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Research article

Effect of harvesting age of plant and pectinolytic selected-fungi in biodegumming ramie performance

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ABSTRACT

Ramie is one of the long natural fiber has strong mechanical properties. To improve the quality of ramie fiber, this study developed a bio degumming method from superior isolates of pectinolytic fungi *Rhizopus* sp. and optimization of raw fiber based on harvesting time. The results of the pectinolytic fungi selection were used as a bio degumming bio starter under optimum conditions of pH and temperature. Also, fiber material harvested at 50-day and 60- day to obtain an increase in the physical quality. The bio degummed fiber was analysed to determine the tenacity and fineness, the functional groups contained, thermal analysis, moisture regain and content, material polymers, and degree of crystallinity. Based on the results, the finest ramie properties with 50-day harvested fiber are as follows: strength 24.54 ± 0, 02 g/tex, elongation 12.04 ± 2,90%, fineness 1.33 ± 0,17 tex, moisture regain 8.23 ± 0,18 %, and moisture content 8.96 ± 0,21 %. Ramie fibers at initial conditions and after bio degumming at 50 and 60-day harvested had the same pattern of thermal stability. The dyeability test showed that the degummed 60-day harvested fibers has the greatest dye fixing ability. The bio degumming process with this method can improve the quality and dyeability of the rami fiber which can be used for future applications.

1. Introduction

Ramie (*Boehmeria nivea* (L) Gaud) is a multipurpose cellulose-polymer long fiber that are used in various textile industry commodities together with cotton, linen, and others. In the other hand, the structure and morphology of the stems of ramie plants show slightly different characters from other fiber plants [1]. Also, the character of planting and harvesting ramie affects the quality of the fiber produced. The usual ramie harvest period is based on morphological characteristics which are generally between 68-75 days or 10–11 weeks. In Indonesia, several ramie clones showed faster growth and fiber elongation which is indicated by the harvest time based on the determination of when the growth rate slowed at 6–8 weeks. Considering the average time of existing clones and harvesting, it is necessary to determine the optimum harvest time to observe an increase in the fiber quality [2].

Ramie has a morphology in form of macro fibrils bundles that is composed of fine fibers bound with plant sap components known as pectin compounds. The chemical content of natural fibers generally shows the composition of lignin 6–7% and hemicellulose 13–16% [3], while the rigid and strong structure of ramie is due to the high pectin content of 3–27% [4]. Therefore, efforts are required to reduce the pectin content to become a material suitable for spinning.

The key to effective dissociation of cellulose microfibril from pectin bonds is by chemical and biological degumming processes [5, 6]. Since the processing with conventional chemical degumming requires high energy consumption which increases the risk of production costs, there is a considerations to adopt environmentally friendly technologies have led to a trend towards the development of bio degumming biotechnology. Biological degumming has the advantage of being environmentally friendly because it does not produce hazardous waste and also saves energy as well as water when compared to other technologies [7].

The application of microbes in degumming has led to several advances in separating the cellulose from pectin. As the pectin degrades, the ramie fiber is released from the gum which later it becomes smooth and well decomposed. A development of biological degumming technology (bio degumming) is carried out by various methods such as the use of enzymes that hydrolyze pectin fast enough 2–24 h, with a controlled

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process that causes no damage and the price of commercial enzymes that burdens the production costs.

Hydrolysis of pectin to a simpler galacturonic acid is a source of carbon and nutrients for the growth of microorganisms and the production of pectinase during the degumming process [8, 9]. In the principle of bio degumming, microorganisms used decompose non-cellulosic materials that are used for growth during the degumming process. These microorganisms produce pectinase, hemicellulase, ligninase [10]. The biodegradation of pectin form polysaccharides, mainly from (1–4)-linked d-galacturonic acid and its methyl ester occurs as a synergistic product and the action of different extracellular enzymes [8, 11]. Moreover, two different classes of pectinolytic enzymes depolymerize pectin or form non-esterides by transilluminative cleavage (lyases) or by hydrolysis (hydrolases). In addition, the ester bonds in pectin are hydrolyzed by pectinesterase, while the pectinolytic enzymes found in plants and microorganisms include bacteria, yeast, and fungi [5].

