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Natural Fibers From the Bark of Mulberry Branches for Textile Application

Abstract
Fibers with low gum content were extracted from the bark of mulberry branches by a combination of bacteria and peroxide treatments. The bark of mulberry branches, with 30% cellulose, is a copious and inexpensive source of natural fibers. However, fibers extracted by microwave, enzyme or alkali had a high gum content (15.5% for hemicellulose and 8.6% for lignin), which rendered them difficult to be made into high- value textiles. In this research, strains with high polygalacturonase activities and subsequent hydrogen peroxide decreased the hemicellulose content to 2.5% and lignin content to 2.4%. Mulberry fibers in our study could be spun into yarns with a fineness of 18.2 tex. Compared to flax yarns, mulberry fiber yarns had a tenacity 20% higher, an elongation 18% higher and an unevenness 30% lower. Cotton/mulberry fiber fabrics had softer and smoother hand than cotton/flax fabrics. Overall, the fibers in our study show better potential for industrial textile applications than those in previous studies.

Key words: *mulberry fibers, cellulose, pectin, spinning, bacteria.*

■ Introduction

Mulberry branches are important agricultural byproducts which are copious and inexpensive but have limited applications. Mulberry trees are extensively cultivated in China to provide leaves as food for silkworms. Mulberry cultivation in China reached 12 million acres in 2014 and it has been estimated that nearly 4 tons of branches are generated per acre of mulberry trees grown each year. The bark of mulberry branches accounts for about 20% of the total mass of the branches. The potential of utilising the bark of mulberry branches as a source of pectin [1], medicinal ingredients [2] and nano-whiskers [3] has been investigated. However, most mulberry branches are currently used as firewood or discarded as agricultural waste.

Fibers from the bark of mulberry branches could be used for pulp or paper [4, 5], but they do not meet the requirements for traditional textile applications mainly due to the high proportion of gum substances or too short fiber length. Qu and Wang obtained fibers from the bark of mulberry branches using a combination of microwaves, enzymes and alkali (AM-BET method) [6]. The fibers had a good fineness (2.2 dtex) and high hemicellulose content (15.5%). Moreover the fibers also showed excellent antimicrobial activities; however, no detailed information could be found on their textile application [7]. Yarns with a coarse fineness of 7-11 Ne from mulberry-fiber papers could be processed into traditional ethnic garments but were limited in conventional textile applications [8].

Moreover, a combination of enzyme, alkali and peroxide treatments was also studied to extract mulberry fibers with a fineness of 2.6 dtex, but the pectin content was as high as 16.4% [9]. Fibers with a high gum content usually have a high Young's modulus and low cohesion forces, which renders them difficult to be blended with cotton in the spinning process. Based on our best knowledge, no reports are currently available on mulberry fibers for industrial textile applications.

Bacteria degumming is considered an economical and environmentally friendly method for extracting traditional bast fibers. Bacteria and actinomycetes can produce a variety of enzymes that degrade the gum inside jute, flax, hemp, ramie, kenaf or coir fibers [10-14]. It was found that polygalacturonase, pectin lyase and xylanase from bacteria played an important role in the degumming process. Moreover the synergistic effect was also observed among different bacteria and their enzymes in the removal of residual gum [10-13]. The bacterial degumming method has many advantages, including energy conversation, low pollution and low cost compared to enzyme or chemical treatments. However, no reports are now available on extracting mulberry fibers using the bacteria method.

In this research, strains found on retted mulberry were applied to separate fibers from the bark of mulberry branches, and then the fibers were treated with hydrogen peroxide to further improve the fineness (bacteria-H_2O_2 method). Mulberry fibers and cotton were processed into

blended yarns and knitted fabrics. Properties of the blended yarns/fabrics were analysed and compared to those of 100% cotton and cotton/flax blended yarns/fabrics.

■ Experimental

Materials

The mulberry branches used were from cultivar HU197, grown in Yancheng city, Jiangsu Province, China. The mulberry branches were collected approximately three years after tree plantation. The outer bark was stripped manually and air dried before fiber extraction. Pectin (CAS 9000-69-5) and xylan (CAS 9014-63-5) were purchased from Sigma Co. Ltd, USA. Other chemicals were reagent grade and purchased from Nantong Jingke Instrument Co. Ltd, PRC. Cotton and flax fibers used for spinning were obtained from Jiangsu Dasheng Group Co., Ltd., China. The cotton fibers were 29.2 mm in length, had an average fineness of 1.5 dtex and breaking tenacity of 2.7 cN/dtex. The flax fibers were 45.1 mm long, 15.2 dtex and with an average breaking tenacity of 4.2 cN/dtex.

