Effect of Growth Period and Sampling Section on the Chemical Composition and Microstructure of Raw Hemp Fibers

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Hemp fiber's origin has a large impact on the physical properties of degummed fibers. In this research, the effect of hemp fiber origin on the treated fibers was studied mainly by evaluation of chemical composition and microstructure, as well as by the evaluation of the degummed fiber's properties under a biochemical process. The samples were chosen from the stem with two different growth periods (an early and a late harvest time) and three different sections along the hemp stem (top, middle, and bottom). The results showed a vast variety of chemical compositions and microstructures for raw hemp fibers in different growth periods. The results of the chemical composition analysis indicated that the cellulose content in raw fibers ranged from 41.9±2.3% to 44.8±0.8% and that the lignin content ranged between 12.0±1.5% and 16.5±0.9%. Both the Fourier transform infrared (FT-IR) and X-ray diffraction (XRD) results demonstrated the close relation of the raw fiber's character to the growth period and sampling section. The physical properties of degummed fibers with different origins were also tested, and large variations in length and linear density were found, which ranged from 42 mm to 67 mm for length and from 11.36±0.37 dtex to 21.55±0.54 dtex for linear density.

Keywords: Hemp fiber; Sampling; Microstructure; Mechanical properties; Physical properties

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INTRODUCTION

Hemp (*Cannabis sativa* L.) is one of the earliest widespread plants first cultivated with the aim of obtaining seeds for food production. With the recent rapid development of its usage in many ways, including textiles (Soljacic and Cunko 1994), paper (Barberà *et al*. 2011), heat insulation (Yates 2006), and composite materials (Thomsen 2006), hemp fiber has attracted increasing attention. As a renewable textile fiber source, its good mechanical and antibacterial properties, potential sustainability (Batog and Przepiera 2011), and biodegradability (Islam *et al*. 2010) make hemp fiber as irreplaceable as fibers such as cotton, flax, jute, and ramie.

Hemp is an annual bast fiber plant ranging in height from 1.2 to 5 m. Synthetic chemical fibers typically have a consistent quality due to their specific nature of production. In comparison, plant fibers lack homogeneity and their properties can be influenced by various aspects of their growing conditions (Mediavilla *et al*. 2001), such as soil quality, weather, and use of fertilizer. Even with the same meteorological conditions (Struik *et al*. 2000), the fiber morphology and chemistry are dependent on the growth stage and the section of stem from which the fibers are produced (Keller *et al*. 2001). Unlike cotton, in which each fiber consists of an individual cell growing out of the epidermis, bast hemp fibers are arranged in bundles of individual fibers running from the top to the bottom of the stem that are glued to each other by a noncellulosic adhesive material. The separation of the cellulosic part from the raw hemp fibers during the subsequent degumming process is required. To optimize the fibers for textile use purposes, the degummed hemp fibers need to be as fine and homogeneous as possible without added damage to their mechanical properties (Sharma and Van Sumere 1992). Various techniques for the hemp degumming have been explored, and the relationship between properties and the chemical composition of the degummed fibers have been discussed. In general, dyeing properties are related to the cellulose, lignin, and hemicellulose content of the fibers (Wang and Ramaswamy 2005; Tan and Wang 2011). The capillary properties of hemp fibers have been found to be improved by increasing the moisture sorption and decreasing water retention by removing hemicellulose from hemp fibers obtained from chemical treatment (Pejic *et al*. 2008).

In view of the importance of chemical composition on the properties of hemp fibers, many studies have been done in recent years to characterize and evaluate the various hemp plants and their raw fiber quality. The growing year and the variety have a significant effect on the hemp yield. Sausserde *et al*. (2013) also evaluated the relationship between industrial hemp cultivars and biomass potential and defined a key specific requirement for hemp-harvesting machines (Sausserde *et al*. 2013). The growth conditions (Amaducci *et al*. 2008) were investigated, and numerous conclusions were obtained. High-quality textile hemp fibers could be obtained through organic cultivation (Bengtsson 2009) and by prolonging the length of daylight illumination to a certain extent (Van der Werf *et al.* 1994). The stem thickness and plant height were found to be inversely proportional to the planting density, but the relationship between the proportion of stem weight as fiber and the sowing density was not apparent in Hall *et al*.'s study (2014). In addition, the research of the impact of the growth stage revealed that a harvest time at the beginning of seed maturity leads to easier decortication without any effect on the tensile strength of the bast fiber (Keller *et al.* 2001). The research teams of Ming Liu (Liu *et al.* 2015a,b) and Antoine Duval (Duval *et al.* 2011) separately studied the effects of different harvest times and stem sections of hemp fibers on the fiber quality. However, the combined effect of both factors was not discussed. Moreover, all previous studies focused on the hemp plant cultivated in Europe, which might be not applicable to the hemp fibers planted in China, given the differences in variety and cultivation area.

In view of the influence of the properties of raw hemp fibers on the downstream processing procedures, an in-depth investigation was conducted in this study to provide an improved understanding of the chemical composition and the microstructure of the raw hemp fibers with different growth periods and sampling sections. In addition, the physical properties of the degummed hemp fibers were also evaluated to verify the effect of the disparity of the raw materials on the physical properties of the degummed hemp fibers.