An alternative biodegumming as a biotechnological step has been reported using bacteria [11] from various species of *Clostridium, Pseudomonas*, and *Bacillus* have been identified as retting agents [12] which include *B. clausii* [13], *B. pumilus* DKS1 [14], *B. cereus* P05, and *Pseudomonas* sp. X12 [15], *B. cereus* hn1-1 [16], *B. tequilensis* SV11-UV37 for degumming hemp fiber by solid-state fermentation [17], *B*. sp. HG-28 [18], *B. licheniformis* HDYM-04 [19], and hemp fiber that is effectively fermented within 9 days by *B. polymyxa* [20].

Screening fungi for potential bio degumming have been stated with the selected microbe *Penicillium chrysogenum* IFO 4626 (Q 176) which was mutated to have the good pectinolytic performance to be used for degumming textile fibers [21]. Furthermore, fungal applications for enzyme production in retting kenaf fibers have been reported with *Aspergillus fumigatus* R6 [22], and for flax with *A. niger* [23]. In this study, pectinolytic fungal isolates were screened to determine the optimal pectinase enzyme activity to improve the quality of ramie fiber. Therefore, a bio degumming method was developed using fungal isolates that have beeb selected as superior pectinolytic fungus *Rhizopus* sp. and also optimization of raw fiber based on harvesting time.

2. Methods

2.1. Materials

The material ramie fiber was obtained from ramie farm CV RABERSA, Wonosobo, Central Java. The sample 50-day and 60-day harvested were collected and decorticated using a decorticating machine with a capacity of 3HP. The pectinolytic fungi consisting of *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Rhizopus* sp. as a collection of the Microbiology Laboratory, Biology Department, University of Padjadjaran, Indonesia. The use of chemicals in form of citrate buffer pH 4, 5, 6, 7, 8; Citrus Pectin, Dinitrosalycilate (DNS), 0.85% sterile physiological NaCl, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), $(NH_4)_2SO_4$, K_2HPO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$.

2.2. Tools

In this study, the equipment used is the Tabai 6PHH-200 oven, analytical balance. Meanwhile, the instruments were employed to investigate the characteristics of the fibers includes Perier Elmer's Fourier transform infrared (FTIR) spectrophotometer, type light microscope, FESEM Thermo Scientific QuattroS, UV-Vis spectrophotometer mode PS-2600, Shimadzu XRD-7000 MAXima-X, angle 10–35° scan speed 2°/s 40 kV 30 mA, TGA Perkin Elmer Diamond TG/DTA 4000 instrument at 25–750 °C.

2.3. Fungal inoculum preparation

Activation of isolates: Fungal isolates were activated for 2 × 24 h in a tube with PDB nutrient broth with a shaker at a speed of 120 rpm and a temperature of 25–27 °C. The spores that grow were used as a biostarter for the degumming process and the fungal growth was measured directly or DC (Direct Count) with TPC (Total Plate Count) according to [24] through a series of dilutions and palting on Potatoes Dextrose Agar (PDA) medium in duplicate. Observations were made every 24 h for 10 days of fermentation and the parameter measured was the number of fungal cells/spores (cfu/ml). During fermentation, fungal growth was incubated at room temperature (25–27 °C) for 3 × 24 h and calculated using the Total Plate Count (TPC) method.

2.4. Biodegumming process

The bio degumming process for selection and fiber production is shown in Figure 1. Pre-treatment process for the decorticated of the bark of was carried out by a press machine, followed by soaking in water for 1 × 24 h in an open bath. The bundles of ramie were immersed in a batch fermenter in a submerged state with a liquid ratio of medium: bio starter isolate: ramie biomass of 10:1:1 (v/v). Furthermore, the degumming process was carried out at room temperature, medium pH 7, and incubated for 10 × 24 h to screen isolates, while the production of fiber was carried out only 2 × 24 h.

In addition, using of fungi during degumming was different in two conditions of fermentation: The screening *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Rhizopus* sp. each in a separate batch with degumming conditions at 25–27 °C, pH 6 and incubated for 10 × 24 h; while for enzymatic production, selected-isolate fungus with conditions at 25–27 °C, medium pH 7 and incubated for 2 × 24 h. The bleaching process using 0.4% hydrogen peroxide at a pH of 7.0 ± 0.2 was maintained for 60 min. Furthermore, the fibers were rinsed 3 times under running water, dried, combed, and prepared as samples to be tested for characterization.

