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ENZYMATIC DEGUMMING USING XYLANASE AND PECTINASE TO IMPROVE BRIGHTNESS AND FINENESS QUALITY OF RAMIE FIBER (*Boehmeria nivea* L.) AS TEXTILE RAW MATERIAL

Degumming Enzimatis menggunakan Xilanase dan Pektinase untuk meningkatkan Kualitas Kehalusan dan Kecerahan dari Serat Rami (*Boehmeria nivea* L.) sebagai Bahan Baku Tekstil

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ABSTRACT
Ramie (Boehmeria nivea *L.*) *fiber is an alternative to cotton fiber, and the degumming process is crucial for preparing it as a textile raw material. This study investigates the enzymatic degumming of ramie fiber using a combination of xylanase enzyme from* Bacillus halodurans *CM1 and commercial pectinase enzyme. The objective is to assess the impact of enzymatic degumming on the physical properties (weight loss, whiteness index, tenacity, elongation, and fineness) of ramie fiber. The degumming process was conducted in a shaker incubator at a temperature of 50 °C, pH 9, and 150 rpm. The degumming treatment involved 3% v/v xylanase, 1% v/v pectinase, 1% v/v surfactant, and 0.05% v/v* $NaClO_2$*. The results show that the bleaching treatment (S6) resulted in higher fiber weight loss (9.52%), whiteness index (87.87%), tenacity (20.08 g/Tex), and fineness (1.05 denier) compared to the non-bleaching treatment.*

Keywords: *Enzymatic degumming, fiber fineness, pectinase, ramie fiber, xylanase*

ABSTRAK
Serat rami (*Boehmeria nivea* L.) merupakan salah satu serat alternatif untuk mensubstitusi kebutuhan kapas. Proses penting dalam membuat produk siap pintal dan jadi dari serat rami adalah proses degumming. Pada penelitian ini, proses degumming enzimatik menggunakan kombinasi enzim xilanase dari *Bacillus halodurans* CM1 dan enzim pektinase komersial. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh degumming enzimatik dengan kombinasi enzim xilanase dan pektinase terhadap perbaikan sifat fisik (*pengurangan berat, derajat putih, kekuatan tarik, kemuluran dan kehalusan*) serat rami yang dibandingkan antara dengan *proses pemutihan* dan tanpa *proses pemutihan*. Proses degumming dilakukan dalam inkubator shaker pada suhu 50°C, pH 9 dan 150 rpm. Variabel perlakuan degumming adalah xilanase 3%v/v, pektinase 1%v/v, surfaktan 1%v/v dan $NaClO_2$ 0,05%v/v. Hasil penelitian menunjukkan bahwa perlakuan dengan *proses pemutihan* (S6) memberikan kehilangan *pengurangan berat* (9,52%), *derajat putih* (87,87%), *kekuatan tarik* (20,08 g/Tex) dan *kehalusan* (1,05 denier) lebih tinggi dibandingkan dengan perlakuan tanpa *proses pemutihan*.

Kata Kunci: Degumming enzimatis, kehalusan serat, pektinase, serat rami, xilanase

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INTRODUCTION

Ramie (*Boehmeria nivea* L.) is a perennial herb and an important bast fiber plant. Known as the queen of natural fibers, ramie fiber possesses attractive luster, high tenacity, enhanced strength, and good microbial resistivity (Cheng et al. 2020). The chemical composition of ramie consists of 68.6-76.2% cellulose, 13.1-16.7% hemicellulose, 1.9% pectin, 0.6-0.7% lignin, and 0.3% wax. The high cellulose content makes ramie fiber one of the strongest natural fibers (Chaudhuri et al. 2020). Decorticated ramie fiber contains a significant amount (19-30%) of gummy matter, which is a heterogeneous mixture of carbohydrates, primarily pectin and other polysaccharides such as mannose, galactose, rhamnose, arabinose, and xylans (Mitra et al. 2014). Pectin and hemicellulose, specifically mannan and xylan, can be easily hydrolyzed in a high-concentration alkali solution, while cellulose remains unaffected.