EXPERIMENTAL

Materials

The industrial hemp (*Cannabis sativa* L.) used in this study was cultivated in Lu’an, in the Anhui province in China. The hemp plants were harvested at two developmental stages: (1) early harvest at the beginning of flowering (on July 23, 2016) and (2) at late harvest at the beginning of seed maturity (on September 15, 2016). Three sampling sections were defined on one hemp stem: bottom (one third above the base of the stem), top (one third below the inflorescence base), and middle (between the bottom and top). In this study, six samples were analyzed, referred to as early-top (E-T), early-middle (E-M), early-bottom (E-B), late-top (L-T), late-middle (L-M), and late-bottom (L-B). The bark, including bast fibers, was manually separated from the fresh stems and dried at 40 °C to obtain raw hemp fibers for the following tests.

The sodium hydroxide (NaOH), sodium hypochlorite (NaClO), sodium bromide (NaBr), sodium pyrophosphate ($Na_4P_2O_7$), and anhydrous sodium sulfate (Na_2SO_3) used in this study were purchased from Aladdin Biological Technology Co., Ltd. (Shanghai, China). All of the chemicals were analytical reagent grade and were used without further purification. Additionally, laccase (activity of 600 IU/L) was purchased from Ruiyang Biotech Co., Ltd. (Jiangsu, China). The activity of laccase was measured by monitoring the oxidation rate of 2,2-azinobis-(3-ethylbenzyl thiozoline-6-sulfonate) (ABTS) (Zhang *et al*. 2016). The emulsified oil was purchased from Wong Butter Tea Development Co., Ltd. (Fuzhou, China). Deionized water was used to prepare all solutions.

Methods

Chemical composition analysis

The main chemical composition of the hemp fibers was adopted according to the Chinese standard GB/T 5889-86 (1986) by averaging the experimental results of the six samples.

Five g raw hemp fibers for each sample was dried in the oven. Then, the dried fibers were processed under boiling alkali solution (150 mL, 20 g/L) for 1 h and re-boiled for 2 h with the new alkali solution. After the boiling treatment and the washing process, the fibers were dried to constant weight. The cellulose content of hemp fibers was calculated as follows,

$$\text{cellulose content (\%)} = 1 - \frac{G_0 - G}{G_0} \times 100 \tag{1}$$

where G_0 is the dry weight of raw hemp sample and G is the dry weight of the sample after treatment.

To remove the resins, oils, fats, and waxes, raw hemp fibers were firstly degreased with an alcohol-benzene mixture (2:1 ratio by volume). Then 1 g of degreased raw hemp fibers for each sample was placed in weighing bottles and dried at 105 °C for 3 h in the oven. The oven-dried specimens were weighed and transferred to a 50 mL beaker with a glass cover, followed by slow addition of 30 mL concentrated H_2SO_4 (72%). The specimen was mixed well with the acid by constantly stirring for at least 1 min and allowed to stand for 24 h. The materials were transferred into a 500 mL beaker, 270 mL of deionized water were added, and the mixture was boiled for 1 h. After allowing the insoluble material to settle, it was filtered into a weighted sand core funnel that had been dried. The residue was washed free of acid with hot water, then the sand core funnel with the lignin was dried at 105 °C for 3 h, and weighed. The lignin content of raw hemp fibers was calculated as follows,

$$\text{lignin content (\%)} = \frac{G_L - G_F}{G_D} \times 100 \tag{2}$$

where G_D is the dry weight of degreased raw hemp fibers, G_L is the dry weight of sand core funnel with lignin, and G_F is the dry weight of empty sand core funnel.

Microscopic observation

Samples of transverse sections were obtained by cutting from the middle of each section using a Harrington slicer (Laizhou, China). Each sample slice was placed on a glass slide with one drop of glycerol. Cover slips were placed on the sample slices, mounted in the glycerol, and observed immediately using a Novel light microscope (XSZ-N107CCD, Yongxin, Ningbo, China) with a Panasonic digital camera (WV-CP480LA, Longzhou, Guangzhou, China) that could record digital images.

Fourier transforms infrared spectroscopy (FT-IR)

The use of computational methods in infrared spectroscopy has been a revolution in lignin analysis (Abreu *et al*. 1999). The FT-IR spectra were examined using a Nicolet 6700 spectrometer (Thermo Fisher Scientific, Waltham, USA). A total of 30 scans were taken over a range from 4000 cm^{-1} to 600 cm^{-1} with a resolution of 0.4 cm^{-1}. The baseline correction and smoothing were accomplished prior to further analysis.

X-ray diffraction (XRD)

The XRD analysis was conducted on a Rigaku diffractometer (D/max 2550 PC, Rigaku, Tokyo, Japan) equipped with a Cu tube (40 kV, 200 mA). The patterns were recorded within an angle range of 2θ from 5° to 60° with a scanning rate of 0.05°/s.

Physical properties tests

Before the physical properties tests, the raw hemp fibers were subject to a biochemical degumming process to dissolve and remove the gum substances between the elementary fibers. The process involved the following steps: raw hemp fibers were subjected to alkali pretreatment, then washing, laccase treatment, washing again, alkali boiling treatment, washing, oil finishing, drying, and finally carding, which produced the degummed hemp fibers. The flowchart is shown in Fig. 1.