2.5. Enzyme assay

Enzyme pectinase was carried out by DNS assay according to [25] by measuring the activity unit (U) of degumming pectinase crude extract on a spectrophotometer with a wavelength of 550 nm. The parameter measured was the number of activity units (U) of pectinase produced by isolates fungi during the fermentation process. Furthermore, fermented sample was 3 mL, centrifuged for 20 min at a speed of 1000 rpm, and 0.2 ml of supernatant, 0.1 ml of pectin, and 0.7 ml of pH 6 buffer were added. This enzyme solution was incubated in a water bath at 27 °C for 10 min. Also, the supernatant was transferred to a cuvette and 1 ml of Dinitrosalycilate (DNS) was added. The incubation results were analysed by spectrophotometer at a wavelength of 550 nm and observations were made every 24 h for 10 days. Each sample was tested triple and the conversion of galacturonic acid activity values from the results of spectrometric absorbance analysis was conducted using a standard galacturonic acid curve. The degumming stage was carried out to measure the number of activity units (U) of pectinase produced by the tested-fungi during the degumming process [8].

2.6. Optimization of pH and temperature in bio degumming

Optimization of pH was carried out by varying the value (4, 5, 6, 7, 8) of the citrate buffer on the crude extract of the enzyme produced by the fungi isolate in the degumming process. Temperature optimization was carried out by supernatant with each different pH buffer incubated in a water bath at 25 °C, 30 °C, 35 °C, 40 °C, and 45 °C for 10 min. Furthermore, the supernatant was put into a cuvette, 1 ml of DNS was added, and the absorbance was measured with a spectrophotometer at a wavelength of 550 nm. Meanwhile, observations on each sample were tested triple.

2.7. Fiber characterization after bio degumming

Degummed ramie fiber was tested at Laboratory Test of Centre for Textile Bandung, Indonesia with the parameters of the strength level

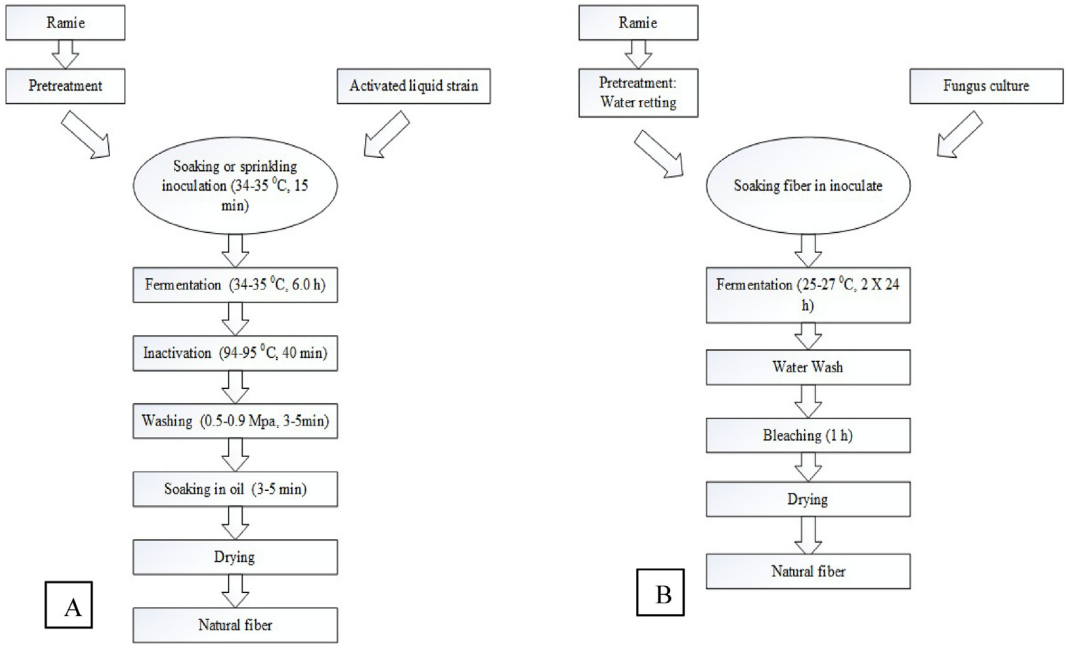

Figure 1. Schematic of the fiber production process: (A) comparative pathway of bio degumming with bacteria [26], (B) bio degumming process pathway with fungi.