In this study, sodium hydroxide was solely used to adjust the pH of the degumming solution, as ramie fiber degummed with a NaOH solution exhibited poorer tensile properties (Lu 2017). In order to utilize these natural fibers, they need to be released from the stem. Traditional dew retting, the method used for fiber extraction, has several disadvantages and often fails to ensure consistent fiber quality. A potential solution to overcome these issues is the replacement of the traditional retting process with a biocatalytic process involving a combination of pectinase and hemicellulase activities (de Prez et al. 2020). Enzymatic treatment effectively removes noncellulosic compounds from the fiber surface, resulting in a clean and highly hydrophilic surface (Zolriasatein and Yazdanshenas 2014). It is also useful in eliminating hygroscopic pectic and hemicellulose materials, thereby producing more homogeneous fiber surfaces (George et al. 2014).

Moreover, enzymatic degumming significantly improves the fineness of ramie fiber during the degumming process, as observed by Nandyawati et al. (2021). Electron microscopic analysis of enzymatically and chemically degummed ramie fiber revealed slight differences in their surface characteristics. The enzymatically degummed ramie fiber exhibited a relatively smoother surface compared to the chemically degummed fiber. Hanana et al. (2015) employed mechanical and chemical treatments to separate the natural fibers and break down fiber bundles, facilitating the penetration of enzyme solutions into the fibers. Due to the complex composition of the gum, effective degumming of ramie requires multiple enzyme systems capable of degrading the complex gummy matter, including hemicellulose, pectin, and lignin.

The non-cellulosic gummy constituents, namely pectin and lignin, exhibit different chemical natures, suggesting that a single enzyme component cannot effectively degum ramie fiber (Biswas et al. 2016). A study by Ding et al. (2014) demonstrated that using a mixture of pectinase and xylanase enzymes for degumming ramie fiber results in improved removal of gum-like materials while retaining fiber strength. Additionally, crude xylanase-pectinase enzymes have been successfully employed as biobleaching agents for soda anthraquinone pulp in the plywood industry (Sharma et al. 2017).

Degumming processes involving pectinase, mannanase, and xylanase have been shown to reduce residual gum content and enhance the tenacity and whiteness of ramie fiber (Shu et al. 2020). During degumming, enzymes are uniformly distributed in the degumming liquid. However, as degumming progresses, degradation products released from the fibers gradually accumulate in the liquid, inhibiting enzyme activity (Fan et al. 2015). Enzyme mixtures, particularly pectinase and hemicellulose, effectively remove the gum from decorticated ramie fiber. Singh et al. (2020) also found that degumming with xylanase and pectinase enzymes can substitute the environmentally unsustainable and energy-consuming chemical scouring technique, reducing the consumption of harmful alkaline scouring chemicals. In this study, enzymatic degumming using a combination of xylanase and pectinase enzymes was employed to improve the physical properties (weight loss, whiteness index, tenacity, elongation, and fineness) of ramie fiber. Additionally, the enzymatic degumming process was compared to bleaching with the addition of a small amount

of sodium chlorite ($NaClO_2$), serving as a greener approach for industrial-scale bleaching. The objective of this research was to evaluate the effects of enzymatic degumming, both with and without bleaching, on ramie fiber quality.

MATERIALS AND METHODS

Location and time

The research was conducted from September to December 2020. Most of the research was conducted in the laboratory of the Center for Bioindustrial Technology BPPT, while some other analyses were conducted at the accredited laboratories of Politeknik STTT Bandung and the Center for Polymers Technology BPPT.

Materials

The chemical materials used for enzymatic assay were as follows: 0.5% xylan beechwood, 2 mg/mL xylose, 0.05 M Tris-Cl buffer, and DNS reagent containing 1% w/v dinitrosalicylic acid, 20% w/v potassium sodium tartrate, 1% w/v sodium hydroxide, 0.05% w/v sodium sulfite, and 0.2% w/v phenol. The materials used for enzymatic ramie degumming were decorticated ramie fibers (CV. Rabersa, Wonosobo, Central Java, Indonesia), 3% xylanase (produced by *B. halodurans* CM1 (Wibowo et al. 2016) from the Center for Bioindustrial Technology), 1% pectinase (product of PT. Chemira), and the following chemicals: 0.1 M sodium hydroxide, 1% surfactant (SB Chem 9201 from PT. Sadya Balawan, Bogor, West Java, Indonesia), and 0.05% sodium chlorite ($NaClO_2$).