Extracting fibers from the bark of mulberry branches

Isolation and screening of strains
The collecting and screening of the strains that have high polygalacturonase activities were conducted using the method of Jacob et al (2008) with minor modifications [15]. Sediment samples were collected aseptically from a retting pond of mulberry branches and enriched for about 10 times in culture broths. The broth medium contained

(g L^{-1}) K$_2$HPO$_4$-1, MgSO$_4$-0.5, NaNO$_3$-3, FeSO$_4$-0.01 and Pectin-2, and had a pH adjusted to 7.0 using 1mol/L NaOH solution. The supernatant was serially diluted and then plated onto pectin agar that contained (g·L^{-1}) K$_2$HPO$_4$-1, MgSO$_4$-0.5, NaNO$_3$-3, FeSO$_4$-0.01, Pectin-4 and Agar-20. After incubation for 72 h at 30 °C, 0.02% Congo red solution was coated on the pectin-agar plate. About 4 hours later, a small amount of sterilised water was added to wash away the residual Congo red on the agar surface. Magenta hydrolysis zones could then be seen against the light, indicating high pectinase activity. Cultures with the largest magenta hydrolysis zones were selected, purified and maintained on pectin agar slants.

Bacterial degumming
Cultures that could produce magenta hydrolysis zones were subjected to submerged fermentation. Each 5 g of bark of mulberry branches was sterilised and added into a 250 ml flask, which contained a 100 ml growth medium of (g·L^{-1}) K$_2$HPO$_4$-1, MgSO$_4$-0.5, NaNO$_3$-6 and had pH adjusted to 7.0 using 1 mol/L NaOH solution. An inoculum of 2.5×10^4 CFU/mL was used. The inoculated flasks were incubated at 37 °C on a rotary shaker at 180 rpm. After degumming for 24 to 60 h, fibers separated from the bark of mulberry branches were thoroughly rinsed and dried at room temperature.

Peroxide treatment
Mulberry fibers after degumming for 42h were treated with 0.5% H$_2$O$_2$ at 90 °C for 30 min with a pH of 10.5 using a liquor to fiber ratio of 30:1. The fibers were then rinsed, dried and combed manually. Fiber samples after bacteria and peroxide treatments were finally mixed together for spinning application.

Enzyme assay
The activities of polygalacturonase, xylanase and cellulase were determined to analyse the role of various enzymes in the bacterial degumming process of mulberry bark. After degumming for 24 to 60 h, bacterial cells and mulberry bark debris were removed from the fermentation solution by centrifugation (4000 rpm), and the supernatant was used as crude enzyme solution. All enzyme activities were determined at 37 °C.

Polygalacturonase (PGase) activity was determined according to the method described by Kobayashi et al. (2001) [16]. Crude enzyme solution was mixed with 0.5% polygalacturonic acid (Sigma) solution. One unit (U) of the enzyme activity was defined as the amount of enzyme releasing 1 μmol of galacturonic acid equivalent min^{-1}.

Xylanase activity was determined by the DNS-stopping method [17], described by Bailey et al. (1992). Crude enzyme solution was mixed with 0.8% oat spelt xylan (Sigma). One unit (U) of xylanase activity was defined as the amount of enzyme that produced 1 μmol of xylose equivalent min^{-1}.

Cellulase activity was detected by measuring the release of reducing sugar from 1% carboxymethylcellulose (sodium salt) with DNS reagent [18]. One unit (U) of enzyme activity was defined as the amount of enzyme which liberated 1μmol of glucose equivalent min^{-1}.

Determination of fiber dimension
About 200 fibers were tested for the length of mulberry fibers, which was given in terms of mm. Fiber fineness was determined three times in terms of dtex, which was defined as the conditioned weight of the fibers in grams per 10.000 m. All data were reported as the average with standard deviation.