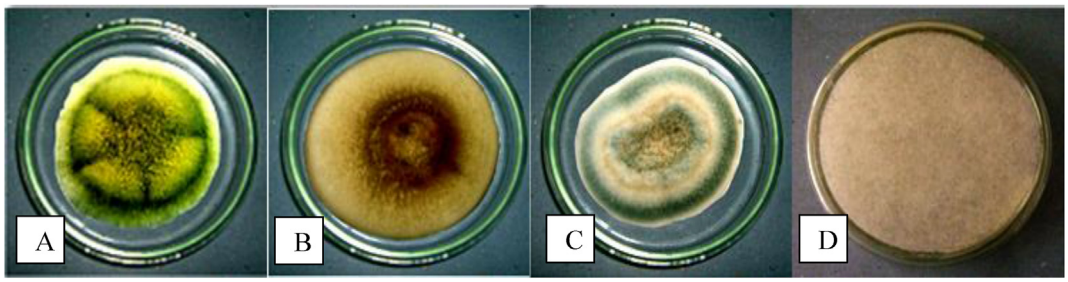

Figure 2. The Pectinolytic fungus from ramie plant (A) *Aspergillus* sp.; (B) *Fusarium* - sp.; (C)*Penicillium* sp.; (D) *Rhizopus* sp.

(tensile and the elongation) and expressed by tenacity based on the quality test of SNI 08-1112-1989 using MESDAN Tensolab 5000. Meanwhile, the fiber fineness was expressed by the denier based on the test quality SNI 08-1111-1989. The all mechanical testing replicated 4 times. Furthermore, ramie fiber testing was carried out at the Biomaterials Laboratory of the Indonesian Institute of Sciences (LIPI), Jakarta, Indonesia in form of a Scanning Electron Microscope (SEM), Fourier-transform infrared spectroscopy (FTIR) with ATR-FTIR Spectrum Two specs, Perkin Elmer, 4000-400 cm^{-1}, resolution 4 cm^{-1}, 16 scans, and x-ray diffraction (XRD) spectra. TGA was performed using TGA Perkin Elmer diamond TG/DTA instrument in LIPI. Fibers in solid form were investigated in nitrogen environment to a maximum temperature of 450 °C at the rate of 100 °C/min. The relative mass change was recorded along the increase in temperature.

The evaluation hands feel were performed using method for sample of fabrics [27] with some modification. The samples fiber with difference

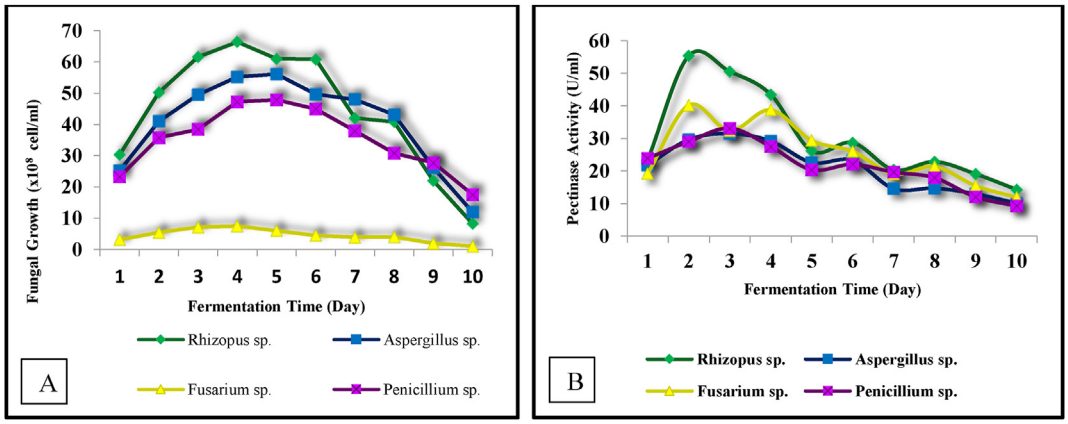

Figure 3. Profile of: (A) pectinolytic-fungi growth; (B) pectinase activity.