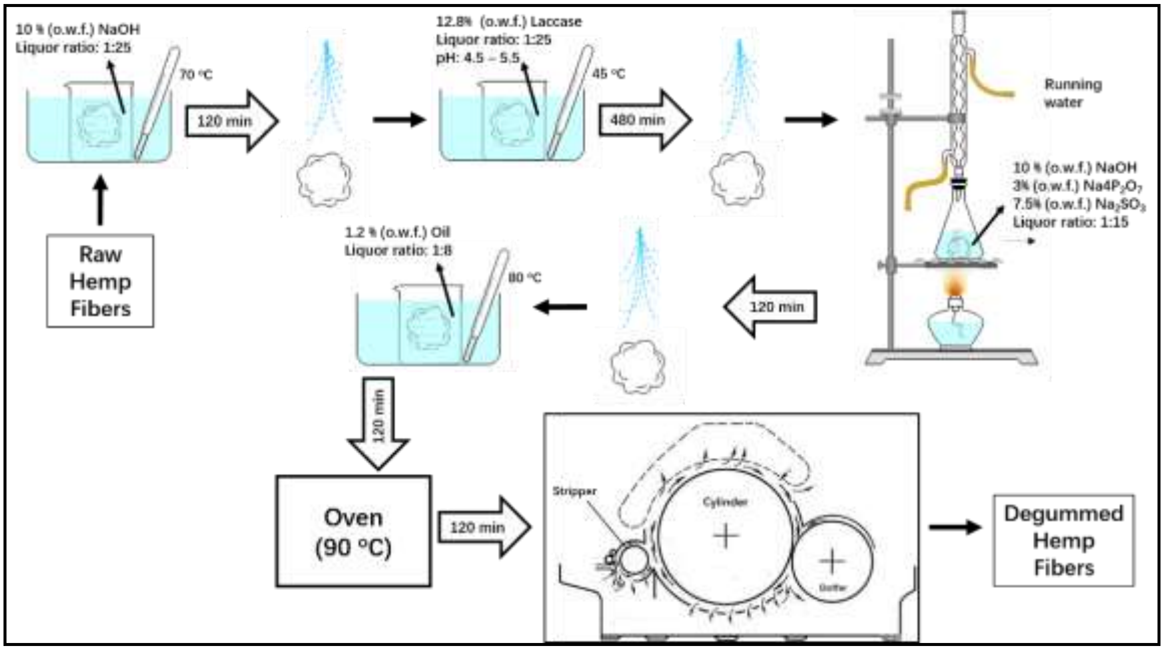

Fig. 1. Schematic of degumming and carding processes

The test method for the length of the degummed fibers was an Array diagram method according to the Chinese standard GB/T 18147.3 (2000). All the samples were conditioned in a standard atmospheric condition (temperature: 20±2 °C, relative humidity (RH): 65±2%) for 24 h before the test. The fibers were hand-sorted by length on black velvet. The parameters as effective length and short fiber content were directly measured by ruler.

The linear density was determined by the middle part cut and weight method, according to the Chinese standard GB/T 18147.4 (2000). All the samples were conditioned in a standard atmospheric condition (temperature: 20±2 °C, relative humidity (RH): 65±2%) for 24 h before the test. The linear density of fiber was determined by the cut middles method. A certain length of fiber was cut from the middle of the fiber bundles, then weighed in the torsion balance and the number of fibers was counted. The linear density was calculated as follows,

$$N_t = 1000G_f/nL \tag{3}$$

where L is the cutting length (30 mm), n is the number of fibers and G_f is weight of the fiber (mg).

RESULTS AND DISCUSSION

Microstructure Analysis

The original hemp stems were organized in layers consisting of the epidermis, cortex (containing primary and secondary fibers), cambium, and xylem from the surface to the pith (Garcia *et al*. 1998), which is shown schematically in Fig. 2. The bast fibers consisted of epidermis and cortex, which was situated outside the vascular cambium. The hemp fibers used for textiles mainly consisted of the primary fibers, which contain cellulose. The formation of the lignin component occurs *via* lignification in the secondary fibers.

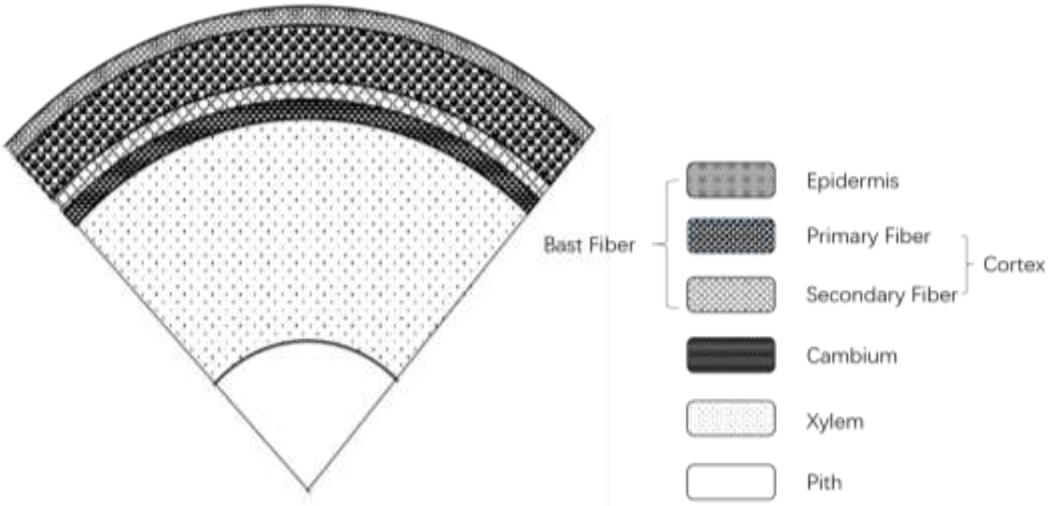

Fig. 2. Schematic of transverse section of hemp stems

As the optical micrographs illustrate in Fig. 3, the thickness of the epidermis layer across the six samples was almost the same. However, the layer thicknesses of the primary and secondary fibers increased from the top to the middle and to the bottom sections in the raw fibers harvested in different growth periods. When comparing the influence of growth period on the layer thickness in the same sections, the differences between the early and late harvest times became apparent from the bottom upwards.