Aspect ratios of the fibers were calculated according to *Equation (1)*.

$$Aspect\ ratio = \frac{L}{0.03568'\sqrt{T_t/\delta}} \qquad (1)$$

Where L is the fiber length in mm, T$_t$ is the linear density of the fibers, which is defined as the conditioned weight of the fibers in grams per 1000 m, and δ is the density of mulberry fibers. Cotton, flax and mulberry fibers have densities of 1.55, 1.49, 1.49 g/cm^3, respectively [19].

Determination of the gum content of mulberry fibers
The contents of pectin, hemicellulose and lignin in mulberry fibers were determined according to China National Standard GB5889-86. All contents were tested three times and data reported as the average with standard deviation.

Determination of tensile properties of mulberry fibers
The tensile properties of mulberry fibers including tenacity, elongation at break and Young's modulus were determined on a tensile tester (LLY-06B; Laizhou Electron Instrument Co. Ltd, PRC). The fibers were conditioned at 21 °C and 65% relative humidity for 24 h before the test. A gauge length of 10 mm with a crosshead speed of 10 mm/min was selected. About 200 fibers were tested for the determination of tensile properties. All data were reported as the average with standard deviation.

Determination of crystalline structure
The crystalline structures of mulberry fibers were determined on a SMART APEX II model X-ray diffractometer (Bruker Co. Ltd, Germany). The Cu target X-ray tube was set at 40 kV and 30 mA. Diffraction intensities were recorded with 2θ ranging from 5 to 50°. Peak decomposition and profile fitting was performed in the diffraction patterns after subtracting the background and air scatter with JADE software. The ratio of the crystalline area to the total diffracted area was taken as the % crystallinity.

Spinning of mulberry fibers
Mulberry fibers extracted from combined treatments with bacteria and peroxide were blended with cotton with a weight ratio of 70/30 (mulberry fiber/cotton), and then processed into yarns on traditional ring spinning machines following the procedure of carding-sliver-roving-spinning. All yarns had a fineness of 18.2 tex and twist coefficient unified as 400. (The fineness of the yarns was determined in terms of tex, which was defined as the conditioned weight of the yarns in grams per 1.000 m).

Determination of tensile properties of blended yarns
The tenacity and elongation at break of the blended yarns were determined according to China National Standard GB/T3916 on an electron tensile tester of yarns (YG061F, Laizhou Electron Instrument Co. Ltd, PRC). A gauge length of 500 mm with a crosshead speed of 500 mm/min was selected. About 200 yarns were tested for each datum. All data were given as the average with standard deviation.

Determination of yarn hairiness
The hairiness factor of yarns was determined according to China Textile Industry Standard FZ/T 01086 on a yarn hairiness tester (YG171L, Laizhou Electron Instrument Co. Ltd, PRC). The hairiness
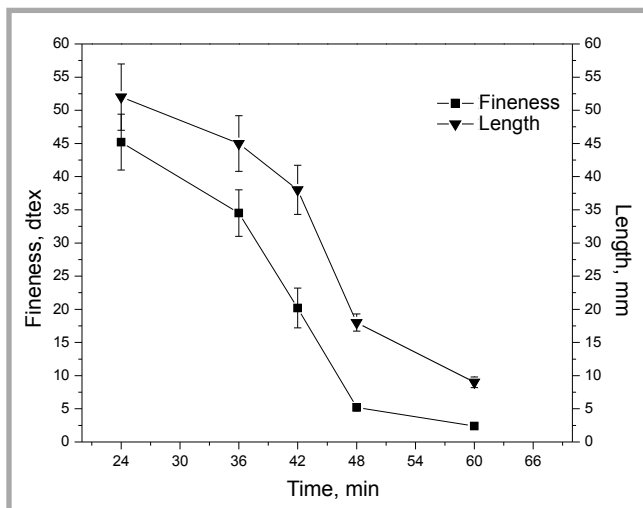
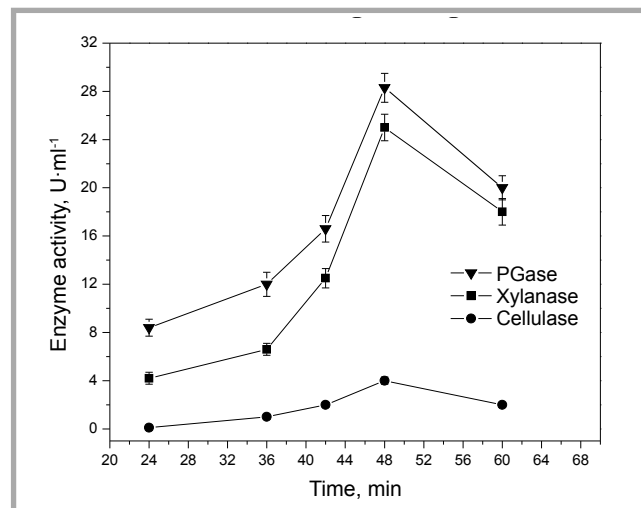**Figure 1.** *Dimension of mulberry fibers in the process of bacteria degumming.*