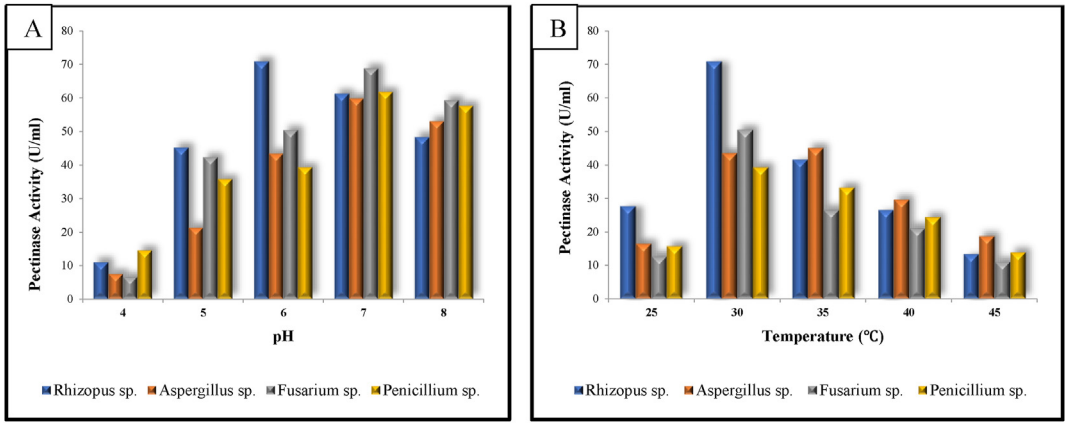

Figure 4. Production of pectinase from fungi based on pH and temperature treatments (A) pH and (B) temperature.

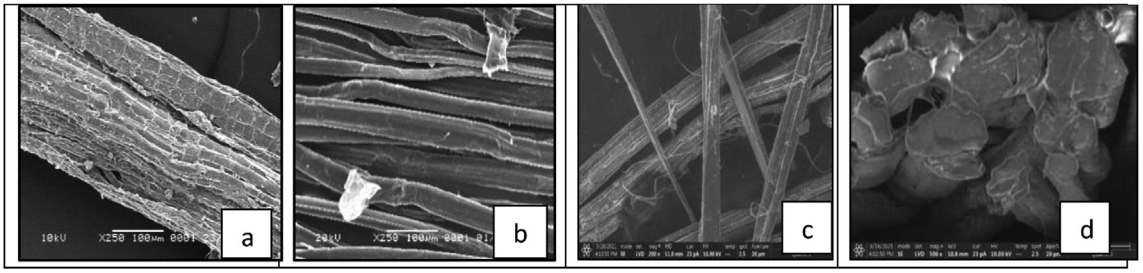

Figure 5. Microstructure of ramie fiber: (a) decorticated-fiber; (b) biodegummed-fiber; (c) longitudinal-section fiber (M: 200X), (d) Cross-section fiber (M: 500X).

appearance were ranked according to their softness, smoothness, and whiteness by a panel consisting of ten persons unaware of sample composition. The fabrics were evaluated on a scale of 1–5, where score 5 indicates a fiber with the best hands feel and score 1 indicates a fiber with the worst.

Dyeability test was done by modification of method [34]. Control ramie fiber and the results of the bio degumming process are dyed with reactive dyes by immersion at room temperature (reactive dyes are textile dyes that have the ability to react and bind covalently to the cellulose molecular chains present in flax fiber). The dye used in this test is classified as a highly reactive type based on dichlorotriazine so it can be used at room temperature. At the end of the dyeing process, the absorbance of each remaining solution (spent dye bath) was measured using a visual spectrophotometer. The more dye that is absorbed and binds to the fiber, the remaining dyeing solution will be younger and also indicated by a smaller absorbance value.

3. Results and Discussion

3.1. Selected pectinolytic fungus and optimization of bio degumming

The macroscopic colonies of the pectinolytic fungal genera *Rhizopus*, *Aspergillus*, *Fusarium*, and *Penicillium* are shown in Figure 2.

Figure 3(a) shows the growth pattern of the four fungi that grew exponentially until day 4, however for Fusarium sp., it did not show optimal growth when inoculated in the PDA. Figure 3 (b) showed that *Rhizopus* sp. had the highest pectinase activity compared to other fungi, The pectinase activity of this isolate hydrolyzed pectin in approximately two days of fermentation with 55.37 U/ml. This result provide recommendation for *Rhizopus* selected has high activity of pectinase for bio degumming of ramie fiber.

Figure 4 shows the optimum pH conditions for fungal fermentation medium and growth in the degumming process of ramie fiber. These results indicate that medium with pH 6 provide the highest level of degradation and hydrolysis of pectin by *Rhizopus* sp.; this can also be observed when the hydrolysis fermentation conditions are set at 30 °C. This is in line with the results of [21] which stated that the production of pectinase in fermentation with the liquid medium is optimum using pectinolytic fungi at a temperature of 30 °C.