The equipment used for this study included an incubator shaker, oven, analytical balance, micropipette of 200 µL and 1,000 µL, thermomixer, water bath, and spectrophotometer.

Methods

The analysis of enzymatic volumetric activity was conducted using the Bailey method. Xylan beechwood substrate was dissolved in 10 mL of buffer solution, resulting in a 0.5% xylan solution. The standard curve of xylose (2 mg/mL) was prepared by diluting it in ddH_2O to obtain concentrations of 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, 1 mg/mL, 1.2 mg/mL, 1.4 mg/mL, 1.6 mg/mL, 1.8 mg/mL, and 2 mg/mL. The xylanase volumetric activity assay was performed by adding 450 µL of 0.5% xylan substrate solution in buffer solution (pH 9, the optimum pH for enzyme activity (Ulfah et al. 2011)) to which 50 µL of enzyme solution was added, followed by incubation for 5 minutes. Enzymatic reactions were stopped by adding 750 µL of DNS solution. The reaction mixture was heated in boiling water for 5 minutes and then diluted with 250 µL of ddH_2O. Absorbance was measured at a wavelength of 540 nm.

The process of ramie degumming was conducted on seven samples, as presented in Table 1, which consisted of a control and two different treatment groups: with bleaching (S2, S4, and S6) and without bleaching (S1, S3, and S5). The degumming process of ramie fiber was carried out in 1,000 mL Erlenmeyer flasks containing 20 g of decorticated dried ramie fiber and 400 mL of degumming solution (material to liquid ratio of 1:20). The samples were incubated in a reciprocal shaking incubator at 50 °C (Chuliveri 2016), 150 rpm for 180 minutes. The degumming solution was adjusted to pH 9 using NaOH (the optimum pH for xylanase enzyme activity (Ulfah et al. 2011) and the optimum pH for pectinase enzyme activity in slightly alkaline conditions (Yadav et al 2022)). The samples were analyzed for xylanolytic activities using the Bailey method assay. Degummed fiber samples

Table 1. Formulation of the degumming solution

Materials	Sample Code						
	C	S1	S2	S3	S4	S5	S6
3 % Xylanase	-	v	v	-	-	v	v
1 % Pectinase	-	-	-	v	v	v	v
1 % Surfactant	-	v	v	v	v	v	v
0.05 % NaClO2	-	-	v	-	v	-	v

were then rinsed with running water and oven-dried at 50 °C. The weight loss percentage of the dried samples was determined by comparing the fiber weight before and after degumming.

Characterization of ramie fiber was initially determined by measuring weight loss (%) and whiteness index (%). The weight changes of the fibers after degumming were determined by calculating the weight loss (%) according to the formula provided by Hanana et al. (2015):

$$Weight\ loss\ (\%) = [(W2 - W1)/W1] \times 100$$

where W1 (g) represents the weight of the dry fiber before degumming, and W2 (g) represents the weight of the dry fiber after degumming. Each experiment was repeated three times. The whiteness index was observed using the Color Muse colorimeter, which combines a colorimetric sensor with light from 3 to 4 different LEDs and employs an interference filter that closely follows the transmission characteristics of the tristimulus curves of the CIE standard observer (Kirchner et al. 2018). Each experiment was conducted at three points and repeated three times.

The sample with the highest result was then tested for tenacity (g/Tex), which is an important mechanical property of ramie fiber (Hendra 2017), as well as elongation (%), fineness (denier), and surface morphology using scanning electron microscopy (SEM). Tenacity was measured using the SNI 08-1112-1989 standard and an Instron Universal Testing System 3380 Series at Politeknik STTT Bandung. Surface morphology analysis was performed using scanning electron microscopy at the Center for Polymers Technology BPPT.