Figure 2. *Changes in the activities of polysaccharide-degrading enzymes in the degumming process.*

factor was defined as the amount of hairiness per meter in the length direction of the yarns. Ten replications were made for the determination. All data were reported as the average with standard deviation. Considering that hairiness with a length above 3 mm was detrimental to the application of yarns, only that had a length above 3mm was included in the calculation of the hairiness factor.

Determination of yarn unevenness

Yarn unevenness was determined according to ASTM D1425 on a CT200 Model unevenness tester (Shanxi Changling Textile Mechanical & Electronic Technology Co., Ltd., China). A testing speed of 200 m/min and time of 10 min was selected. Yarn evenness was expressed as CV%. Each datum was tested 3 times and reported as the average with standard deviation.

Preparation of knitted fabric

Yarns with a linear density of 18.2 tex were processed into 1+1 rib fabric on a Z652C Model hand-knitting machine (Guosheng knitting machine Co. Ltd, China). The fabric had 36 wales and 21 courses per 50 mm, and a mass of 89.6 grams per square meter.

Determination of fabric hand

The hand of the fabrics, including the relative hand value (RHV), resilience, softness and smoothness, was measured according to AATCC TM 202-2012 on a PhabrOmeter® system (Nu Cybertek, Inc, USA). Pure cotton fabric was selected as a reference sample. Circular knitted fabric samples were inserted/

extracted through a specially designed nozzle. Fabric samples were deformed under a very complex low stress. Forces were then recorded as a function of time and a load-displacement extraction curve was generated which contains all the information of the fabric hand [20]. RHV is an overall hand value relative to a given reference fabric. A lower RHV means a hand of a sample more close to that of the reference fabric. Resilience is used to describe how easy it is to bend a product with fingers. A larger resilience value means a strong sense of stiffness. Softness is the compressibility judged by squeezing a piece of fabric in hands. A larger softness value indicates a softer fabric. Smoothness is used to describe the resistance when one slides one's fingertip across the fabric surface. A larger smoothness value means a smoother fabric surface.

Results and discussion

Mulberry fibers after bacteria degumming

Fineness and length of mulberry fibers
Figure 1 shows the dimension of mulberry fibers in the process of bacteria degumming. As seen from **Figure 1**, an increase in the degumming time decreased the fineness of mulberry fibers but caused a reduction in the fiber length as well. When the time increased from 42 h to 48 h, fiber fineness decreased from 20.2 to 5.2 dtex, but the fiber length decreased drastically from 38 to 17 mm. Further prolonging the time would aggravate the reduction in the fiber length until the bark of mulberry branches was com-

pletely disintegrated into paste. Since mulberry fibers had fewer convolutions and lower cohesion forces compared to cotton, a relatively high fiber length was required for their spinning applications. Therefore a time of 42 h was considered more desirable than others for extracting mulberry fibers with favorable dimensions.