Figure 5 showed that degradation and hydrolysis of pectin in surface of the ramie fiber was confirmed by Scanning Electron Microscope (SEM). Figure 5(a) showed a decorticated-fiber as a control fiber that is still as a bundle. It is observed that the defibrillation of ramie fiber is due to the loss of the pectin component in the bio degumming treatment process (Figure 5 (b)). This image also features a longitudinal section of the fiber and a cross section after bio degumming (Figure 5 (c) and (d).

Table 1. The quality of physical character of ramie fiber.

Fiber Sample	Tenacity (g/tex)	Fiber Elongation (%)	Fiber Fineness (tex)	Moisture Content (%)	Moisture Regain (%)
Control of 50-day harvested	19.77 ± 6.94	15.48 ± 1.96	2,64 ± 0.19	9.32 ± 0.02	10.66 ± 0.54
Control of 60-day harvested	20.97 ± 2.52	11.08 ± 2.55	2.92 ± 0.82	9.74 ± 0.02	10.79 ± 0.02
Biodegum of 50-day harvested	24.54 ± 0.02	12.04 ± 2.90	1.33 ± 0.17	8.23 ± 0.18	8.96 ± 0.21
Biodegum of 60-day harvested	21.76 ± 1.20	10.88 ± 1.26	1.32 ± 0.39	9.25 ± 0.46	10.19 ± 0.56
Ramie Biodegum [16]			6.25 ± 0,18		
Chemical Degum [16]			8.2 ± 0,24		

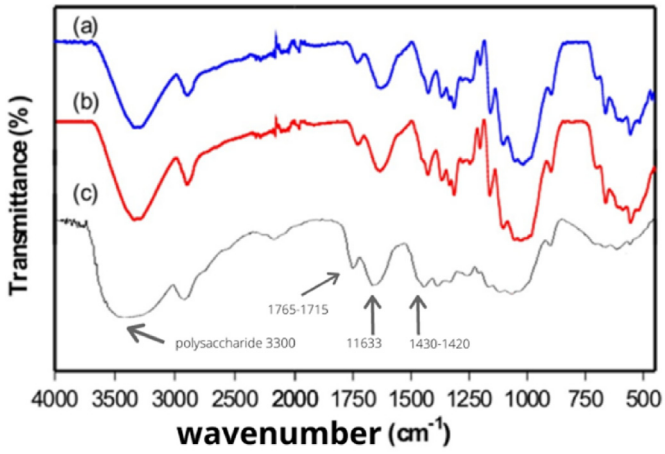

Figure 6. FTIR analysis of ramie fibers (a) bio degumming of 50-day harvested fibers; (b) bio degumming of 60-day harvested fiber; (c) control fiber.

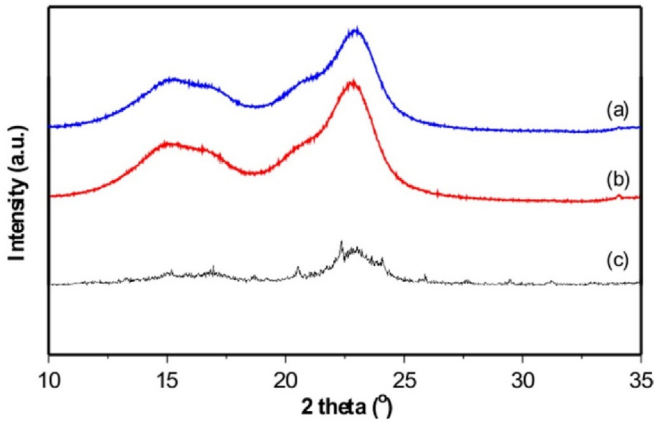

Figure 7. XRD analysis of ramie fibers: (a) bio degumming of 50-day harvested fibers; (b) bio degumming of 60-day harvested fiber; (c) control fiber.

3.2. Fibers characteristics

Degummed-fibers of ramie were then analysed for further characterization based on their physical and mechanical properties which were also confirmed by changes in molecular structure, thermal effects, hand fell character, and dyeability with dyes. The qualitative physical characters as shown in Table 1.

The bio degumming treatment showed that there was an increase in the tenacity of the ramie after the treatment at the age of 50 days and 60 days. The 50-day harvest can show a higher level of tenacity than the 60-day harvested fiber. The elongation character seems to be affected by the degumming process with a decrease in the elongation rate. The level of fineness of ramie fiber after bio degumming was able to produce very fine thing both at the use of 50 and 60 days of harvested. Although it is possible that the condition of the control fiber might be different, the results of this bio degumming can show a very high fineness of the fiber than has been reported [16]. The effect of moisture only seems to be different for the 50-day harvest, with lower moisture content and moisture regain than other fibers.