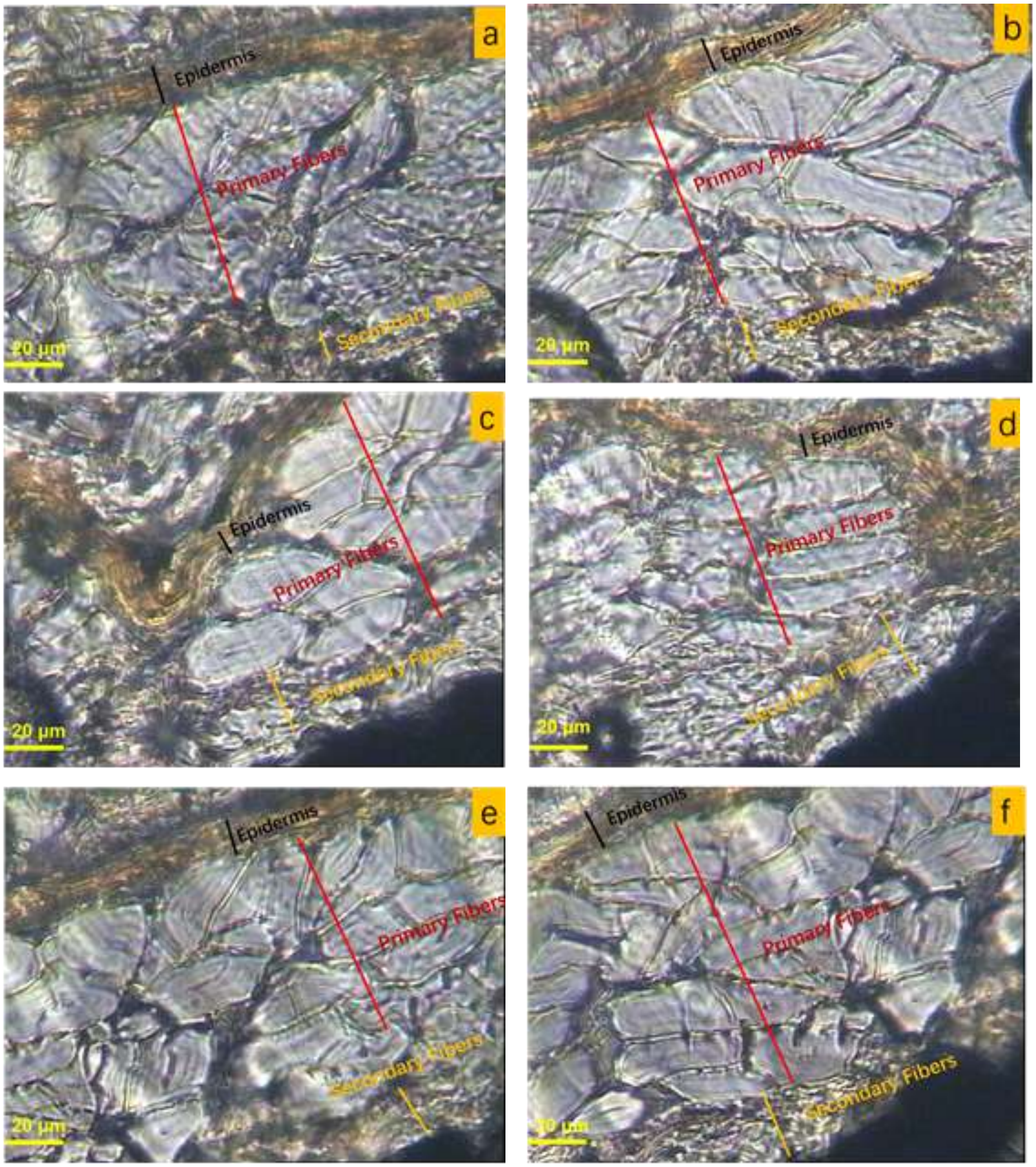

Fig. 3. Light micrographs of transverse sections from different samples (a: E-T; b: E-M; c: E-B; d: L-T; e: L-M; and f: L-B)

The primary fibers were formed and filled during vegetative growth before flowering (Mediavilla *et al*. 2001). The hemp stem contained several internodes, and the number of primary fibers was constant throughout one internode. These fibers were extended along the length of their own internode, so that the total content of the primary fibers continuously increased as the hemp plant grew in height. The secondary fibers were formed as soon as the elongation of internodes ceased. With the lignification of the secondary fibers, the quality of the hemp bark decreased because the lignin was difficult to remove during the degumming process. The cellulose content of the raw hemp fibers depended primarily on the proportion of primary fibers, and the lignin content depended mainly on the proportion of secondary fibers. In the textile industry, it is common knowledge that a high cellulose content and low lignin content are advantageous for the production of textiles (Mediavilla *et al*. 2001; Bengtsson 2009). In other words, more primary fibers and fewer secondary fibers were wanted in the cortex.

Chemical Composition Analysis

The foremost influencing factors relative to the utilization of raw hemp fibers in textiles were the cellulose and lignin contents, which can be observed clearly in Fig. 4. The content of cellulose varied slightly according to both the growth period and the sampling section. In contrast, the lignin content of the fibers had a greater increase with higher maturity, especially in the top and middle sections.