Enzymes in relation to bacteria degumming
Figure 2 shows changes in the activities of polysaccharide-degrading enzymes in the process of bacteria degumming. As seen from **Figure 2**, the activities of PGase, xylanase and cellulase increased initially but then decreased with an increase in time. Single cells in the bark of mulberry branches are stuck together with gum substances such as pectin, hemicellulose and lignin, etc. With the release of pectin and hemicellulose at the initial stage of degumming, the abilities of bacteria to secrete PGase and xylanase increased gradually. However, the removal of gum rendered cellulose microfibrils in the bark of mulberry branches exposed to bacteria directly, which led to an increase in cellulase activity. After a time of 48 h, the activities of cellulase enzymes reached the maximum. An increase in cellulase activities caused a drastic decrease in the polymerisation degree of cellulose, and eventually led to the complete disintegration of mulberry fibers. The results were in accordance with the changes in the dimension of mulberry fibers shown in **Figure 1**, indicating that PGase and xylanase played an important role in the bacterial degumming of mulberry bark.
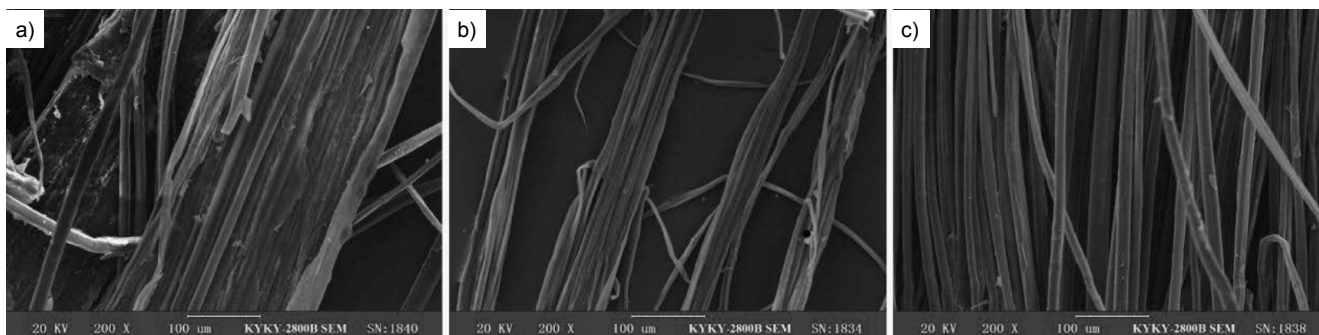
Figure 3. SEM images of (a) fibers in raw bark of mulberry branches, (b) mulberry fibers after bacteria treatment, and (c) mulberry fibers after bacteria-H_2O_2 treatment.

Mulberry fibers extracted by bacteria-H_2O_2 method

Morphology and dimension of mulberry fibers

SEM images of mulberry fibers extracted by different methods are shown in **Figure 3**. As seen from **Figure 3.a**, untreated mulberry fibers had an average diameter of above 300 μm and a rough surface, on which non-cellulose impurities had attached. After bacterial degumming (seen in **Figure 3.b**), mulberry fibers showed a relatively smooth surface and had an average diameter reduced to about 50 μm. Fibers after bacteria-H_2O_2 treatment exhibited cleaner and smoother surfaces, and had an average diameter further reduced to 11 μm, as shown in **Figure 3.c**.

Table 1 shows the dimension of mulberry fibers extracted by different methods. As seen from **Table 1**, fibers in raw bark of mulberry branches had a coarse fineness of 54.0 dtex. After bacteria treatment, the fineness and length of mulberry fibers decreased by 66% and 15%, respectively, whereas their aspect ratio increased by 407%. Peroxide treatment further reduced fiber fineness by 87% but did not cause a drastic decrease in fiber length. Mulberry fibers extracted by the bacteria-H_2O_2 method have the best fineness of 2.3 dtex, which is close to that of cotton but far lower than that of flax. The dimensions of mulberry fibers are influenced by the variety of mulberry trees and the extraction conditions used to obtain the fibers. Compared to mulberry fibers in other reports, the fibers in our study have a similar fineness but better length.

Content of gum in mulberry fibers

Table 2 shows the composition of mulberry fibers and bark of mulberry branches. As seen from **Table 2**, content of pectin and hemicellulose in the bark of mulber-

ry branches is reduced by 87% and 71%, respectively, but the lignin content is decreased by only 43% after bacterial treatment. Strains with high PGase and xylanase activities could remove pectin and hemicellulose efficiently but were limited in the removal of lignin. Subsequent peroxide treatment further decreased the content of pectin, hemicellulose and lignin in mulberry fibers to 0.4%, 2.5% and 2.4%, respectively. Compared to mulberry fibers in the references, fibers in this study had a lower hemicellulose and lignin content, which rendered them softer and easier to be processed on spinning machines.

Tensile properties of mulberry fibers

Table 3 shows the tensile properties of mulberry fibers compared with those of cotton and flax. As seen from **Table 3**, mulberry fibers extracted by the bacteria-H_2O_2 method had a tenacity and elongation similar to those of flax, but a lower Young's modulus. Compared to mulberry fibers in the references, those in this research had a modulus 39% lower, which should be attributed to a more thorough removal of gum inside the fibers. Fibers with a low Young's modulus are softer and thus have higher cohesion forces.