RESULTS AND DISCUSSION

The volumetric activity of xylanase was analyzed before the degumming process, with a standard curve of xylose (Figure 1). The results of xylanase volumetric activity, as presented in Figure 2, showed that the volumetric activity of xylanase without bleaching (S1, S3, and S5) was 22.12 U/mL, 2.9 U/mL, and 25.40 U/mL, respectively. Meanwhile, the samples with bleaching (S2, S4, and S6) showed xylanase volumetric activity of 22.43 U/mL, 3.29 U/mL, and 25.40 U/mL, respectively.

The enzymatic degumming process of ramie fiber was compared with bleaching (with 0.05% $NaClO_2$) and non-bleaching. The samples S1, S3, and S5 underwent enzymatic degumming without bleaching, while the samples S2, S4, and S6 underwent enzymatic degumming with bleaching. In another study (Subash et al. 2021), the characterization of ramie fiber was determined by hemicellulose and cellulose content, density, moisture, ash content, lignin quantification, and tensile analysis. However, in this study, the initial characterization of ramie fiber was determined by weight loss and whiteness index.

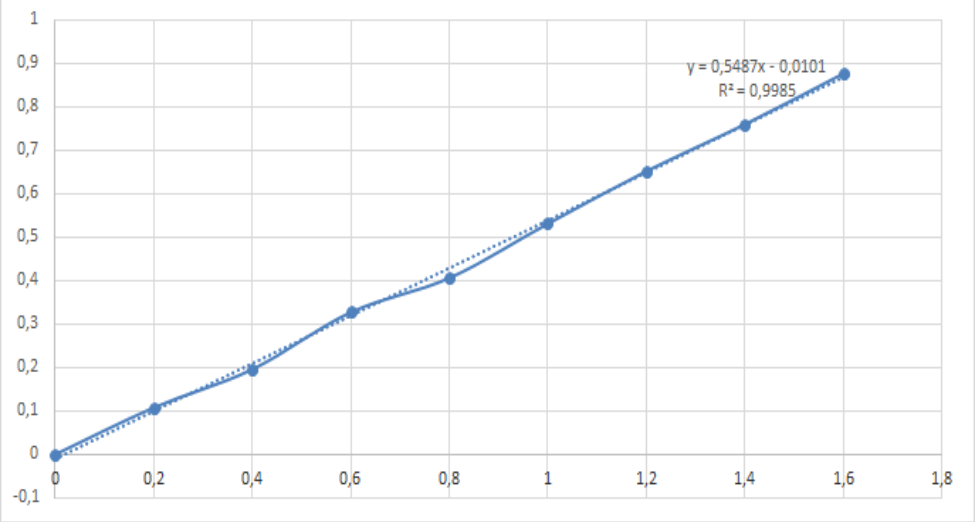

Figure 1. Standard curve of xylose

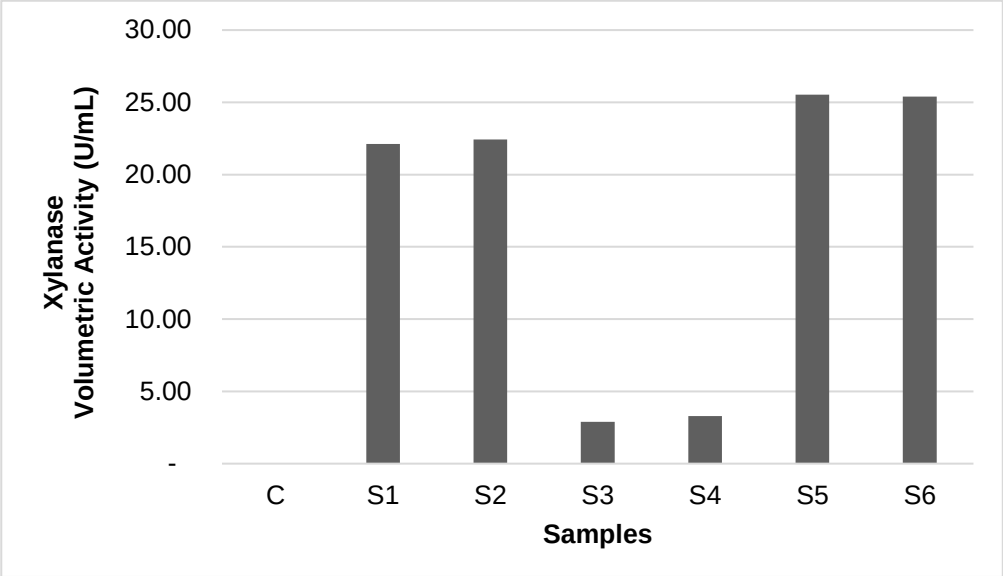

Figure 2. Volumetric activity of xylanase

The weight loss data, as presented in Figure 3, showed that the weight loss of the control sample (C) was 5.06%. The weight loss for 3% xylanase (S1) was 8.82%, slightly higher compared to 3% xylanase with bleaching (S2) at 8.74%. The sample with 1% pectinase (S3) showed a weight loss of 8.70%, lower than 1% pectinase with bleaching (S4) at 9.04%. The weight losses for 3% xylanase + 1% pectinase without bleaching (S5) and with bleaching (S6) were 8.54% and 9.52%, respectively. The highest weight loss for non-bleached samples was 8.82% (S1), and for bleached samples was 9.52% (S6), which is supported by the study of de Prez et al. (2020), indicating that when pectinases and hemicellulases are combined, the polymer network surrounding the elementary fibers within the technical fiber bundle should be degraded more thoroughly, resulting in a finer fiber.

The bleaching process performed with $NaClO_2$ also provides an advantage with less weight loss on the fabric (Dursun and Yıldız 2022). Enzymatic treatments also contribute to the high controllability of the retting process, implying less fiber damage and a higher fiber yield (Prez et al. 2019). The data in Figure 4 show the L* (lightness) value, which is stated as the whiteness

