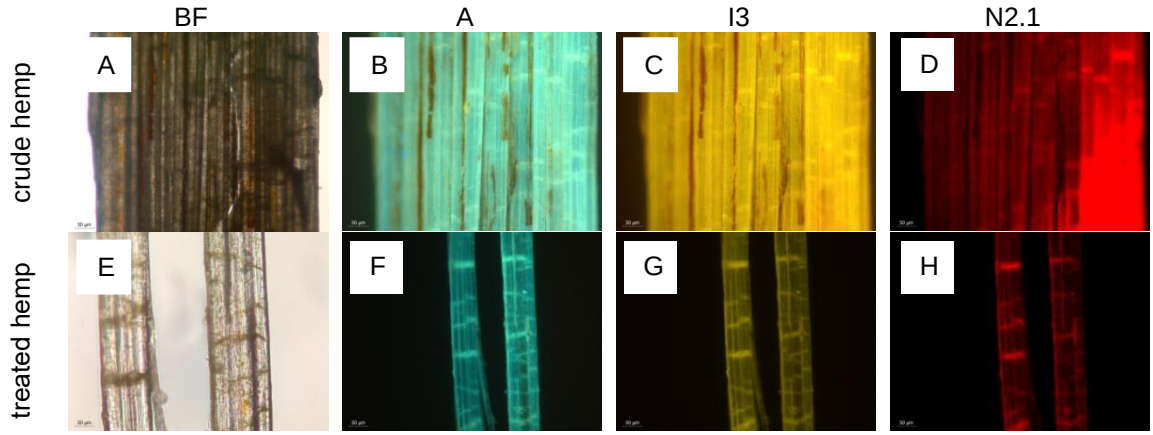

Fig. 6. Fluorescence microscopy of longitudinal views of untreated hemp fibers (A-D) and treated hemp fibers (E-H) under the bright field (BF), UV (A), blue (I3), and green (N2.1) filters

FT-IR Analyses

FT-IR was used to investigate changes in the surface composition of hemp fibers before and after degumming. The FT-IR spectra of crude hemp (Fig. 7A) and hemp treated by enzymatic hydrolysis (Fig. 7B), alkali treatment (Fig. 7C), and bleaching (Fig. 7D) showed the typical cellulose bands at 899 cm^{-1} (symmetric stretch, in-plane(C–O–C) of β-(1→4)-glycosidic linkages), indicating that the cellulose structure had few changes during degumming (Stevulova *et al*. 2014). The band at 1730 cm^{-1} was attributed to the C=O stretching of ester groups and carbonyl in pectin and hemicellulose (Sisti *et al*. 2016). Fibers after alkaline pectinase treatment (Fig. 7B) showed a weakened peak at 1730 cm^{-1} compared with crude fibers (Fig. 7A), indicating that the pectin was removed by alkaline pectinase. The characteristic peaks of lignin appeared at 1510 cm^{-1} and 1550

cm^{-1} (both attributed to stretching of the C=C of the aromatic ring), but the intensity of the lignin peaks was weaker for alkali treatment hemp fibers (Fig. 7C). Consequently, bleached hemp fibers (Fig. 7D) indicated the removal of lignin. However, the band at 1730 cm^{-1} (attributed to the C=O stretching of ester groups and carbonyl in pectin and hemicelluloses) was absent in the alkali treatment hemp fibers (Fig. 7C) and bleached hemp fibers (Fig. 7D), signifying that hemicellulose was removed. In particular, the signal at 2849 cm^{-1}, typical of waxes and oils disappears. The bleach hemp fiber spectra (Fig. 7D) indicated that the characteristic peaks were well related to the standard cellulose spectra according to the Sadtler file No 232 for Paper Materials Library. These observations confirmed that the pectin, waxes, lignin, and hemicellulose were removed.