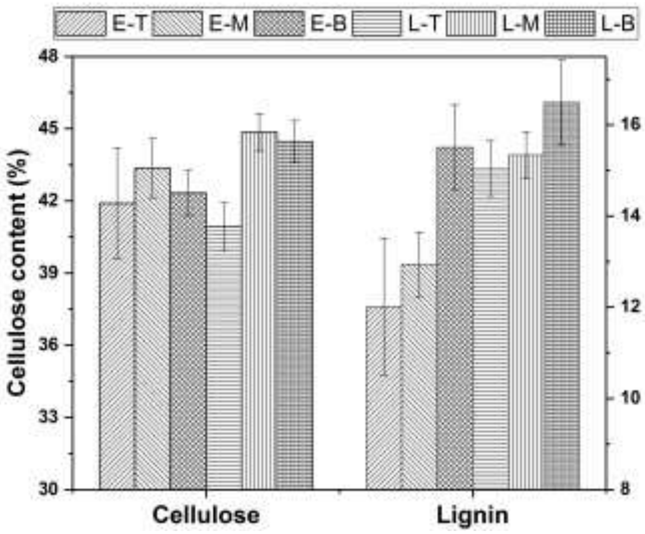

Fig. 4. The cellulose and lignin contents of raw hemp fibers

The formation of raw hemp fibers is complex and also strongly related to the growth pattern of the plant (Clarke 1998). The primary fibers were first created during vegetative growth and expanded until flower formation. The secondary fibers were formed at the induction of the generative phase and spread along the stem from the bottom upwards. With an early harvest, raw hemp fibers contained less lignin, especially in the top and middle sections. This was because in that growth stage, the formation and lignification of the secondary fibers had just begun in the internodes of the top and middle sections. However, the elongation of internodes in the bottom section ceased first, so that the content of the secondary fibers was considerably greater than those in the other two sections. Both aspects explained why the lignin content increased remarkably with the plant growth, which was greatest in the bottom section. The differences in cellulose content across the six samples were less notable than those of lignin. A larger cellulose content in the middle section was observed for both harvest times. Moreover, the cellulose content had a slight increase in the middle and bottom sections with the maturity of the hemp plant. This was due to the fact that while the number of primary fibers remained constant, the content in the different samples varied with the elongation of the internodes. In addition, the calculation method of the cellulose content should also be taken into account.

For the other non-cellulosic materials, there were small differences in the contents of hemicellulose (varied from 19.81% to 21.56%), pectin (varied from 7.16% to 8.74%), fats and wax (varied from 2.22% to 2.72%), across the six samples. A decrease in the content of water-soluble matter could be observed with the increase of growth periods (E-T: 13.47%, E-M: 12.80%, E-B: 12.47%, L-T: 12.08%, L-M: 8.88%, L-B: 8.40%).

Chemical Structure Analysis

Comparison of different stem sections

The FT-IR analysis of the hemp fibers of different stem sections is depicted in Fig. 5.