Table 1. Dimension of mulberry fibers compared with that of cotton and flax.

Materials	Extraction method	Fiber fineness, dtex	Fiber length, mm	Aspect ratio	References
Mulberry fibers	Untreated	54.0±4.8	45.0±5.2	150	This paper
	Bacterial	18.2±3	38.1±3.7	760	This paper
	Bacteria-H_2O_2	2.3±0.2	32.2±3.0	2909	This paper
	AMBET	2.2±0.2	23.3±2.5	2074	Qu and Wang [6]
Cotton	–	1-4	15-56	863-5864	Dong et al [19]
Flax	–	1-20	20-140	1573-3403	Dong et al [19]

Table 2. Composition of mulberry fibers and bark of mulberry branches.

Materials	Extraction method	Cellulose, wt%	Pectin, wt %	Hemicellulose, wt %	Lignin, wt %	References
Bark of mulberry branches	–	32.7±2.9	6.9±0.5	17.7±1.6	16.9±1.4	This paper
Mulberry fibers	Bacteria	65.2±4.5	0.9±0.1	5.2±0.5	9.6±1.0	This paper
	Bacteria-H_2O_2	82.2±5.8	0.4±0.1	2.5±0.3	2.4±0.3	This paper
	AMBET	41.4	–	15.5	8.6	Qu and Wang [6]

Table 3. Tensile properties of mulberry fibers compared with those of cotton and flax.

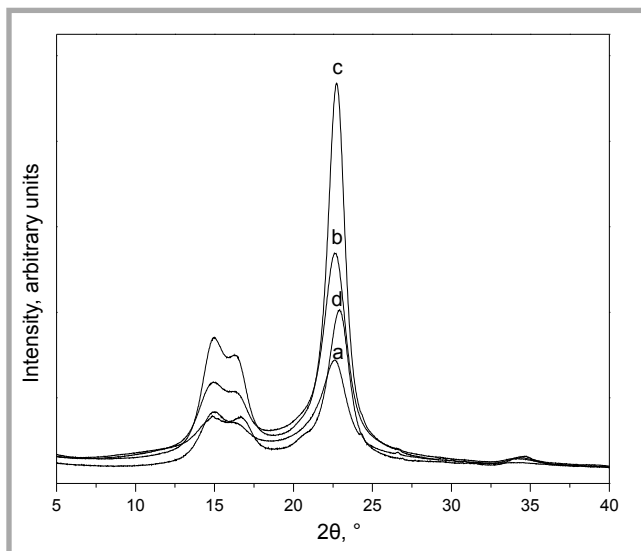| Materials	Extraction method	Tenacity, cN·dtex⁻¹	Elongation at break, %	Young's modulus, cN·dtex⁻¹	References
Mulberry fibers	Bacteria-H_2O_2	4.6±0.4	3.0±0.2	100.6±11.5	This paper
	AMBET	6.18±0.57	3.76±0.33	165.2±18.9	Qu and Wang [6]
Cotton	–	2.4-3.2	6.0-9.0	49.5-81.0	Reddy and Yang [21]
Flax	–	4.1-5.5	1.6-3.3	175.5-184.5	Reddy and Yang [21]

Figure 4. X-ray diffraction intensity patterns of (a) the bark of mulberry branches, (b) mulberry fibers after bacteria pretreatment, (c) mulberry fibers after bacteria-H₂O₂ treatment, and (d) cotton

Crystalline structure of mulberry fibers

Figure 4 shows the X-ray diffraction patterns of bark of mulberry branches and mulberry fibers extracted by different methods. As seen from **Figure 4**, the bark of mulberry branches and mulberry fibers both had the typical crystalline structure of cellulose I. Peaks at 2θ angles of approximately 22° correspond to the (002) lattice planes, whereas those at 14.9° and 16.6° correspond to the (1-10) and (110) lattice planes, respectively [22-23]. These peaks turn out to be sharper after bacteria and peroxide treatment, indicating that gum substances in mulberry fibers were removed gradually [24]. **Table 4** shows t the crystallinity of mulberry fibers after bacteria and alkali treatments.