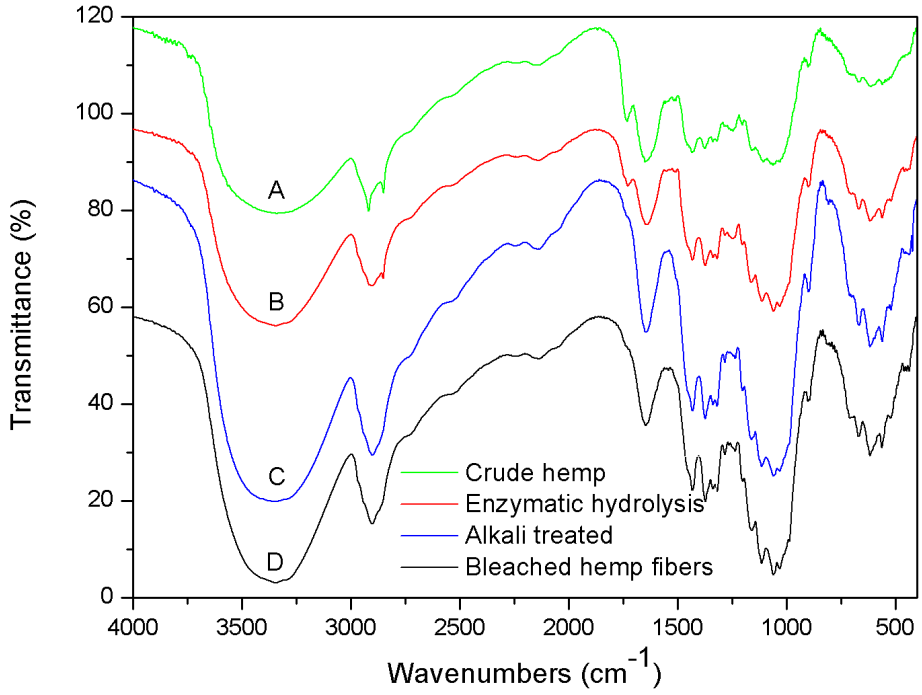

Fig. 7. FT-IR spectra of hemp fiber samples: crude hemp (A), enzymatic hydrolysis (B), enzyme followed by alkali treatment (C), and bleached hemp fibers (D)

Chemical Composition and Degree of Polymerization Analyses

The chemical compositions and DP of hemp fiber of various stage of degumming was obtained (Table 2).

Table 2. Chemical Composition and Degree of Polymerization of Hemp Fiber at Various Stages of Degumming

Process	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Pectin (%)	Fat and wax (%)	Water extractives (%)	DP
Crude hemp	57.1	17.4	6.8	6.2	1.1	9.6	5420
Enzyme scoured	67.6	17.1	6.8	1.7	0.9	4.1	4988
Alkali refined	89.4	3.1	3.1	1.1	0.3	1.2	2746
Bleached	91.9	2.4	2.3	0.8	0.2	0.6	2150

The pectin content in the crude hemp was 6.2%, while in the enzyme-resulted fiber it was 1.7%, suggesting that the pectin can be effectively removed by the enzyme treatment process.

Although the pectin was not fully removed, great changes have taken place in the molecular structure of gum in crude hemp because of the role of the enzyme. Thus, most of the remaining gum can be easily degraded by the following dilute alkaline treatment process. After alkali refining, the content of hemicellulose, lignin, pectin, fat plus wax, and water extractives were reduced to 3.1%, 3.1%, 1.1%, 0.3%, and 1.2% as compared to the original 17.4%, 6.8%, 6.2%, 1.1%, 9.6%, respectively. Regarding lignin, under alkaline conditions, it can react with alkali, then the ether bonds of α-phenyl ether, α-alkyl ether, β-aryl ether, and aryl ether in lignin would be attacked by nucleophile of OH^- and broken. After the lignin was removed by alkali, hemicellulose depolymerization occurred, since the reducing end-group of the hemicellulose chain have been exposed and oxidized. And the hemp fibers lost most of their non-cellulosic constituents through the last step of bleaching extractions.

The fiber DP of crude hemp was 5420. The DP was slightly decreased by enzyme scouring; however, it was reduced markedly after alkali refining and bleaching (Table 2). Maybe this was due to a certain extent of alkaline hydrolysis together with the possible oxidization, which led to the drop in fiber DP. As the degree of polymerization has a strong relation with the cellulose chain length in hemp and fiber strength, this was the reason for the decreases of the fiber breaking strength with the degumming progress.

Bio-chemical and Traditional Alkali Degumming Method Analyses

Table 2 shows the quality features of hemp fiber and chemical oxygen demand (COD) under different bio-chemical and traditional alkali degumming method. The DP was 2150 following bio-chemical treatment, which was higher than the traditional alkali degumming method (1944). The residual gum content of bio-chemical and traditional alkali of degumming process was 4.8% and 4.2%, respectively. Compared with the traditional alkali of degumming process, the bio-chemical process in the present study reduced the NaOH consumption by 75% and increased the breaking tenacity from 37.1 cN/tex to 49.8 cN/tex. Hence the bio-chemical combined degumming can be regard as an economic and environmentally-friendly technology.

Table 3. Quality of Hemp Fiber and Wastewater Indicators in Different Processes

Method	Breaking tenacity (cN/tex)	Residual gum content (%)	Add pectinase content (%)	NaOH (mol/L)	DP
Bio-chemical	49.8	4.8	15	0.0625	2150
Alkali	37.1	4.2	0	0.25	1944

CONCLUSIONS

1. An efficient hemp bio-chemical combined degumming method has been developed in which gums can be effectively removed using 15% (v/v) pectinase solution followed by 0.0625 mol/L NaOH treatment, resulting in high quality final fibers with an ideal residual gum content of 4.8% and good breaking tenacity of 49.8 cN/tex.

2. Compared with the traditional chemical method, this bio-chemical degumming technology reduced the NaOH consumption by 75% and markedly increased the breaking tenacity of the final fiber.
3. The present bio-chemical combined degumming method is an economic and environmentally-friendly technology. It can be easily industrially utilized in hemp textile industry.

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