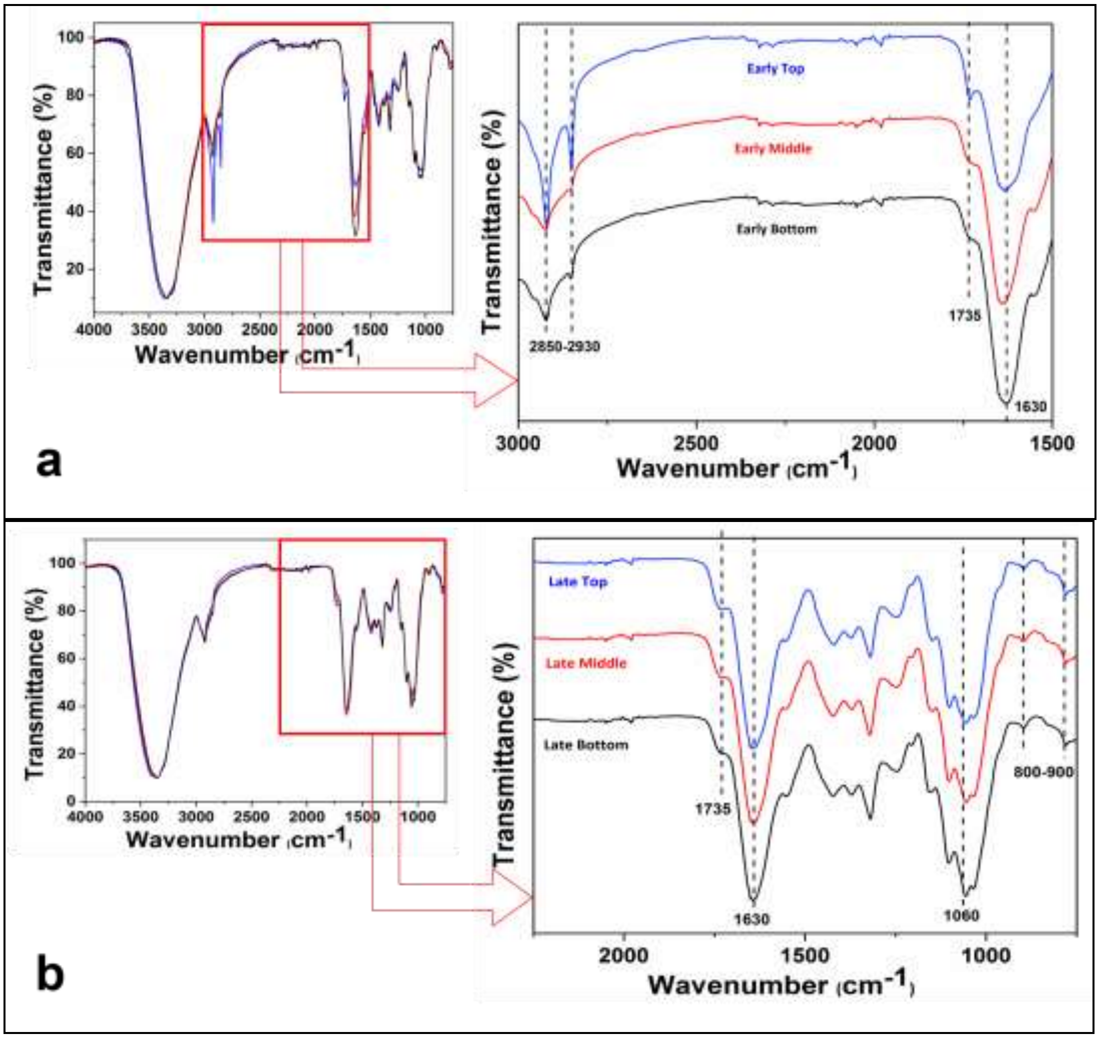

Fig. 5. FT-IR spectra of hemp fibers from different stem section (a: early harvest; and b: late harvest)

There were some differences among the bottom, middle, and top, especially in the region at 3000 cm^{-1} to 1500 cm^{-1}(Le Troedec *et al*. 2008), that were marked and magnified, as can be seen in Fig. 5a. The band at 1630 cm^{-1} was specific for the C=O stretching vibration of the lignin component in the hemp fiber where the peak intensity of the E-B hemp fiber was strongest, followed by the E-M and E-T hemp fibers. This indicated that the lignin content in the bast hemp fiber along the stem decreased from the bottom up. The formation of lignin is a process of accumulation. Before the flowering of the hemp plant, the bottom section of the hemp plant was obviously more robust than the middle and the top sections. In the meantime, the accumulation of lignin was accomplished first at the bottom because of the growth characteristics. The peaks at 1735 cm^{-1} that corresponded to the C=O stretching from the ketones and/or esters of hemicelluloses (Li *et al.* 2016) were almost the same as the bottom and middle sections of the hemp with a short growth period. However, there was an apparent enhancement in the top section, which led to a high hemicellulose content of the E-T hemp fiber that was in good accordance with the results of the chemical compositions. The same situation appeared in the region between 2850 cm^{-1} and 2930 cm^{-1}, representing the C-H bond vibrations, which indicated that the E-T hemp fibers had the largest peak intensity.

As is apparent in Fig. 5b, there were no notable differences between the bottom, middle, and top. With a long growth period, the homogenization of hemp fibers was almost accomplished, and only a tiny discrepancy was observed among all the three sections.

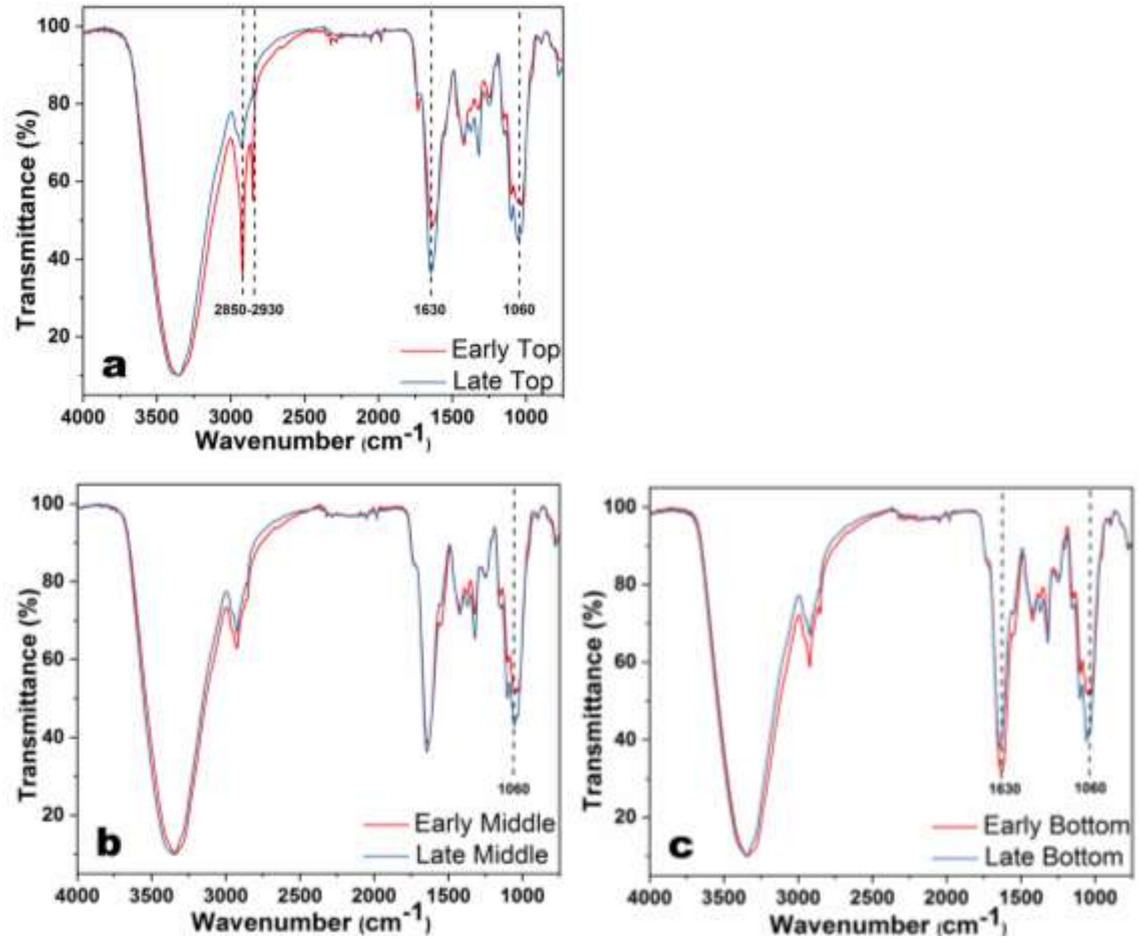

Fig. 6. FT-IR spectra of hemp fibers *vs.* harvest time (a: top section; b: middle; c: bottom)