As seen from **Table 4**, the bark of mulberry branches had a low crystallinity of only 40.2%, mainly due to the high proportion of amorphous gum inside. The crystallinity of mulberry fibers extracted by the bacteria-H₂O₂ method was 9.8% higher than that of cotton.

Properties of blended yarns from mulberry fibers

Table 5 shows the properties of blended yarns from mulberry fibers compared with those of flax yarns. As seen from **Table 5**, blended yarns from mulberry fibers had an unevenness 30% lower and hairiness factor 19% lower than those of flax yarns, but had tenacity 20% and elongation 18% higher than those of flax

yarns. A finer fineness and lower Young's modulus rendered mulberry fibers easier to be blended with cotton fibers than flax. Therefore blended yarns from mulberry fibers have a better structure and fewer yarn defects compared to flax blended yarns.

Properties of blended fabrics from mulberry fibers

Table 6 shows the hand of cotton/mulberry fiber blended fabric compared with that of pure cotton as well as cotton/flax blended fabric. Compared to cotton/flax fabric, cotton/mulberry fiber fabric had RHV closer that of to pure cotton fabric. Moreover cotton/mulberry fiber fabric had smaller resilience as well as a larger softness and smoothness value compared to cotton/flax fabric. Softer and smoother hand could be attributed to the lower Young's modulus of mulberry fibers and more perfect yarn structure of blended yarns from mulberry fibers.

■ Conslusions

Bacteria and peroxide treatments in succession were used to extract mulberry fibers for high value textile applications. Increasing the bacterial degumming time could improve the removal of gum substances and reduce the fineness of mulberry fibers. However, an excessively long time caused a rapid decrease in the fiber length until the mulberry fibers were completely disintegrated into paste. Fibers with a fineness of 20.2 dtex and length of 38 mm were extracted after an optimum time of 42 h. This optimum time was varied depending on the cultivars and age of the mulberry trees. Subsequent peroxide treatment further reduced the fineness to 2.3 dtex and length to 32.2 mm. Compared to mulberry fibers in the references, fibers extracted by the bacteria-H₂O₂ method had a similar fineness but the fiber length improved by 44%. Moreover the content of hemicellulose and lignin in the fibers was 84% and 72% lower than that of mulberry fibers in the references, respectively. Mulberry fibers in this research were spun into blended yarns with a fineness of 18.2 tex. Compared to cotton/flax yarns, cotton/mulberry fiber yarns had a 20% higher tenacity and 18% higher elongation , but a 30% lower unevenness and 19% lower hairiness coefficient. Cotton/mulberry fiber fabric had RHV close to pure cotton fabric. Compared to cotton/flax fabrics, cotton/mulberry fiber fabrics had a softer and smoother hand. Overall, the method

Table 4. *Crystallinity of mulberry fibers compared with that of cotton.*

Fiber sample	Crystallinity, %
Bark of mulberry branches	40.2±2.0
Mulberry fibers after bacterial pretreatment	60.2±4.5
Mulberry fibers after bacteria and alkali treatments	76.3±6.6
Cotton	69.5±5.8

Table 5. *Properties of blended yarns from mulberry fibers.* **Note:** *All yarns had a fineness of 18.2 tex and twist coefficient unified as 380.*

Yarns	Unevenness, %	Hairiness factor, numbers·m⁻¹	Tenacity, cN·dtex⁻¹	Elongation at break, %
100% cotton	10.2±1.1	11.6±1.1	14.8±1.3	6.8±0.6
Cotton/mulberry fibers 70/30	13.2±1.2	18.8±1.5	12.2±1.2	4.7±0.4
Cotton/flax 70/30	18.8±1.6	23.1±2.1	10.2±1.0	4.0±0.4

Table 6. *Hand of knitted fabric of pure cotton, cotton/flax and cotton/mulberry fiber blends. Both blended fabrics have the same blending ratio with 70% cotton.*

Fabrics	Blending ratio	RHV	Resilience	Softness	Smoothness
Cotton	–	0	29.2	91.8	88.6
Cotton/mulberry fiber	70/30	3.6	38.7	82.7	77.2
Cotton/flax	70/30	10.2	47.3	76.3	68.6

of bacteria-H_2O_2 can be used to extract mulberry fibers for producing high value textiles.

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