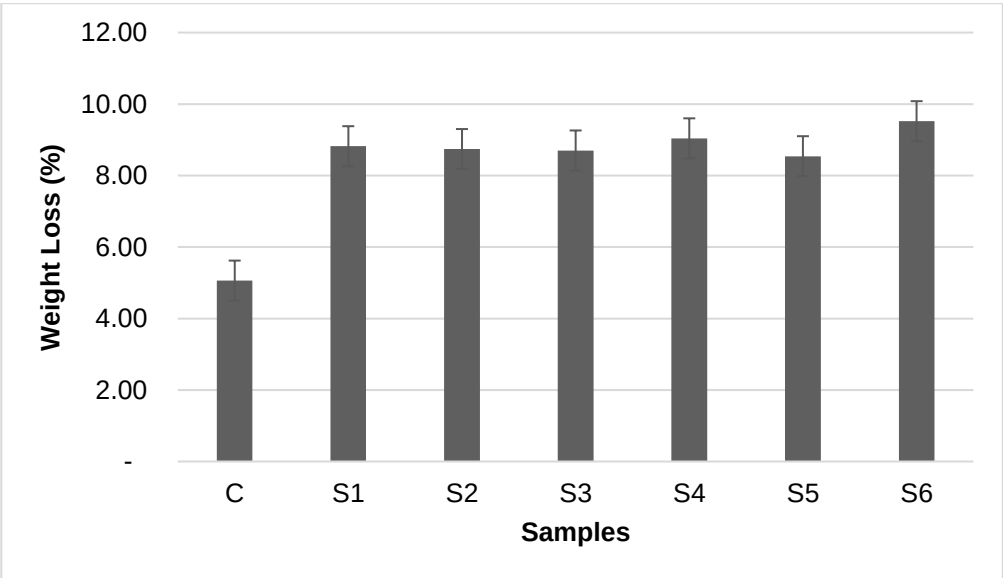

Figure 3. Average weight loss in the degumming process of ramie fiber

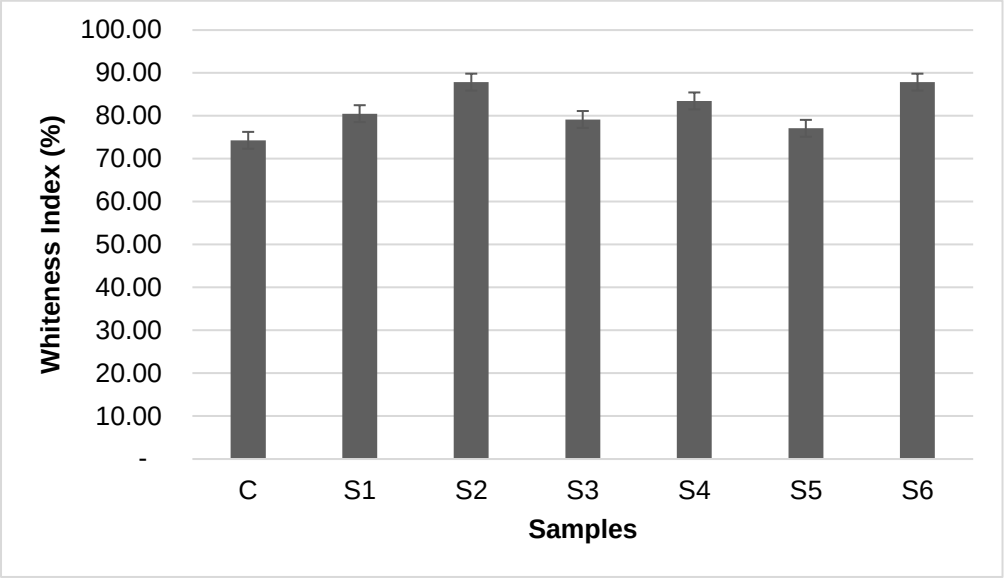

Figure 4. Average whiteness index in the enzymatic degumming process of ramie fiber

index. The control sample of decorticated ramie fiber showed a whiteness index of 74.30%, with higher percentages indicating greater lightness of the ramie fiber. After bleaching, the optical characteristics were further enhanced, resulting in higher fiber brightness and whiteness (Singh et al. 2020). The whiteness index for non-bleached samples, which were 3% xylanase (S1), 1% pectinase (S3), and 3% xylanase + 1% pectinase (S5), were 80.52%, 79.16%, and 77.10%, respectively. Meanwhile, the samples with bleaching, which were 3% xylanase (S2), 1% pectinase (S4), and 3% xylanase + 1% pectinase (S6), were 87.30%, 