Comparison of different harvest times

As illustrated in Fig. 6, the top section showed the most remarkable differences based on growth period. The composition of the top section changed to a higher degree than did that of the other two sections with the different growth stages of the hemp plant (Keller *et al.* 2001). This was because the volume of nutrients assimilated reached its peak at the beginning of seed maturity.

Guaiacyl/Syringyl (G/S) ratio

The lignin in the raw fibers was the most stable non-cellulosic material to be removed in the degumming process. The intensive research was conducted with the introduction of G/S ratio as a parameter. Lignin can be described as a three-dimensional macromolecule with a high molecular weight, and its precursors are three aromatic alcohols (monolignols), namely p-coumaryl (1), coniferyl (2), and sinapyl alcohols (3) (Buranov and Mazza 2008) (Fig. 7). The respective aromatic constituents of these alcohols in the polymer are called p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) moieties (Lewis and Yamamoto 1990).

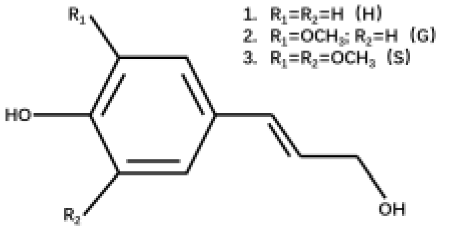

Fig. 7. Lignin monolignols (Lewis and Yamamoto 1990)

The lignin in hemp fibers was classified as a softwood lignin that contained mostly guaiacyl (G) and syringyl (S) units in different ratios. The peak intensities at the bond of 1287 cm^{-1} and 1349 cm^{-1}, which represented G and S lignin, were used to calculate the G/S ratio. The results are shown in Fig. 8.

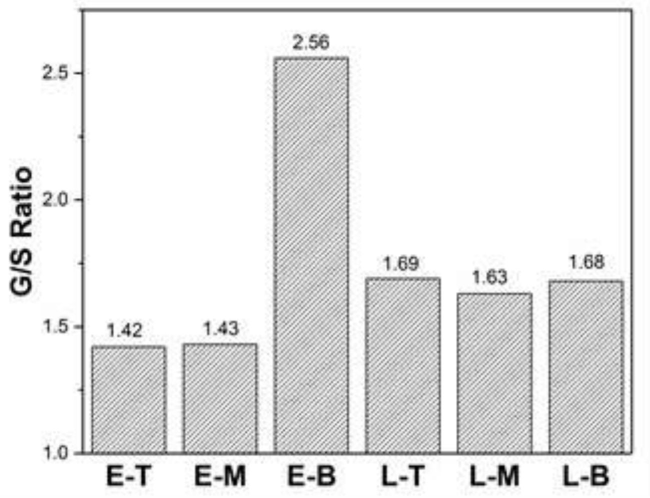

Fig. 8. G/S ratio of the raw hemp fibers

As can be concluded from Fig. 8, for the early harvest hemp fibers, the fibers obtained from the middle and the top sections had almost the same G/S ratio, which was the smallest among the six samples. This phenomenon was in high accordance with the theory mentioned above that in the early growing period, the secondary fibers were newly formed, which resulted in a lower lignin content. With a long growing period, the lignin accumulation and distribution were completed in all sections, and the differences in G/S ratio in the three sections of the late harvest hemp fibers were hardly seen. Notably, the E-B hemp fibers had the largest value of G/S ratio of all six samples, which indicated a high G-lignin content in the raw fibers. Compared with the S-lignin group, the G-lignin group had a free position of C-5, which could be used to form the C-C bonds between groups, increasing the resistance of lignin depolymerization (Abreu *et al.* 1999). In contrast, The S-lignin unit had fewer branches and lower condensation than did the G-lignin unit. The characteristics of fewer branches and lower condensation resulted in good reactivity with the chemical reagent, which was of great importance in the following degumming process.

Crystal structure analysis

The crystalline form of the hemp fibers was investigated by XRD, as shown in Fig. 9. The position of the diffraction peaks was exhibited by all six samples because the chemical compositions of the different samples were qualitatively the same, although they originated from different growth periods and stem sections. Nonetheless, the intensity of the diffraction peaks was not the same, and an apparent decline was observed in the curve of L-T hemp fibers compared with that of the other samples. Specifically, the diffraction peaks around 14.8° and 16.4° are characteristic of the cellulose crystal assignments (Okano and Sarko 2010). Moreover, a major diffraction peak for 2θ ranging between 22° and 23°, corresponding to the crystallographic plans of cellulose I, could be noticeably observed in all curves (Le Troedec *et al*. 2008). The intensity of the inferior peaks of the L-T raw hemp fibers indicated a lower cellulose content, which was in accordance with the results of the chemical composition.