These results to confirm that the tenacity of ramie fiber is higher than that of bamboo (17 g/tex), kenaf (12.9 g/tex), and cotton (10 g/tex). Furthermore, the tenacity of the ramie fiber is twice that of cotton which allows it to be an alternative for textile manufacture [31]. The moisture return of flax has also been shown to be greater than that of cotton (8.5%), ramie (8%), and kenaf (10%) indicating that crude and degumming fibers have good water absorption ability and are used as textile materials [32]. Meanwhile, the good absorption ability of ramie is utilized for hydrophilic textiles because it can moisturize the body [33].

Figure 6 showed the FTIR spectra of the cellulose and non-cellulose compounds profile from the control fiber compared to the fiber after the degumming process. Furthermore, the spectral transmission of the hydroxyl group of cellulose was seen from the increasing peak intensity of 3315 cm^{-1} in the bio degumming fiber at the 50-day harvested fiber. At the spectrum at 1765-1715 cm^{-1}, the chemical bonds of non-cellulose components are known as the C=O vibrational strain area of the acetyl and ester groups which belong to the pectin group such as hemicellulose or carboxylic (ferulic) bonds and p-coumaric lignin or hemicellulose showed no deformation. This peak was present at 1633 cm^{-1} in the spectrum corresponding to the raw fiber due to C=O link presence which is a characteristic group of lignin that changes from the control fiber compared to the bio degummed one. From the comparison of the 3 FTIR spectra, there is a change in the sharpness of the peak spectrum which shows that a peak spectrum is sharpened due to the degumming process.

The image shows diffraction x-ray (XRD) spectra as shown in the three spectra which indicated that there is no change in crystal structure and cellulose type before and after bio degumming. These results show the character pattern of cellulose as natural fibers with a primary peak at a diffraction angle of $2\theta = 23.12^{\circ}$ and two peaks at $2\theta = 15.70^{\circ}$ and 16.68°. Meanwhile, the XRD review shows that the same diffraction pattern with high intensity is different, in terms of crystallinity between the control and bio degummed fiber (Figure 7).

This study complements data on comparative thermal thermogravimetric behavior of other natural fiber sources are from jute, sisal, cotton [31]. Table 2 shows a summary of the thermal character data of ramie by comparing the weight loss and DTG data with other natural fibers. The thermogravimetric behaviour of natural fiber has a three-stage thermal decomposition (Stage I is frying stage, Stage II is active pyrolysis stage, and stage III is passive pyrolysis stage) pattern and other plant polymer characters by providing a degradation ratio of the cellulose fraction in the range of 200–400 °C [28]. Moreover, weight loss in the first stage is the process of losing water content in the fiber, while the thermal degradation associated with the lignocellulosic structure begins at the second

Table 2. Thermogravimetric parameters of ramie and some natural fibers.

Natural fiber	Stage 1		Stage 2					Stage 3
	Weight loss (%)	DTG peak (°C)	Stage on set to (°C)	Weight loss (%)	DTG shoulder (°C)	DTG main peak (°C)	DTG tail (°C)	Weight loss (%)
Ramie 50-d harvested fiber (this study)	10	55	340	60	300	370	400	20
Ramie 60-d harvested fiber (this study)	10	50	310	65	300	360	390	28
Biodegummed ramie 50-d harvested fiber (this study)	12	55	310	58	300	370	390	15
Biodegummed ramie 60-d harvested fiber (this study)	12	50	320	70	300	360	390	10
Jute [29]	8	60	260	89	290	340	470	15
Sisal [29]	9	52	250	76	275	345	465	13
Cotton [29]	4	55	265	91	280	330	410	5
Wood [30]	2	107	290	85	270	367	400	15

Table 3. Hand feel score of the ramie fibers.

Characteristics	Control of 50-day harvested fiber	Control of 60-day harvested fiber	Biodegum of 50-day harvested fiber	Biodegum of 60-day harvested fiber
Hand feel score	1.45 ± 0.4	2.2 ± 0,2	3.75 ± 0,3	3.65 ± 0.6
Fiber characteristics	Rigid, coarse, brownish	Coarse, rigid, light brownish	smooth, micro fibrils shown, whiter	smooth, micro fibrils shown, whiter

decomposition stage. Furthermore, the main peak of the DTG curve indicates the presence of thermal decomposition of cellulose, while the shoulder and tail peaks indicate the presence of hemicellulose and lignin decomposition, respectively. Also, the weight loss at the third stage is described as another residue.