83.49%, and 87.87%, respectively. The highest whiteness index for non-bleached samples was 80.52% (S1), and for bleached samples was 87.87% (S6). The improvement in whiteness index after bleaching is mainly due to the delignification of the fiber surface. Enzymatic bleaching of fibers with glucose oxidase may yield better results in terms of whiteness index (Shahid et al. 2016). The study by Zolriasatein and Yazdanshenas (2014) showed that the mixture of three enzymes (cellulase, xylanase, and pectinase) improved the whiteness index and facilitated the penetration of oxidizing whitening agents into the fiber structure.

Based on the weight loss and whiteness index data, the samples of ramie

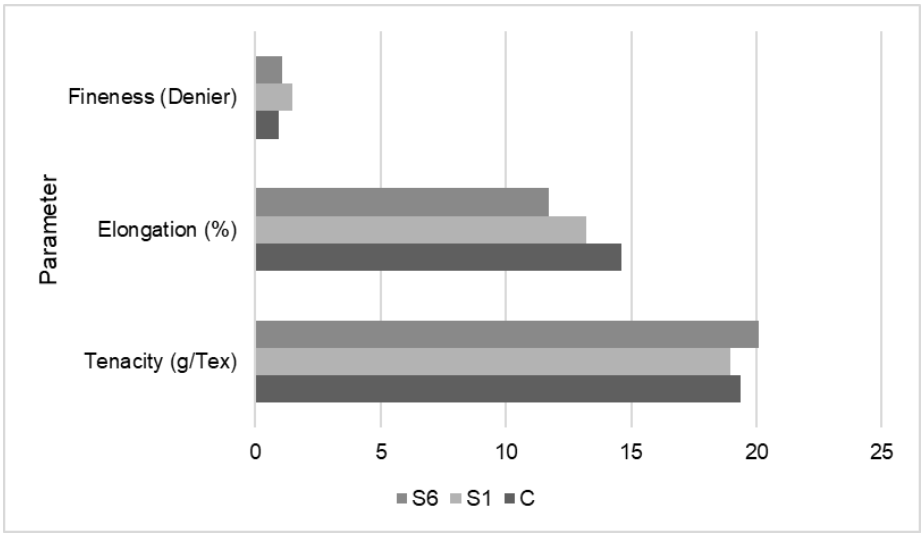

Figure 5. Characterization of ramie fiber (fineness, elongation, and tenacity) by enzymatic degumming process