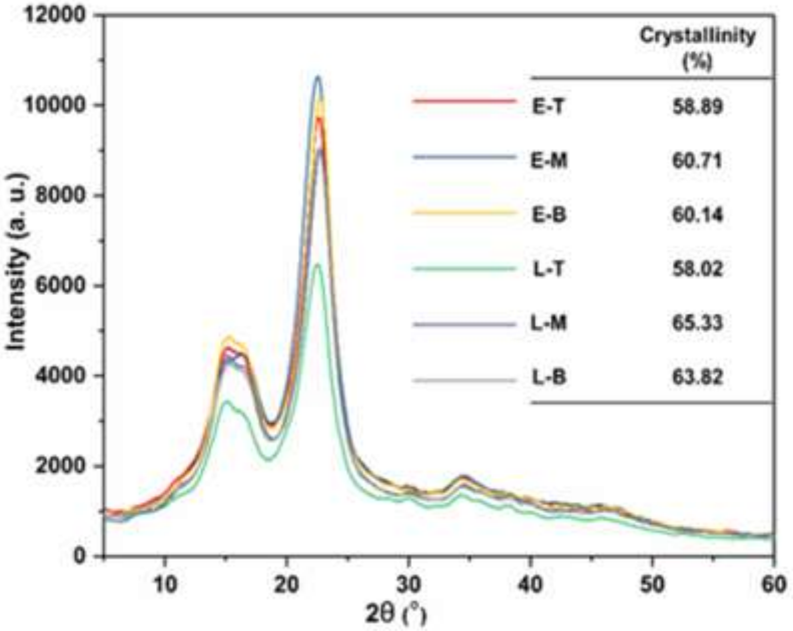

Fig. 9. XRD patterns of raw hemp fibers

The crystallinity index of the raw fibers is shown in Fig. 9. The crystallinity of L-T fibers, which had inferior peak intensity, was undoubtedly the smallest. This was because the crystallinity is greatly influenced by the cellulose content (Li *et al*. 2015; Meng *et al*. 2016). With a higher cellulose content, the content of non-cellulosic materials, which contained lots of amorphous materials and impurities, is lower (Ebringerová *et al.* 2005), resulting in a larger crystallinity index.

Physical Properties Tests

To evaluate the physical properties of the degummed fibers, the linear density, length, short fiber content, and coefficient of variation were tested. Considerable differences were found between the six degummed hemp fibers, as shown in Table 1. The two top sections of the degummed hemp fibers had the shortest length and lowest fiber contents. Among all of the non-cellulosic materials, which the degumming process aimed to remove, the hemicellulose and water-soluble materials reacted most easily with the degumming solutions and became soluble in water during the degumming process. Thus, the raw hemp fibers in the top section that had the largest hemicellulose content and the water-soluble materials in this section were excessively degummed, resulting in undesirable physical properties (Li *et al.* 2016). The fibers from the bottom section had better properties in the three parameters of length, but their linear density was too large to obtain high-value textile products. Taken together with this biochemical degumming process, the hemp fiber of the middle section, especially the E-M hemp fibers, had the most favorable properties overall. A segmented degumming process should be developed to obtain high-homogeneity and high-quality degummed fibers, which would improve the efficiency and reduce the cost in both the degumming and spinning processes.

Table 1. Length, Short Fiber Content, and Linear Density of Degummed Hemp Fibers

	Length (mm)	CV (%)	Short Fiber Content (%)	Linear Density (dtex)
E-T	42	63.50	64.42	11.36±0.37
E-M	67	71.15	62.00	20.66±0.42
E-B	60	68.33	58.76	21.55±0.54
L-T	52	67.16	57.46	15.03±0.45
L-M	60	65.00	46.25	18.45±0.31
L-B	63	73.80	48.78	19.57±0.33

Previously, all raw hemp fibers, no matter when they were harvested and which stem section they came from, had been subject to the same degumming procedure. As a consequence, the wide variation in the compositions of bast hemp fibers with different growth periods and sections had slowed down the degumming efficiency, rendering great disparities in the composition and physical properties of the degummed fibers, which had been a stumbling block for high-end use in the textile industry.

To improve the degumming efficiency and physical properties of hemp fibers, the selection of fibers with fewer variations should be considered. Moreover, differences in the starting materials might be overcome by adjusting the subsequent degumming process to achieve the homogeneity in the raw fibers from one hemp stem to maximize the end use value of hemp fibers.

CONCLUSIONS

1. An in-depth investigation was conducted on the influence of harvest time and sampling section on hemp fiber chemical composition and microstructure. The results revealed that raw fibers with a late harvest time at the beginning of the seed maturity were highly lignified compared to the fibers with an early harvest time. This difference was more noticeable in the raw fibers sampled from the top and middle sections. Within the same growth period, the content of lignin in the bottom section was obviously larger than that of the other two sections. The cellulose content was the same among the six samples. However, a greater value was observed in the fibers from the two middle sections. The content of the other components in raw hemp fibers also varied over a small range.
2. All of these phenomena were confirmed with the results of microstructures, which indicated that there was a vast variety in the chemical compositions and microstructures of the raw hemp fibers with different harvest times and sampling sections. This variety was attributed to the combined effect of the formation and increase in the proportion of secondary fibers, and to the growth pattern of the primary fibers.
3. The consequence of the inhomogeneity in the raw fibers was that these fibers were not desirable or favorable for degumming, which was confirmed by the subsequent physical properties tests. When using the same degumming process for the raw fibers of different origins, the test results showed great disparity in both the length and linear density of the degummed fibers. Various degumming technologies for hemp of different origin might result in better fiber quality and it would be useful to investigate this issue in the future
4. Considering the hemp fiber yield and properties, the hemp plant should be harvest at the beginning of flowering for the possible best results. Moreover, to obtain very high quality textile products of hemp fibers, it worth to use only the middle part of the hemp stem.

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