Ramie fibers at initial conditions and after bio degumming at 50 and 60-day harvested had the same pattern of thermal stability. There is no significant change before 100 °C, after that a slight mass loss happened when the temperature raised up to 310 °C, while a fast mass loss occurred between 300-400 °C. Stage I of thermal analysis start between 50-55 °C showed the volatilization of low weight compound and absorbed water. All the samples were having mass loss at the stage II that occurred on 300–400 °C, all component such as hemicellulose, cellulose, and lignin were degraded in this stage. All the samples of ramie fiber has greater DTG main peak compared to other natural fiber listed. It showed that ramie fiber has greatest thermal stability among other natural fibers.

3.3. Hand feel analysis

The fiber character using the hand feel test showed scores of 1.45 ± 0.4 and 2.2 ± 0.2 on ramie fiber 50-day and 60-days harvested with no bio degumming treatment are rigid, coarse, and brownish in color due to the presence of gum. In contrast, the scores for degummed fibers were 3.75 ± 0.3 and 3.65 ± 0.6, respectively, giving a hand feeling that they were smoother because the microfibril shown and whiter than untreated fibers (Table 3).

3.4. Dyeability of Ramie Fibers

The potential of ramie fiber to become a fabric requires good dyeability for commercial purposes. Dyeability was tested by analysing the absorbance of them after dyeing for both untreated and treated by biodegumming at both harvesting times.

Figure 8 shows the results of the spectrum at a length of 400–700 nm to investigate the high color intensity of the fiber. The results showed that 60-day harvested had a lower absorbance than 50-day harvested; while the degummed-material in old 60 days had the lowest absorbance among other specimen. Those, in this period of plant growth with proses degumming has a great dyeability. This result shows that degumming technology can increase the dyeability of the fiber, which are caused by removing the gum. It also might be several hydroxyl groups played an important role in the fixation of the dye.

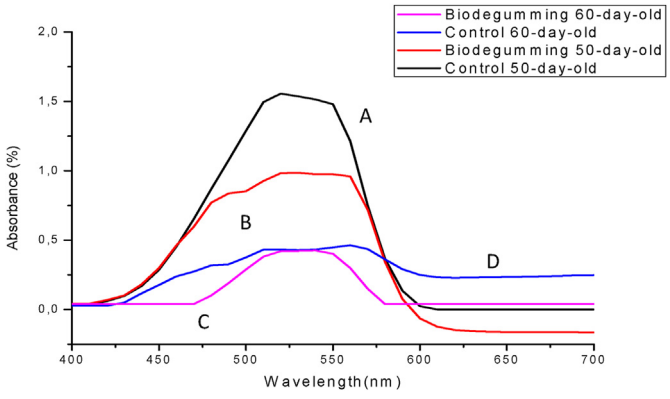

Figure 8. Dyeability of Ramie Fibers: (A) 50-day harvested fiber; (B) bio degummed of 50-day harvested fiber; (C) 60-day harvested fiber; (D) bio degummed of 50-day harvested fiber.

4. Conclusion

The development of a novel green energy procedure with a bio degumming process using: selected *Rhizopus* sp. under the optimum conditions of pH 6, temperature of 30 °C for 2 × 24 h with submerged fermentation; using 50-day harvested stem of ramie succeed to improve the quality fiber. The finest properties of the fiber showed that tenacity 24.54 (g/tex), elongation 12.04 (%), fineness 1.33 (tex), moisture content 8.23 (%), and moisture regain 8.96 (%). Meanwhile, the fiber at initial conditions and after bio degumming at 50 and 60-day harvested had the same pattern of thermal stability with the main peak of DTG showed that cellulose of ramie had the greatest thermal stability among other natural fibers. Fiber testing with dyeability test showed that the degummed-fiber harvested 60 days had the greatest dye fixation ability. The bio degumming process is proven to improve fiber quality and increase the dyeability of the fiber which can be used for future applications.

Declaration

Author contribution statement

Asri Peni Wulandari: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Junaedy Raganzan Purba, Budi Irawan: Performed the experiments.

Nanang Masruchin, Maya Ismayanti, Rr. Srie Gustiani: Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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