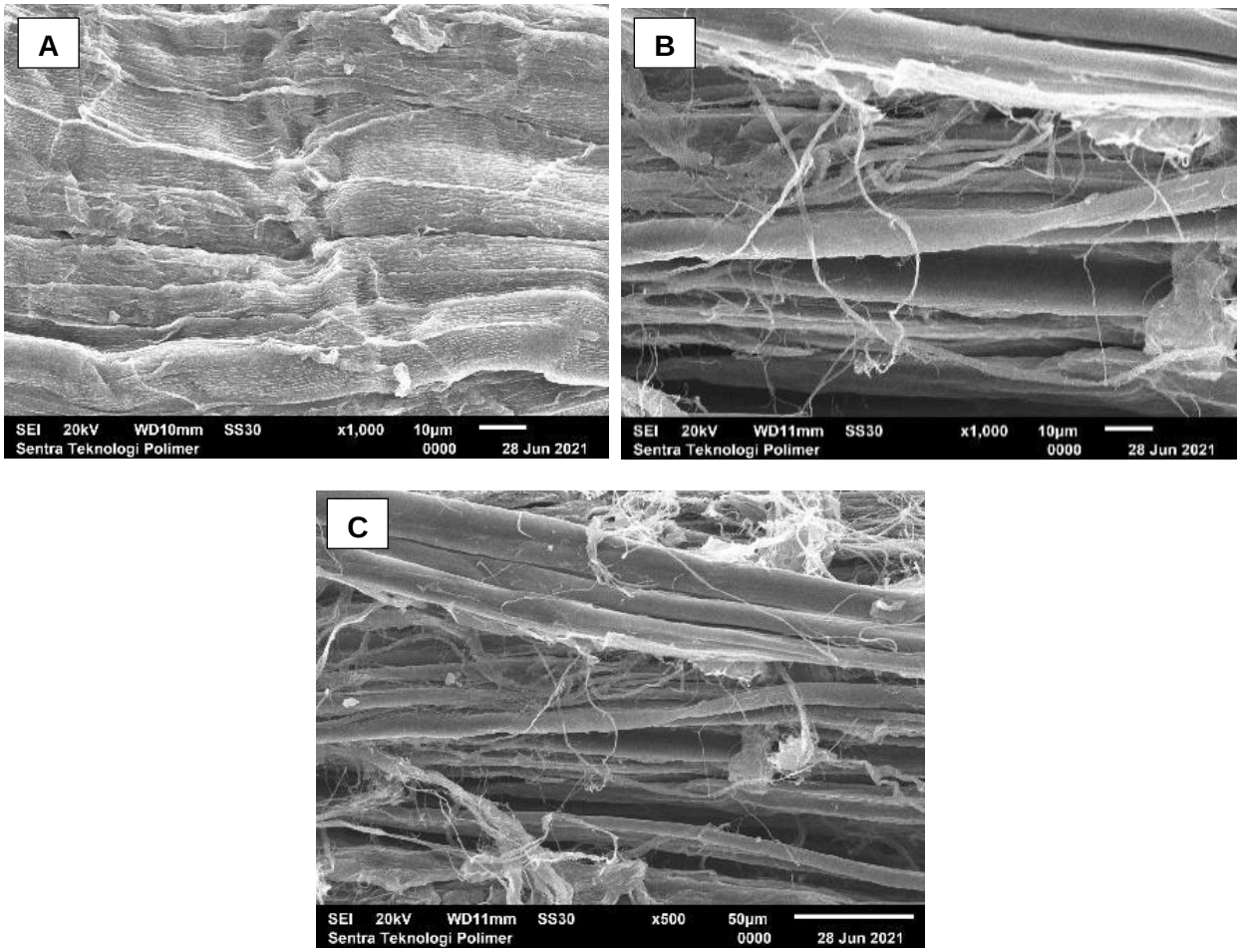

Figure 2. Morphology of ramie fiber observed through scanning electron microscopy (SEM). (A) SEM of ramie fiber decortication, (B) SEM of ramie fiber degumming, (C) SEM of ramie fiber degumming and bleaching

fiber from S1 and S6 were further analyzed for tenacity (g/Tex), elongation (%), fineness (denier), and surface morphology (SEM), as presented in Figures 5 and 6. The characterization of 3% xylanase + 1% pectinase with bleaching (S6) showed higher tenacity of 20.08 g/Tex and fineness of 1.05 denier compared to 3% xylanase + 1% pectinase without bleaching (S1), which had values of 18.96 g/Tex and 1.47 denier, respectively. Compared to the control treatment (19.37 g/Tex and 0.95 denier), enzymatic treatments resulted in improved strength but no enhancement in fineness. The study by Prez et al. (2019) also found that enzymatic-retted flax fibers were coarser but stronger compared to water-retted flax fibers. The content of polysaccharides in ramie fiber may affect the strength of the fibers due to different bonding forces between fibrils. Ramie fiber tenacity increases as the contents of galactoglucomannan, glucomannan, and xylan decrease. This means that the removal of these components can improve the fiber tenacity (Li et al. 2016). Although the lignin on the fiber bundle surface had been removed during the degumming process, the study by Jiang et al. (2017) indicates that a large portion of lignin remains inside the fiber cell. Therefore, a stronger degumming treatment or pretreatment process is necessary. The elongation of 3% xylanase + 1% pectinase with bleaching (S6) showed the lowest value of 11.72% compared to 3% xylanase + 1% pectinase without bleaching (S1) at 13.2% and the control at 14.63%. This differs from the study by Dursun and Yıldız (2022), which stated that components such as cellulose and pectin in raw fabric fibers are removed from the fiber by the bleaching process and cause an increase in the percentage of elongation.

The results of the surface morphology showed that decorticated ramie fiber (A) was covered with gums containing pectin, hemicellulose, and lignin substances. The removal of these components resulted in an

increase in individual bundle exposure, as observed from the SEM micrographs (Lu 2017). The surface morphology of (B) and (C) showed clear longitudinal channels between the entities of ramie, which are not apparent in the decorticated ramie samples (A). On the other hand, the surface morphology of decorticated ramie showed a rough and coarse texture, and enzymatic degumming removed the encrusting materials, resulting in fibers with a smoother surface. The degumming solution, which contained 3% xylanase (S1), released some gums from the fiber surface, as well as with bleaching (S6). The morphological changes of the treated fibers with 3% xylanase + 1% pectinase showed the presence of cellulose microfibrils, indicating that lignin and other non-cellulosic materials were greatly removed from the fiber surface. The change in fiber structure indicates that enzyme treatment could enhance the permeability of bleaching chemicals and dissolve lignin in subsequent bleaching operations (Zolriasatein and Yazdanshenas 2014).

This study has some limitations, such as the lack of observation of the chemical content of ramie fiber and the absence of calculations for degumming yield and residual gums of the degummed fiber. However, according to Jiang et al. (2018), major indexes used to evaluate the degumming quality of textile fibers include gum content, fiber fineness, fiber tenacity, and whiteness, which supports the validity of our results.

CONCLUSION

Enzymatic degumming resulted in less fiber damage and a higher fiber yield. The treatment of 3% xylanase + 1% pectinase with bleaching (S6) showed higher weight loss, whiteness index, tenacity, and fineness of ramie fiber compared to non-bleaching (S1). However, the treatment of 3% xylanase + 1% pectinase without bleaching (S1) showed higher fiber elongation compared to the bleaching treatment. Based on tensile properties, elongation, and fiber fineness, enzymatic degumming with bleaching did not yield exceptional results compared to the control, but it produced competitive results compared to conventional degumming. It is also important to characterize the functional groups and crystal properties of ramie fiber before and after the degumming process. A higher concentration of enzymatic degumming treatment can have an impact on the whiteness index and weight loss of raw ramie fiber, improving its physical condition and expanding its utilization. Mechanical and physical pretreatment processes should be considered to further improve the fiber quality of ramie in future studies.

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